ISSN 0973-3930

Volume 37 | Issue 3 | July–September 2017

International Journal of Diabetes in Developing Countries

Official Publication of
Research Society for the
Study of Diabetes in India

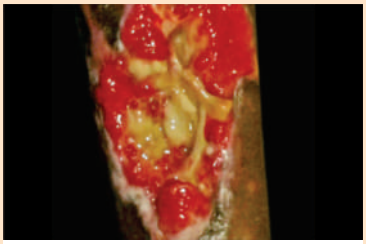
Springer

In Chronic Wound Management

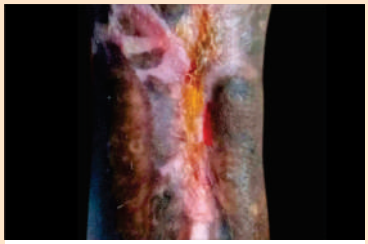
ZiFi TURBO

LINEZOLID 600MG + CEFIXIME 200MG **Tablets**

A Case of Chronic Wound



Before Treatment



After Treatment

Case : 38 year old Female H/o Mandibulectomy.

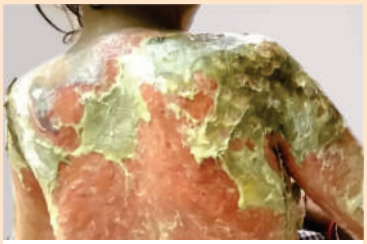
Treatment : ZiFi TURBO b.i.d. for 10 days.

In Acute Wound Management

ZiFi TURBO 600

LINEZOLID 600MG **Tablets**

A Case of Acute Wound



Before Treatment

After Treatment

Case : 45 year old Female H/o **Thermal injury**.

Treatment : ZiFi TURBO 600 b.i.d. for 7 days

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Incorporating Diabetes Bulletin

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International Journal of Diabetes in Developing Countries is published 4 times a year. Volume 37 (4 issues) of will be published in 2017.

ISSN 0973-3930 print version
ISSN 1998-3832 electronic version

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International Journal of Diabetes in Developing Countries

Volume 37 · Number 3 · July–September 2017

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):227–228
DOI 10.1007/s13410-017-0573-2

EDITORIAL

Hypogonadism and diabetes mellitus - Implications for cardiovascular risk

Madhu SV[1]

Published online: 4 August 2017

It is well recognized that hypogonadism is common in diabetes mellitus (DM) [1–3]. However, most of the earlier reports have been in the context of sexual dysfunction particularly resulting in erectile dysfunction (ED) in diabetic men [2]. While ED is an important cause of morbidity in these individuals, there could be other health consequences that also require urgent attention. Testosterone deficiency can affect several facets of the well-being of diabetic patients including sexual performance, mental health, and quality of life [4]. More recently, studies have also focussed on issues that could impact the cardiovascular (CV) and bone health of those affected with DM. Several studies have suggested that hypogonadism particularly in diabetic men is associated with insulin resistance (IR) [5] and a higher occurrence of metabolic syndrome [6]. Clamp studies in patients of DM with testosterone deficiency have revealed a significant reduction in insulin-mediated glucose uptake that has been shown to improve with gonadal hormone replacement in these hypogonadal individuals [7]. These observations suggest that hypogonadism could be associated with significant CV risk among diabetic subjects.

It is well known that DM enhances CV risk manifold and that several factors associated with DM such as abdominal obesity, dyslipidemia, and hypertension drive diabetes-related atherosclerosis and CV risk. However, it is also known that a large part of the CV risk associated with DM remains unexplained by conventional risk factors and the search for newer risk factors is still on. There have been recent reports of hypogonadism in 30–40% of male subjects with DM [1–3] particularly in those with evidence of coronary artery disease (CAD) [8]. A significant positive correlation has also been reported between hypogonadism and CAD [8] and atherosclerosis [9]. Coupled with its documented association with insulin resistance and metabolic syndrome, these findings would suggest that testosterone deficiency is not only an important CV risk factor but could also be a major contributor to the CV burden associated with diabetes. The CV risk associated with testosterone deficiency is believed to be related to its known effect on body fat and lean body mass [10] as well as to other independent effects [11] which are not fully understood.

Other studies in men without DM have also reported an association of low testosterone levels and increased CV risk [11] and increased carotid intima media thickness (CIMT) [12]. Testosterone is believed to improve coronary blood flow in men and has been shown to induce vasodilatation in isolated rabbit aorta [13].

The study by Young et al. [14] from Nigeria on the current issue reinforces the findings of earlier studies from Asia and America and supports a similar contribution of testosterone deficiency to CV burden in countries from the African continent as well. In a cross-sectional study that attempts to determine the prevalence, types, and association of hypogonadism in 108 Nigerian men with established diabetes mellitus, the authors found a tenfold higher prevalence of hypogonadism (38.9 vs 3.6%) among male patients with type 2 DM in Nigeria. A high HbA1C predicted the occurrence of hypogonadism among them.

Testosterone replacement therapy in hypogonadal diabetic men has also resulted in improvements in insulin resistance [15], glycemia [15], and metabolic syndrome [16] and a significant lowering of CV risk [17] particularly the risk of acute myocardial infarction and stroke [17]. Studies have also reported improved survival in them after testosterone

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replacement [18]. A recent study by Dandona et al. [3] has also confirmed the beneficial effects of testosterone therapy on insulin resistance and inflammation in patients with type 2 DM.

These findings indicate that replacement of testosterone can be considered an important CV risk reduction strategy in diabetic men particularly in view of its reported contribution to a significant CV burden in them. Well-designed long-term randomized control trials in this direction are the need of the hour.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):229–239
DOI 10.1007/s13410-016-0534-1

REVIEW ARTICLE

Genetic variation, biological structure, sources, and fundamental parts played by CXCL12 in pathophysiology of type 1 diabetes mellitus

Mojgan Noroozi Karimabad[1] · **Hossein Khoramdelazad**[1] · **Gholamhossein Hassanshahi**[1]

Received: 24 January 2016 / Accepted: 14 November 2016 / Published online: 8 December 2016

Abstract Type I diabetes (TID) is defined as an autoimmune disease resulting from the destruction of insulin-producing beta-cells by autoreactive T cells. It is believed that TID resulted from the immune-mediated destruction of insulin-producing β-cells in pancreatic islets of Langerhans. Chemokines are small glycoproteins (weighing 8–10 kDa) that are attractive chemotactive factors for a wide variety of cell types, especially immune system cells and their target cells which express appropriate G protein receptors. It has been well established that chemokines as the main arms of the immune system play key roles in the regulation of immune responses which is evidenced to be important in the pathogenesis of the diseases. Several other environmental and genetic components of the immune systems also confirmed to influence the onset of immune-related diseases. The CXC chemokine CXCL12 is involved in the development of immune responses. Previous studies reported that the known genetic variation SDF1–3′A regulates the expression of CXCL12. Hence, the aim of this study was to address the latest findings regarding the relation between the serum concentrations and CXCL12 genetic variation, in SDF1–3′A in T1D.

Keywords CXCL12 · Polymorphism · Type-1 diabetes · Autoimmune · SDF1–3′A

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Introduction

Type 1 diabetes mellitus (T1D) is described as a chronic autoimmune disease state that is caused by the selective destruction of pancreatic β-cells, followed by hyperglycemia, oxidative stress, and consequent extensive impairment of immune cell functions, a phenomenon responsible for the development of chronic diabetic complications [1–3]. Therefore, a potent regulatory system is required to prevent these types of diseases. It is now evidenced that cytokines play key roles in the regulation of immune responses involved in the pathogenesis of hypersensitivity and autoimmune-associated diseases caused by running immune system non-self and self antigens for hypersensitivity, autoimmune response, and receptivity [4, 5]. The prevalence of T1D is increasing globally by an annual rate with considerable variation in different geographical regions [6–9]. Finland with the highest rate of 64 cases per 100,000 of population of T1D diagnosed per year and Sweden with more than 40 cases per 100,000 of population of new cases annually stand on the top word countries [10]. These highly T1D zone countries are in contrast to China and Venezuela with less than one case per 100,000 of populations, yearly [11]. Compelling evidences revealed that T1D is carrying most of the characteristics of an immune disorder, as highlighted by the pattern of variation in the profile of chemokine expression observed during the progression of the disease [12]. The most recent investigations evidenced that chemokine/chemokine receptor axes are crucially involved in the pathogenesis of T1D, due to their paramount parts in diabetes and its specific complications [13]. T1D is a serious disease, which usually occurs in children at a young age and is accompanied by autoimmune-facilitated destruction of the insulin-expressing pancreatic β-cells which consequently lead to insulin deficiency [14]. The most frequent complications of T1D vary from retinopathy, nephropathy, and neuropathy to

atherosclerosis [15]. T1D is also associated with increasing microvascular complications and inflammation [16] as studies demonstrated that it is a pro-inflammatory state in which elevated quantities of C-reactive protein (CRP), sCD40L, and pro-inflammatory chemokines and cytokines are observed [17–20]. are described as a subgroup of chemotactive cytokines with several biological activities varying from recruitment of leukocytes to the sites of injury, infection, and inflammation to angiogenesis and angiostasis [21, 22]. Chemotactive mediators are subdivided according to the position of cysteine motifs in their structure into CC, CX3C, C, and CXC subdivisions [23–26]. The stromal cell-derived factor 1α (SDF-1α), which in the last categorization is nominated CXCL12, fits into the CXC subdivision [6]. This chemoattractant mediates are active in the chemoattraction and migration of T lymphocytes and monocytes, but not neutrophils. The CXC chemokine receptor CXCR4, as a receptor for CXCL12, induces a rapid and transient rise in the level of intracellular calcium ions and further chemotaxis of target cells. CXCL12 also binds to another CXC chemokine receptor (CXCR7), which activates the beta-arrestin pathway [27, 28]. CXCR7 has positive effects on monocyte migration but negative effects on the adhesion of these cells throughout LYN kinase. In other words, CXCL12 stimulates migration of monocytes and T lymphocytes via its receptors, CXCR4 and CXCR7, and inversely decreases the adhesion of monocytes to the surfaces coated with intracellular adhesion molecule 1 (ICAM-1) that is a ligand for beta-2 integrins. The CXCL12/CXCR4 signaling axis prevents beta-2 integrin LFA-1-mediated adhesion of monocytes to ICAM-1 throughout LYN kinase. The key roles of CXCL12 as an inhibitory chemokine in autoimmunity and inflammation raise questions concerning the impacts of this chemokine in the pathogenesis of immune-related diseases [29–32]. Cumulative studies evidenced that the expression of CXCL12 can be affected by its polymorphism at position +801 regions (the SDF1–3′A) [33, 34]. More recently accumulating reports documented that the serum and/or the tissue expression of CXCL12 is increased in autoimmune diseases, including T1D [17, 18]. Therefore, it seems that the genetics of CXCL12, as an immunoregulatory chemokine, may be altered in the T1D; thus, in this article, we have focused on the crucial role(s) played by CXCL12 along with its polymorphic form (SDF1–3′A) in the pathogenesis of T1D. Therefore, we tried our best to collect the most recent reports regarding the relation between the serum concentrations and the genetical variant of CXCL12 in T1D.

CXCL12 and its receptors

CXCL12 was initially isolated from a bone marrow stromal cell line ST-2 [35] and in subsequent investigation by expression cloning as a stimulating factor for pre-B stromal cells in the generating B cells in vitro [36]. CXCL12 was conserved across the evolution, and only one amino acid difference was found between murine, rat, and human homologs of CXCL12, allowing for the action of this chemokine across species [37–39]. Presently, there are two alternatively spliced SDF-1 messenger RNAs (mRNAs), SDF-1α and SDF-1β, to date, with polypeptide variants encoded by 68 and 72 amino acids, respectively [40]. Regardingly, CXCL12 is an 8-kDa protein which exists in both monomeric and dimeric forms [41]. The coding regions in the nucleotide sequences of human and mouse variant CXCL12 genes are 99% identical, the fact that CXCL12 is regarded as the most highly conserved chemokine described to date [37, 42]. Mature human and murine CXCL12 are various only at position 18 (valine in the human protein and isoleucine in the murine protein) [40]. In spite of CXC chemokines, the CXCL12 gene is clustered located on chromosome 10 in the vicinity of cc chemokine genes [40], whereas genes encoding the other CXC chemokines are situated on chromosome 4 [43]. The 5′ flanking region of the CXCL12 gene is TATA-less, while it is GC-rich (three boxes), which means that CXCL12 is a constitutive gene [44]. Three GC boxes and one CAAT box are binding elements for general transcription factors Sp1 and CTF [40]. It appears that the constitutive expression of CXCL12 is due to CpG islands (a transcription-binding motif specific for housekeeping genes). Binding motifs for NF-κB and AP-1 are lacking in the CXCL12 promoter [40, 42]. In addition to involvement in inflammatory cell trafficking, this chemokine and its receptors (CXCR4 and CXCR7) have been reported to incorporate in the development of the normal heart and cerebellum [45], B cell development and trafficking [46], and vascular development and vessel formation (angiogenesis) [47]. CXCL12 is at high levels expressed by bone marrow stromal cells in a constitutive faction [48]. The wide tissue distribution of CXCL12 proposes that it may play a role in immune surveillance rather than inflammation as it has been demonstrated to function principally in trafficking and export and homing of bone marrow cells [49]. Furthermore, the interaction of CXCL12 and its receptor CXCR4 is believed to play fundamental parts in homeostasis, vascular development, and homing of naïve T cells into lymphoid tissues [50–52].

CXCL12 pathway

Activation of CXCR4 or CXCR7 signaling may affect several major signaling pathways related to cell survival, proliferation, and migration (see Fig. 1). For example, CXCL12 activates PI3K/Akt, IP3, and MAPK pathways via CXCR4, thus regulating cell survival, proliferation, and chemotaxis. CXCR4 signaling can be modulated by β-arrestin-mediated internalization of the receptor. Much less is known about CXCL12 signaling via CXCR7, which was initially thought

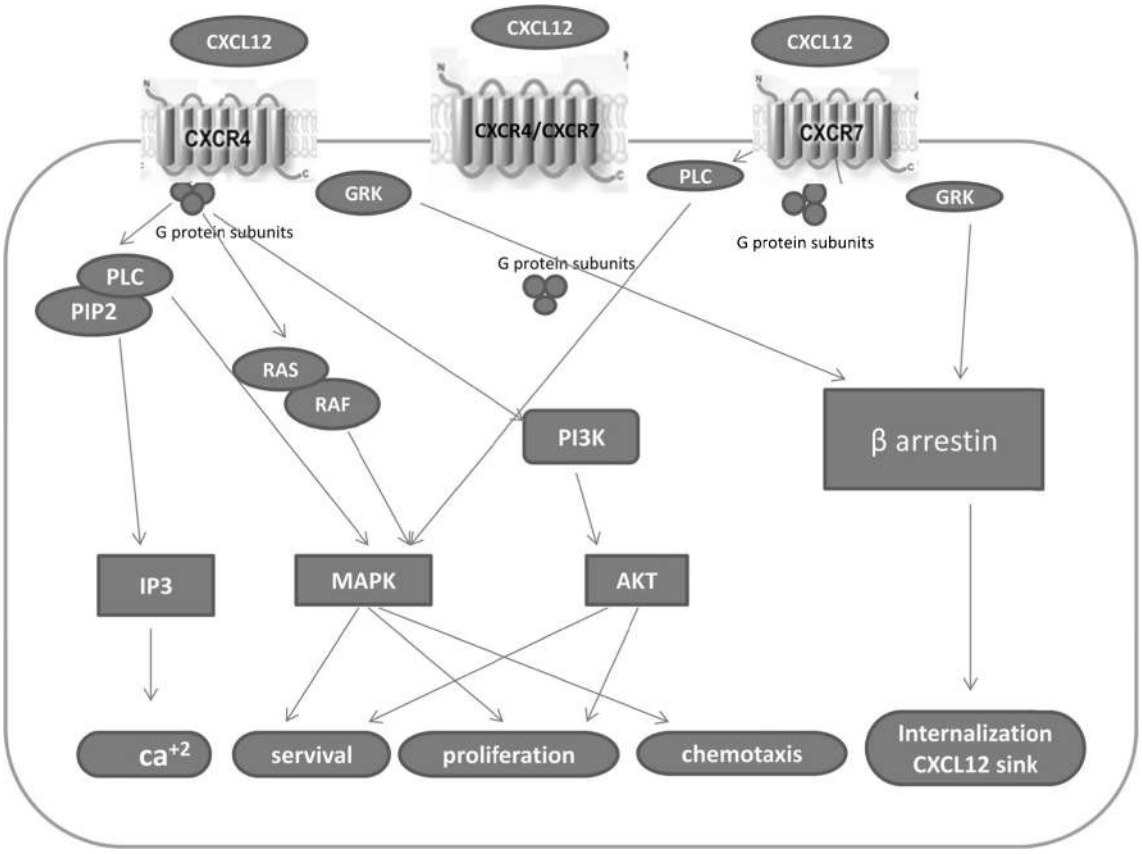

Fig. 1: CXCL12 binds to CXCR4 and CXCR7, which are G protein-coupled receptors (GPCR) that can form homodimers or heterodimers. In the latter case, CXCR7 changes the conformation of the CXCl12/G-protein complexes and abrogates signaling. Activation of CXCR4 by CXCL12 leads to G protein-coupled signaling through PI3K/Akt, IP3, and MAPK pathways, which subsequently promotes cell survival, proliferation, and chemotaxis. The β-arrestin pathway can also be activated through GRK to internalize CXCR4. When CXCR7 binds CXCL12, the classical GPCR mobilization of Ca^{2+} does not occur, and activation of the β-arrestin pathway may lead to scavenging of CXCL12. In certain cancer cells (e.g., glioma), CXCR7 can also signal through PLC/MAPK to increase cell survival [[53]]

to serve primarily as a sink for CXCL12 [53]. Indeed, when CXCR7 is activated by CXCL12, the classical GPCR mobilization of Ca2+ is not observed. Rather, the β-arrestin pathway is activated and scavenges CXCL12 [22, 25]. CXCR4 and CXCR7 can also form heterodimers, whereby CXCR7 changes the conformation of the CXCR4/G-protein complexes and abrogates its signaling. Finally, CXCR7 can signal through the PLC/MAPK pathway and increase cell survival [53].

The multiple faces of CXCL12 during ongoing inflammatory autoimmunity

In the light of aforementioned introductory comments, it is thought that CXCL12 plays an important role in the attraction of T cells to the specific sites. It was suggested that CXCL12 could play a pro-inflammatory role in various autoimmune diseases, particularly rheumatoid arthritis (RA) and nephritis, in murine lupus erythematosus and therefore could be a favorable target for neutralization of signs and symptoms of these syndromes [54, 55]. Accumulating evidences demonstrated that in physiologic and stable states within the healthy CNS, CXCL12 mediates the migration of CXCR4-expressing neural and oligodendrocyte precursors [56]. Unregulated expression of CXCL12 within the astrocytes in the central nervous system (CNS) in multiple sclerosis (MS) is well documented; however, its role in the regulation of this disease is not fully understood [15, 57, 58]. Additionally targeted neutralization of CXCL12 in encephalomyelitis (EAE) model of MS showed to suppress the disease. Meiron and co-workers indicated that CXCL12 neutralization at different time-points following active EAE was induced and contradictory effects were found and therefore a CXCL12-Ig fusion protein was administered to mice after the induction, but before the onset of symptoms of EAE, the onset of the disease was delayed for 2–3 days. They also observed a rapid remission of the disease when the fusion protein was administered after the onset of disease [59]. Overall, they found that endogenous CXCL12 is firstly produced by astrocytes within the CNS and other cells and is involved in modulating the recruitment of leukocytes to the CNS. Accordingly, during the event of inflammatory

process within the CNS which later enters into an accelerating phase, CXCL12 acts as an anti-inflammatory chemokine that directs the polarization of CD4_ T cells and macrophages to become IL-10 high-producing regulatory T cells [59]. Altogether, these suggest CXCL12 as a promising target which could be used as a potential drug only during advanced stages of inflammatory and autoimmunity, only due to its pleiotropic characteristics. In the study of Karin N et al., they elaborate the role of cytokines in directing the polarization of effector and regulatory T cell subset and the plasticity of this process. Then, we extend this notion to chemokines while focusing on CXCL12 and the CXCR3 ligands. Finally, we elaborate the potential clinical implications of these studies for therapy of autoimmunity, graft-versus-host disease, and cancer. In the study of Wang, they investigated that the SDF-1–CXCR4 signaling pathway plays a pro-inflammatory role in an experimental rat model of temporomandibular joint osteoarthritis (TMJOA). The bicyclam derivative AMD3100 can alleviate the severity of experimental TMJOA, and there might be a potential relation between the SDF-1–CXCR4 axis and the ERK signaling pathway. In the study of Hanaoka, their objective was to evaluate the roles of circulating B cells in the pathogenic process of systemic lupus erythematosus (SLE) by measuring the expression of chemokines and their receptors. Flow cytometric analysis revealed that the expression level of CXCR4 on circulating B cells was significantly higher in patients with active disease than in those with inactive disease or controls. Serum CXCL12 concentration was not different between these groups. In addition, the migratory ability of B cells toward CXCL12 was enhanced in active SLE patients. Finally, CXCR4-expressing B cells were more frequently observed in the renal biopsy specimens of lupus nephritis. Up-regulated CXCR4 expression on circulating B cells in active SLE may enhance their chemotactic response toward CXCL12, which may promote infiltration of these cells into inflamed renal tissue and contribute to the development of SLE

The CXCL12 protein level in T1D

T1D is an autoimmune disease triggered by environmental factors in genetically susceptible subjects. According to the previous reports, CXCL12 is believed to be strongly associated with pathogenesis T1D. Chemokines and their receptors are part of polarized T helper (Th1 and Th2)-mediated immune responses which control trafficking of immune-compatible cells to the inflammatory regions [60]. A number of various immune-related cell types varying from $CD4^+$ T cells, to dendritic cells, to B lymphocytes express CXCR4. In a similar pattern as other autoimmune disorders, the form of cytokine and chemokine expression is profoundly changed in T1D [61]; thus, T1D and its complications are regarded as immune disease. In our previous studies, we have documented that circulating CXCL12 was augmented in T1D patients compared to healthy subjects. Clinicians may be able to speculate the approximate time of the T1D event, according to the CXCL12 serum levels. Moreover, CXCL12 demonstrated both CXCL8 and CCL2, both of which are pro-inflammatory and angiogenic chemokines; hence, CXCL12 may facilitate the development of an inflammatory response in T1D patients (our unpublished data). Importantly, it is possible that oriented migration and recruitment of CXCR4-expressing leukocytes in response to CXCL12 aid the development of T1D [21, 28, 62]. Most of the complications of T1D follow a pro-inflammatory pattern, and elevation of the chemokine probably is as result of inflammatory response which occurs during T1D [28, 62]. The serum levels of CXCL12 could be used as a key biomarker in T1D diagnosis and prognosis, and it may be concluded that elevated CXCL12 levels assist in the progression of T1D and a relation could be assumed between the the chemokine increased levels and the degree of autoimmunity in T1D patients [63]. The beneficial effects of CXCL12 could be explained by its several properties specific to this chemokine. CXCL12 induces bi-directional movement of T cells, toward lower concentration and away from higher CXCL12 concentration [64]. It also exerts a type of chemorepulsive effect on diabetogenic T cells and mediates firm adhesion of normal T cells [65]. Moreover, the expression of CXCL12 in islets was shown to lead to the selective repulsion of autoreactive T cells and retention of Tregs within the site [66]. Tregs play a fundamental part in suppressing autoimmunity, and literature data support their relevance to the T1D pathogenesis (Table 1) [14]. It has been indicated that pancreatic lymph nodes (PLNs) of non-obese diabetic (NOD) mice lack Tregs, while the recovery of euglycemia in these mice was related to the restoration of the Treg population in PLNs [67, 68]. The locally decreased expression of CXCL12 is associated with migration of Tregs, suggesting that improved function of the CXCL12/CXCR4 axis followed by retention of Tregs in the PLNs could serve as the basis for an alternative therapeutic approach for treating T1D. The selective repulsion of autoreactive T cells along with attraction of Tregs has been suggested as a beneficial mechanism for a recently reported novel strategy in islet transplantation. It has also been revealed that the immune-mediated fashion of rejection of transplanted islets could be delayed throughout their local immune-isolation achieved via coating or encapsulation of islets with CXCL12, thus excluding the necessity for systemic immunosuppression [69]. In spite of the evident importance of this finding for T1D treatment, it should be noted that local immunosuppression achieved through CXCL12 has also been observed in cancer models where this type of mechanism protects cancer cells from immune attack [68, 70]. Furthermore, Khairul Matin et al. indicated that in the

Table 1 A Summary of the literature reviewed in relation to the immune-related diseases reported

Country	Racial information	Sample size	Sex (M/F)	Serum levels of CXCL10	Ref. no.
Japan	Japanese	225	225 females	SDF-1-Ig group tended to develop mild insulitis (grade 0, 22·8 ± 5·3%; grade 1, 32·8 ± 5·2%; grade 2, 23·8 ± 2·8%; grade 3, 14·8 ± 3·3%; and grade 4, 5·7 ± 2·2%))	[58]
France	French	NOD Thy-1,2 mice	1% in females and 15–20% in males	0·12 ± 0·003 at 4 weeks versus 0·09 ± 0·01% at 12 weeks	[60]
China	Chinese	Ser 473	–	Up-regulated expression of SDF-1α and CXCR4	[63]
USA	American	–	–	The elevated CXCL12 expression	[64]
France	French	328	Were similar in 208 unrelated Caucasian patients with type 1 diabetes and in 120 Caucasian control subjects	SDF1–3′A allele was associated with a 5-year reduction in the age at onset of diabetes ($P = 0.0067$)	[72]
Iran	Iranian	334	72 patients and 262 cases of normal healthy control	Increasing	[74]
Japan	Japanese	129	60 men 69 women	–	[50]
		Twelve-week-old female NOD were used	Twelve-week-old female NODs were used	CXCR4 protein expression was significantly decreased in NOD/LtJ T cells, and inhibition of CXCR4 activity significantly reversed SDF-1 chemorepulsive effects	[17]
USA	American	Six-week-old female BALB/c (H2d), 6-week-old female C57BL/6 (H2b), and 4-week-old female NOD/LtJ (H2g7) mice were used in this study	Six-week-old female BALB/c (H2d), 6-week-old female C57BL/6 (H2b), and 4-week-old female NOD/LtJ (H2g7) mice were used in this study	CXCL12 from 1.5% alginate capsules during the first 24 h was 1.75 0.01 ng/mL/h and after 4 days stabilized at a release rate of 0.18 0.002 ng/mL/h	[23]
USA	American	15	8 men 7 women	SDF-1 increased NOD T cell adhesion to recombinant adhesion molecules, a phenomenon that was reversed by recombinant SLIT2	[80]
Italy	Italian	Diabetic mice	–	In diabetic mice, OSM neutralization prevented CXCL12 induction and improved granulocyte colony-stimulating factor and ischemia-induced mobilization	[81]

presentation of anti-CXCL12 antibodies, an effective inhibition of diabetes and insulitis without affecting autoimmune sialadenitis was observed in NOD mice [71]. Moreover, Zhao Y et al. reported that the selective destruction of pancreatic beta islet cells was mediated by human T lymphocytes following an initial trigger which was supplied by the injection of irradiated spleen mononuclear cells (SMCs) from diabetic NOD mice[72]. This has led to severe insulitis, a remarkable loss of total beta-cell mass, and other related phenotypes of T1D. The human T cell chemoattration to pancreatic islets was controlled by the beta cell-generated highly conserved chemokine and its receptor, as demonstrated by in vivo blocking experiments using antibody against CXCR4 [72]. Aboard et al. revealed that the CXCR4 mRNA level was increased in pancreatic lymph nodes of NOD mice in comparison to Balb/C mice. However, a significant reduction of CXCR4 was noticed at 12 weeks (both at the mRNA and protein levels) while its expression was increased in the inflamed islets. The percentage of CXCL12 that attracted splenocytes in a transwell chemotaxis assay was significantly increased in NOD versus Balb/c mice [73]. Immunofluorescence studies indicated that AMD3100 reduced the number of CXCR4- and CXCL12-positive cells in the inflamed islets. Thus, it could probably be concluded that the CXCL12/CXCR4 axis has protective effects against autoimmune diabetes. Noh et al. indicated that the most effective umbilical cord blood-mesenchymal stem cells (UCB-MSCs) secreted higher levels of CXCL12 and CCL5 chemokines [74]. Ferraro et al., in mouse models of T1D and T2D diabetes (streptozotocin-induced and db/db mice, respectively), reported that response to G-CSF treatment release of murine HSPCs from the bone marrow was impaired. Furthermore, HSPCs were aberrantly localized in the marrow niches of the diabetic mice, and abnormalities in both number and biological functions of sympathetic nerve terminus were associated with this mislocalization [75]. In a study, Jie et al. found that CXCL12, CXCR4, and proliferating cell nuclear antigen (PCNA) all were up-regulated in tunica media of thoracic aortas by streptozotocin-induced hyperglycemic Sprague Dawley rats [76]. Treatment of primary vascular smooth muscle cells (VSMCs) with high dose of glucose (25 mM) caused an up-regulation of the expression of both CXCL12 and CXCR4, activated phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt) signaling, and subsequent promotion of the proliferation and chemotaxis of VSMCs [76]. Interestingly, the administration of the CXCL12 siRNA or neutralizing antibody against CXCL12 abrogated high glucose-induced up-regulation of CXCR4 [76]. Moreover, employing a neutralizing antibody, a specific inhibitor of CXCR4 (e.g., AMD3100) or one PI-3K inhibitor (e.g., LY294002), also abolished the high glucose-potentiated proliferation and chemotaxis in VSMCs. Evidences are in favor of the fact that high glucose levels activate the CXCL12/CXCR4/PI-3K/Akt signaling pathway in VSMCs in an autocrine fashion, which consequently considerably enhanced the proliferation and chemotaxis of VSMCs [76]. Leng et al. reported that the level of CXCL12 transcripts enhanced in the bone marrow of NOD mice as compared to Balb/c and C57BL/6 mice. Accordingly, naïve T cells, regulatory T cells, and HSC are accumulated in the bone marrow of NOD mice. Where NOD mice receive AMD3100, an antagonist of CXCR4, it mobilizes T cells and HSC from the bone marrow to the circulatory blood and concomitantly inhibits insulitis along with delayed onset of diabetes. Interestingly, previous investigations have shown that the elevated CXCL12 expression promotes TID in NOD mice by altering T cell and HPSC trafficking, which together may highlight the potential tendency of AMD3100 for treatment or prevention of the development of TID [77]. In a study, Al Ghamdi and co-workers demonstrated that the induction of diabetes in mice was associated with hyperglycemia and significant decreases in the insulin level and the lymphocyte count. In this context, diabetic mice displayed severe diabetic complications, as indicated by a remarkable decrease in the levels of IL-2, IL-4, and IL-7, prolonged elevation of the levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and reactive oxygen species, and altered lipid profiles compared with control non-diabetic mice. Furthermore, antigen stimulation of B and T with antigenic reagent lymphocytes markedly reduced the proliferative capacity and chemotaxis of these cells towards CCL21 and CXCL12 in diabetic mice [78]. In a study, Glawe showed that CXCR4 protein expression was induced in mouse and human T cells. Diabetogenic splenocyte transfer was performed in NOD/LtSz Rag1(−/−) mice and the effect of CXCL12 mimetic CTCE-0214 on adoptive transfer of diabetes. CXCR4 and ROBO1 protein expression was elevated in diabetic NOD/ShiLtJ T cells over time and coincided with the onset of hyperglycemia. CXCR4 and ROBO1 expression was also increased in human type 1 diabetic T cells, with ROBO1 expression maximal at less than 1 year post-diagnosis. Cell detachment studies revealed that immunoneutralization of ROBO1 prevented CXCL12-mediated chemorepulsion of NOD T cell firm adhesion to TNFα-stimulated islet endothelial cells. CXCL12 increased NOD T cell adhesion to recombinant adhesion molecules, a phenomenon which inversely changed by recombinant SLIT2. Finally, they found that a CXCL12 peptide mimetic prevented NOD T cell adhesion in vitro and markedly delayed adoptive transfer of autoimmune diabetes in vivo. In a study [79], Albiero showed that patients with diabetes have increased M1 macrophages, whereas diabetic mice have increased CD169(+) BMMΦ with SC-retaining activity. Depletion of BMMΦ restored SC mobilization in diabetic mice. They showed that CD169 labels M1 macrophages and that conditioned medium (CM) from M1 macrophages, but not from M0 and M2 macrophages, induced expression by mesenchymal stem/stromal cells. It was

demonstrated that oncostatin M (OSM) as the soluble mediator contained in M1 CM induces CXCL12 expression via a purse pathway as a member of mutagen-activated protein kinase-p38 signal pathway and activator of a transcription 3-dependent pathway. In diabetic mice, it was shown that OSM neutralization inhibited CXCL12 induction and improved granulocyte colony-stimulating factor and ischemia-induced mobilization, SC homing to ischemic muscles, and vascular recovery. In patients with diabetes, BM plasma OSM levels were higher (GCSF) and correlated with the BM-to-PB SC ratio. Accordingly, BMMΦ prevents SC mobilization by OSM secretion, and OSM antagonism is a strategy to restore BM function in diabetes, and this in turn can translate into protection mediated by BMSCs [80]. Hence, regarding these data in future chemokines, including CXCL12 may possibly be used for monitoring the clinical course and the development of TID and its associated complications. Consequently, this may aid in identifying patients with a high degree of likelihood of achieving a therapeutic response, and therefore, CXCL12 may even exhibit potential properties as an advantageous therapeutic reagent.

The CXCL1–3′A genetic variation in T1D

Accumulating evidences documented the involvement of the immune system in T1D and its associated complications [28]. Therefore, T1D and its complications, such as nephropathy, are considered as immune-related disease. Recently, the important regulatory effects of CXCL12 on the other members of the CXC subfamily in the development of diabetes in NOD mice were well elucidated [71]. Furthermore, the CXCL12 gene is located on chromosome 10q11.1 in the vicinity of T1D susceptibility locus IDDM10, suggesting a contributory role for CXCL12 in progression of diabetes. A transition at position +801 (G to A) (SDF1–3′A) is described as a common polymorphism in the 3′-untranslated region of the CXCL12 gene [60]. It has been reported that the SDF1–3′A variant is related to the early onset of T1D in a French population [81]. In a previous report, we have demonstrated that there exists a significant association between the CXCL12 (SDF1–3′A) polymorphism and T1D in an Eastern Iranian regional population. We showed that 7.3% of T1D patients had A/A genotype, 81.5% had A/G, and ultimately 11.2% emerged with a G/G genotype variant. Despite that there are only few studies to report this genetic variation in different diseases, the association between SDF1–3′A polymorphisms and T1D [60, 82] and lung cancer was reported. [83]. Hence, such types of polymorphism are able to participate in the susceptibility to clinical presentations of T1D [81]. The SDF1–3′A allele frequency reported in a Caucasian population was 25.8% in patients with early onset of diabetes compared to 15.8% in patients with age at onset >15 years. The association between early onset diabetes and the SDF1–3′A variant was significant in a dominant type (A/A + A/G versus G/G, where A and G indicate the variant and wild-type alleles) but not in a recessive one, as authors stated [81]. These gene variations were in patients who did not express the HLA class II DR3/DR4 combination that confers the highest risk for T1D and is in parallel with early onset of the disease [81]. The CXCL12 functions as a potent chemoattractant which mediates the attraction of monocytes and naive T cells. However, studies in the NOD mouse, a model for T1D, have emphasized on the fundamental parts played by both dendritic cells and macrophages at the onset of insulitis and β-cell destruction. Evidences are supporting the fact that the severity of pancreatic islet infiltration correlates with the rate of progression to diabetes [84]. It is not well defined whether CXCL12 is involved in the insulitis process; to date, however, in addition to its role in T lymphocyte migration, CXCL12 is also a co-stimulator for CD4+ T cells [85]. Regardingly, one could speculate that the SDF1–3′A variant modulates the degree of islet infiltration and the age at onset of T1D mononuclear cells. This variant is quite closely to related highly conserved sequences that may regulate the expression of CXCL12 at the protein level [86]. A known biological consequence for this genetic variation was not observed in in vitro transfection studies [87–89]. Alternatively, mutations in CXCL12 or other genes located in the same region could be involved in association with linkage disequilibrium with SDF1–3′A. We have also reported an association between the SDF1–3′A polymorphisms and post-transfusion occult hepatitis B infection, type 2 diabetes, and MS. This gene variant is reported to be associated with other autoimmune disorders including type 1 diabetes and autoimmune thyroid disease. Finally, the overexpressed values of CXCL12 protein levels in T1D patients which was reported in several studies could possibly be due to SDF1–3′A polymorphism, as we reported in other autoimmune-type disorders [90, 91]. Furthermore, Kawasaki et al. claimed that the allele and genotype frequencies of SDF1–3′A polymorphism were significantly different between T1D patients with autoimmune type-1 diabetes (AITD) and controls and between non-diabetic patients with AITD and healthy control subjects [60]. Ide et al. demonstrated, however, that CXCL12 gene polymorphism is associated with the age at onset of T1D in a Japanese population but CXCL12 chemokine gene polymorphism is not associated with the onset of age in T1D Japanese patients [92]. In contrast, Shigihara et al. reported that in "Caucasian" T1D, the SDF1–3′A variant was not associated with early onset of the disease in Japanese T1D [93]. Again, Dhamodharan et al. reported the genetic association of IL-6, TNF-α, and SDF-1 polymorphisms with serum cytokine levels in diabetic foot ulcer. They showed an association between SDF1–3′A and its correlation with the serum levels of chemokine along with other clinical risk factors they genotyped. Normal glucose tolerance T2DM without DFU,

T2DM with neuropathy, and T2DM with PVD subjects by PCR-RFLP and the serum CXCL12 levels were determined by ELISA. They reported that the SDF-1 "A" allele conferred significant protection against both DM and DFU-DN and not DFU-PVD and alleles significantly affected the serum cytokine/chemokine levels in diabetic patients. Djuric assessed the distribution of the SDF1–3′A genotype in 130 diabetic patients with retinopathy. In T1D patients suffering from proliferative retinopathy, the frequency of the homozygous SDF1–3′A genotype was significantly higher than in patients with non-proliferative retinopathy. This correlation was confirmed when T2D patients were analyzed separately. The finding that homozygous carriers of the SDF1–3′A genotype are more frequent in diabetes patients with proliferative retinopathy suggests a possible role of this genotype in the development of sight-threatening diabetic retinopathy.

Conclusion

Overall, according to the latest information that we formularized in the present article, the serum CXCL12 level (as a Th1 response chemokine) is defined by many as a favorable candidate either as a predictive prognostic parameter or as a marked biological marker reflecting the disease 70 and situation of the pancreatic lymph node in TID; thereupon, the measurement of serum CXCL12 levels may plausibly serve as a useful clinical pathophysiologic procedure for examination of the disease course in TID. It is now broadly accepted that the treatment of T1D is in accordance with the regulational immune system and, generally, chemokines and CXCL12 especially as arms of the immune system could be considered as useful therapy and diagnostic tools. The B cell regeneration processes are also treated as an important strategy for the treatment of T1D. Accumulating evidences arising from several studies are proposing that the CXCL12/CXCR4 axis plays a crucial part in the autoimmune processes and in ß-cell destruction in TID. Thus, accordingly, due to the close relation between the CXCL12/CXCR4 axis and its involvement in both of the above strategies, inhibition of the CXCL12 expression at the onset of diabetes seems to be a possible means for TID therapy. This therapeutic method in combination with other regulatory intervention strategies, including GAD autoantigen sensitization, could also incorporate to a successful curative treatment for TID. Accordingly, based on the latest scientific reports collected in this review article, we can conclude that the measurement of serum CXCL12 concentration is useful either as a predictive factor for the development of TID and/or progression of its complications or even as a therapeutic target in T1D. This approach may also be considered as an important tool in delineating the pathogenesis of other autoimmune diseases as well. SDF1–3′A is well identified as a common polymorphism in the 3′-untranslated region of the SDF-1α (CXCL12) gene. However, it exists only to address a role for these few studies existing on the polymorphisms in different diseases, and the association of SDF1–3′A polymorphisms and different diseases has been reported in our previous study. This gene variant is reported to be associated with other autoimmune disorders including type 1 diabetes and autoimmune thyroid disease. Finally, the overexpressed values of CXCL12 protein levels in T1D patients which were reported in several studies could possibly be due to SDF1–3′A polymorphism, as we reported in other autoimmune-type disorders. Due to the complexity of neuroinflammation and the chemokine network, we suggest that more studies are needed to clarify the exact role of CXCL12 and its corresponding receptors (e.g., CXCR4) in different stages and forms of T1D. Based on these findings, we will be able to design novel therapeutic strategies such as preservation of the CXCL12 level or development of inhibitors.

Acknowledgements This project was supported by a grant from the Rafsanjan University of Medical Sciences.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and for national research committee.

Informed consent No informed consent.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):240–247
DOI 10.1007/s13410-016-0479-4

ORIGINAL ARTICLE

Dual X-ray absorptiometry body composition and its associated factors in children and adolescence with type 1 diabetes mellitus in South of Iran, a case-control study

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Received: 29 November 2015 / Accepted: 9 March 2016 / Published online: 17 March 2016

Abstract To date, limited studies have been conducted for evaluation of the body composition with the dual X-ray energy absorptiometry (DEXA) method in children with type 1 diabetes mellitus (T1DM). Also, there are lack of data about factors associated with body composition parameters in T1DM children. This case-control study was performed on T1DM children whom had referred to Diabetes Clinics of Shiraz University of Medical Sciences, 2013–2014. Weight, height, physical activity, sun exposure, insulin regimen, and the Tanner stage of children were recorded by a trained physician. Serum lipids and glycemic tests were assessed. Body composition was assessed by the DEXA Hologic system. Statistical analysis was carried out using SPSS 18.0 software. Eighty-seven T1DM children (39 male and 48 females) and 87 age- and sex-matched healthy controls with a mean age of 12.4 ± 4.2 years were enrolled in this study. Fat mass index was more in T1DM (P = 0.012), and lean mass index was more in non-diabetic children (P = 0.013). The android/gynecoid fat ratio in T1DM children was less than that of controls (P = 0.002). On multiple regression analysis, there was an independent effect of Tanner stage (P < 0.001) and FBS (P = 0.045) on total fat, an independent positive effect of age onset of T1DM (P < 0.001) on total lean mass. This study revealed an increase in the body fat index and a decrease in the lean mass index in T1DM children.

Keywords Type 1 diabetes · Children · Iran · Fat mass · Lean mass · Body composition

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder accompanied with an inability to maintain appropriate insulin production and hyperglycemia due to pancreatic β-cell destruction [1]. Many studies have extensively studied a close association between progress and development of diabetes complications (nephropathy, neuropathy, and cardiovascular) and glucose control [2]; however, little is known about the effect of long-standing T1DM on body composition of T1DM children and its possible associated factors. The changes in fat or lean body composition may be primarily mediated by metabolic changes in hyperglycemia or may be due to its other micro- or macro-vascular complication [3]. Some previous reports have showed excessive weight gain in female T1DM adolescents [4–6], increased abdominal circumference in both T1DM and type 2 diabetes mellitus (T2DM) after insulin therapy [7, 8], especially in intensive insulin therapy for type 1 diabetes [9], and increase in waist circumference and waist-to-height ratio indexes in T1DM [10]. However, it remains a question whether weight gain or increasing in body measurement (such as waist circumference) is due to an increase in fat mass alone or an increase in muscle mass

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[11–15]. In addition, less is known about the differences in both sexes and the association of the age of onset, level of HbA_1c, daily dosage of insulin, and type of insulin therapy in changes of body composition in T1DM children [3, 7, 9, 10, 16, 17]. To date, limited studies have been conducted to evaluate the body composition with the DEXA method in T1DM children, and there is lack of data about factors associated with body composition parameters in T1DM children. DXA is increasingly used for body composition assessment because of its high precision and low dose of radiation [16, 18]. The present study appears to be the first to have assessed the body composition in a relatively large group of T1DM children, which evaluates multiple factors associations with body composition parameters.

The aim of the present study is to compare the body composition parameters of lean and fat indexes of T1DM children in south of Iran with non-diabetic control group and to further assess the body composition association of these parameters with the age onset of T1DM, HbA_1c, daily insulin usage, blood sugar, lipid profile, sun exposure, Tanner stage of puberty, type of insulin therapy, BMI, and exercise.

Material and methods

Study population

This case-control study was performed on children with T1DM whom had referred to the pediatric clinic of diabetes affiliated to the Shiraz University of Medical Sciences, July 2013 to August 2014. Inclusion criteria were age < 18 years, have pretreatment FBS > 125 mg/dl along with diabetes symptoms (such as polyuria and polydipsia), insulin dependency for maintaining blood sugar in normal range, and duration of T1DM more than 2 years. Diagnosis of T1DM in children was confirmed by the presence of autoantibodies (glutamic acid decarboxylase, islet–cell antibody and insulin autoantibody) in blood samples [19]. We excluded all diabetic children who had a known chronic liver disease, kidney or heart diseases, or other chronic disease accompanied with edema or change in the body compositions [3]. The control subjects were age- and sex-matched healthy children selected by randomized systemic sampling from local schools, who did not have any chronic or systemic disease and were normoglycemic.

Anthropometric measurements, puberty, physical activity, sun exposure, and insulin regimen

Weight, height, and Tanner stage of children were recorded by a trained physician. Weight was measured with a single standard scale with the child wearing light cloths, rounded to the nearest 0.1 kg (Seca, Germany). Height was measured with a standard wall-mounted meter with the child standing upright without shoes, rounded to the nearest 0.5 cm. Body mass index (BMI) was calculated by dividing weight (in kilogram) by height (in centimeters) per square meter. Puberty was determined by the five-stage Tanner classification [18, 20]. According to the American College of Sports Medicine which recommends at least 3 days of physical activity per week for children [21], we divided the children in to two groups according to whether they performed physical activities fewer than or more than three times per week [20]. We classified the children according to their average exposure to sunshine per day into three groups, those who are exposed for <15 min/day, 15–30 min/day, and those exposed for >30 min/day [20]. Daily insulin usage was mentioned as unit/day and unit/kg/day.

Biochemical studies

Serum triglyceride (TG) mg/dl, total cholesterol (TC) mg/dl, and fasting blood glucose (FBS) mg/dl were assessed with a Kodak Ektache m 702 Analyzer with the on enzymatic method (Eastman Kodak, Rochester, NY). Glycated hemoglobin (HbA_1c) was measured with a commercial kit (Unimate HbA_1c; Roche Diagnostics, Basel, Switzerland) by a single lab technician.

Body composition

The Hologic system (Discovery QDR, USA) was used to measure total fat (g), trunk fat percent (%), total body fat percent (%), total lean mass (g), android fat percent (%), gynecoid fat percent (%), android/gynecoid fat ratio, trunk/leg fat ratio, and trunk/limb fat ratio. We calculated fat mass index (FMI) by dividing total fat mass (kg) by height^2 (m^2) and lean mass index by dividing total lean mass (kg) by height^2 (m^2). This study was done with the children wearing special clothing. Plots of daily spine phantom scans were used to check the scanner stability through the course of study. The coefficient of variation (CV) in our laboratory was 0.7 % for fat mass and 1.9 % for fat percentage and lean mass.

Ethics This study was approved by the Shiraz University of Medical Sciences (SUMS) ethics committee. All children and parents who participated in our study provided signed informed consents.

Statistics

Data were shown as mean ± SD. Normality of data distribution was evaluated with the Kolmogorov-Smirnov test. Student's *t* test and Mann-Whitney test were used for comparison of normally and non-normally distributed data, respectively. Correlations between normal distributed parameters

were assessed by Pearson's test and Spearman's ranking test for non-normally distributed data. Comparison of qualitative data was carried out using chi-square test. ANOVA was used to compare qualitative data with more than two categories; however, when the equality of variance was significant, Kruskal-Wallis test was used for this analysis. Variables with significant correlation with body composition parameters in univariate analysis were entered using multivariate binary logistic regression analysis to assess their independent predictive effect on these parameters. Collinearity was assessed by variance inflation factor (VIF) and VIF less than 5 was considered as non-collinearly. The linear regression analysis used data that was normally distributed (assessed by Q-Q plots), with homogeny of variance and reported independence of predictive effects. Adjusted T squared and VIF were mentioned in all regression analysis.

Statistical analysis was carried out using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Eighty-seven T1DM children (39 male and 48 females) and 87 healthy control ones aged 12.4 ± 4.2 years were enrolled in this study. The clinical characteristics of case and control subjects are summarized in Table 1. There was no significant difference between weight, height, and BMI of the case and control groups. A summary of the disease information and laboratory data in patients of both sexes is given in Table 2; however, there was no significant difference in this regard in both sexes. Twenty-three (26.4 %) T1DM children had family history of T2DM. Tanner stage distribution of the patients was 39.1, 6.9, 11.5, 12.6, and 29.9 % in stages 1, 2, 3, 4, and 5 respectively; 16.1 % of the children had physical activity more than three times per week. There was no significant difference in Tanner stage of patients and controls ($P = 0.065$). About sun exposure, 8, 12.6, and 79.3 % of T1DM children were exposed to <15, 15–30, and 30–60 min of sun light per day, respectively. Of the children, 41 % used NPH added to regular insulin twice daily, and 52 % used daily glargine added to 3 Aspart dose per meal. There was no significant difference in family history of T2DM ($P = 0.81$), Tanner stage ($P = 0.69$), sun exposure ($P = 0.135$), and insulin regimen ($P = 0.275$) between both sexes of T1DM children; however, boys had more physical activity ($P = 0.008$) per week.

The body composition of T1DM children compared to normal controls has been summarized in Table 3. The total body fat percent and trunk fat percent were more in T1DM subjects (both $P < 0.001$), and the total lean mass in T1DM was less than the control ones ($P = 0.01$). Both android and gynecoid fat was more in T1DM subjects (*P*, 0.026 and <0.001, respectively); however, android/gynecoid fat ratio in T1DM children was less than that of controls ($P = 0.002$). Fat mass index was more in T1DM ($P = 0.012$), and lean mass index was more in non-diabetic children ($P = 0.013$). Trunk-to-leg fat ratio and trunk-to-limb fat ratio in T1DM children were less than the normal subjects (*P*, 0.044 and 0.016 respectively).

Table 1 Descriptive data of case and control group and *p* value of their comparison

Variable	Case[a]		Control[a]		*P* value
	Mean	SD	Mean	SD	
Age (year)	12.4	4.2	12.4	4.2	1
Weight (kg)	39.7	15.3	41.27	13.8	0.31
Height (cm)	146.2	20.1	151.1	16.4	0.258
BMI (kg/m^2)	17.8	3.2	17.5	3	0.718
FBS (mg/dl)	228	108	75.5	11.4	<0.001

[a] Case and control groups are age- and sex-matched (both groups included 39 male and 48 females)

Body composition of T1DM children compared to controls considering their sex is summarized in Table 4. T1DM girls had more total body fat percent ($P = 0.015$) and more gynecoid fat percent ($P = 0.009$), compared to the girls in the control group. However, the total lean mass, android/gynecoid fat ratio, lean mass index, trunk/leg fat ratio, and trunk/limb fat ratio of T1DM girls were less than the control subjects (*P*, 0.007, <0.001,0.029–0.002 and 0.004, respectively). T1DM boys had more total fat ($P = 0.008$), more total trunk fat percent ($P < 0.001$), more total body fat percent ($P < 0.001$), more android fat ($P < 0.001$), more gynecoid fat ($P < 0.001$), and more fat mass index ($P < 0.001$) than the control boys.

The association of body composition parameters and some disease factors of T1DM is summarized in Table 5. The total body fat was associated with age onset of DM ($P < 0.001$), total daily insulin ($P < 0.001$), Tanner stage ($P < 0.001$), and BMI ($P < 0.001$). Total body lean mass had significant association with age onset of DM ($P < 0.001$), total daily insulin ($P < 0.001$), Tanner stage ($P < 0.001$), physical activity ($P = 0.046$), and BMI ($P < 0.001$). Android/gynecoid fat ratio was associated with age onset of DM ($P < 0.001$), total daily insulin ($P = 0.011$), daily insulin per kg of body weight ($P = 0.023$), Tanner stage ($P < 0.001$), and BMI ($P < 0.001$).

Fat mass index was associated with age onset of T1DM ($P = 0.027$), total daily insulin ($P = 0.019$), Tanner stage ($P = 0.003$), and BMI ($P < 0.001$). Lean mass index was associated with age onset of disease ($P < 0.001$), total daily insulin ($P < 0.001$), Tanner stage ($P < 0.001$), and BMI ($P < 0.001$). Trunk/leg fat ratio was associated with age onset of disease ($P < 0.001$), total daily insulin ($P = 0.017$), serum FBS ($P = 0.019$), Tanner stage ($P = 0.013$), and BMI ($P = 0.001$).

Table 2 General characteristics of T1DM children in both sexes and *P* value of male and female univariate comparison

Variables	Total		Boys		Girls		*P* value
	Mean	SD	Mean	SD	Mean	SD	
Age (year)	12.37	4.21	12.97	4.4	11.87	4	0.228
Age onset of DM (year)	8	4	8.6	4.4	7.5	3.6	0.195
HbA_1c	10.2	2.2	10.1	2.2	10.2	2.3	0.731
Insulin use (u/day)	21.8	13	22.7	12.6	21.1	13.4	0.585
Insulin use (u/kg/day)	0.7	0.27	0.7	0.3	0.72	0.25	0.332
Duration DM (year)	4.4	2.8	4.4	2.1	4.4	3.4	0.246
FBS (mg/dl)	228	108	207	105	247	109	0.094
TG	123	50	128	42	118	56	0.069
Total cholesterol	152	36	157	41	147	32	0.204
Weight (kg)	39.7	15.3	42.67	16.6	37.3	13.9	0.067
Height (cm)	146.2	20.1	149.6	22.5	143.4	17.6	0.107
BMI (kg/m^2)	17.8	3.2	18.2	3.2	17.4	3.13	0.356

On multiple regression analysis, there was an independent effect of Tanner stage($P < 0.001$) and FBS ($P = 0.045$) on total fat (g) ($R^2 = 0.549$, VIF = 1–2.85); an independent positive effect of age onset of T1DM ($P < 0.001$) on total lean mass (g) ($R^2 = 0.811$, VIF = 1.2–4.9); an independent positive effect of FBS on android/gynecoid fat ratio ($P = 0.019$, $R^2 = 0.368$, VIF = 1.01); an independent positive effect of Tanner stage on fat mass index ($P = 0.022$, VIF = 2.8, $R^2 = 0.167$); an independent positive effect of age onset of T1DM ($P = 0.008$) on lean mass index ($R^2 = 0.439$, VIF = 3.2–4); and an independent positive effect of FBS on fat trunk/fat leg ratio ($P = 0.01$, $R^2 = 0.247$, VIF = 1).

Discussion

This study revealed a remarkable increase in body fat index and decrease in lean mass index in T1DM children. However, the increase in gynecoid fat was more than the increase in android fat, and it was sex dependent. Total fat was independently associated with Tanner stage and FBS; total lean mass and lean mass index were independently associated with age onset of T1DM; android/gynecoid fat ratio and trunk/leg fat ratio were independently associated with FBS; and fat mass index was independently associated with the Tanner stage.

Insulin therapy, especially with an intensive regimen, has been linked to weight gain [22]; also, excessive intake results in elevated body fat and obesity [23]. However, some studies in children have failed to demonstrate a similar link [17, 24]. The result of a cohort study in the Pittsburgh epidemiology of diabetes complications (EDS) on 589 T1DM adult patients in the last 20 years revealed that obesity (BMI > 30 kg/m^2) increased to 22.7 % and overweight population (BMI 25–30 kg/m^2) rose from 26 to 42 % [25]; however, body composition with DEXA was not studied. Ingberg et al. showed that T1DM adults almost invariably had higher skin fold thickness values

Table 3 Body composition of case and control groups and *p* value of their comparison

Body composition parameter	Case		Control		*P* value
	Mean	SD	Mean	SD	
Total fat (g)	11,007	5094	9849	5128	0.148
Trunk fat %	23.7	5.5	19.9	7.8	<0.001*
Total body fat %	28.4	5.8	23.4	8.1	<0.001*
Total lean mass (g)	26,316	10,256	30,488	10,280	0.01*
Android fat (%)	24.7	6.6	21.4	9.2	0.026*
Gynecoid fat (%)	35.2	6.9	27.5	9.1	<0.001*
Android/gynecoid fat ratio	0.7	0.12	0.78	0.14	0.002*
Fat mass index	4.9	1.6	4.1	1.9	0.012*
Lean mass index	12.2	2.1	13.3	2.17	0.013*
Trunk/leg fat ratio	0.68	0.08	0.72	0.12	0.044*
Trunk/limb fat ratio	0.66	0.1	0.71	0.14	0.016*

* These values are statistically significant

Table 4 Body composition of T1DM children in both sex (Mean ± SD) and *P* value of the comparison between case and control in each sex group

Body composition parameters	Boys			Girls		
	Case	Control	*P* value	Case	Control	*P* value
Total fat (g)	10,653 ± 4752	7928 ± 3811	0.008*	11,310 ± 5408	11,409 ± 5553	0.932
Trunk fat %	21.5 ± 5.3	15.1 ± 4.5	<0.001*	25.7 ± 4.7	23.8 ± 7.6	0.088
Total body fat %	23.1 ± 0.22	18.7 ± 0.26	<0.001*	31 ± 4.7	27.7 ± 7.5	0.015*
Total lean mass (g)	30,172 ± 11,678	34,179 ± 12,004	0.148	23,011 ± 7543	27,425 ± 7425	0.007*
Android fat (%)	22.7 ± 7.1	15.9 ± 6.4	<0.001*	26.5 ± 5.6	29.3 ± 6.5	0.104
Gynecoid fat (%)	31.1 ± 7	22.2 ± 7.2	<0.001*	38.8 ± 4.5	34.9 ± 5.7	0.009*
Android/gynecoid fat ratio	0.73 ± 0.14	0.75 ± 0.16	0.608	0.68 ± 0.1	0.83 ± 0.09	<0.001*
Fat mass index	4.5 ± 1.6	3.1 ± 1.2	<0.001*	5.3 ± 1.6	5.4 ± 1.9	0.734
Lean mass index	13.1 ± 2.1	13.8 ± 2.5	0.26	11.5 ± 1.8	12.6 ± 1.3	0.029*
Trunk/leg fat ratio	0.7 ± 0.09	0.7 ± 0.14	0.996	0.66 ± 0.08	0.74 ± 0.1	0.002*
Trunk/limb fat ratio	0.7 ± 0.1	0.7 ± 0.15	0.518	0.64 ± 0.1	0.73 ± 0.11	0.004*

* These values are statistically significant

compared with healthy controls, a finding which was not confirmed in corresponding measurements with DEXA [3]. The diabetes control and complication trial research group performed a study to evaluate the influence of intensive diabetes treatment on body weight and composition revealed an increased in fat-free mass [9]. However, Carlson and Campbell study [26] found that increase in body weight was accounted for by an increased in fat mass. In addition, Goodship et al. found no difference in fat-free mass in 31 T1DM adults compared with age and sex matched non-diabetic volunteers [27]. All these studies did not include T1DM children, and except for the Goodship et al. studied, they did not compare there results with a control group.

Gomez et al. who assessed body composition in T1DM patients over 15 years old demonstrated differences in anthropometry and body composition in T1DM patients, especially those with lower waist/hip ratio in males and higher fat-free mass in males in relation to controls [19]. Our study which was the first case-control study for evaluation of body composition with DEXA in a relatively large group of T1DM children revealed that T1DM children had more body fat percent and fat mass index and less body lean mass and lean mass index. In addition, we showed that both android and gynecoid fat increased in T1DM children; however, the increase in gynecoid fat was more than that of android fat. Also, trunk/leg fat ratio and trunk/limb fat ratio were decreased in T1DM children. There were some differences in both sexes; decrease in total lean mass and lean mass index was more prominent in girls, who had lesser physical activity compared to the boys. In addition, android fat was much more increased in boys and gynecoid fat was much more increased in girls. Puberty and hormonal factors might have an influence on these changes. However, another study in girls older than 16 years old [16] showed an increase in the abdominal to leg fat ratio which had no association with age or hormonal factors. Genetic factors, a relatively sedentary life style in older children of that study, and unfavorable dietary habits may all also associate to the tendency of abdominal fat accumulation in the Swedish adolescent girls [16]. It is known that the android/gynecoid ratio is a good predictor of both insulin resistance and cardiovascular risks, even if a child is at normal weight [21]. Interventions in both children and adults have been shown to decrease android fat, which could improve insulin resistance [28]. So, our study revealed that increase in the fat mass index in T1DM children is accompanied with a decrease in android/gynecoid fat and could not increase cardiovascular risks.

We do not know yet the exact mechanism by which insulin therapy or T1DM pathogenesis increases body fat mass. Some theories proposed the role of inflammatory cytokines secreted by the adipose tissue, like in type 2 diabetes [29–31]. Others have proposed that the increasing insulin concentration could stimulate 11β-hydroxy steroid dehydrogenase, which modulates the fibroblasts differentiation to adipocytes [32], and some studies discussed it by the antilipolytic effect of insulin [15]. Serum resistin concentrations was decreased in fasting T1DM children and adolescents and has a negative correlation with body fat mass, which was not observed in healthy children. These findings might be the result of broken physiological adipo-insular regulations in T1DM children, independent of disease duration, its metabolic control, and insulin supply [33]. The decreasing lean mass in our T1DM patient treated at least 2 years with insulin has been demonstrated in other clinical situations with hyperinsulinism as in myotonic dystrophy [34, 35]. Another interesting explanation for increasing fat mass and decreasing lean mass in type 1 diabetes was alternation in Wnt signaling pathway in T1DM which has a promoting role in human β-cell proliferation [36]. Recent studies have demonstrated that WNT/β-catenin signaling has a key role in the balance between myogenesis and adipogenesis. Wnt10b is an important inhibitor of adipogenesis and should

Table 5 Association of body composition parameters with some factors in T1DM children: *P* value, (correlation of coefficent)

Body composition parameter	Age onset of DM	HbA1c	Total daily insulin (unit)	Daily insulin per kg (u/kg)	FBS (mg/dl)	TG	TC	Sun exposure	Tanner	Excersie	Family hx of DM2	Insulin type (NPH/glargin)	BMI
Total fat (g)	<0.001* (0.58)	0.304 (0.119)	<0.001* (0.468)	0.14 (−0.173)	0.197 (−0.151)	0.877 (0.018)	0.601 (−0.06)	0.342	<0.001*	0.591	0.161	0.854	<0.001* (0.813)
Trunk fat %	0.65 (0.052)	0.927 (−0.011)	0.782 (0.032)	0.977 (−0.003)	0.309 (−0.119)	0.89 (0.016)	0.974 (−0.004)	0.68	0.456	0.283	0.488	0.468	<0.001* (0.402)
Total body fat %	0.879 (−0.018)	0.916 (0.012)	0.886 (−0.017)	0.856 (−0.021)	0.768 (−0.035)	0.928 (−0.01)	0.803 (0.029)	0.621	0.598	0.151	0.355	0.311	0.622 (0.057)
Total lean mass (g)	<0.001* (0.735)	0.195 (0.149)	<0.001* (0.598)	0.063 (−0.218)	0.336 (−0.113)	0.716 (0.042)	0.49 (0.08)	0.541	<0.001*	0.046*	0.453	0.771	<0.001* (0.67)
Android fat (%)	0.073 (0.204)	0.615 (−0.058)	0.595 (0.061)	0.134 (−0.176)	0.249 (0.061)	0.589 (0.06)	0.54 (0.07)	0.325	0.173	0.492	0.473	0.545	<0.001* (0.503)
Gynecoid fat (%)	0.2 (−0.147)	0.927 (−0.01)	0.14 (−0.16)	0.85 (0.02)	0.62 (0.05)	0.76 (0.03)	0.85 (0.02)	0.527	0.5	0.466	0.392	0.312	0.316 (0.115)
Android/gynecoid fat (%)	<0.001* (0.485)	0.46 (−0.08)	0.011* (0.285)	0.023* (−0.264)	0.413 (0.09)	0.61 (0.06)	0.41 (0.09)	0.649	<0.001*	0.291	0.943	0.745	<0.001* (0.597)
Fat mass index	0.027 (0.25)	0.56 (0.07)	0.019* (0.27)	0.45 (−0.09)	0.38 (−0.104)	0.78 (0.03)	0.85 (−0.02)	0.587	0.003*	0.915	0.164	0.478	<0.001* (0.718)
Lean mass index	<0.001* (0.488)	0.24 (0.13)	<0.001* (0.53)	0.2 (−0.15)	0.42 (−0.09)	0.41 (0.09)	0.64 (−0.05)	0.758	<0.001*	0.387	0.338	0.976	<0.001* (0.758)
Trunk/leg fat ratio	0.001* ((0.369)	0.67 (−0.04)	0.017* (0.886)	0.88 (−0.02)	0.019* (−0.27)	0.42 (0.09)	0.53 (−0.07)	0.628	0.013*	0.791	0.822	0.538	0.001* (0.458)
Trunk/limb fat ratio	0.79 (0.03)	0.78 (0.03)	0.67 (0.04)	0.25 (0.13)	0.208 (−0.14)	0.8 (0.03)	0.27 (−0.12)	0.962	0.473	0.856	0.447	0.377	0.372 (0.01)

* These values are statistically significant

be suppressed for preadipocytes differentiation. Also, disruption of WNT signaling causes spontaneous adipocyte conversion. So, loss of Wnt10b causes increasing adipogenic potential of myoblasts and the acquisition of adipocyte characteristics during muscle regeneration [37].

In this study, we found that the increase in body fat mass in T1DM children was independently associated with Tanner stage and FBS of disease (combination of pubertal hormonal charges and glycemic control); however, the decrease in body lean mass was independently associated with age onset of T1DM. Increased fat located in the abdominal region, expressed as the trunk/leg fat ratio, was independently associated with FBS, an index of a higher insulin requirement and poor glycemic control. Similar to our report results of Ingberg et al. [16] study, which evaluated body composition of 18 post-menarchal T1DM female adolescents, adolescents aged 16–19 years in Sweden revealed a higher fat deposit in diabetic patients, which was mostly located in the abdominal region. Abdominal obesity seems to be associated with poor glycemic control [38]. However, due to limitation in pubertal stage of patients in the Ingberg study, association of Tanner stage with body fat mass was not evaluated. Another study by Gomez and his colleagues, which assessed body composition in T1DM patients over 15 years old, also revealed that an increase in body fat was related to the disease itself, dietary or energy intake, and insulin treatment [18]. However, an investigation in Greece failed to find any correlation between glycemic control and anthropometric variables [17]. To our knowledge, these researches were the only ones who studied the association of DEXA-evaluated body composition and factors of T1DM disease; however, both did not include children under 15 years old. Yet we did not find any independent association with body composition and lipid profile, sun exposure, and type of insulin therapy.

The limitation of this study was our inability to check the various genetic factor axes (known to contribute independently to growth and probably also to pubertal status) because these might be possible relevant factors. Also, we have checked that the sun exposure and physical activity with subjective methods might have some bias in this study.

Conclusions

This study revealed an increase in body fat index and a decrease in lean mass index in T1DM children. We found that the increase in body fat mass in T1DM children was independently associated with Tanner stage and FBS of patients; however, decreasing body lean mass was independently associated with age onset of T1DM. Improvement in controlling blood sugars might prevent increasing of the body fat mass; also, emphasis on doing regular physical activity is needed to attenuate the loss of lean body mass especially in patients with earlier onset of disease. Further investigation is needed to find out other important factors in body composition.

Acknowledgments The authors would like to thank Dr. Asma Erjaee at Center for Development of Clinical Research of Nemazee Hospital for editorial assistance.

Conflict of interests The authors declare that they have no conflicts of interest.

Financial support There is no financial support.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):248–253
DOI 10.1007/s13410-016-0480-y

ORIGINAL ARTICLE

The predictive value of diabetes-related antibodies in children with type 1 diabetes mellitus and their siblings

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Received: 5 November 2015 / Accepted: 9 March 2016 / Published online: 30 March 2016

Abstract The purpose of this study is to screen for diabetes-related antibodies in newly diagnosed type I diabetes Saudi children, and their non-diabetic siblings. We studied 69 newly diagnosed type 1 diabetic Saudis (35 girls, 34 boys), 60 non-diabetic siblings (1 to 17 years), and 42 age- and sex-matched controls not having type 1 diabetes. Their sera were tested for insulin autoantibodies (IAA), antibodies to glutamic acid decarboxylase (GAD65A) and protein tyrosine phosphatase-2 antibodies (IA-2) using ^{125}I radioimmunoassays. Fifty-two percent of patients were significantly positive for IAA compared to controls (4.9 %). A total of 50.7 % of patients were also significantly positive for IA-2 compared to controls (4.8 %) ($p < 0.0001$). GAD65 antibody was detected in 81.2 % of the patients and in none of the controls ($p < 0.0001$). Both IAA and IA-2 are significantly higher among younger age group, unlike GAD65A, which is significantly higher among older age groups. In non-diabetic siblings, the frequencies of IAA, IA-2, and GAD65A were 6.8, 5.2, and 10.2 % (0.039), respectively, which were higher than in controls. IAA and IA-2 titers were significantly high among younger age group (<0.027), and GAD65A is significantly higher among older age group. A total of 21.6 % of diabetics were positive for all the three antibodies and 3.4 % in siblings. A total of 35.3 % were positive for two antibodies and none in siblings, while 39.2 % were positive for one antibody and 11.9 % in siblings. The combined GAD65 and IA-2 was positive in 81.3 % of young age, 80 % of middle, and in 100 % of the old age group. The screening of type 1 diabetes among Saudis showed the presence of diabetes-related antibodies in 96.1 % of all newly diagnosed patients, compared to 11.9 % of controls (non-diabetic siblings). IAA and IA-2 were significantly higher among younger age groups; GAD65A was significantly higher among older age. The combined GAD65 and IA-2 tests can be considered as a sensitive marker for predicting the occurrence of the disease in individuals at risk of type 1 diabetes in Saudis.

Keywords Insulin autoantibodies · Glutamic acid decarboxylase · Protein tyrosine phosphatase-2 antibodies

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Introduction

Type 1 diabetes mellitus (insulin-dependent diabetes mellitus (IDDM)) is a chronic disease characterized by autoimmune destruction of pancreatic B cells and complete insulin deficiency [1]. There is a large body of evidence indicating that inherited genetic factors can influence both susceptibility and resistance to the disease [2]. Genetic susceptibility is clearly dependent on the degree of genetic identity with the proband, and the risk of diabetes in families has a non-linear correlation between the numbers of alleles shared with the proband [3]. There is also a possibility that additional genetic factors or their expression may be acquired after birth, perhaps through environmental exposures. Such factors may play a key role in triggering B cell autoimmunity in susceptible individuals [4].

Autoantibodies to islet antigens, such as islet cell antibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase antibodies (GAD65A), and protein tyrosine phosphatase-2 (IA-2) antibodies, are considered to be markers

of islet autoimmunity in most children at the time of diagnosis of type 1 diabetes and prior to diagnosis in some of the first-degree relatives [5].

These ICAs antibodies were the first to be identified in 70 to 80 % of type 1 diabetes at or prior to disease onset [6]. They were present in other polyendocrine disorders, but at much lower frequencies [7]. The role of ICAs as predictive markers was studied extensively more than any other type 1 diabetes-associated autoantibodies [8]. In addition, early studies in first-degree relatives of type 1 diabetic patients showed that ICA was detected several years prior to the onset of the clinical disease [9]. Later, the probability of developing the disease was directly related to the titer of ICA [10].

The association of IAA with type 1 diabetes was first reported in 1983 when Palmer and colleagues established their presence in 18 % of untreated newly diagnosed diabetic patients [11]. The prevalence of insulin antibodies is almost 100 % in very young individuals and is almost absent in patients with adult onset type 1 diabetes [12]. Type 1 diabetes relatives with IAA have an increased probability of developing type 1 diabetes, and this probability is greater when IAA were detected in combination with ICA [13].

IA-2 antibody to an intracellular fragment of protein tyrosine phosphates-2 was found in 50 to 70 % of type 1 diabetic patients at or prior to disease onset [14]. IA-2 antibodies play an important role in predicting the disease especially when detected in combination with other markers such as ICA, GAD65, and insulin antibodies [15].

Antibodies to glutamic acid decarboxylase (64-kD islet cell proteins) were described in association with type 1 diabetes [16]. GAD65 was known to be one of the major antigens recognized by autoantibodies in sera obtained from patients with type 1 diabetes. GAD65 antibodies were found in 70 to 80 % of patients at or prior to the onset of the disease [17]. First-degree relatives who are positive for ICA and GAD65 antibodies had a higher probability of developing type 1 diabetes than those with ICA alone [18].

Although nearly 90 % of new cases of type 1 diabetes occur sporadically, studies of first-degree relatives are indispensable [19]. Extended follow-up of individuals at risk of developing type I has proved to be useful in establishing the predictive value of the different markers and the subsequent application of preventive measures.

In the current study, we describe for the first time, the prevalence of IAA, IA-2, and GAD65 antibodies among newly diagnosed Saudi children and their non-diabetic siblings at the time of diagnosis of the probands. We also analyzed the combined analysis of IAA, IA-2, and GAD65A to find out whether they could replace the histochemical ICA test. The autoimmune pathogenesis of type 1 diabetes was not studied before in Saudi from the central province of Saudi Arabia. Despite the fact that Saudi Arabia is considered one of the places with low incidence of type 1 diabetes (T1DM), there is a general clinical impression of an increased trend in the incidence of the disease in this area following the gulf war 1991.

Subject and methods

Children with new onset type 1 diabetes ($n = 69$), which were recruited from the Sulimania Pediatric Hospital, Riyadh, Saudi Arabia, were chosen as the subject of this study. The patients (34 girls and 35 boys) were all of Saudi origin, developed diabetes before the age of 15 years, and were dependent on insulin from the time of diagnosis. The mean age of disease onset was 6.6 ± 0.48 years (range 1.17 to 12.58 years). The study group also included 60 non-diabetic siblings of the recruited subjects, age ranged between 1 and 17 years. A third group of 42 healthy Saudi children served as controls who are sex- and age-matched to the patients and with no family history of type 1 diabetes. The Institutional Review Board (IRB) and the ethical committee at the College of Medicine, King Khalid University Hospital, approved the study. A written consent obtained from the parents of the participants. Patients were divided into the following three groups of age range: younger than 5 years, 5 to 10 years, and older than 10 years.

Detection of the autoantibodies

Serum samples from the patients, controls, and non-diabetic siblings were tested for the presence of the following diabetic antibodies: IAA, IA-2, and GAD65A using ^{125}I-Radioimmunoassay kits from Diagnostka GMBH (DLD) Germany.

Insulin-autoantibodies

IAA were measured using radiobinding assay as described previously [20]. Serum samples incubated overnight with ^{125}I-labeled mono iodinated insulin. This was followed by the addition of anti-human IgG to precipitate the labeled insulin-anti-insulin complexes. After centrifugation, the precipitate counted with Wizard 1470 automatic gamma counter (Wallac, Finland). The results converted to arbitrary units by the use of a standard curve. A titer of ≥5 U/ml was considered positive.

Protein tyrosine phosphatase-2 and glutamic acid decarboxylase 65 antibody

The sera were incubated overnight with ^{125}I-labeled human recombinant IA-2 or ^{125}I-labeled human recombinant GAD65, followed by the addition of solid-phase protein A, to precipitate the labeled IA-2 and the IA-2 antibodies

complexes or the labeled GAD65 and GAD65 antibodies complexes. After centrifugation, the precipitate counted for radioactive ^{125}I. The results converted to concentration units by the use of a standard curve. Antibody titer ≥1 U/ml was considered positive.

Statistical analyses

All statistical analyzes were performed using the SPSS statistical software version 13 (SPSS, Chicago, IL). Data were expressed as frequencies, mean + SD. Antibody titers were presented as mean ± SEM. Student's *t* test was used to analyze normally distributed continuous variables, and the Mann-Whitney *U* test analysis was used in case of skewed distributions. Spearman's non-parametric correlation analysis was used to analyze the relationship among the levels of various antibodies. Differences in the distribution of individual among groups were tested with chi-square statistics unless any expected value was less than five when Fisher's exact test used. A two-tailed *p* value <0.05 was considered significant.

Results

Insulin antibodies

The prevalence of IAA among patients was 52 % (36/69) which is significantly higher compared to controls 4.9 % (2/42) (*X* = 31.67, OR 53.5, 95 % CL 7.2–1099.4, *p* 0.0001) (Table 1). The frequency of IAA among different age groups was as follows: 55.6 % (20/36), 27.8 % (10/36), and 16.7 % (6/36) in young <5 years), middle (5 to 10 years), and old (>10 years) age groups, respectively. The frequency of IAA in the young age group is significantly higher compared to that in the older age group (*X* 10.17, OR 6.25, 95 % CL 1.86–20.88, *p* 0.001). In addition, it was significantly higher compared to the frequency of IAA in the middle age group (*X* 4.6, OR 3.25, 95 % CL 1.09–9.82, *p* 0.03) Table 2.

The mean concentration of IAA among the positive cases was 28.4 ± 8.1 U/ml, which is significantly higher compared to controls (0.5 U/ml) (*p* 0.0001).

Protein tyrosine phosphatase-2 antibody

A total of 50.7 % (35/69) of patients were tested positive for IA-2 antibody which is significantly higher compared to healthy controls 4.3 % (2/42) (*X* 24.66, OR 16.13, 95 % CL 4.24–71.96, *p* 0.0001) (Table 1). IA-2 frequencies in different age groups are as follows: 45.71 % (16/35), 42.86 % (15/35), and 11.43 % (4/35) in young, middle age, and old age groups, respectively. The frequency of IA-2 among the young age is significantly higher compared to the old age group (*X* 8.47, OR 6.526, 95 % CL 1.68–27.43, *p* 0.004) (Table 2). The mean IA-2 concentration among positive cases was 4.5 ± 1.03 U/ml, which is significantly higher compared to controls (0.3 ± 2) (*p* = 0.001).

Glutamic acid decarboxylase antibody

A total of 81.2 % (56/69) patients were positive for glutamic acid decarboxylase (GAD65) antibody, which was significantly higher compared to control (zero/42) (*X* 44.88, OR 68.6, 95 % CI 13.6–139.57, *p* 0.0001) (Table 1). GAD65 antibody frequency in different age groups was as follows: 17.9 % (10/56), 28.6 % (6/56), and 53.6 % (30/56) in the young, middle, and old age groups. The frequency of GAD65A among the older age group was significantly higher compared to that among the middle age group (*X* 6.23, OR 2.9, 95 % CL 1.23–6.82, *p* 0.013), also significantly higher compared to the younger age group (*X* 5.28, OR 2.65, 95 % CL 1.14–6.2, *p* 0.02) (Table 2). The mean concentration of GAD65 antibodies among positive cases was 41.7 ± 11.63, which is significantly higher compared to control (zero) (*p* = 0.0001)

There is no correlation found between sex of the patients and the frequency of the three antibodies (IAA, IA-2, and GAD65A).

Multiple antibodies in newly diagnosed type 1 diabetic patients

The prevalence of multiple antibodies in newly diagnosed type 1 diabetes was as follows: 21.73 % (15/69) of patients were positive for the three antibodies (IAA, IA-2, GAD65) at the time of diagnoses; 33.33 % (23/69) of patients were positive for two antibodies; and 39.13 % (27/69) of patients tested positive for one antibody. All in all, 94.2 % (65/69) of the patients were positive for one or multiple antibodies.

The combined IA-2 and IAA antibodies were present in 58.6 % (10/16) of the young age, in 57.14 % (20/35) of the middle age, and in 83.3 % (15/18) of the older age group. While, both IA-2 and GAD65 antibodies were present in 81.3 % (13/16) of young age, 80 % (28/35) of middle, and in 100 % (18/18) of the old age group. Lastly, GAD65 and IAA were present in 93.8 % (15/16) of the young age, in 82.9 % (29/35) of the middle, and in 94.4 % (17/18) of the old age group.

Autoantibodies in non-diabetic siblings

Insulin antibodies

The prevalence of insulin autoantibodies (IAA) among non-diabetic sibling was 6.67 % (4/60) (Table 1), compared to 4.8 % (2/42) in controls.

Table 1 The prevalence of IAA, IA-2, and GAD65 antibodies among type 1 diabetes patients, non-diabetic siblings, and controls

	IAA *n* (%)	I A-2 *n* (%)	GAD65A *n* (%)
Controls (*n* = 42)	4.8 % (2/42)	4.8 % (2/42)	0 (0.0 %)
Patients (*n* = 69)	52 % (36/69)*	50.7 % (35/69)*	81.2 % (56/69)*
	p < 0.0001	*p < 0.0001*	*p < 0.0001*
	X = 31.7	*X* = 24.7 OR = 16.13	*X* = 44.9
	OR = 53.5	(95 % CL 4.2–71.9)	OR = 68.6
	(95 % CL 7.2–109.9)		(95 % CL 13.6–139.6)
Siblings (*n* = 60)	6.8 % (4/60)	5.1 % (3/60)	10 % (6/60)

Italicized entries is significant to $P < 0.05$

* Significance compared to corresponding controls

Protein tyrosine phosphatase-2 antibody

Five percent (3/60) of the non-diabetic siblings tested positive for protein tyrosine phosphatase-2 (IA-2) antibody (Table 1), which is slightly higher than IA-2 frequency in controls 4.8 % (2/42).

Glutamic acid decarboxylase 65 antibody

Ten percent (6/60) of the non-diabetic siblings were positive for GAD65 antibody (Table 1).

The prevalence of multiple antibodies among diabetic siblings was as follows: 3.3 % (2/60) of siblings had the three antibodies, and 11.66 % (7/60) were positive for one antibody. Collectively, 15 % (9/60) of the non-diabetic siblings showed evidence of autoimmunity against beta cells at the time of proband diagnosis.

Discussion

The major goal of diabetes research is the prediction and prevention of type 1 diabetes. In the current study, we studied the autoimmune etiology of type 1 diabetes among Saudi children and their non-diabetic siblings for the first time in the region. The frequencies and concentration of three autoantibodies, IAA, GAD65A, and IA-2 were determined in newly diagnosed diabetics and their non-diabetic sibling.

We found the prevalence of IAA among Saudi type 1 diabetes patients was significantly higher compared to control (52 versus 4.8 %, $p = 0.0001$). The IAA frequency in Saudi patients is comparable to the reported range (18 to 69 %) from other countries [21–23]. We found that IAA are significantly higher among the younger age (0 to 5 years) compared to the older age group. It is similar to what was previously reported for increased incidence of IAA among younger age diabetic children [12, 24]. In contrast to other, who found no significant correlation between IAA positivity and age of onset in newly diagnosed patients [25]. Although the role of IAA in predicting diabetes is still a matter of controversy, some establish that the presence of IAA in conjunction with islet cell antibodies in high-risk subjects enhanced the predictive value for the development of type 1 diabetes [26]. In other studies, they were unable to demonstrate a high predictive value for IAA for the development of the diabetes [27].

The wide range of IAA frequencies reported in the different publication may reflect the different assay techniques used for the detection of IAA, which recognized various epitopes on the insulin molecule. Most of these epitopes are available for binding in the fluid phase RIA assay. Additionally, the RIA assay has the advantage of using a very low quantity of the tracer and consequently is sensitized to detect high-affinity antibodies [28].

IA-2 was also assayed using RIA. It was detected in 50.7 % of a newly diagnosed diabetic with type 1 diabetes and in 4.8 % of the controls sera. The prevalence IA-2 among Saudi patients is in the lower range of frequencies reported from the USA and Europe (50 to 80 %) [29]. The lower frequency of IA-2 antibodies in Saudi diabetic patients may be a reflection of the geographical and/or ethnic variations known about this disease. A similar low prevalence reported in Asians living in the UK compared to Europid diabetic subjects

Table 2 The prevalence of IAA, IA-2, and GAD65 antibodies in different ages of onset of disease

	<5 years	5 to 10 years	>10 years
Male/female	10/6	17/18	6/12
IAA (*n* = 36) 52.2 %	55.6 % (20/36)*	27.8 % (10/36)	16.7 % (6/36)
IA-2 (*n* = 35) 50.7 %	45.7 % (16/35)*	42.8 % (15/35)	11.4 % (4/35)
GAD65 (*n* = 56) 81.2 %	17.9 % (10/56)	28.6 % (16/56)	53.6 % (30/56)*

* Significance compared between age groups

[30]. IA-2 autoantibodies have high specificity, sensitivity, and positive predictive value for the developing of diabetes [31]. In addition, other studies found that the presence of IA-2 antibody is associated with a more rapid progression to the clinical onset of type 1 diabetes in first-degree relatives of affected children [32].

The autoantibodies against glutamic acid decarboxylase (GAD 65) were present in individuals at risk for the development of type 1 diabetes, several years before the clinical onset of the disease [9]. Approximately 7 % of patients with initially non-insulin-dependent adult onset diabetes have GAD65 autoantibodies [33]. Almost all of those GAD65-positive individuals became insulin dependent [34].

The glutamic acid decarboxylase antibody was the most prevalent of the three autoantibodies in Saudi children with type 1 diabetes. GAD65A was detected in 81.2 % in newly diagnosed type 1 diabetes among Saudi children, which is in line with similar findings of 50–80 % reported in Europids [35]. In addition to the high prevalence of GAD65 among patients, none of the control sera tested positive for the GAD65 antibody. Thus, the GAD65 antibodies test is considered highly specific for screening newly diagnosed type 1 diabetes among Saudi children [36].

In the current study, the simultaneous presence of antibodies to GAD65 and or IA-2 was detected in 90.2 % of newly diagnosed patients. Therefore, such combination can safely substitute the histochemical ICA test, which is not feasible in Saudi Arabia due to the strict legislation on organ collection.

It is known that children who are first-degree relatives with type 1 diabetes have more than 10-fold higher risk of developing the disease [37]. Thus, there is a pressing need to study non-diabetic sibling of type 1 diabetes at the time of diagnosis of a proband. In the present study, we were able to find evidence of autoimmunity among type 1 diabetes healthy siblings. in which IAA, IA-2, and GAD65 antibodies were detected in 6.67, 5, and 10 %, respectively. The frequency of IAA and IA-2 in non-diabetic Saudi siblings is in agreement with other previous reports [38]. IA-2 antibody-positive young children are considered a marker for disease progression [39]. The follow-up of the IA-2-positive siblings for an extended period (more than 5 years) is the appropriate approach to establish the predictive value of IA-2 in developing the type 1 diabetes in the Saudi population. The prevalence of GAD65 among non-diabetic siblings (10 %) is in the range reported from other countries [40, 41].

The presence of multiple islet-related antibodies identifies subjects at high risk of developing diabetes. In this study, we found that 11.66 and 3.3 % of non-diabetic sibling have one and three antibodies, respectively. Overall, 15 % of non-diabetic siblings show signs of autoimmunity against pancreatic beta cells.

The high frequencies of IAA, IA-2, and GAD65 antibodies, detected in Saudi diabetic children, reflect the role of autoimmunity in beta cells destruction in type 1 diabetes among Saudis. In addition, the presence of these antibodies among non-diabetic siblings is in agreement with reports from countries with high incidence of the disease such Europid studies [30]. The presence of diabetic autoantibodies emphasizes the autoimmune etiology of type 1 diabetes among the Saudi population. However, more important in the prediction of the disease is the presence of multiple antibodies.

Acknowledgments This work was supported by the King Abdulaziz City for Science and Technology (grant reference number MS-4-46). We thank Dr. S. Abdelrahman at the Sulimania Pediatric Hospital for referring patients to the study.

Compliance with ethical standards

Conflict of interest All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The authors declare that they have no competing interests. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):254–261
DOI 10.1007/s13410-016-0481-x

ORIGINAL ARTICLE

Hypogonadism in Nigerian men with type 2 diabetes mellitus

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Received: 14 November 2015 / Accepted: 10 March 2016 / Published online: 19 March 2016

Abstract Hypogonadism in patients with diabetes mellitus (DM) has been associated with insulin resistance and poor glycaemic control. This study was conducted to determine the prevalence, types, and associations of hypogonadism in Nigerian men with established type 2 diabetes mellitus. The study was a cross-sectional observational work, which was conducted at the Lagos University Teaching Hospital (LUTH). The participants consisted of 108 men with type 2 DM and 56 non-diabetic controls. A questionnaire was used to obtain demographic data while the Androgen Deficiency in Aging Male (ADAM) questionnaire was administered to elicit symptoms of hypogonadism. The enzyme-linked immunosorbent assay (ELISA) method was used to test for serum-free testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and estradiol. Other investigations done included fasting lipid profile, fasting plasma glucose, and glycated haemoglobin (HbA1c). Statistical analysis was done with SPSS v20. Tests of normality were carried out on the data to determine appropriate statistical analytic methods. The mean (±SD) ages of the DM patients and controls were 51.7 ± 5.9 and 50.9 ± 4.6 years, respectively ($p = 0.349$), with a mean duration of diabetes 93.6 ± 6.29 months. Among the DM patients, 17 (15.7 %) were obese, while 6 (10.7 %) controls had obesity. Hypogonadism (predominantly secondary in (78.5 %)) was present in 42 (38.9 %) of the DM patients. In the controls, only 2 (3.6 %) had hypogonadism. Predictors of hypogonadism were a high HbA1c. Hypogonadism, which was predominantly secondary hypogonadism, was prevalent in the patients and was associated with HbA1c levels.

Keywords Hypogonadism · Type 2 diabetes · Glycaemic control · Testosterone · Nigeria

Introduction

Hypogonadism in persons with diabetes mellitus (DM) is an area where very little was known until recently. Hypogonadism in DM has generated a lot of interest in the light of recent publications associating it with insulin resistance, poor glycaemic control, erectile dysfunction (ED), osteoporosis, increased cardiovascular risk, impaired cognition, and depression [1].

In patients without DM, published estimates of the frequency of hypogonadism range from 30 to 40 % in men older than 65 years to as high as 70 % in men 80 years or older [2, 3]. Comorbidities associated with male hypogonadism include ED, dyslipidaemia, metabolic syndrome, and DM [4].

Insulin resistance is a major feature of type 2 DM. It has been observed that low testosterone levels in men are associated with reduced insulin sensitivity and type 2 diabetes [5]. An inverse relationship exists between testosterone levels and insulin concentrations in healthy men [6]. Recent studies have also linked the development of type 2 diabetes and insulin resistance with low testosterone levels [7, 8].

The primary symptoms of hypogonadism in persons with DM include reduced libido, ED, fatigue, reduced physical

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strength, and mood changes. These symptoms are mostly non-specific and are similar to symptoms seen in many other illnesses. Questionnaires, such as the Androgen Deficiency in Ageing Male (ADAM) questionnaire have been developed to help in the clinical assessment of hypogonadism [9]. This questionnaire has a sensitivity of 86 % and a specificity of 66 %, implying that it should not be used alone for the diagnosis of male hypogonadism without biochemical confirmation [9].

In a Nigerian study, hypogonadotropic hypogonadism was common among men with diabetes who were evaluated for erectile dysfunction [10], in contrast to the findings among non-diabetic Nigerian men where a high prevalence of hypogonadotropic hypogonadism (primary hypogonadism) was reported [11–13]. Recently, Ogbera et al. [14] in a study on male hypogonadism recorded a prevalence rate of 35 % among Nigerian men with type 2 DM. This finding is similar to that published by Dhindsa et al. [15] but is far higher than the 7.3 % prevalence rate reported among Nigerian men without diabetes who were evaluated for infertility [13].

From literature search, most of the studies on hypogonadism in diabetes had involved Caucasians with very few African/Nigerian studies. Also, the earlier studies had measured serum total testosterone [10, 14], unlike in our study, which we measured free testosterone levels. Free testosterone levels are more representative of total testosterone in patients who have factors that affect the levels of sex-hormone binding globulin (SHBG). These factors include the presence of liver disease, thyroid disease, and the use of drugs such as corticosteroids [16]. This study has helped in expanding the inadequate knowledge on this subject in Nigeria.

Subjects and methods

The study was conducted at the Diabetes Clinic of the Lagos University Teaching Hospital (LUTH) situated in Lagos state in South Western Nigeria. Lagos has an estimated population of about 21 million and a male to female ratio of 1:0.7. Ethical approval for the study was obtained from the hospital ethics committee.

The minimum sample size for the study was calculated using the method of Leslie and Kish [19], obtaining a sample size of 89. Men with type 2 DM aged between 30 and 60 years and age-sex-matched controls were recruited into the study after obtaining written informed consent from them. The DM patients were selected by simple random sampling from the clinic attendance sheet on each clinic day according to the way they reported. An average of 30 men attended the clinic each clinic day. The selection process for the DM patients was done over 10 weeks leading to the recruitment of 150 DM patients as potential participants. One hundred and eight DM patients completed the study.

The controls for the study were consenting men without diabetes selected from the members of the staff of LUTH. The controls were selected through personal contact and public notice. Their fasting blood glucose was done, and only those with fasting plasma glucose <5.5 mmol/l who satisfied the inclusion criteria were recruited as controls. The selection process was concluded over 4 weeks, and a total of 80 controls were selected. Fifty-six controls eventually participated in the study. A questionnaire was administered to obtain sociodemographic data, followed by administration of the ADAM questionnaire [9]. Physical examination and anthropometry were done. Blood samples were collected for fasting plasma glucose, glycated haemoglobin (HbA1c), and fasting plasma lipids, hormonal assays for free testosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, and estradiol.

Fasting plasma glucose was done using the glucose oxidase method, after collecting blood samples in fluoride oxalate tubes. The glycated haemoglobin assay was carried out using the ELISA method. The ELISA technique was employed for the hormonal assays. The participant samples, standards, and controls were all run in duplicates. The mean absorbances of the duplicated assays were determined. The respective standard curves were then plotted to allow for the concentration of the different analytes to be derived.

Definition of terms

A positive ADAM score was any score between 6 and 10. Hypogonadism was defined as serum-free testosterone level <25 pg/ml with or without a positive ADAM score. Primary hypogonadism was defined as low serum-free testosterone (<25 pg/ml) and elevated LH (>18 mIu/ml) with or without elevated FSH (>18 mIu/ml). Secondary hypogonadism was defined as low serum-free testosterone (<25 pg/ml) and low LH with or without low FSH (<4 mIu/ml). Normogonadotrophic hypogonadism was defined as low testosterone with normal (4–18 mIu/ml) gonadotrophins. The reference levels for the hormones were as follows: LH 4–18 mIU/ml, FSH 4–18 mIU/ml, oestradiol 0–120 pmol/L, prolactin 45–375 mU/ml. Poor glycaemic control was defined as HbA1c >7 % [20].

Data analysis

Data analysis was done using SPSS version 20. The normality of data was tested using the Shapiro-Wilk test. Data was summarized using frequencies, means, and standard deviations for normally distributed data, medians, and interquartile range for skewed data. Chi-square or Student's *t* test was used for differences in proportions or means, respectively, while the Kruskal-Wallis or Mann-Whitney *U* test was used to test the difference in skewed data. A *p* value of <0.05 was regarded as significant.

Results

Characteristics of study participants

The mean (±standard deviation) ages of the DM patients and control subjects were 51.7 ± 5.9 and 50.9 ± 4.6 years, respectively ($p = 0.349$). The duration of DM among the patients ranged from 5 to 360 months with a mean of 93.6 ± 6.29 months. History of DM in a first-degree relative was present in 49(45.4 %) of the DM patients compared to 6 (10.8 %) in the controls ($P < 0.01$). History of hypertension was more common among the DM patients (50; 46.3 %) compared to the controls (14; 25.0 %) ($P < 0.01$). The anthropometric and clinical indices of the DM patients and controls were evaluated. Among the DM patients, 17 (15.7 %) were obese while 51 (47.2 %) were overweight. Similarly, among the controls, 6 (10.7 %) were obese while 34 (60.7 %) were overweight ($p < 0.01$).

Significant differences were observed between the DM patients and controls in their HbA1C ($p < 0.01$), fasting plasma glucose ($p < 0.01$), LDL cholesterol ($p = 0.01$), and HDL cholesterol ($p = 0.04$) (Table 1).

The frequency of poor glycaemic control in the patients with DM was 48.1 %.

Prevalence and pattern of hypogonadism in the study population

The gonadal function of the study participants was assessed using serum-free testosterone, oestradiol, prolactin, LH, and FSH levels. The DM patients had significantly lower free testosterone, LH, and FSH levels. The results are as summarized in Table 2. Hyperprolactinaemia was present in 6 (5.6 %) of the diabetic patients while none of the controls had hyperprolactinaemia. There was no significant difference in the ADAM score of those with hyperprolactinaemia and those without ($p = 0.43$).

Hypogonadism was present in 42 (38.9 %) of the DM patients. Three patterns of hypogonadism were seen namely hypogonadotropic hypogonadism, hypogonadotropic hypogonadism, and noromo gonadotrophic hypogonadism (Table 3).

Secondary hypogonadism was the predominant type in the DM patients and occurred in 33 (78.5 %) of the 42 DM patients who had hypogonadism.

The clinical characteristics of participants with hypogonadism were assessed. Table 4 shows the clinical features of the DM patients with hypogonadism compared to DM patients with normal gonadal functions (eugonadal). Participants with hypogonadism were further assessed to define their biochemical characteristics. The result is as shown in Table 4. For the DM patients, glycaemic control was significantly worse among those with hypogonadism than those without.

Patients with hypogonadism had higher levels of serum prolactin than those without though it was not statistically significant ($p = 0.67$). However, the level of serum beta-estradiol was significantly lower in the patients with hypogonadism ($p = 0.03$).

There were significant differences between the free testosterone and LH levels of patients who had poor glycaemic control and those who were well controlled. There was, however, no statistical difference in their FSH, estradiol, and prolactin levels as shown in Table 5.

The correlations between testosterone and some clinical and biochemical variables were explored in all the study participants using Spearman's correlation. The results are as shown in Table 6. There were statistically significant correlations between testosterone levels and age, HbA1c, and ADAM score in the DM patients, but not in the control group.

Predictors of low testosterone in the diabetic patients

Multiple logistic regression analysis was carried out to determine the predictors of low testosterone in the diabetic patients. Variables entered into the model include age, DM duration, blood pressure, FPG, HBA1c, serum prolactin, LH, FSH, oestradiol, and ADAM score. The only significant predictors in the model were HbA1c, LH, and FSH (Table 7).

Discussion

The study was carried out on male diabetic patients and non-diabetic controls, who were age- and sex-matched. The aim was to determine the frequency and pattern of hypogonadism among the subjects and to identify possible clinical predictors of low testosterone in them.

The mean age of the study population showed that they consisted of mainly middle-aged men. Gonadal function in men is known to decline with increasing age as was apparent in the study. There was a significant negative correlation between age and serum testosterone levels, although this was more pronounced in all the study subjects than in either group. Low serum-free testosterone levels were found in 42 (38.9 %) of the DM patients, representing more than a third of them. Diabetes mellitus is known to be associated with low testosterone levels. Recent publications have associated low serum testosterone levels in type 2 DM patients with insulin resistance, poor glycaemic control, erectile dysfunction, osteoporosis, increased cardiovascular risk, impaired cognition, and depression [15, 18]. The prevalence of hypogonadism among DM patients in this study was comparable to findings from other similar studies [10, 14]. Using total testosterone, a Nigerian study recorded a 36 % prevalence of hypogonadism

Table 1 Clinical characteristics and biochemical parameters of the study participants

Variable	Number (%) DM		X^2	*P* value
	N = 108	Controls *N* = 56		
Hypertension present	50(46.3)	14(25.0)	6.593	<0.01*
Ever smoked (yes)	25(23.1)	11(19.6)	0.143	0.14
Body mass index (kg/m^2) ≥30	17(15.7)	6(10.7)	0.056	0.26
Waist circumference (cm) ≥94	40(37.0)	20(35.7	0.014	0.50
Variable	Mean ± SD DM	Controls	Student's *t* test	*P* value
Age (years)	51.7 ± 5.9	50.9 ± 4.6	0.62	0.35
BMI (kg/m^2)	26.3 ± 3.6	26.6 ± 4.6	0.45	0.65
WC (cm)	90.3 ± 13.3	87.7 ± 10.5	0.83	0.17
Pulse rate (/min)	78.4 ± 9.1	74.9 ± 10.1	2.22	0.03*
Systolic BP (mmHg)	142.7 ± 20.8	133.4 ± 15.8	2.94	0.01
Diastolic BP (mmHg)	84.7 ± 9.5	77.9 ± 9.0	4.41	<0.01
Positive ADAM score	94(87 %)	26(46.4)	120(73.2)	<0.01
FPG (mmol/l)	6.5 ± 2.8	4.2 ± 0.4	5.19	<0.01*
HbA1c% (mmol/mmol)	7.6 ± 1.8 (59.6)	5.3 ± 0.5 (34.4)	4.36	<0.01*
Total cholesterol (mmol/l)	4.4 ± 1.1	4.2 ± 0.8	0.29	0.38
LDL cholesterol (mmol/l)	2.9 ± 0.1	2.5 ± 0.2	2.14	0.01*
HDL cholesterol (mmol/l)	1.0 ± 0.1	1.8 ± 0.1	1.52	0.04*
Triglyceride (mmol/l)	0.9 ± 0.3	0.8 ± 0.3	0.87	0.07

BMI body mass index, *WC* waist circumference, *ADAM* Androgen Deficiency in Ageing Male, *FPG* fasting plasma glucose

*Significant difference

among DM patients [14]. The result of this study is also not too different from the result obtained among DM patients by Dhindsa et al. [15] where a prevalence of 33 % was recorded in a study involving Caucasians while a study in Korea reported a prevalence of 34.9 % [19].

Testosterone replacement therapy is known to improve glycaemic control, erectile function, and the overall well-being in persons with diabetes mellitus [19].

Secondary hypogonadism was the most frequent pattern of hypogonadism among the DM patients (79 %). Other less common patterns were primary hypogonadism and noromogonadotropic hypogonadism. Among the controls, one of the two persons who had hypogonadism had primary hypogonadism while the other had normal gonadotrophins. The high frequency of secondary hypogonadism seen in the DM patients in this study suggests a hypothalamic or pituitary defect. Although the exact cause of this defect is not clear, it could have resulted from DM-associated inflammatory processes. The pituitary or hypothalamic defect could be isolated or occur in combination [5]. This finding is similar to the report by Dhindsa et al. where a frequent occurrence of secondary hypogonadism was reported [17]. Olarinoye et al. [12] also reported a similar finding among Nigerian men with diabetes evaluated for erectile dysfunction though with a lower

Table 2 Hormonal indices of the study participants

Variable	DM patients	Controls	[a]*Z*	*p* value
Free testosterone (pg/ml)	24.74 (15–31)	28.80 (18–34)	5.39	<0.01*
Luteinizing hormone (miu/ml)	6.20 (1.65–8.95)	8.78 (3.70–12.55)	4.87	<0.01*
Follicle-stimulating hormone (miu/ml)	6.68 (1.9–9.5)	8.16 (2.5–11.0)	4.14	<0.01*
Prolactin (miu/l)	213.6 (78–269)	218.9 (86–281)	1.86	0.19
Oestradiol (pmol/l)	81.0 (32–105)	79.2 (29–101)	0.59	0.24

Median (IQR) used for all variables

[a] Mann-Whitney *U* coefficient

*Significant difference

Table 3 Pattern of hypogonadism in the study participants

Variables	Number (%)		X^2	*p* value
	DM patients			
	N = 108	Controls *N* = 56		
Eugonadism	66 (61.1)	54 (96.4)	20.9	<0.01*
Hypogonadotrophic hypogonadism (secondary hypogonadism)	33 (30.6)	0 (0)**	20.1	<0.01*
Hypergonadotropic hypogonadism (primary hypogonadism)	4 (3.7)	1 (1.8)**	2.1	0.19
Normogonadotropic hypogonadism	5 (4.6)	1 (1.8)**	0.8	0.34

*Significant values. **Fisher's exact test

prevalence. Kapoor et al. [20] reported a higher prevalence of primary hypogonadism (22 %) among men with diabetes compared with 7 % prevalence of secondary hypogonadism.

Symptoms of hypogonadism were assessed in this study using the ADAM questionnaire. Diabetes mellitus is known to cause erectile dysfunction [21], a key determinant of the overall ADAM score; hence, more DM patients are expected to have abnormal score than the controls as seen in this study. A positive ADAM score (more abnormal) was more frequent in the DM patients with hypogonadism than in those without ($p = 0.04$), and this was an expected finding because erectile dysfunction is one of the components of the ADAM score and a major manifestation of hypogonadism. Erectile dysfunction in diabetes is multifactorial, resulting not only from hypogonadism but also other factors such as atherosclerosis, hypertension, age, and side effects of medication.

Glycaemic control was poor in almost half of the DM patients with 52 (48.1 %) of them having HbA1c ≥7 %. Attaining good glycaemic control is usually quite challenging for most DM patients in a developing country like Nigeria. Multi-centre studies in Nigeria such as the DIABCARE study have also reported such abysmal figures [22]. Patients with DM often have other co-morbidities and are placed on several medications including anti-hypertensive drugs, anti-platelets, and lipid-lowering drugs in addition to the glucose-lowering agents. This has substantial financial implications leading to poor drug compliance and also to the absence of robust health insurance systems makes diabetes treatment a challenge in our

Table 4 Comparison of clinical and biochemical characteristics of DM patients with and without hypogonadism

Variable	DM without hypogonadism (*N* = 66)	DM with hypogonadism (*N* = 42)	Student *t* test	*P* value
Age (years)	50.9 ± 6.0	52.8 ± 5.7	−1.63	0.11
BMI (kg/m^2)	26.3 ± 3.4	26.2 ± 3.9	0.17	0.87
Waist circumference (cm)	90.5 ± 10.7	92.1 ± 12.7	−0.69	0.49
Positive ADAM score	61 (92.4)	33 (78.6)	4.37**	0.04*
Systolic BP (mmHg)	143.1 ± 21.2	142.2 ± 20.5	0.21	0.83
Diastolic BP (mmHg)	84.9 ± 9.4	84.5 ± 9.7	0.21	0.84
Fasting plasma glucose (mmol/L)	6.2 ± 2.3	6.9 ± 3.4	−1.5	0.14
Glycated haemoglobin (%)	6.9 ± 0.9	8.9 ± 1.9	−7.6	0.000*
Total cholesterol (mmol/l)	4.3 ± 0.3	4.5 ± 0.2	−0.81	0.42
LDL (mmol/l)	2.94 ± 0.72	2.91 ± 0.78	0.23	0.82
HDL (mmol/l)	0.67 ± 0.24	0.69 ± 0.22	−0.57	0.57
Triglyceride (mmol/l)	0.87 ± 0.49	1.12 ± 0.97	−1.8	0.08
LH***	8.5 ± 1.5	3.4 ± 5.5	7.2	0.000
FSH***	7.7 ± 2.4	6.0 ± 5.8	2.1	0.000
Prolactin***	208.1 ± 70.9	222.2 ± 124.8	−0.75	0.67
Beta-estradiol***	84.4 ± 18.2	75.7 ± 21.5	2.25	0.03

Results are represented as mean ± standard deviation. *BMI* body mass index

*Statistically significant difference. ** Chi-square. ***Independent samples Kruskal-Wallis test

Table 5 Comparison between hormonal indices of patients with good glycaemic control and poor glycaemic control

Hormone	Good control $N = 56$ Mean rank	Poor control $N = 52$ Mean rank	Z	p
Free testosterone	67.9	40.1	−4.64	0.000*
LH	63.5	44.8	−3.12	0.002*
FSH	59.2	49.4	−1.64	0.101
Estradiol	55.9	53.1	−0.48	0.628
Prolactin	53.6	55.4	−0.30	0.763

Asterisk Significant values

Table 7 Multiple logistic regression showing predictors of low testosterone

Parameter	B coefficient	S.E.	*P*	Exp B	CI
FPG	0.05	0.14	0.73	1.05	0.79–1.38
HbA1c	−1.33	0.38	0.000	0.26	0.13–0.55
ADAM score	−1.85	1.18	0.12	0.016	0.02–1.58
Triglyceride	0.43	0.66	0.51	0.65	0.18–2.37
LH	0.65	0.18	0.000	1.92	1.34–2.74
FSH	−0.38	0.17	0.03	0.68	0.49–0.95
Oestradiol	0.006	0.02	0.72	1.01	0.98–1.04

setting. Poor glycaemic control is known to affect testicular function adversely [19, 23, 24]. The findings from our study are in keeping with this widely held position. Compared with fasting plasma glucose, glycosylated haemoglobin correlated negatively and more strongly with free testosterone. HbA1c also emerged as the strongest predictor of low serum testosterone levels in the multivariate model.

Although elevated serum prolactin has been said to be associated with erectile dysfunction, its prevalence in men with diabetes has been found to be as low as 3.6 % [25]. Elevated prolactin was also uncommon in our study (5.6 %) and was not significantly associated with ADAM score ($p = 0.43$) or hypogonadism ($p = 0.16$).

Corrales et al. [24] had also reported similar correlations of serum testosterone with HbA1c, but not fasting glucose or insulin sensitivity. The study in Korea found a strong relationship between both fasting glucose and HbA1c with testosterone [19]. They speculated that endogenous testosterone levels might influence glycaemic control in subjects with type 2 DM. Glycosylated haemoglobin is known to be a better marker for glycaemic control than fasting plasma glucose. The pathophysiology of low testosterone and hypogonadism due to hyperglycaemia is likely due to deposition of advanced glycated end products (AGEs), and thus, the poor function of testosterone-secreting cells. It has been suggested that testosterone replacement improves insulin sensitivity and glycaemic control in subjects with type 2 diabetes and hypogonadism [26] although this has not been widely substantiated [23]. It is thus very likely that testosterone deficiency and poor glycaemic control are related in men with type 2 diabetes. It is, however, unclear which precedes the other, and larger prospective studies may be needed to elucidate this. This relationship between glycaemic control and testosterone has not been reported with other hormones such as prolactin and estradiol, similar to the findings in our own study (Table 5).

Table 6 Relationship between testosterone and clinical and biochemical variables in the study participants

Variable	DM only $N = 108$		Controls only $N = 56$	
Testosterone vs	Rho	*P* value	Rho	*P* value
Age (years)	−0.15	0.11	−0.05	0.73
WC (cm)	−0.041	0.67	−0.019	0.36
BMI (kg/m^2)	−0.059	0.54	−0.024	0.48
HbA1c (%)	−0.629	<0.01*	−0.014	0.82
Total cholesterol (mmol/L)	0.024	0.77	0.068	0.49
Triglyceride (mmol/L)	0.051	0.61	0.047	0.53
LDL cholesterol (mmol/L)	0.076	0.32	0.038	0.62
HDL cholesterol (mmol/L)	0.063	0.45	0.081	0.28

WC waist circumference, *BMI* body mass index

*Significant relationship

Low testosterone levels are also said to be common in patients who are overweight and obese. Testosterone causes an inhibition of lipoprotein lipase, an enzyme, which is responsible for the uptake of free fatty acids in adipocytes [27]. Hence low testosterone levels will encourage the accumulation of free fatty acids through the non-inhibition of lipoprotein lipase resulting in obesity. Body mass index, and waist circumference were found to correlate inversely, though not significantly with free testosterone in our study as was similarly reported by the Korean study in which testosterone did not correlate with BMI and other indices of insulin resistance such as HOMA-IR [21].

There was a high prevalence of hypertension in the DM patients (46.3 %), which is similar to other studies, which have reported figures ranging from 50 % to as high as 75 % [28]. The mean systolic or diastolic blood pressures, however, did not significantly differ between the DM patients with and without hypogonadism. This was a similar finding by Ogbera et al. [12].

Dyslipidaemia was more frequent in the DM patients than in the control subjects. Diabetes is associated with dyslipidaemia, especially high triglyceride and LDL cholesterol levels, and this greatly contributes to the increased cardiovascular risk seen in them. None of the lipid fractions was significantly related to testosterone. This is not unexpected, as there is no direct relationship between dyslipidaemia and testicular function.

In conclusion, this study has demonstrated a relatively high prevalence of hypogonadism in patients with type 2 diabetes, when compared to age and sex-matched subjects without diabetes. Hypogonadism was closely related to glycaemic control using the HbA1c, but was not influenced by obesity or dyslipidaemia. The ADAM score was not a good predictor of hypogonadism. One of the major limitations of the study was the small sample size, which may not be truly representative of patients with diabetes. Also, the type of drugs being used by the patients was not determined, as the use of drugs such as beta-blockers may contribute to sexual dysfunction in them. However, drugs are not likely to influence the prevalence of hypogonadism or the levels of the other sex steroids.

Measures aimed at improving glycaemic control should be further strengthened since poor glycaemic control, which was seen in this study in almost half of the DM patients were found to be a predictor of hypogonadism. Improved glycaemic control will likely help in reducing the frequency and burden of hypogonadism in men with DM. It is suggested that further studies of the effect of testosterone replacement (in diabetic patients with hypogonadism) on glycaemic control should be done.

Acknowledgments The authors are grateful to the nurses and staff of the Lagos University Teaching Hospital for their assistance during the research. They also wish to thank the staff of the Clinical Chemistry laboratory of the same institution.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the Lagos University Teaching Hospital research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding This was by personal funding from the authors.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):262–266
DOI 10.1007/s13410-016-0482-9

ORIGINAL ARTICLE

Regular exercise with an active lifestyle improves the lipid profile of individuals with diabetes mellitus

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Received: 19 December 2015 / Accepted: 21 March 2016 / Published online: 30 April 2016

Abstract Incidence of diabetes with its associated morbidities is increasing worldwide and hence needs major lifestyle modifications. Hence, this study was designed to compare the effect of moderate intensity exercise for a shorter period of time with an active lifestyle to a low-intensity exercise for a longer period of time and sedentary lifestyle on the lipid profile of diabetic men. The outpatients of MV Hospital were screened using a structured questionnaire to evaluate their lifestyle and exercise pattern over a period of 12 months. The data of 293 men and women were divided into three groups based on their activity level. Group 1 led a totally sedentary lifestyle with no exercise. Group 2 included individuals who were active throughout the day and walked at moderate intensity for a period of 20–30 min, and group 3 exercised at a low intensity for a period of 45–60 min with a sedentary lifestyle. The anthropometric measurements and the lipid profiles of the three groups were compared. A total 41.8 % of the group which led an active lifestyle as well as a moderate intensity of exercise had good glycemic control. The non-HDL levels were 131 ± 38.4 which was significantly lower than the other groups. Hence, the group which led an active lifestyle with moderate intensity exercise fared better than the sedentary group. An active lifestyle throughout the day with an exercise schedule of moderate intensity maintains the lipid parameters more effectively than a low-intensity exercise for a much longer period of time.

Keywords Active lifestyle · Exercise · High-density lipoproteins · Low-density lipoproteins · Very low-intensity lipoproteins

Introduction

Physical inactivity is a state of concern as it leads to major health problems like obesity, hypertension, and various metabolic disorders. Exercise is recommended as a therapeutic lifestyle change as it leads to various health benefits. It is also known to bring about changes in lipid parameters [1–3].

Epidemiological evidence suggests that physically active individuals have a 30–50 % lower risk of developing type 2 diabetes or cardiovascular disease (CVD) than do sedentary persons. Moreover, habitual physical activity (HPA) confers a similar risk reduction for coronary heart disease [4].

Physical exercise without diet restriction or weight loss has evidenced improvements in blood lipid profiles and to decrease fat mass [5]. It is unknown which is the most efficient mode of exercise to improve the response on lipid profile. High-intensity strength training showed evidence that improved blood lipid profile [6, 7]. Endurance training also has shown improvements on blood lipid profile without diet restriction [8].

Recent evidence suggests that multiple short bouts of exercise combined with dietary change may be more effective in improving exercise adherence and weight loss in the short term [9]. However, the long-term implications of short bouts of exercise have not been examined.

Recently, high-intensity intermittent exercise has been highlighted for the purpose of weight reduction and lowering

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atherogenic index [10, 11]. Abby et al. [9] has stated that regular short-term exercise results in a better long-term adherence rate. Conflicting results exists about low- to moderate-intensity exercise as against high-intensity exercise for improving the lipid profile, lipoprotein levels, and blood pressure.

The important factors that have to be considered during an exercise session are its intensity and duration which has to be determined to produce major health benefits. Low-intensity exercise done for longer periods uses fat as the substrate for energy whereas high-intensity exercise uses carbohydrate rather than fat [12].

Therefore, this cross-sectional study was aimed at comparing the effect of moderate-intensity exercise, i.e., brisk walking for 20–30 min and an active lifestyle, to 45–60 min of slow-paced walking everyday on the lipid profile of diabetic patients in contrast to a totally exercise lacking schedule.

Method and material

The outpatients visiting the MV Hospital of diabetes were screened using a structured questionnaire evaluating the lifestyle pattern over a period of 12 months. The data of 293 men and women diagnosed with diabetes mellitus over the last 2 years were recruited as subjects, and their pattern of exercise was evaluated. Patients who were on lipid-lowering drugs were excluded from the study. Patients who were on metformin were included in the study. Individuals who spent a large proportion of their time in activities like walking throughout the day, climbing stairs, gardening, shopping, type of employment, and other physical activities were considered as leading an active lifestyle as against individuals who spent their time mainly watching TV, daytime sleeping, etc. were considered as sedentary. Based on the activity pattern, the patients were classified into three categories. The first group (group 1) included those subjects who do not walk or do any other form of exercise and led a completely sedentary lifestyle. The second group (group 2) consisted of individuals who walked daily and briskly for a period of 20 to 30 min and with an active lifestyle for the rest of the day. The third group (group 3) walked sedately for 45 to 60 min every day but led a very sedentary retired life the rest of the day. The anthropometric parameters, fasting and post prandial blood glucose levels, and HbA1c levels were estimated. The lipid profile was evaluated and compared to the exercise and lifestyle pattern.

Statistical analysis

Statistical analysis was done for the collected data using SPSS software version 12. Frequency, percentage, mean, STD deviation, and STD error were reported appropriately. The chi-square test was used as a test of significance for all categorical variables. One-way ANOVA was applied for continuous variables.

Result

The characteristics are shown in Table 1.

The mean ages of the three groups were 51.2 ± 9.8 for group 1, 52.9 ± 10.2 for group 2, and 53.3 ± 10.2 for group 3, with the age ranging from 26 to 76 years. Of the subjects, 0.3, 33.1, 46.1, and 20.5 % were underweight, normal weight, overweight, and obese, respectively, as seen from the classification of BMI according to WHO. The overall BMI ranged from 16.5 to 45.2 with the mean BMI 26.98 ± 4.06. A total of 81.2 % had an elevated waist circumference and 18.8 % within the prescribed limit of <90 cm for men and <80 cm for women. The waist circumference ranged from 67.5 to 137. The mean waist circumference was 94.14 ± 9.3. Of the 293 subjects recruited, 54.6 % walked regularly and the rest did no exercise. Only 27.0 % had good glycemic control while the remaining 73 % had poor control based on their HbA1c values (Table 1).

Table 2 shows the mean BMI and the standard error of the three groups, i.e., the group which did no exercise, the 20- to 30-min brisk walking group with an active lifestyle and the 45- to 60-min slow walking group.

It can be seen very clearly that the glycemic control was the best in group 2. The fasting and post prandial blood glucose levels were lowest in this group and the difference was statistically significant. The HbA1c values were 7.96 in group 2 and 8.5 and 8.8 in the group 3 and group 1, respectively. It is also noteworthy that 41.8 % of the individuals in group 2 had

Table 1 Demographic and anthropometric characteristics of the overall subjects (n = 293)

Variables		Frequency	Percent
Gender	Male	188	64.2
	Female	105	35.8
Exercise	None	133	45.4
	Yes	160	54.6
Increased waist circumference	No	55	18.8
	Yes	238	81.2
BMI	Underweight	1	0.3
	Normal	97	33.1
	Overweight	135	46.1
	Obese	60	20.5
Glycemic control	Good (<7 HbA1c)	79	27.0
	Poor (>7 HbA1c)	214	73.0

Table 2 Comparison of anthropometric values and glycemic status

Variable	Group 1 mean(SE)	Group 2 mean(SE)	Group 3 mean(SE)	*P* value
BMI	27.43(0.38)	26.78(0.41)	26.33(0.42)	0.18
Waist circumference	94.31(0.83)	93.88(0.97)	94.17(1.07)	0.942
Fasting plasma glucose	204.9(7.02)	178.8(7.02)	194.5(9.8)	0.038*
Post prandial	306.7(9.5)	276.17(10.4)	296.5(13.2)	0.095
HbA1c	8.8(0.17)	7.96(0.19)	8.5(0.25)	0.008*

*Significant

good glycemic control while 21.3 % and only 18.7 % in group 3 and group 1, respectively, could maintain blood glucose levels (Fig. 1).

The mean triglyceride values of the three groups, i.e., group 1, group 2, and group 3 were 171.9 ± 115.3, 130.7 ± 60.1, and 165.03 ± 127.6, respectively. The lowest value was seen in group 2. This difference is statistically significant. (*P* value = 0.009). A similar trend was observed in both total cholesterol and non-HDL levels. Cholesterol levels were 188.7 ± 47.3, 175.9 ± 40, and 174.6 ± 40.3 in group 1, group 2, and group 3, respectively (*P* value = 0.034). The non-HDL values were 145.7 ± 41.8, 131.8 ± 38.4, and 134.9 ± 36.9 for group 1, group 2, and group 3, respectively (*P* value = 0.022). The differences in these values are significant. However, the HDL value for the group 1 was 43.9 ± 13.4, group 2 was 45.02 ± 15.2, and group 3 was 43.09 ± 8.9. There was no significant difference in these value. The interval plots of triglycerides, total cholesterol, and non HDL show the advantage of walking briskly with an active lifestyle (Fig. 2).

Discussion

The main finding of the present study was that moderate intensity of brisk walking even for a time period of 20 to 30 min with an active lifestyle throughout the day (group 2) was effective in significantly maintaining lipid profile.

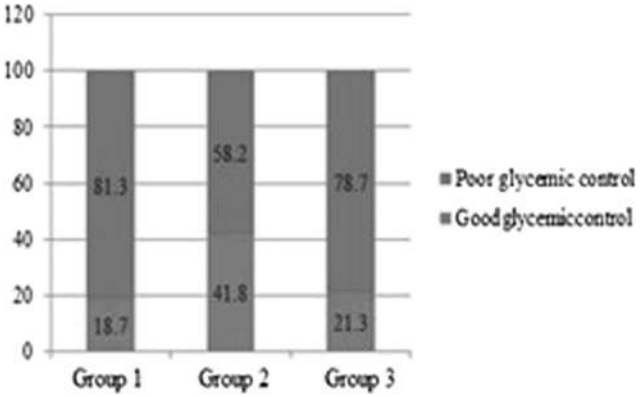

Fig. 1 Glycemic control among the three groups

A second observation from the study was that even though the lipid parameters in group 3 were higher than those in group 2, they are still lower than those in group 1. Therefore, irrespective of the duration and intensity of exercise, the benefits from a pattern of daily physical activity are still beneficial in spite of the sedentary lifestyle.

Duncan et al. [13] has reported a study in which three walking groups (strollers, brisk walkers, and aerobic walkers) exercised the same amount (approximately 13 mi [20.8 km] per week) for 24 weeks. The strollers and aerobic walkers had improvements of 6 % and brisk walkers an improvement of 4 % in the HDL cholesterol concentration. Several studies have shown that low-intensity exercise can result in improvements in lipoproteins [9, 13–15]. This finding reported by Duncan contrasts with the present study. The literature has shown that HDL levels start increasing only above an exercise threshold of acute and high-intensity exercise [9, 16].

Our data, taken together with those of others, suggest that any effect on lipids of the intensity of exercise is as important as leading an active lifestyle. A larger duration of exercise has little value and is negated if the individual otherwise leads a very sedentary lifestyle.

Several studies concerning the effects of exercise on plasma lipid profile have suggested that there is an intensity threshold for eliciting changes in these parameters [17, 18]. In all these studies, moderate-intensity exercise has resulted in a reduction of the triglyceride, total cholesterol, and very low-density lipoproteins. Our data has also proved that diabetic subjects have been able to maintain their lipid parameters below the safe level by moderate-intensity exercise even for a lesser period of time.

It is well documented that there is an important lipid profile adaptation, especially in regard to VLDL secretion, promoted by both acute and chronic exercise [19–21].

Such adaptation is usually related to an increased fatty acid delivery and oxidation in skeletal muscle promoted by lipoprotein lipase and the carnitine palmitoyl transferase (CPT) system adaptation even under intermittent high-intensity training [22].

Observational studies in human suggest that increased habitual activity is inversely associated with intrahepatic TG

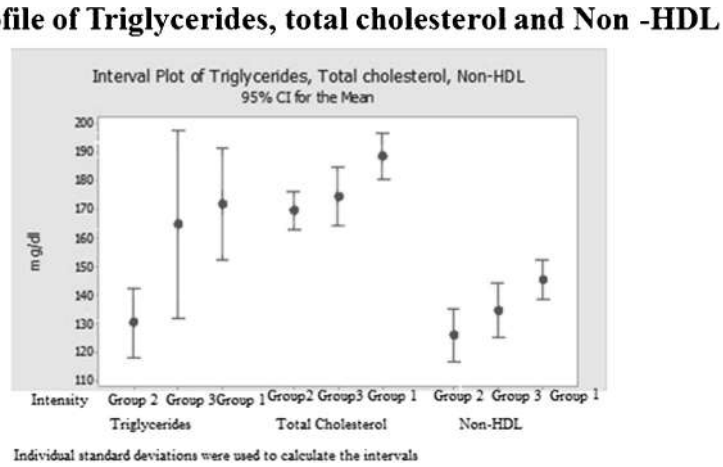

Fig. 2 The profile of triglycerides, total cholesterol, and non-HDL

content [23] and endurance training in animals reduces liver fat accumulation [24, 25].

Several studies show a clear effectiveness imposed by training intervention upon hepatic lipid content and a significant adaptation of hepatic metabolism to regular physical activity [26].

During acute physical activity, an important interaction between the liver, muscle, and adipose tissue occurs, as to provide and maintain adequate blood levels of glucose, free fatty acids (FFAs), and consequently, ATP levels for the contracting muscle [28]. In addition, it is well known that during exercise, insulin levels are decreased allowing the mobilization of the supracited fuel substrates supporting skeletal muscle contraction [27, 28].

The limitation of the present study is that it is a cross-sectional study and the study includes patients from different age groups, whose exercise pattern may not be similar. Further follow-up investigation is necessary to make a decisive conclusion of the findings.

In conclusion, our study demonstrates that regular exercise has broad beneficial effects on the lipoprotein profile. A clear, biologically consistent association has emerged between the amount of exercise and the degree of improvement in the lipoprotein profile, with the higher intensity of exercise for a shorter duration having a much greater beneficial effect on lipids and lipoproteins (maintaining within the normal limits) than the lower intensity but longer duration of exercise. The lower amount of exercise was clearly more beneficial for the lipoprotein profile than was a sedentary lifestyle.

Finally, the duration of exercise was less important, in terms of lipoprotein responses.

Hence, the literature has proved that during exercise, the metabolism of lipids in the liver is different and the profile of synthesis of various lipoproteins changes and results in a reduced and healthier lipid profile in the blood.

Conclusion

In summary, moderate-intensity brisk walking along with an active lifestyle keeps the lipid parameters in the blood within limits and helps maintain a healthy cardiac system.

Acknowledgments The authors wish to thank Prof. M. Viswanathan Diabetes Research Centre for the data which has been collected from its records and the support to carry out this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):267–271
DOI 10.1007/s13410-016-0489-2

ORIGINAL ARTICLE

Individuals with type 2 diabetes are at higher risk of chronic musculoskeletal pain: a study with diabetes cohort

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Received: 3 June 2015 / Accepted: 29 March 2016 / Published online: 6 April 2016

Abstract The relationship between type 2 diabetes (T2DM) and chronic musculoskeletal complaints (cMSCs) remains largely debated. We investigated the association between T2DM and cMSCs in a cohort of 347 participants. A cross-sectional design was employed using the modified Nordic musculoskeletal questionnaire to investigate the prevalence of chronic musculoskeletal symptoms (cMSS) defined by pain and/stiffness lasting ≥3 months during the past 12 months. Multiple logistic regressions were employed to estimate the odds ratio among the diabetics compared to those without diabetes at 95 % CI. Generally, there was a high prevalence of cMSS among the cohorts, chronic low back pain being the most common complaint both among the diabetics (83; 49.7 %) and non-diabetics (70; 38.9 %).T2DM was associated with a higher prevalence of cMSS in at least one body segment and in all the nine body regions studied. cMSS was 2.5 times more likely among persons with T2DM compared to those without diabetes. Also, individuals with T2DM are 29 times at risk of cMSP of the upper back and knee compared to healthy cohorts. T2DM is associated with a higher risk of cMSP, a risk which is increased for the peripheral system much the same as centrally located musculoskeletal structure.

Keywords Musculoskeletal disorders · Diabetes complications · Musculoskeletal complication

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Background

Diabetes mellitus (DM) is a multi-system condition characterized by persistent hyperglycemia with both acute and chronic biochemical and anatomical sequelae. Type 2 DM represents approximately 90 % of all cases of diabetes [1]. Comorbid chronic pain is very common in type 2 diabetes mellitus (T2DM) due to the presence of diabetic neuropathy and musculoskeletal condition that are associated with prolonged hyperglycemia [2–4].

Although neuropathic pain is the most common type of comorbid pain studied in T2DM, recent studies have shown that several types of chronic musculoskeletal disorder (cMSD) appear to be highly prevalent in T2DM [5–10].

One will ask: is there a documental evidence of association between T2DM and chronic musculoskeletal pain (cMSP)? Despite the fact that both diabetes and cMSD complaints are relatively common, few studies have focused on the relationships between chronic musculoskeletal complaints and diabetes mellitus. To clarify this potential association, a cross-sectional study may be an effective beginning and will provide potential clues to the mechanism of chronicity of MSD among individuals with type 2 diabetes mellitus.

We therefore aim to investigate the prevalence of chronic pain in individuals with T2DM compared to their age-gender-physical activity-matched cohort to establish a possible association between chronic pain and T2DM in a cross-sectional survey.

Method

The research was reviewed and the protocol approved by the University of Nigeria Teaching Hospital Research Ethics Committee (NHREC/05/01/2008B-FWA0002458-

1RB00002323). Participants were recruited from the University of Nigeria Teaching Hospital and Enugu State University of Sciences and Technology Teaching Hospital as well as both universities and hospital communities. For the diabetes patients, this was done in the clinics on days when the patients were seeing their physicians. A patient who expressed interest in participating also completed an informed consent process which included authorization to review their medical record. Healthy cohorts were given a questionnaire at their offices or university quarters. To avoid the inclusion of persons with undiagnosed diabetes among the healthy cohorts, a random non-fasting glucose of ≥200 (mg/dL) [11.1 mmol/L] forms the exclusion criterion [11].

The International Physical Activity Questionnaire (IPAQ) was distributed to individuals who met the inclusion criteria and who consented to participating in the study. Participants' weights to the nearest 0.1 kg and heights to the nearest 1.0 cm were measured with weight and height scales respectively. A modified standardized Nordic questionnaire [12] was used to seek information about chronic musculoskeletal pain. Participants were asked whether they had suffered pain or stiffness in muscles and joints lasting for at least 3 months during the last year. Also, they were asked to indicate the number of days during the last month of such complaints. In this questionnaire, those participants who responded "yes" were then asked to tick off one or several of the following nine areas of the body: neck, shoulders, elbows, wrist/hands, upper back, low back, hips, knees, and/or ankles/feet.

Data analysis

All data analyses were performed using Statistical Package for the Social Sciences, version 15 (SPSS, Chicago, IL, USA). Personal characteristics of participants were presented in a table of frequencies and percentages. Physical activity in met-minute value was used to categorize individuals into low, moderate, and high physical activity. Differences in age and BMI of diabetics and non-diabetics were tested with an independent *t* test. Chi-square was employed to test the differences in the number of males and females, association between diabetes and musculoskeletal pain, as well as association between musculoskeletal pain and age, gender, and BMI. Adjusting for the appropriate covariate, odds for musculoskeletal discomfort in at least one body segment as well as the different body regions were sought by logistic regression using the backward stepwise model including all intercepts and defining subpopulations (musculoskeletal disorder) by factor (diabetic status) and covariates while considering only diabetes status for determining hierarchy (musculoskeletal disorder prevalence) within the covariate effect. In all inferential analyses, a 95 % confidence interval was assumed with the significant level set at $p < 0.05$.

Results

Four hundred questionnaires were given out. A total of 347 subjects (191 (55 %) female and 156 (45 %) male) of whom 167 (48.1 %) are diabetics and 180 (51.9 %) non-diabetics completed the questionnaire, with a response rate of 86.75 %. The age of the participants ranged between 35 and 90 years with means of 46.46 ± 9.21 and 59.23 ± 11.65 for those with and without diabetes respectively. Mean BMI were 26.54 ± 4.24 kg/m^2 for persons with diabetes and 26.54 ± 5.02 kg/m^2 for those without diabetes. The details of the demographic and anthropometric characteristics of the participants are presented in Tables 1 and 2.

Low back symptom was the most reported both by the diabetics (83; 49.7 %) and non diabetics (70; 38.9 %). There were no reports of elbow, wrist, and neck discomfort among the non-diabetics, and chronic elbow discomfort was the least reported among the diabetics. There was a significant association between diabetes and chronic musculoskeletal symptom prevalence in at least one body segment as well as in all the nine body regions studied (details presented in Table 3).

Table 4 demonstrated an association between age, BMI, gender, and chronic musculoskeletal symptoms. Age was significantly associated with chronic musculoskeletal symptom

Table 1 Personal characteristics of participants (n = 347)

	Number of participants	Percentage
Gender		
Male	191	55
Female	156	45
Age		
30–39	71	20.5
40–49	67	19.3
50–59	104	30.0
60–69	69	19.9
70–79	28	8.1
80–89	07	2.0
90–100	01	0.3
BMI		
Underweight	06	1.7
Normal weight	126	36.3
Overweight	148	42.7
Obese	67	19.3
Diabetes status		
Diabetic	167	48.1
Non-diabetic	180	51.9
Physical activity		
Low	109	31.41
Moderate	166	47.84
High	72	20.75

Table 2 Anthropometric characteristics between the diabetes and the non-diabetes groups (n = 347)

Anthropometric characteristics	Diabetics $x \pm SD$	Non-diabetics $x \pm SD$	t	p value
Age	46.46 ± 9.21	59.23 ± 11.65	−11.365	0.043
BMI	26.54 ± 4.24	26.74± 5.02	−0.374	0.393
Demographic characteristics	Diabetics n (%)	Non-diabetics n (%)	X^2	p value
Gender				
Male n (%)	74	82		
Female n (%)	93	98	0.054	0.830

prevalence in at least one body segment as well as in all the nine body regions. Also, BMI was associated with prevalence of chronic musculoskeletal symptoms at the shoulder and hip but not with an overall prevalence or prevalence in the seven body regions other than the shoulder and hip. Gender was not associated with prevalence of chronic musculoskeletal symptoms.

The relative risk estimates by logistic regression showed that individuals with diabetes had 2.5 odds of chronic musculoskeletal symptoms in at least one body part compared to those without. Similarly, with diabetes, individuals are more than 28 times at risk of chronic musculoskeletal symptoms of the knee, and are close to 29 times at risk of chronic upper back musculoskeletal symptoms compared to those without diabetes. Also, chronic low back discomfort was 1.5 times more likely among individuals with diabetes compared to those without diabetes.

Discussion

We studied a cohort of Nigerians to investigate the association between diabetes and MSD pain. A current documentary of the prevalence of diabetes in Nigeria put it at about 5.2 % [13]. Although the prevalence is lower compared to what is obtainable in other countries, the burden is potentially significant due to co-morbidities associated with diabetes including musculoskeletal problems. In this cross-sectional study, prevalence of chronic musculoskeletal symptoms was high for both the participants with diabetes and those without diabetes. Low back symptoms were the most commonly reported for both groups. The elbow was the least reported body region with symptoms among the diabetics whereas there was no report of neck, elbow, and wrist and ankle/foot symptoms among the non-diabetics. Our finding should be interpreted in perspective and may not generalize to other regions given that the differential epidemiology of diabetes and musculoskeletal complaint in our population compared to other regions may modulate the associations between T2DM and the final outcome in terms of musculoskeletal complaints. For instance, the prevalence of diabetes [13] and osteoarthritis [14] in Africa is lower compared to that of other regions of the world. Therefore, our report should be interpreted in terms of a region-specific picture and not a single summary evidence regarding the global picture. That notwithstanding, our finding, although described as a specific developing country scenario, can serve as a foundation for future research with the capacity for conducting cross-regional comparisons.

In the present study, T2DM was associated with an increased prevalence of chronic musculoskeletal symptoms in at least one body region as well as in all nine body regions, lending credence to previous reports of a possible relationship of diabetes to some forms of chronic pain syndromes. For instance, a higher prevalence of fibromyalgia has been reported among

Table 3 12 months prevalence of musculoskeletal symptoms and association with diabetes

Body segments	Normal group (n = 180)	Diabetic group (n = 167)	X^2	p value
At least one body segment, yes (%)	73 (45.6)	105 (62.9)	17.27	<0.001*
Neck, yes (%)	0.0 (0.0)	15 (9.0)	16.90	<0.001*
Shoulder, yes (%)	1 (0.6)	10 (6.0)	8.33	0.004*
Elbow, yes (%)	0 (0.0)	6 (3.4)	8.58	0.01*
Wrist, yes (%)	0 (0.0)	9 (5.4)	9.96	0.02*
Upper back, yes (%)	1 (0.6)	24 (14.4)	24.59	<0.001*
Lower back, yes (%)	70 (38.9)	83 (49.7)	3.93	0.047*
Hip, yes (%)	8 (4.4)	28 (16.8)	14.15	<0.001*
Knee, yes (%)	1 (0.6)	23 (13.77)	23.51	<0.001*
Ankle/foot, yes (%)	0 (0.0)	15 (9.0)	16.17	<0.001*

* Means that p is significant at 0.05

Table 4 Association between musculoskeletal discomfort and personal characteristics of participants

Body segment	X^2	p value
At least one body segment		
Age	30.71	<0.001*
BMI	5.921	0.116
Gender	0.182	0.67
Neck		
Age	15.06	0.02*
BMI	7.816	0.05
Gender	2.12	0.15
Shoulder		
Age	10.77	0.96
BMI	9.352	0.025*
Gender	0.001	0.97
Elbow		
Age	15.80	0.015*
BMI	4.066	0.254
Gender	1.975	0.160
Wrist		
Age	15.80	0.015*
BMI	4.110	0.250
Gender	0.504	0.478
Upper back		
Age	12.71	0.048*
BMI	0.852	0.837
Gender	2.421	0.120
Lower back		
Age	20.53	0.002*
BMI	3.932	0.269
Gender	0.193	0.661
Hip		
Age	23.32	<0.001*
BMI	11.512	0.009*
Gender	2.193	0.139
Knee		
Age	32.00	<0.001*
BMI	5.932	0.115
Gender	2.598	0.107
Ankle/foot		
Age	25.64	<0.001*
BMI	2.257	0.521
Gender	0.013	0.908

* Means that p is significant at 0.05

women with T2DM [15] as well as among 100 patients with diabetes compared to control [4]. Also, an Italian study demonstrated that majority of patients with DM reported chronic musculoskeletal symptoms [16]. Additionally, it has been previously reported by several studies that the hyperglycemic condition in DM is associated with reduced pain thresholds in individuals with known DM [17–19].

Among the most debated issues in the diabetes chronic musculoskeletal relationship is whether diabetes affects all musculoskeletal systems of the body equally irrespective of location. There are arguments that only musculoskeletal structures of the extremities are affected by diabetes, but studies have independently demonstrated that trunk muscle and other postural muscle control during a more strenuous task are hampered in those with T2DM [20–24]. Defective postural control may invariably create an abnormal musculoskeletal system biomechanics conducive for different musculoskeletal sequelae. This study seems to be the first that studied the occurrence of chronic musculoskeletal discomfort in T2DM, looking closer at different body regions. In particular, persons with T2DM were close to 29 times more likely to have chronic musculoskeletal symptoms at the upper back and knee, demonstrating that both centrally and peripherally located musculoskeletal structure may be affected equally.

Although the risk of chronic musculoskeletal disorder was not as high for other body regions as for the upper back and knee, our finding consistently showed that T2DM is associated with increased prevalence of chronic musculoskeletal symptoms and those with T2DM were at higher risk compared to those without. The strong association seen in this study raises a query if T2DM may in some manner mediate chronic musculoskeletal disorder. This research hypothesis needs to be answered by a prospective study.

Particularly, our finding seems to have put to rest the debate of selective association of type 2 diabetes to certain regional musculoskeletal symptoms and not others, and shows that T2DM may increase the risk of having centrally located musculoskeletal structures (e.g., upper back OR 28.9) much the same way as peripheral segments (e.g., knee OR 28.5). Also, all those with T2DM were consistently at higher risk of having chronic musculoskeletal symptom of the low back, shoulder, hip, knee, and foot/ankle compared to those without.

Limitations

Our study has several limitations. We did not investigate the blood sugar level of those with diabetes to understand differential interaction of control/uncontrolled diabetes to chronic musculoskeletal symptoms. Also, we could not estimate the relative risk of musculoskeletal symptoms at the neck, elbow, wrist, and ankle/foot due to the fact that there were no non-diabetic persons who reported symptoms at these regions. Future research should incorporate a larger sample so as to be able to draw a risk estimate of chronic musculoskeletal pain in these body regions. Finally, the cross-sectional design which was utilized in our study at best explained the association between T2DM and chronic musculoskeletal symptoms and does not infer a cause and effect relationship.

Conclusion

Individuals with type 2 diabetes are at increased risk of developing chronic musculoskeletal symptoms both for the centrally and peripherally located musculoskeletal regions. This is insightful because these chronic musculoskeletal pains may result in significant morbidity if overlooked in routine diabetes clinics, with further deterioration in quality of life and independence in activities of daily living among diabetics. As such, physicians and other clinicians managing diabetics should routinely inquire of patients regarding symptoms of musculoskeletal sequelae as well as monitor patients in order to timely intervene or appropriately refer patients for optimum care. Further study is warranted to investigate elements responsible for the increased risk/prevalence including somatic and psychological factors.

Acknowledgments The authors wish to acknowledge the physicians at the diabetes clinics of UNTH Ituku Ozalla and Parklane Hospital Enugu for their wonderful cooperation throughout the duration of the study.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of Nigeria Teaching Hospital Ethics and Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):272–279
DOI 10.1007/s13410-016-0484-7

ORIGINAL ARTICLE

Myocardial functional abnormalities and serum N-terminal pro-brain natriuretic peptide in type II diabetes mellitus patients with cardiovascular autonomic neuropathy

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Received: 12 February 2015 / Accepted: 29 March 2016 / Published online: 6 April 2016

Abstract The aim was to investigate ventricular myocardial functions in patients with type II diabetes mellitus (DM) with cardiovascular autonomic neuropathy (CAN) in correlation with serum N-terminal pro-brain natriuretic peptide (NT-pro-BNP). We studied 56 patients with type II DM of >5 years' duration. Thirty healthy subjects matched for age and sex served as control group. The patients with type II DM were divided into two groups according to the outcome of the autonomic nerve function tests as those with CAN (DM + CAN) and without CAN (DM). Echocardiographic studies were performed to assess ventricular functions. NT-pro-BNP levels were measured in all patients. Subclinical left ventricular diastolic dysfunction was not different between diabetic patients with CAN (84 %) and those without CAN (74.2 %); all of them were classified as impaired relaxation pattern ($p > 0.05$). Subclinical right ventricular diastolic dysfunction was not also different between diabetic patients with CAN (48 %) and those without CAN (32.3 %) ($p > 0.05$). The NT-pro-BNP levels were not different between patient groups and not significantly increased in patients with diastolic dysfunction. Multivariate logistic regression analysis demonstrated that only diabetes mellitus was associated with diastolic dysfunction (OR 5.8, 95 % CI 1.7–19.2, $p = 0.004$). NT-pro-BNP is not significantly elevated in diabetic patients with subclinical mild diastolic dysfunction which is not related to CAN.

Keywords Diabetes mellitus · Cardiovascular autonomic neuropathy · Diastolic dysfunction · Echocardiography · N-terminal pro-brain natriuretic peptide

Introduction

Diabetic heart disease was proposed as a distinct clinical entity with myocardial dysfunction in the absence of ischemic, valvular, or hypertensive heart diseases [1]. Asymptomatic diastolic dysfunction, characterized by impairment in LV relaxation and passive filling, was reported as the earliest manifestation of diabetic heart disease which may progress to heart failure [2]. Heart failure due to diabetic heart disease is related to high morbidity and mortality [3]. Many mechanisms such as microvascular disease, autonomic dysfunction, metabolic disorders, and interstitial fibrosis have been suggested as causative factors [2]. However, the exact etiopathogenesis of diabetic heart disease still remains unclear.

Cardiovascular autonomic neuropathy (CAN) in type II diabetic patients has been implicated in the pathogenesis of diabetic heart disease [3]. CAN encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels resulting in abnormalities in heart rate, stroke volume, and peripheral vascular resistance thus contributing to myocardial dysfunction [4]. However, a matter of debate is whether or not CAN could cause myocardial dysfunction by itself, because other factors such as interstitial myocardial fibrosis and microangiopathic or metabolic changes may also be responsible [5].

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Early diagnosis and management of preclinical ventricular abnormalities has proved to reduce cardiovascular complications in the management of diabetic patients especially those with accompanying CAN [6]. While echocardiography is the cornerstone of diagnostic evaluation of ventricular dysfunction, it is a time-consuming method and has limited availability. Brain natriuretic peptide (BNP) and its biologically inactive fragment N-terminal pro-brain natriuretic peptide (NT-pro-BNP) are peptide hormones secreted from the cardiac ventricles in response to increased pressure and volume [7]. Natriuretic peptides have been suggested as simple and cost-effective test to identify patients with the highest likelihood of myocardial dysfunction who could benefit from further risk evaluation for early detection and treatment of cardiac abnormalities.

In this prospective cross-sectional study, we aimed to investigate myocardial functions in type II diabetic patients with CAN in correlation with serum N-terminal pro-brain natriuretic peptide (NT-pro-BNP).

Material and methods

All subjects gave an informed consent to participate in the study. The hospital ethics committee approved the study protocol. Patients were enrolled in the study if they had type II DM for at least 5 years, treated with oral hypoglycemic agents and/or insulin during their disease course. The diagnosis of type II DM was established by American Diabetes Association criteria [8]. We studied 56 patients with type II DM of >5 years duration. Thirty-one healthy patients matched for age and sex served as control group. Patients with type II DM were divided into two groups according to the outcome of the cardiovascular autonomic nerve function tests as those with CAN (DM + CAN, $n = 25$) and without CAN (DM, $n = 31$).

Laboratory investigations included measurement of overnight fasting (12 h) blood sugar, fasting lipid profile, serum level of urea and creatinine, and urine analysis for proteinuria, which was measured by reagent strip. Hemoglobin A1c (HbA1c) was measured by plasma high-performance liquid chromatography method. Blood samples for NT-pro-BNP level assessment were drawn immediately after echocardiographic study in ethylenediamine-tetraacetic acid (EDTA)-containing tubes, centrifuged to separate the serum, then stored at 20 °C before analysis. Plasma NT-pro-BNP was determined by an electrochemiluminescent sandwich immunoassay (Elecsys 2010 system, Roche Diagnostics).

Evaluation of cardiovascular autonomic nerve function tests

Ewing et al. has suggested five autonomic function tests out of which at least two must be abnormal for a definite diagnosis of CAN [9]. The following Ewing test battery was performed:

- Systolic blood pressure response during postural change
- Diastolic blood pressure response during sustained handgrip
- Beat-to-beat variation of R-R interval on the ECG assessed by expiration/inspiration index
- Valsalva maneuver (Valsalva index, longest R-R interval after maneuver (bradycardia)/shortest R-R interval during maneuver (tachycardia))
- Variation of R-R interval during postural change (30:15 index)

Echocardiographic studies

Two-dimensional and M-mode imaging was performed with commercially available echocardiographic machine (ESAOTE, Genova, Italy) equipped with 2.5-MHz phased-array transducers at left lateral decubitus position. M-mode echocardiography was used to measure cardiac dimensions and wall thickness. Left ventricular ejection fraction (LVEF) was calculated by means of the modified Simpson's method. None of the patients had echocardiographically detectable wall motion abnormalities and each patient had normal ejection fraction (>0.55).

Left (LV) and right ventricular (RV) conventional Doppler parameters of diastolic function, peak early (E) and peak late (A) diastolic filling velocities, E-wave deceleration (EDT), and isovolumic relaxation time (IVRT) in milliseconds and E/A ratio were measured from mitral and tricuspid flow, respectively.

Tissue Doppler imaging (TDI) of the left and right ventricules was performed in the apical four-chamber view from velocities of interventricular septum and right ventricule free wall, respectively. The following TDI variables were evaluated: peak systolic (Sm), peak early diastolic (Em), peak late diastolic (Am) myocardial velocities, isovolumetric contraction time (ICTm), isovolumetric relaxation time (IRTm), and ejection time (ETm) were measured. E/Em ratio which is correlated with ventricular filling pressure was calculated. All measurements were averaged from three consecutive recordings. The myocardial performance index (MPIm) was calculated from the formula: MPIm = (IRTm + ICTm)/ETm. Tricuspid annular plane systolic excursion (TAPSE) was measured from the systolic displacement of the RV free wall-tricuspid annular plane junction in the apical 4-chamber view M-mode recordings.

All Doppler-derived time intervals were adjusted according to bazett formula, and all measurements were analyzed by one observer who was blinded to all patients' data.

Left ventricular diastolic dysfunction was categorized as described previously [10]:

- Mild, defined as impaired relaxation without evidence of increased filling pressures: $E/A \leq 0.8$, EDT > 200 ms, IVRT ≥ 100 ms, $E' < 0.08$ m/s, $E/E' \leq 8$

- Moderate, defined as impaired relaxation associated with moderate elevation of filling pressures or pseudonormal filling: E/A = 0.8–1.5; EDT = 160–200 ms, IVRT >60, <100 ms, E′ < 8, E/E′ 9–15
- Severe, defined as advanced reduction in compliance or restrictive filling: E/A ≥ 2, EDT < 160 ms, IVRT ≤ 60 ms, E/E′ ≥15.
- Right ventricular diastolic dysfunction was categorized as described previously [11]
- Mild, defined as impaired relaxation without evidence of increased filling pressures: E/A ratio < 0.8
- Moderate, defined as impaired relaxation associated with moderate elevation of filling pressures or pseudonormal filling: E/A = 0.8–2.1; E/E′ 6;
- Severe, defined as advanced reduction in compliance or restrictive filling: E/A ≥ 2.1, EDT < 120 ms.

Diastolic dysfunction defined as presence of left and/or right ventricular diastolic dysfunction.

Exclusion criteria

1. History of ischemic heart disease which was determined mainly by history and resting electrocardiogram, valvular heart disease, heart failure, atrial fibrillation, peripheral vascular disease, and hypertension
2. Diabetic microvascular complications (proliferative retinopathy, nephropathy, or microalbuminuria, since mild renal dysfunction influences the optimal cutoff points of NT-pro-BNP [12]
3. Liver, thyroid, cancer, and psychiatric illness, alcoholism
4. Subjects with poor transthoracic echo window and who can not perform cardiovascular autonomic reflex tests.

Statistics

Quantitative variables were expressed as mean (±SD), and qualitative variables were expressed as percentage (%). A comparison of parametric values between groups was made by using ANOVA, and categorical variables were compared using the chi-squared test or Fisher's test. Post hoc analysis (Bonferroni) allowed identification of significant differences in mean values between the groups. Spearman rho and Pearson tests were used for correlation analysis. Binary logistic regression analysis was used to evaluate independent association between diastolic dysfunction and clinical parameters. All statistical analyses were carried out using SPSS version 18.0 (SPSS, Chicago, IL, USA). A p value <0.05 was considered significant.

Results

Clinical characteristics

We included 56 patients with type II DM and 30 controls. Twenty-five (44.6 %) diabetic patients had accompanying CAN. The baseline characteristics were presented in Table 1. Fasting blood glucose and HbA1c levels were higher in diabetic patients compared to control patients ($p < 0.001$, $p < 0.001$; respectively). Systolic and diastolic blood pressure were higher in diabetic patients with CAN compared to control patients ($p = 0.007$, $p = 0.01$; respectively). There was no significant difference between diabetic patients with CAN and those without CAN only except for the duration of diabetes mellitus which was longer in diabetic patients with CAN ($p = 0.009$).

Echocardiographic findings

Left and right ventricular dimensions were not different between groups ($p > 0.05$). Left and right ventricular systolic parameters were normal and not different between groups ($p > 0.05$).

Doppler- and TDI-derived diastolic function indices are shown in Table 2. Mitral E velocity was lower, and mitral EDT and IVRT were longer both in diabetic patients with and without CAN compared to controls ($p < 0.001$), whereas mitral A velocity was higher only in diabetic patients with CAN ($p = 0.002$). E/A ratio was also lower in both diabetic patients with and without CAN compared to controls ($p < 0.001$). Tricuspid A velocity was higher in diabetic patients with CAN ($p < 0.001$). Tricuspid EDT was longer in diabetic patients with and without CAN ($p = 0.001$ and $p = 0.01$, respectively). TDI assessment of left ventricular global longitudinal myocardial function showed lower Em velocity in diabetic patients with and without CAN ($p < 0.001$). Right ventricular TDI-derived diastolic function indices were not different between groups ($p > 0.05$).

Subclinical left ventricular diastolic dysfunction was diagnosed in 21 (84 %) diabetic patients with CAN and 23 (74.2 %) diabetic patients without CAN, where only in 11 (36.7 %) control patients; all of them were classified as impaired relaxation pattern, with a statistical significant difference ($p < 0.001$). Subclinical right ventricular diastolic dysfunction was demonstrated in 12 (48 %) diabetic patients with CAN, 10 (32.3 %) diabetic patients without CAN, and 4 (13.3 %) control patients wtih a statistical significant difference ($p = 0.02$) (Fig. 1). However, diastolic dysfunction was not found different between diabetic patients with and without CAN.

The effects of different variables on diastolic dysfunction were calculated in univariate analysis for each. The variables for which the unadjusted p value was ≤0.05 in logistic regression analysis were identified as potential risk markers and included in the full model. Systolic and diastolic blood

Table 1 Clinical characteristics of the patient groups

	Group 1 (control) $N = 30$	Group 2 (DM) $N = 31$	*Group 3 (DM + CAN)* $N = 25$	*p*	*p**	*p***	*p****
Age (year)	49.4 ± 6.4	50.3 ± 7.6	52.8 ± 7.5	0.2	0.86	0.19	0.41
Sex (female/male)	20/10	20/11	18/7	0.83			
Body mass index (kg/m^2)	29.2 ± 5.1	30.9 ± 4.8	31.1 ± 4.9	0.27	0.47	0.49	0.99
Body surface area (m^2)	1.83 ± 0.1	1.87 ± 0.1	1.89 ± 0.1	0.18	0.75	0.2	0.99
Smoking (*n*, %)	2, 2.3	3, 3.5	4, 4.7	0.52			
Duration of DM (year)		8.9 ± 7.8	10.9 ± 4.2				0.009
Nonproliferative retinopathy (*n*, %)		4, 12.9	5, 20	0.47			
Heart rate (bpm)	70.7 ± 6.8	71.5 ± 7.5	73.2 ± 11.7	0.5	0.36	0.36	0.46
Systolic BP (mmHg)	117.3 ± 14.5	123.3 ± 11.1	126.7 ± 9.3	0.01	0.09	0.007	0.08
Diastolic BP (mmHg)	74.7 ± 8.6	77.7 ± 6.8	79.9 ± 5.5	0.03	0.18	0.01	0.11
Fasting glucose (mg/dL)	95.5 ± 7.7	170.9 ± 28.3	182.2 ± 45.2	<0.001	<0.001	<0.001	0.22
Hb A1C	5.7 ± 0.3	7.8 ± 0.8	8.3 ± 1.1	<0.001	<0.001	<0.001	0.07
Creatinine	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.13	0.27	0.23	0.99
Urinary albümin excretion (mg/24 h)	9,1 ± 6.1	11.4 ± 8.5	13.2 ± 8.9	0.26	0.37	0.1	0.46
Total cholesterol (mg/dL)	187.8 ± 43.5	208.5 ± 40.3	191.8 ± 52.1	0.18	0.24	0.99	0.52
LDL-C (mg/dL)	122.5 ± 26.8	127.2 ± 34.2	115.2 ± 39.6	0.42	0.99	0.99	0.56
HDL-C (mg/dl)	43.6 ± 8.8	45 ± 8.9	48.8 ± 11.9	0.13	0.99	0.15	0.46
Triglycerides (mg/dl)	149.9 ± 88.2	224.5 ± 190.6	164 ± 72.3	0.07	0.09	0.99	0.28
Antidiabetic drugs							
Insulin (*n*, %)		17, 54.8	18, 72	0.19			
Oral antidiabetics (*n*, %)		14, 45.2	7, 28	0.19			
Statins (*n*, %)	5, 16.7	10, 32.3	11, 44	0.09			

DM diabetes mellitus, *HbA1C* hemoglobin A1C, *LDL-C* low-density lipoprotein-cholesterol, *HDL-C* high-density lipoprotein-cholesterol

*p**, between groups 1 and 2; *p***, between groups 1 and 3; *p****, between groups 2 and 3

pressures, body mass index, diabetes mellitus, and CAN were analyzed with multivariate logistic regression model. Multivariate logistic regression analysis demonstrated that only diabetes mellitus was associated with diastolic dysfunction (OR 5.8, 95 % CI 1.7–19.2, $p = 0.004$) (Table 3).

NT-pro-BNP

The NT-pro-BNP levels were normal and not different between groups. Furthermore, in subgroup analysis, NT-pro-BNP levels were not also different between patients with normal diastolic function and those with diasolic dysfunction (Fig. 2).

Observer variability

Intraobserver variability was low (2.4 % ± 1.6 %).

Discussion

Our study showed that diastolic functional abnormalities are frequently encountered in asymptomatic patients with type II DM. However, diastolic functions were not significantly influenced by accompanying CAN in patients with type II DM, and NT-pro-BNP is not significantly elevated in diabetic patients with subclinical mild diastolic dysfunction.

Boyer et al. found that the prevalence of left ventricular diastolic dysfunction in asymptomatic, normotensive patients with type II diabetes mellitus was as high as 75 % [13]. Our results were consistent with these findings.

Previous studies including analysis of left and right ventricular diastolic functions in asymtomatic diabetic patients without overt heart disease showed filling abnormalities on conventional and tissue Doppler imaging, including decreased peak early filling rate (E), greater dependence on the atrial contraction for ventricular filling (A) with a consequent decrease in the E/A, increased deceleration and isovolumic relaxation time, decreased Em, increased Am, and increased E/Em [14–18]. In the present study, most of the diastolic indices were similiarly abnormal.

There is a controversy among previous studies regarding systolic function in diabetic patients. Majority of the previous studies in asymptomatic patients with well-controlled type II DM revealed diastolic dysfunction with normal or near

Table 2 Echocardiographic data of the patient groups

	Group 1 (control) $N = 30$	Group 2 (DM) $N = 31$	Group 3 (DM + CAN) $N = 25$	p	p^*	p^{**}	p^{***}
LVESD (cm)	2.86 ± 0.27	2.94 ± 0.27	2.99 ± 0.25	0.16	0.42	0.15	0.76
LVEDD (cm)	4.53 ± 0.35	4.61 ± 0.36	4.72 ± 0.29	0.12	0.67	0.1	0.41
IVSD (cm)	0.93 ± 0.09	0.98 ± 0.15	0.98 ± 0.11	0.32	0.25	0.15	0.83
PWD (cm)	0.9 ± 0.09	0.95 ± 0.14	0.96 ± 0.11	0.18	0.16	0.08	0.78
LVEF %	0.66 ± 0.04	0.65 ± 0.03	0.64 ± 0.04	0.14	0.38	0.13	0.77
NT-Pro-BNP	25.4 ± 23.4	26.3 ± 29.3	40.9 ± 47.7	0.25	0.93	0.16	0.14
Left ventricular parameters							
E (m/s)	0.81 ± 0.2	0.58 ± 0.16	0.57 ± 0.19	<0.001	<0.001	<0.001	0.99
A (m/s)	0.77 ± 0.13	0.87 ± 0.19	0.94 ± 0.19	0.002	0.07	0.002	0.5
E/A	1.07 ± 0.32	0.73 ± 0.35	0.66 ± 0.31	<0.00.1	<0.001	<0.001	0.99
EDT (ms)	199.43 ± 38.79	242.87 ± 41.24	251.16 ± 46.62	<0.001	<0.001	<0.001	0.99
IVRT	89.1 ± 12.34	106.03 ± 16.93	108.2 ± 17.53	<0.001	<0.001	<0.001	0.99
Em (m/s)	0.1 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	<0.001	<0.001	<0.001	0.99
E/Em	7.66 ± 0.41	7.76 ± 0.42	7.73 ± 0.65	0.99	0.99	0.99	0.99
Am (m/s)	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.42	0.73	0.82	0.99
Sm (m/s)	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.02	0.97	0.85	0.79	0.88
MPIm	0.46 ± 0.14	0.49 ± 0.14	0.5 ± 0.17	0.55	0.64	0.59	0.99
Right ventricular parameters							
E (m/s)	0.56 ± 0.1	0.58 ± 0.11	0.58 ± 0.12	0.72	0.75	0.77	0.99
A (m/s)	0.48 ± 0.08	0.55 ± 0.14	0.56 ± 0.11	0.007	0.08	<0.001	0.66
E/A	1.18 ± 0.22	1.11 ± 0.25	1.07 ± 0.25	0.2	0.45	0.19	0.81
EDT (ms)	197.9 ± 38.95	227.16 ± 36.66	237.36 ± 43.68	0.001	0.01	0.001	0.61
Em (m/s)	0.12 ± 0.03	0.11 ± 0.04	0.1 ± 0.03	0.1	0.38	0.09	0.65
E/Em	4.96 ± 1.31	5.67 ± 1.92	5.72 ± 1.51	0.14	0.26	0.26	0.99
Am (m/s)	0.16 ± 0.05	0.16 ± 0.05	0.17 ± 0.05	0.89	0.99	0.91	0.9
Sm (m/s)	0.15 ± 0.02	0.16 ± 0.03	0.16 ± 0.04	0.98	0.91	0.88	0.82
MPIm	0.4 ± 0.15	0.44 ± 0.16	0.46 ± 0.13	0.3	0.47	0.31	0.93
Tapse (cm)	2.46 ± 0.27	2.35 ± 0.27	2.34 ± 0.32	0.21	0.28	0.28	0.99

LVESD left ventricular end systolic diameter, *LVEDD* left ventricular end diastolic diameter, *IVSD* interventricular septal diameter at end diastole, *PWD* posterior wall diameter at end diastole, *LVEF* left venticular ejection fraction, *NT-pro-BNP* N-terminal pro-brain natriuretic peptide, *E* peak early, *A* peak late Doppler diastolic velocity, *EDT* peak early wave deceleration time, *IVRT* isovolumetric relaxation time, *Em* peak early, *Am* peak late, *Sm* peak systolic myocardial velocity, *MPIm* myocardial performance index, *TAPSE* tricuspid annular plane systolic excursion

p^*, between groups 1 and 2; p^{**}, between groups 1 and 3; p^{***}, between groups 2 and 3

normal left ventricular systolic function [19]. However, some of the previous studies with TDI and strain deformation imaging showed subtle systolic changes of regional left ventricular function despite normal ejection fraction either at rest or during stress [20, 21]. Some of these studies that showed accompanying systolic dysfunction were included in patients with older ages and concominant hypertension [20]. In a recent radionuclide ventriculography study, left ventricular systolic functions were not found different between type II diabetic patients with CAN and those without CAN [22]. There have been few studies analyzing the right ventricular systolic functions. In a previous study, it was shown that biventricular systolic functions were normal despite the impaired relaxation in patients with type I DM [17]. On the other hand, in another study, right ventricular Tei index was found to be correlated to Ewing score in patients with type II DM [23]. In the present study, normotensive diabetic patients with or without CAN had normal biventricular systolic functions.

CAN is a well-recognized complication of diabetes mellitus. The prevalence of CAN varies between 20 and 73 % in patients with type II DM [4]. In keeping with these previous studies, our study showed 44.6 % of diabetic patients had CAN. Although it has been speculated that CAN is associated with diastolic dysfunction which may finally progress to heart failure, mainly diastolic heart failure with preserved systolic function, the pathophysiological role of CAN in diabetic heart disease has not been established. Most of the previous studies suggested an independent association between CAN and ventricular diastolic dysfunction in patients with type II DM [24–26]. However, some of these studies included

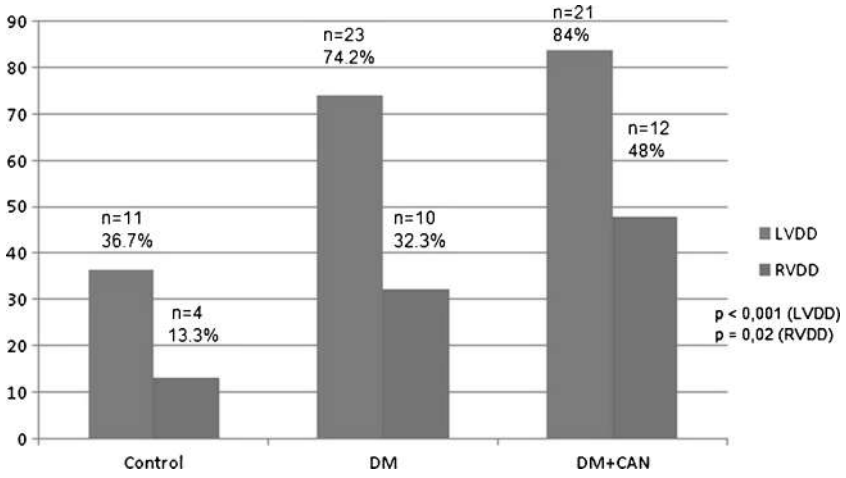

Fig. 1 Graphical representation of diastolic dysfunction between groups. *DM* diabetes mellitus, *CAN* cardiovascular autonomic neuropathy, *LV* left ventricule, *RV* right ventricule, *DD* diastolic dysfunction

patients with nephropathy and cardiovascular diseases, such as hypertension and ischemic heart diseas [24, 26]. Karamitsos et al. suggested that the presence of CAN seems to have an additive effect on diastolic dysfunction in type I DM, but diabetic patients with CAN had poorer glycemic control than those without CAN [27]. In contrast to these studies, Irace et al. demonstrated no correlation between CAN and diastolic dysfunction in a larger type I diabetic patients group [28]. Romanens et al. also found no correlation between CAN and diastolic dysfunction in type I DM [29]. Our study also showed no correlation between CAN and diastolic dysfunction in patients with type II DM. Acute changes in metabolic control and a large number of nondiabetic variables, such as heart rate, respiration, preload, and afterload, are known to affect left ventricular diastolic filling [30, 31]. Thus, the influence of some of these variables on left and right ventricular diastolic filling may partly explain the conflicting results in regard to ventricular diastolic dysfunction in diabetic patients with CAN. The other possible reason may also be patient selection and exclusion criteria.

The diagnostic role of natriuretic peptides for detecting myocardial dysfunction in asymptomatic diabetic patients is still debated [16, 32–34]. Most of the previous studies found correlations between natriuretic peptides, including BNP and NT-pro-BNP, and left ventricular diastolic dysfunction in asymptomatic patients with type II DM [34–36]. However, some of these studies included diabetic patients with untreated hypertension [35]. In the present study, diabetic patients with hypertension were excluded. On the other hand, Kiencke et al. showed that screening with BNP is not useful for diagnosing preclinical diastolic dysfunction in diabetic heart disease [16]. In addition, in a number of large, community-based populations, BNP proved to be a suboptimal screening test to detect preclinical left ventricular dysfunction [37]. Furthermore, two other studies reported that BNP was not sufficiently sensitive to identify mild diastolic dysfunction in asymptomatic patients with type II DM [33, 38]. It has been well known that significantly higher NT-pro-BNP levels were found in patients with advanced diastolic dysfunction [39]. In a study, it was also demonstrated that BNP is independently related to moderate to severe diastolic dysfunction in type II diabetic patients, but

Table 3 Univariate and multivariable analyses of predictors of diastolic dysfunction

	OR (95 % CI)	*p*
Univariate analysis		
Systolic blood pressure	1 (1–1.1)	0.025
Diastolic blood pressure	1.1 (1–1.2)	0.02
Body mass index	1.1 (0.9–1.2)	0.05
DM	7.9 (2.9–21.8)	<0.001
CAN	3.7 (1.1–11.9)	0.03
Multivariate analysis		
DM	5.8 (1.7–19.2)	0.004

DM diabetes mellitus, *CAN* cardiovascular autonomic neuropathy

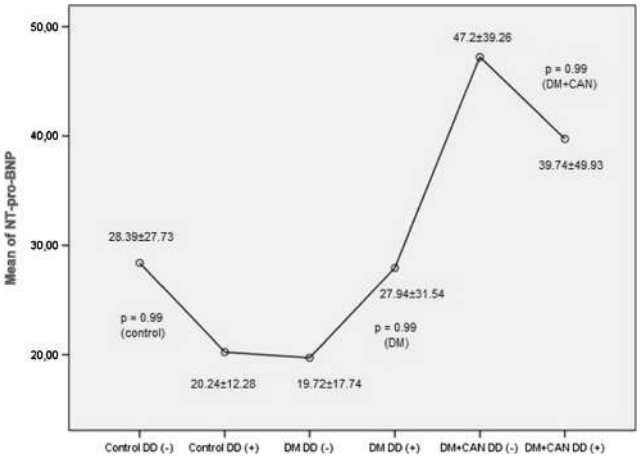

Fig. 2 Comparison of NT-pro-BNP levels between groups subdivided according to diastolic dysfunction. *DD* diastolic dysfunction, *DM* diabetes mellitus, *CAN* cardiovascular autonomic neuropathy, *NT-pro-BNP* N-terminal pro-brain natriuretic peptide

no significant correlation was found with mild diastolic dysfunction [32].

Some of the previous studies suggested that neuropepetides play an important role in diabetic heart disease in patients with autonomic neuropathy [40]. A previous study showed that elevated BNP level is correlated with CAN, but it is not correlated with left ventricular diastolic dysfunction in type II DM [41]. In this study, urinary albümin excretion was higher in high BNP group and this mild nephropathy may have caused volume overload and may result in increased BNP levels. In the present study, diabetic patients with nephropaty were excluded. In another study in type I diabetic patients with sympathetic myocardial dysinnervation, impaired diastolic function is associated with ANP but not with BNP levels [42]. In the present study, NT-pro-BNP levels were normal and not different between diabetic patients with CAN and without CAN.

In our study, we found that NT-pro-BNP is not significantly elevated in diabetic patients with subclinical mild diastolic dysfunction which is not related to CAN. One of the possible explanation of these results can be the presence of just impaired relaxation in our population which represents the mild diastolic dysfunction showing normal filling pressures [32, 33]. Furthermore, natriuretic peptides in plasma have been reported to lower in patients with obesity and insulin resistance than in nonobese and insulin-sensitive patients [43]. This could also be an explanation for normal NT-pro-BNP levels in our study group which included obese diabetic patients.

This study had several limitations. First, the major limitation of this study is small sample size. Second, myocardial systolic and diastolic functions were determined only by echocardiography. Third, pulmonary and hepatic venous flow Doppler analysis and strain deformation were not recorded. Fourth, since coronary artery disease was assessed solely by history and resting ECG, presence of such disease as the underlying cause of diastolic dysfunction could not be completely ruled out. Finally, ambulatory blood pressure monitoring was not performed.

Conclusions

In conclusion, asymptomatic type II DM patients without overt cardiovascular disease have a high prevalence of diastolic dysfunction as compared with healthy subjects. DM is predictive of diastolic dysfunction, irrespective of CAN. BNP is not useful to detect preclinical mild diastolic dysfunction in diabetic patients without any micro- or macrovascular complications.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):280–285
DOI 10.1007/s13410-016-0485-6

ORIGINAL ARTICLE

Incidence of type-2 diabetes among industrial Workers in Kerala, India

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Received: 4 January 2016 / Accepted: 29 March 2016 / Published online: 6 April 2016

Abstract Kerala is the most advanced Indian state in epidemiological transition and has the highest type 2 diabetes prevalence. However, data on incidence of diabetes in Kerala are limited. We studied the incidence of diabetes and pre-diabetes among industrial workers. We measured fasting plasma glucose (FPG) among 326 workers (mean age 51 years, men 76 %) from two major industries in Kerala in the years 2009 and 2011 using standard protocol. Individuals with FPG ≥126 mg/dl or on medications for diabetes were considered to have diabetes mellitus, FPG ≥100 mg/dl and ≤125/dl mg as pre-diabetic, and FPG <100 mg/dl as normal. Among the 326 workers, 26.1 % (95 % CI 21.6–31.1) were diabetic, 32.8 % (CI 28.7–37.2) were pre-diabetic, and the remaining 41.1 % were having normal FPG at baseline. At year two, 13.3 % of the 241 workers who were either normal or had pre-diabetes at baseline developed diabetes providing an incidence rate of 6.65 % per year. Among the 134 workers with normal FPG at baseline 28.4 % progressed to pre-diabetes, 5.2 % developed diabetes, and among the 107 pre-diabetics, 23.4 % developed diabetes at year two. The odds of progressing to diabetes from pre-diabetes were five times higher compared to those from normal FPG (OR 5.53; CI 2.28–13.37). Progression to pre-diabetes and diabetes occurred at a very fast rate in this population indicating the need for preventive measures to slow down this fast progression.

Keywords Incidence · Type 2 diabetes · Pre-diabetes · Industrial workers · Kerala · India

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Introduction

The Indian state of Kerala with a population of 33 million [1] is the most advanced in terms of epidemiological and demographic transition [2] and is reported to have the highest prevalence of type 2 diabetes in India: 20 % among those aged 15–64 years in rural areas [3]. Life expectancy in the state during the period of 2009–13 was 75 years compared to 68 years for India as a whole [4], and the additional life expectancy of 8 years partly explains the high prevalence of most non-communicable diseases (NCDs), including type-2 diabetes. The state has also been reported to have high prevalence of NCD risk factors such as tobacco use, alcohol consumption, overweight, hypertension and hypercholesterolemia, and a harbinger of what the rest of India is going to face in the near future with regard to NCDs such as type 2 diabetes [3].

In 2015, India was reported to have more than 69 million adults with type 2 diabetes, the second largest population in the world after China, and more than one million deaths in India were attributed to diabetes [5]. However, incidence data on diabetes from India are limited. A recent study from the neighboring state of Tamil Nadu reported an incidence rate of 22.2 per 1000 person years among adults with normal glucose tolerance [6]. This study also reported a very high incidence rate of 78.9 per 1000 person years among those with pre-

diabetes. There are no comparable diabetes incidence data from Kerala. The objective of the present study was to find the incidence of type 2 diabetes and pre-diabetes among industrial workers with normal glucose tolerance in Kerala, South India. We also looked at the progression from pre-diabetes to diabetes in this population.

Methods

The present study was part of a pilot study on the Community Interventions for Health (CIH). Detailed methodology of CIH and results from the adult community sample has been published [7]. CIH was an international collaborative study that took place between 2008 and 2011 in communities in China, India, and Mexico and was designed to reduce the risk of NCDs by targeting the three main risk factors of tobacco use, physical inactivity, and unhealthy diet. The aim of CIH was to evaluate culturally specific strategies to (i) decrease the prevalence of smoking and smokeless tobacco use, (ii) improve diet by increasing intake of fruits and vegetables and reducing use of salt, and (iii) increase levels of physical activity. CIH was conducted in three communities: Kerala state in India, Hangzhou city in China, and Mexico City in Mexico, and was undertaken in four settings: health centres, workplace, schools, and the general community.

In this present study, we analyzed the data related to the workplace in Kerala state of India. Using a quasi-experimental study design, two major industries were selected from two southern districts of Kerala: one acted as the intervention and the other as a control site. Both the industries were under the public sector with around 1500 workers each. About 70 % of the workers were men. One industry was manufacturing condoms and the other titanium dioxide. Both industries were comparable in the nature of work. Culturally adapted structural interventions were provided in the intervention industry [8]. The sample size for the workplace was fixed for each country site as 2000 based on the methodology published previously [7]. The interview schedule used for the survey incorporated questions from previously validated surveys including World Health Organization (WHO) STEPS, international physical activity questionnaire (IPAQ), and the global adult tobacco survey (GATS) [7]. We collected data from 2426 workers aged 18–64 years from the two selected industries in Kerala in 2009 (1008 from control industry and 1418 from intervention industry) and from 1748 workers after 2 years using the same procedure from the same industries (765 from control industry and 983 from intervention industry). Using the unique worker's code, we identified 326 workers who participated in both surveys and their data were analyzed for obtaining incidence of diabetes. Details of the sample selection process are given in Fig. 1. Cumulative incidence rate was calculated as the proportion of people who were diabetic at the 2-year follow-up among those who were non-diabetic at baseline. Incidence of diabetes was also calculated per 1000 person years with the number of persons who developed diabetes during follow-up as numerator and the total person years as denominator.

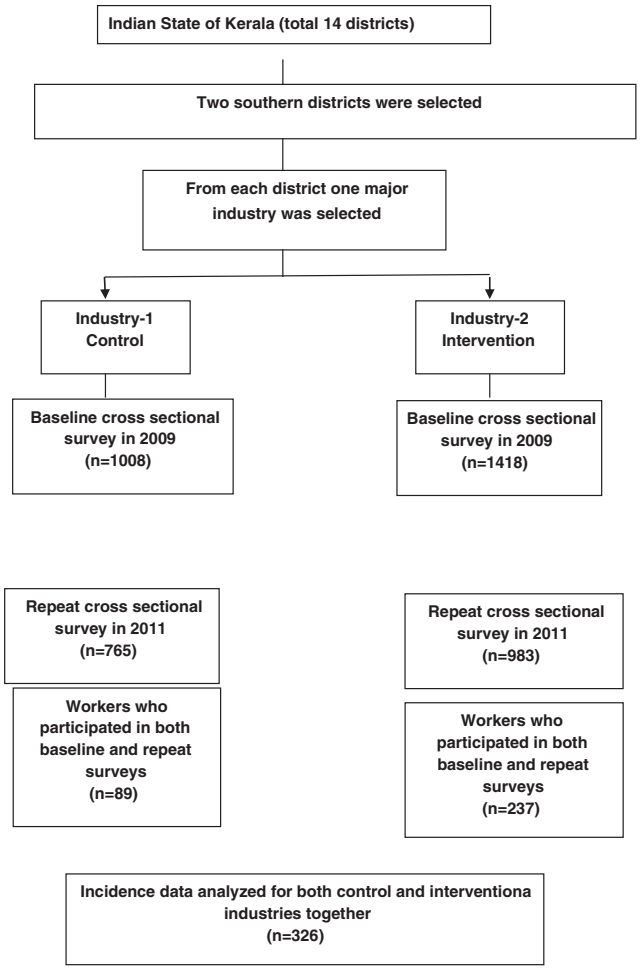

Fig. 1 Sample selection procedure

Fasting plasma glucose (FPG) and plasma lipids were measured using Cholestech LDX System [9]. In both surveys, diabetes was defined as FPG ≥126 mg/dl or on medication (modern medicine) for diabetes, pre-diabetes as FPG ≥100 mg/dl and ≤125 mg/dl and normal (normoglycemia) as FPG <100 mg/dl [10]. We did not do two hour glucose tolerance test or HBA1C because of logistical and economic reasons. WHO has recommended that fasting plasma glucose alone is enough to assess diabetes status for epidemiological studies [11]. Weight, height, waist circumference, and blood pressure were measured using WHO STEPS protocol [12]. Abdominal obesity was defined as waist circumference ≥90 cm for men and ≥80 cm for women [12], Low HDL (high-density lipoprotein) cholesterol as <40 mg/dl for men and <50 mg/dl for women, hypertriglyceridemia as triglycerides ≥150 mg/dl, hypercholesterolemia as total cholesterol ≥200 mg/dl [13] and hypertension as systolic blood pressure

(SBP)≥ 140 mmHg and or diastolic blood pressure (DBP) ≥90 mmHg or on medication for hypertension [14]. Information on age, sex, tobacco use, alcohol use, and family history of diabetes was collected using the interview schedule.

Table 1 Baseline characteristics of the study sample ($N = 326$)

Baseline characteristics	*N* (%)
Age (years)	
<50	180 (55.2)
≥50	146 (44.8)
Sex	
Men	246 (75.5)
Women	80 (24.5)
Family history of diabetes	
Yes	96 (29.4)
No	230 (70.6)
Marital status	
Single	16 (4.9)
Currently married	288 (88.3)
Others	22 (6.6)
Abdominal obesity[a]	
Yes	206 (63.2)
No	120 (36.8)
Low HDL cholesterol[b]	
Yes	216 (66.3)
No	110 (33.7)
Hypertriglyceridemia[c]	
Yes	112 (34.4)
No	214 (65.6)
Hypercholesterolemia[d]	
Yes	166 (50.9)
No	160 (49.1)
Hypertensive[e]	
Yes	119 (36.5)
No	207 (63.5)
Current tobacco use[f]	
Yes	78 (23.9)
No	248 (76.1)
Current alcohol use[g]	
Yes	118 (36.2)
No	208 (63.8)

[a] Waist circumference ≥90 cm for men and ≥80 cm for women

[b] High-density lipoprotein (HDL) cholesterol <40 mg/dl for men and <50 mg/dl for women

[c] Triglycerides ≥150 mg/dl

[d] Total cholesterol ≥200 mg/dl

[e] Systolic blood pressure ≥140 mmHg and or diastolic blood pressure ≥90 mmHg or on medication for hypertension

[f] Any use of tobacco in the last 1 month

[g] Any alcohol use in the last 1 month

Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL). Chi-square test and Fisher's test were used for comparison between categorical variables, and *t* test was used to compare continuous variables. The results are presented as unadjusted odds ratios (ORs) with 95 % confidence intervals. The minimum statistical significance level was fixed as $p < 0.05$.

Results

The baseline characteristics of the 326 workers are given in Table 1. Mean age was 51 years. Prevalence of current tobacco use (either smoking or smokeless tobacco) was 23.9 % (men 30.6 %, women 2.6 %). Current alcohol consumption was reported by 36.2 % (men 44.4 %, women 10.3 %). Mean and standard deviation of continuous variables are presented in Table 2.

Among the 326 workers, 85 were diabetic (26.1 %, 95 % CI 21.6–31.1), 107 were pre-diabetic (32.8 %, CI 28.7–37.2), and the remaining 134 (41.1 %) had normal FPG levels at baseline. Glycemic status of the 241 non-diabetic workers at baseline and at 2-year follow-up is given in Table 3. Among the 134 workers with normal FPG levels at baseline, 28.4 % progressed to pre-diabetes, 5.2 % developed diabetes, and among the 107 pre-diabetics, 23.4 % developed diabetes at year two. The odds of progressing to diabetes from pre-diabetes were five times higher compared to those from normal FPG (OR 5.53; CI 2.28–13.37).

Factors associated with incident diabetes among those with pre-diabetes, and dysglycemia among workers with normal glucose tolerance (NGT) are given in Table 4. Progression to dysglycemia from NGT was significantly higher among men compared to women. Progression from pre-diabetes (IFG) to diabetes was 36.4 % among current tobacco users compared to 18.9 % among non-users of tobacco, but did not reach statistical significance. However, when we analyzed the

Table 2 Mean and standard deviation of continuous variables at baseline

Variables	Mean ± SD
Body mass index (kg/m^2)	24.9 ± 3.5
Waist circumference (in cm)	85.5 ± 9.9
HDL cholesterol (mg/dl)	38.6 ± 13.8
Triglycerides (mg/dl)	139.9 ± 79.3
Total cholesterol (mg/dl)	202.1 ± 38.7
Systolic blood pressure (mmHg)	131 ± 18
Diastolic blood pressure (mmHg)	80 ± 10
Fasting plasma glucose (mg/dl)	121.9 ± 49.7

Table 3 Glycemic status of workers at baseline and at 2-year follow-up ($N = 241$)

Baseline status	Status at 2-year follow-up	New cases	Incidence rate per 1000 person years
NGT ($n = 134$)	Diabetes	8	30.7
NGT ($n = 134$)	IFG	38	165.2
NGT ($n = 134$)	IFG or diabetes	46	207.2
IFG ($n = 107$)	Diabetes	26	138.2
NGT or IFG ($n = 241$)	Diabetes	34	75.8

NGT normal glucose tolerance, *IFG* impaired fasting glucose

progression of 241 non-diabetic (IFG and NGT combined) workers to diabetes at 2-year follow-up, the incidence of 23.7 % among tobacco users was significantly higher compared to the incidence of 11.0 % among non-users of tobacco ($p = 0.019$). Similarly, the incidence of 21.2 % among alcohol users was significantly higher compared to the 10.6 % among non-users of alcohol ($p = 0.031$).

Discussion

This study provides the first incidence data on type 2 diabetes from the Indian State of Kerala. Incidence from normal glucose tolerance to diabetes, from pre-diabetes to diabetes, and from normal glucose tolerance to dysglycemia (IFG or diabetes) is also provided. Incidence of diabetes among pre-diabetes in our cohort was 138.2 per 1000 person years which was higher than the 78.9 per 1000 person years reported from the neighboring state of Tamil Nadu [6] and the Pima Indians (87.3 per 1000 person years) [15].

Incidence of diabetes among workers with normal glucose tolerance at baseline was 30.7 per 1000 person years, which was also higher than the 22.2 per 1000 person years reported from Tamil Nadu [6]. However, incidence of pre-diabetes among workers with baseline normal glucose tolerance was 165.2 per 1000 person years which was significantly higher than the 29.5 per 1000 person years reported from Tamil Nadu. Some of this difference could be explained by the fact that our sample was industrial workers with access to subsidized canteen food, whereas the Tamil Nadu sample was from the general community.

Male workers were three times more likely to progress to diabetes compared to women in our study. Incidence of diabetes was reported to be more among men compared to women in the MONICA study in Germany where the incidence of diabetes was 5.8 per 1000 person years for men and 4.0 per 1000 person years for women [16]. One explanation for the high incidence of diabetes among men in our study could be the higher use of current tobacco and alcohol by men. Current tobacco use was associated with higher incidence of diabetes in our study, similar to the findings from a cohort study in South Korea where smoking was reported to increase the risk of diabetes incidence and mortality [17]. Current alcohol use was significantly associated with incidence of diabetes in our

Table 4 Factors associated with incident diabetes and dysglycemia: results of bivariate analysis

Variables	Progression to diabetes ($N = 107$) (IFG to DM)		Progression to dysglycemia ($N = 134$) (NGT to DM/IFG)	
	(%)	OR (95 % CI)	(%)	OR (95 % CI)
Age (years)				
<50	20.8	Reference	31.3	Reference
≥50	30.3	1.63 (0.06–4.01)	38.0	1.34 (0.64–2.83)
Sex				
Women	21.0	Reference	17.1	Reference
Men	25.5	1.25 (0.37–4.16)	41.9	3.50 (1.40–8.73)
Family history of diabetes				
No	27.1	Reference	34.7	Reference
Yes	13.6	0.42 (0.11–1.57)	33.3	0.94 (0.41–2.16)
Abdominal obesity[a]				
No	21.0	Reference	35.8	Reference
Yes	28.9	1.53 (0.63–3.72)	30.8	0.79 (0.35–1.77)
Low HDL cholesterol[b]				
No	27.0	Reference	39.5	Reference
Yes	22.9	0.80 (0.32–1.99)	31.9	0.71 (0.33–1.52)
Hypertriglyceridemia [c]				
No	24.6	Reference	32.3	Reference
Yes	23.8	0.95 (0.38–2.37)	40.0	1.39 (0.62–3.09)
Hypercholesterolemia[d]				
No	25.0	Reference	36.2	Reference
Yes	23.7	0.93 (0.38–2.26)	32.3	0.84 (0.41–1.71)
Hypertensive[e]				
No	23.1	Reference	30.4	Reference
Yes	26.2	1.18 (0.48–2.90)	46.9	2.02 (0.89–4.55)
Current tobacco use[f]				
No	18.9	Reference	30.6	Reference
Yes	36.4	2.44 (0.97–6.12)	50.0	2.27 (0.95–5.43)
Current alcohol use[g]				
No	19.7	Reference	33.3	Reference
Yes	30.8	1.81 (0.72–4.50)	36.6	1.17 (0.54–2.54)

[a] Waist circumference ≥90 cm for men and ≥80 cm for women

[b] High-density lipoprotein (HDL) cholesterol <40 mg/dl for men and <50 mg/dl for women

[c] Triglycerides ≥150 mg/dl

[d] Total cholesterol ≥200 mg/dl

[e] Systolic blood pressure ≥140 mmHg and or diastolic blood pressure ≥90 mmHg or on medication for hypertension

[f] Any use of tobacco in the last 1 month

[g] Any alcohol use in the last 1 month

study, similar to the findings from the USA where men with high alcohol intake was reported to have higher incidence of type 2 diabetes [18]. A recent systematic review and meta-analysis reported that moderate intake of alcohol may reduce risk of diabetes among men in non-Asian population [19]. However, the pattern of drinking in our population is not moderate and the per capita consumption of alcohol is one of the highest in India. Alcohol intake was also associated with twofold higher incidence of diabetes in the Tamil Nadu study [6]. Kerala reported the second highest prevalence of alcohol consumption (45 %) after Andhra Pradesh (47 %) [20] which may partially explain the high diabetes prevalence in the state.

Limitation

The survey was conducted among industrial workers and thus did not exactly represent the general population of Kerala. The lack of long-term prospective follow-up data was another limitation. Findings of our study need to be contextualized as risk factors, incidence, and progression to diabetes may be different in other parts of India. Detailed multivariate analysis was not done because of the moderate sample size of the study. However, since there was no other data on incidence of diabetes from the Indian state of Kerala, we thought this will be a useful contribution to the literature.

Conclusions

Incidence of diabetes in this population is one of the highest in developing countries**.** Progression to pre-diabetes and diabetes occurred at a very fast rate in this population. Urgent measures are required to halt this progression by population and individual level approaches.

Acknowledgments Community Interventions for Health (CIH) was supported by a registered UK charity, the Oxford Health Alliance (OxHA), and facilitated by MATRIX Public Health Solutions. For a full list of donors supporting OxHA, please go to http://www.oxha.org.

Authors' contributions The study was conceptualized by KRT and RPV. Data analysis was performed by GKM and PSS. All authors contributed to writing and editing this paper and have read and approved the final manuscript.

Compliance with Ethical Standards All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was approved by the Institute Ethics Committee of Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. Written informed consent was obtained from all the participants before the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):286–297
DOI 10.1007/s13410-016-0503-8

ORIGINAL ARTICLE

Interactive role of endothelial nitric oxide synthase gene polymorphisms in T2D with CAD and CAD patients of Punjab (North-West India)

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Received: 2 November 2015 / Accepted: 27 May 2016 / Published online: 6 June 2016

Abstract Dysregulation of endothelial nitric oxide synthase (eNOS) activity causes the reduction in the production of nitric oxide (NO) which is an early indicator of type 2 diabetes (T2D) and cardiovascular complications. The present study evaluates the association of −786 T > C, 894 G > T, and 4a/b polymorphisms of *eNOS* gene in total of 1223 individuals enrolling 307 coronary artery disease (CAD) cases, 486 T2D cases with ($n = 170$) and without ($n = 316$) CAD as the secondary macrovascular complication, and 430 healthy controls from Punjab (North-West India). Genotyping of −786 T > C and 894 G > T polymorphisms was done with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the genotyping of 4a/b insertion/deletion polymorphism was done by PCR. The minor allele frequency (MAF) of −786 T > C polymorphism was higher in CAD (21.8 %), T2D + CAD (22.4 %), and T2D cases (18.4 %) as compared to healthy controls (17.6 %). However, in single-locus analysis, no significant results were obtained for *eNOS* polymorphisms in any of the studied groups. Significant association of C-b-G haplotype with the risk of both CAD [$P = 0.003$, odds ratio (OR) = 1.89 (1.04–3.45)] and T2D [$P = 0.019$, OR = 1.69 (0.92–3.13)] was observed. Diplotype analysis showed that TbG/CbG haplotype combination conferred risk towards CAD and T2D [$P = 0.01$, OR = 2.04 (1.18–3.57); $P = 0.006$, OR = 2.13 (1.22–3.57), respectively]. Furthermore, phenotypic parameters like waist circumference, high-density lipoprotein, and waist to height ratio are significantly associated with 894 G > T genotypes among CAD patients. In conclusion, the *eNOS* polymorphisms did not provide any conclusive result individually; however, their interactive effect gives some insights towards *eNOS* role in the population of Punjab.

Keywords Haplotype · SNP-SNP interaction · Association · Polymorphism

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Introduction

Endothelial dysfunction is the earliest functional abnormality in the blood vessels seen in diabetic patients and is also considered as an early marker for atherosclerosis [1–4]. Endothelial dysfunctioning is mainly due to deregulation of endothelial nitric oxide synthase (eNOS) enzymatic activity and inactivation of nitric oxide (NO) through oxidative stress [5]. Endothelium-derived NO is synthesized from L-arginine to L-citrulline by NO synthase enzyme encoded by the endothelial NO synthase (*eNOS* or *NOS3*) gene [6]. Endothelium-derived NO plays a vasoprotective role by removing super-oxide radicals, suppressing platelet aggregation, leukocyte adhesion, smooth muscle cell proliferation, regulation of vascular tone, and angiogenesis [7–11]. Reduced bioavailability of NO is involved in the initiation, progression, and complications of atherosclerosis [12, 13]. NO participates in inhibiting the adhesion and aggregation of platelet, migration, and growth of vascular smooth muscle cell. Low NO release can cause several cardiovascular diseases such as atherosclerosis, hypertension, and thrombosis, while high circulating NO concentration is generally toxic [14].

The *eNOS* gene is mapped to chromosome 7q36 (*eNOS*; Online Mendelian Inheritance in Man (OMIM) 163729; http://www.ncbi.nlm.nih.gov/OMIM/searchmorbid/), and it has been evidenced that genetic variants of *eNOS* gene effect the activity of *eNOS* and NO production [15]. The −786 T > C (rs2070744) in promoter region, 894 G > T (rs1799983; Glu298Asp) in exon 7, and 4a/b intron 4 are the most clinically relevant polymorphisms in the *eNOS* gene [16]. Studies have reported that −786 T > C promoter polymorphism reduces the promoter activity and thus affects eNOS protein expression and *eNOS* activity [17]. The 894 G > T (Glu298Asp) polymorphism causes a structural change of the eNOS protein and reduces *eNOS* activity [18]. The 4a/b VNTR polymorphism in the intron 4 of *eNOS* gene has also been associated with the reduced plasma concentration of NO [19]. Many studies have depicted the association of −786 T > C, 894 G > T, and 4a/b polymorphisms in the *eNOS* gene with coronary artery disease (CAD) [16, 20, 21] and type 2 diabetes (T2D) [22, 23] in different ethnic groups. The c allele of eNOS −786 T > C polymorphism is reported to provide 1.3 fold risk towards CAD [P = 0.041; OR = 1.31 (1.01–1.69)]. However, the results have been contradictory among different populations around the world [24–26]. Few studies have been reported on the association of −786 T > C, 894 G > T, and 4a/b polymorphisms with CAD and T2D in the Indian population. In North-West Indian population, especially in Punjab, due to the consumption of high-calorie and fat-rich diet and physical inactivity, the prevalence of CAD, T2D, and obesity has been increasing proportionally. Therefore, to fill the existing lacunae, the present study was aimed to investigate the association of −786 T > C, 894 G > T, and 4a/b polymorphisms with CAD, T2D + CAD, and T2D in the population of Punjab.

Materials and methods

Study subjects

In the present case control study, a total of 1223 individuals comprising 307 CAD patients without history of T2D, 316 T2D cases, 170 cases with T2D + CAD, and 430 gender-matched healthy controls above the age of 40 years were enrolled from various hospitals and localities of Punjab after obtaining informed consent and approval from institutional ethics committee. The details of diagnosis, sample collection, along with inclusion and exclusion criteria for cases and controls have been reported earlier [27]. The clinical and demographic details, and anthropometric measurements like height, weight, waist circumference (WC), and hip circumference (HC), were measured for calculating body mass index (BMI), waist-hip ratio (WHR), and waist to height ratio (WHtR) from each individual.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by inorganic method of DNA isolation [28] with some lab modifications. Three polymorphisms from *eNOS* gene, −786 T > C from the promoter region, 894 G > T from the exon 7, and 4a/b insertion/deletion (ins/del) from the intron 4, were selected on the basis of information available in the public databases like dbSNP and literature. Genotyping of −786 T > C and 894 G > T polymorphisms in *eNOS* gene was done with PCR-restriction fragment length polymorphism (PCR-RFLP) [29], and the genotyping of 4a/b ins/del polymorphism was done by PCR followed by genotyping on 2.5–3 % agarose gel [25]. The amplification conditions were modified according to the laboratory conditions.

Statistical analyses

Statistical analysis was performed using SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). Continuous data was represented as mean ± standard deviation (SD). Difference between continuous variables was evaluated by two-tailed Student's *t* test with Bonferroni's correction. Power of the study was calculated using CaTS power calculator software [30]. The distribution of genotype (scored by gene-counting method) and allele frequencies in cases and controls was compared using chi (χ^2) squared test. Association analysis was performed on different genetic models, and association was determined in terms of odds ratio (OR) at 95 % confidence interval (CI). Corrected values for OR were obtained by binary logistic regression analysis after correction with age, gender, BMI, WC, WHR, and family history of CAD/T2D. Haplotype frequencies for these polymorphisms were calculated using the *Haploview* (version 4.2) software that uses expectation-maximization (EM) algorithm. Haplotypes with low frequencies (<5 %) were discarded for further analysis. Measures of linkage disequilibrium (LD) were analyzed with the LD-plot function of this software. The benefit of a haplotype-based analysis is that it captures all of the variation across a region, which may improve the ability to detect an association [31]. Further, diplotype analysis was performed using MedCalc online software. Multifactor dimensionality reduction (MDR) was used to assess allele-allele interaction among all the SNPs [32, 33]. The benefit of allele-allele-based analysis by MDR is that it gives testing balance accuracy (TBA) which predicts the risk/disease status in independent testing sets generated through cross-validation consistency (CVC) [34]. Statistical significance was evaluated using a 1000 permutation test to compare observed testing-balanced accuracies [33]. To understand the genotype-phenotype correlation, one-way analysis of variance (ANOVA) test was performed and post hoc Bonferroni's correction was

applied. One-way ANOVA graphs were prepared using GraphPad Prism (version 6.0). A P value <0.05 was considered as significant.

Results

Clinical data

The comparison of various demographic, anthropometric, and clinical characteristics between cases and controls has been reported earlier [27].

Genetic association data

There was no deviation from HWE in the controls for the *eNOS* −786 T > C ($P = 0.302$), 894 G > T ($P = 0.710$), and 4a/b ($P = 0.171$). The power of study was found to be more than 80 % among different cases for all the three polymorphisms under the dominant mode of inheritance. Tables 1, 2, and 3 document the genotype and allele frequency distribution of 894 G > T, −786 T > C, and 4a/b polymorphisms of *eNOS* gene among CAD, T2D + CAD, T2D cases, and control groups. The minor allele frequency (MAF) of −786 T > C polymorphism was higher in CAD (21.8 %), T2D + CAD (22.4 %), and T2D cases (18.4 %) as compared to healthy controls (17.6 %). The c allele of *eNOS* −786 T > C polymorphism is reported to provide 1.3-fold risk towards CAD [$P = 0.041$, OR = 1.31 (1.01–1.69)]. However, no significant results were obtained for *eNOS* −786 T > C, 894 G > T, and 4a/b polymorphisms in any of the studied groups.

Haplotype analysis

Table 4 depicts the frequencies of haplotypes of 894 G > T, −786 T > C, and 4a/b polymorphisms of *eNOS* gene in CAD, T2D + CAD, and T2D when compared with control groups. The frequency of C-b-G haplotype is significantly higher in CAD ($P = 0.003$) and T2D ($P = 0.019$) cases as compared to controls, and this haplotype is associated with risk towards CAD [OR = 1.89 (1.04–3.45)] and T2D [OR = 1.69 (0.92–3.13)]. However, C-a-G haplotype is providing protection towards T2D [$P = 0.044$, OR = 0.64 (0.34–1.20)]. No association was observed with respect to other haplotypes. For determining the LD structure of the *eNOS* gene, LD was measured between SNP pairs using the D' and r^2 values (Table 5 and Fig. 1). In the present study, −786 T > C and 4a/b were in slight LD among CAD ($D' = 0.433$), T2D ($D' = 0.254$), and T2D + CAD ($D' = 0.364$) cases; however, in controls, this pattern was disrupted. Table 6 shows the association of various diplotype combinations towards the risk of CAD, T2D + CAD, and T2D in comparison to the controls. When CAD

Table 1 Genotype and allele frequency distribution with genetic model analysis for *eNOS* −786 T > C polymorphism among cases and controls

Genotype (total)	CAD ($n = 307$)	T2D + CAD ($n = 170$)	T2D ($n = 316$)	CN ($n = 430$)	CAD vs CN	T2D + CAD vs CN	T2D vs CN	CAD vs T2D + CAD	T2D + CAD vs T2D
TT	59.9 % (184)	60.6 % (103)	66.4 % (210)	68.1 % (293)	$P = 0.067$	$P = 0.165$	$P = 0.871$	$P = 0.624$	$P = 0.307$
TC	36.5 % (112)	34.1 % (58)	30.4 % (96)	28.6 % (123)					
CC	3.6 % (11)	5.3 % (9)	3.2 % (10)	3.3 % (14)					
Allele					$P = 0.041$*	$P = 0.056$	$P = 0.692$	$P = 0.850$	$P = 0.135$
T	78.2 %	77.6 %	81.6 %	82.4 %	OR = 0.76 (0.59–0.99)	OR = 0.74 (0.54–1.01)	OR = 0.95 (0.73–1.24)	OR = 1.03 (0.75–1.42)	OR = 0.78 (0.56–1.08)
C	21.8 %	22.4 %	18.4 %	17.6 %					
Dominant model (TC + CC vs TT)	–	–	–	–	$P = 0.176$	$P = 0.078$	$P = 0.628$	$P = 0.889$	$P = 0.198$
					OR = 1.25 (0.90–1.73)	OR = 1.39 (0.96–2.0)	OR = 1.08 (0.79–1.47)	OR = 1.03 (0.69–1.52)	OR = 1.28 (0.88–1.89)
Codominant model (TC vs TT + CC)	–	–	–	–	$P = 0.149$	$P = 0.185$	$P = 0.599$	$P = 0.606$	$P = 0.398$
					OR = 1.28 (0.92–1.78)	OR = 1.29 (0.88–1.89)	OR = 1.09 (0.79–1.50)	OR = 1.11 (0.75–1.64)	OR = 1.19 (0.80–1.77)
Recessive model (CC vs TT + TC)	–	–	–	–	$P = 0.809$	$P = 0.241$	$P = 0.944$	$P = 0.372$	$P = 0.248$
					OR = 1.09 (0.49–2.44)	OR = 1.67 (0.70–3.85)	OR = 0.97 (0.43–2.22)	OR = 0.67 (0.27–1.64)	OR = 1.72 (0.68–4.35)

OR odds ratio, *CAD* coronary artery disease, *T2D* type 2 diabetes, *CN* controls

*P value <0.05 was considered to be statistically significant and corrected for age, gender, body mass index, waist circumference, and waist-hip ratio

Table 2 Genotype and allele frequency distribution with genetic model analysis for *eNOS* 894 G > T polymorphism among cases and controls

Genotype (total)	CAD ($n = 307$)	T2D + CAD ($n = 170$)	T2D ($n = 316$)	CN ($n = 430$)	CAD vs CN	T2D + CAD vs CN	T2D vs CN	CAD vs T2D + CAD	T2D + CAD vs T2D
GG	68.1 % (209)	61.2 % (104)	66.8 % (211)	65.8 % (283)	$P = 0.453$	$P = 0.519$	$P = 0.955$	$P = 0.127$	$P = 0.415$
GT	30.9 % (95)	35.9 % (61)	31.3 % (99)	32.1 % (138)					
TT	1.0 % (3)	2.9 % (5)	1.9 % (6)	2.1 % (9)					
Allele					$P = 0.399$	$P = 0.274$	$P = 0.774$	$P = 0.088$	$P = 0.206$
G	83.6 %	79.1 %	82.4 %	81.9 %	OR = 1.13 (0.85–1.48)	OR = 0.84 (0.61–1.15)	OR = 1.04 (0.80–1.36)	OR = 1.34 (0.96–1.88)	OR = 0.81 (0.58–1.13)
T	16.4 %	20.9 %	17.6 %	18.1 %					
Dominant model (GT + TT vs GG)	–	–	–	–	$P = 0.520$ OR = 0.90 (0.66–1.23)	$P = 0.258$ OR = 1.22 (0.85–1.75)	$P = 0.785$ OR = 0.96 (0.70–1.29)	$P = 0.129$ OR = 0.74 (0.50–1.09)	$P = 0.218$ OR = 1.28 (0.87–1.89)
Codominant model (GT vs GG + TT)	–	–	–	–	$P = 0.741$ OR = 0.95 (0.69–1.30)	$P = 0.374$ OR = 1.18 (0.82–1.72)	$P = 0.825$ OR = 0.97 (0.71–1.32)	$P = 0.271$ OR = 0.80 (0.54–1.19)	$P = 0.308$ OR = 1.23 (0.83–1.82)
Recessive model (TT vs GG + GT)	–	–	–	–	$P = 0.238$ OR = 0.46 (0.12–1.72)	$P = 0.535$ OR = 1.41 (0.47–4.35)	$P = 0.852$ OR = 0.91 (0.32–2.56)	$P = 0.109$ OR = 0.33 (0.08–1.39)	$P = 0.461$ OR = 1.56 (0.47–5.26)

P value <0.05 was considered to be statistically significant and corrected for age, gender, body mass index, waist circumference, and waist-hip ratio

OR odds ratio, *CAD* coronary artery disease, *T2D* type 2 diabetes, *CN* controls

Table 3 Genotype and allele frequency distribution with genetic model analysis for *eNOS* 4a/b polymorphism among cases and controls

Genotype (total)	CAD ($n = 307$)	T2D + CAD ($n = 170$)	T2D ($n = 316$)	CN ($n = 430$)	CAD vs CN	T2D + CAD vs CN	T2D vs CN	CAD vs T2D + CAD	CAD vs T2D	T2D + CAD vs T2D
bb	72.3 % (222)	68.8 % (117)	75.3 % (238)	72.1 % (310)	$P = 0.986$	$P = 0.118$	$P = 0.421$	$P = 0.109$	$P = 0.443$	$P = 0.140$
ba	24.8 % (76)	30.6 % (52)	23.1 % (73)	25.1 % (108)						
aa	2.9 % (9)	0.6 % (1)	1.6 % (5)	2.8 % (12)						
Allele					$P = 0.983$	$P = 0.818$	$P = 0.228$	$P = 0.815$	$P = 0.271$	$P = 0.240$
b	84.7 %	84.1 %	86.9 %	84.7 %	OR = 1.00 (0.75–1.34)	OR = 0.96 (0.68–1.36)	OR = 1.20 (0.89–1.61)	OR = 1.04 (0.73–1.50)	OR = 0.84 (0.61–1.15)	OR = 0.80 (0.55–1.16)
a	15.3 %	15.9 %	13.1 %	15.3 %						
Dominant model (ba + aa vs bb)	–	–	–	–	$P = 0.948$ OR = 0.99 (0.71–1.37)	$P = 0.426$ OR = 1.18 (0.79–1.72)	$P = 0.325$ OR = 0.85 (0.61–1.18)	$P = 0.421$ OR = 0.85 (0.56–1.27)	$P = 0.394$ OR = 1.16 (0.82–1.67)	$P = 0.124$ OR = 1.39 (0.92–2.08)
Codominant model (ba vs bb + aa)	–	–	–	–	$P = 0.911$ OR = 0.98 (0.70–1.38)	$P = 0.172$ OR = 1.31 (0.89–1.95)	$P = 0.526$ OR = 0.90 (0.64–1.26)	$P = 0.169$ OR = 0.75 (0.49–1.13)	$P = 0.628$ OR = 1.10 (0.76–1.58)	$P = 0.072$ OR = 1.47 (0.97–2.23)
Recessive model (aa vs bb + ba)	–	–	–	–	$P = 0.909$ OR = 1.05 (0.44–2.5)	$P = 0.095$ OR = 0.21 (0.03–1.59)	$P = 0.274$ OR = 0.56 (0.19–1.61)	$P = 0.087$ OR = 5.00 (0.64–50.0)	$P = 0.256$ OR = 1.89 (0.62–5.56)	$P = 0.344$ OR = 0.37 (0.04–3.23)

P value <0.05 was considered to be statistically significant and corrected for age, gender, body mass index, waist circumference, and waist-hip ratio

OR odds ratio, *CAD* coronary artery disease, *T2D* type 2 diabetes, *CN* controls

Table 4 The distribution of haplotype frequency for the three polymorphisms of the *eNOS* gene and their comparison in cases and controls

CAD vs control	Haplotype[a]	Haplotype frequency	Frequency CAD ($n = 307$)	Frequency controls ($n = 430$)	*P* value	OR (95 % CI)
	T-b-G	0.627	0.611	0.638	0.305	0.91 (0.67–1.22)
	T-b-T	0.113	0.105	0.119	0.385	0.86 (0.54–1.39)
	C-a-G	0.079	0.081	0.077	0.765	1.06 (0.62–1.82)
	C-b-G	0.063	0.085	0.047	0.003*	1.89 (1.04–3.45)
	T-a-G	0.057	0.058	0.057	0.944	1.01 (0.54–1.89)
T2D + CAD vs control	Haplotype[a]	Haplotype frequency	Frequency T2D + CAD ($n = 170$)	Frequency controls ($n = 430$)	*P* value	OR (95 % CI)
	T-b-G	0.628	0.590	0.642	0.093	0.80 (0.56–1.15)
	T-b-T	0.114	0.112	0.115	0.900	0.98 (0.56–1.72)
	C-a-G	0.075	0.073	0.076	0.875	0.92 (0.46–1.82)
	C-b-G	0.051	0.068	0.045	0.095	1.64 (0.78–3.45)
	T-a-G	0.057	0.059	0.056	0.807	1.05 (0.49–2.27)
	C-b-T	0.052	0.070	0.045	0.071	1.64 (0.78–3.45)
T2D vs control	Haplotype[a]	Haplotype frequency	Frequency T2D ($n = 316$)	Frequency controls ($n = 430$)	*P* value	OR (95 % CI)
	T-b-G	0.636	0.632	0.639	0.796	0.34 (0.26–0.47)
	T-b-T	0.113	0.108	0.117	0.613	0.92 (0.58–1.45)
	C-a-G	0.063	0.048	0.073	0.044*	0.64 (0.34–1.20)
	C-b-G	0.060	0.077	0.047	0.019*	1.69 (0.92–3.13)
	T-a-G	0.063	0.067	0.059	0.496	1.15 (0.63–2.08)

T2D type 2 diabetes, *CAD* coronary artery disease, *OR* odds ratio, *CI* confidence interval

*$P < 0.05$ is statistically significant

[a] Order of SNPs in eNOS haplotypes: −786 T > C, 4a/b, and 894 G > T

Table 5 Measure of LD, observed in a pair-wise comparison for the three polymorphisms of the *eNOS* gene among cases and controls

Variant 1	Variant 2	Controls			CAD patients			T2D + CAD patients			T2D patients		
		D′	LOD	r^2	*D′*	LOD	r^2	*D′*	LOD	r^2	*D′*	LOD	r^2
−786 T > C (rs2070744)	4a/b	0.479	18.48	0.196	0.433	7.79	0.121	0.364	2.6	0.087	0.254	2.85	0.043
−786 T > C (rs2070744)	894 G > T (rs1799983)	0.164	2.12	0.026	0.093	0.33	0.006	0.228	1.57	0.048	0.178	1.79	0.03
4a/b	894 G > T (rs1799983)	0.134	0.06	0.001	0.633	0.83	0.014	0.268	0.11	0.004	0.254	0.12	0.002

T2D type 2 diabetes, *CAD* coronary artery disease, *D′* Lewontin's coefficient, r^2 Pearson's correlation, *LOD* logarithm of odds

cases and T2D cases were compared with controls, only TbG/CbG diplotype conferred nearly 2-fold risk towards CAD [$P = 0.01$, OR = 2.04 (1.18–3.57)] and T2D [$P = 0.006$, OR = 2.13 (1.22–3.57)], respectively; however, no significant results were obtained with other diplotype combination among any group.

The interaction analysis of the tested SNPs revealed significant interactions towards CAD and T2D + CAD susceptibility. A two-locus interaction between −786 T > C and 4a/b SNPs was significant when CAD patients ($P = 0.01$) and T2D + CAD patients ($P = 0.007$) were compared with healthy controls. This model had a TBA of 0.5029 and a CVC of 5 of 10 for CAD cases and 0.5482 TBA and 10 of 10 CVC for T2D + CAD cases (Table 7 and Fig. 2).

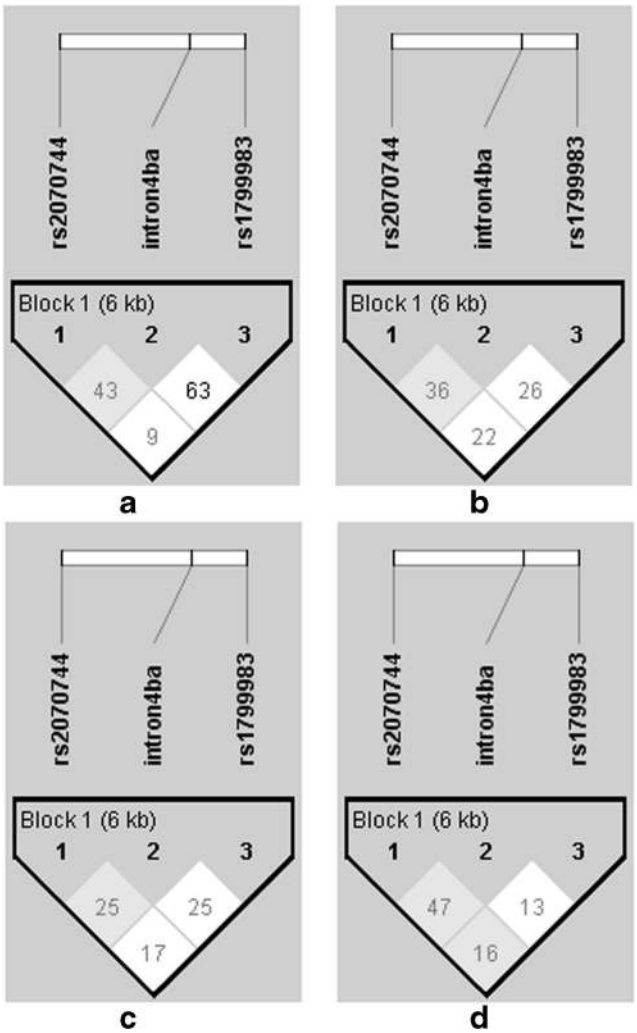

Fig. 1 Linkage disequilibrium plot with the help of *Haploview* for the *eNOS* polymorphisms (rs2070744, intron 4a/b, rs1799983) in **a** CAD cases, **b** T2D + CAD cases, **c** T2D cases, and **d** healthy controls. The *boxes* contain *D′* values multiplied by 100 to indicate strength of linkage disequilibrium between pairs of SNPs. Shades of *pink*/*red* represent LOD > 2 and *D′* < 1, and *white squares* represent LOD < 2 and *D′* < 1

Genotype-phenotype analysis

The genotype-phenotype analysis revealed a significant association of WC, WHtR, and high-density lipoprotein (HDL; Fig. 3) with the 894 G > T genotypes among CAD patients. No significant association was observed between other parameters and studied SNP genotypes among T2D and T2D + CAD cases.

Discussion

There is considerable amount of evidence suggesting that impairment of NO production causes endothelial dysfunction, which contributes to the development of insulin resistance; T2D; chronic renal failure; and cardiovascular complications including hypertension, hypercholesterolemia, and atherosclerosis [35, 36]. Several polymorphisms in the *eNOS* gene affect the bioavailability of NO. However, the MAF of *eNOS* gene polymorphisms varies among different global population groups (Table 8). Various studies have documented some contradictory results. Therefore, the present study is designed to explore the association of −786 T > C, 894 G > T, and 4a/b polymorphisms with predisposition to CAD, T2D + CAD, and T2D in North-West Indian population (Punjab).

In our population, we did not find any significant association of *eNOS* SNPs in the entire studied groups. However, we found a significantly higher frequency of "C" allele among CAD patients ($P = 0.041$) of −786 T > C polymorphism when compared with healthy controls. Previous studies documented that the risk of developing CAD is higher for individuals with

Table 6 Diplotype frequency distribution of *eNOS* gene polymorphisms among cases and controls

Diplotype[a]	No. in CAD cases	No. in control	*P* value	OR (95 % CI)	No. in T2D + CAD cases	No. in control	*P* value	OR (95 % CI)	No. in T2D cases	No. in control	*P* value	OR (95 % CI)
TbG/TbG	109	177	0.13	0.79 (0.58–1.06)	58	177	0.12	0.74 (0.51–1.08)	120	177	0.41	0.88 (0.65–1.18)
TbG/TbT	46	68	0.84	0.93 (0.63–1.41)	23	68	0.53	0.83 (0.50–1.39)	45	68	0.61	0.88 (0.59–1.33)
TbG/TaG	20	30	0.88	0.93 (0.52–1.69)	14	30	0.60	1.19 (0.62–2.33)	30	30	0.22	1.39 (0.83–2.38)
TbG/TaT	8	11	1.00	1.02 (0.40–2.56)	6	11	0.59	1.39 (1.03–3.85)	8	11	1.00	0.99 (0.39–2.50)
TbG/CbG	33	24	0.01*	2.04 (1.18–3.57)	9	24	1.00	0.94 (0.43–2.08)	35	24	0.006*	2.13 (1.22–3.57)
TbG/CbT	26	31	0.58	1.19 (0.69–2.04)	18	31	0.19	1.52 (0.83–2.78)	30	31	0.28	1.35 (0.80–2.27)
TbG/CaG	33	40	0.53	1.18 (0.72–1.92)	18	40	0.65	1.15 (0.64–2.38)	20	40	0.17	0.66 (0.38–1.15)
TbG/CaT	11	18	0.71	0.85 (0.39–1.82)	9	18	0.66	1.28 (0.56–2.94)	9	18	0.43	0.67 (0.29–1.52)

OR odds ratio, *CI* confidence interval, *T2D* type 2 diabetes, *CAD* coronary artery disease

*$P < 0.05$ is statistically significant

[a] Order of SNPs in eNOS diplotypes: −786 T > C, 4a/b, and 894 G > T

Table 7 Allele-allele interaction analysis by the multifactor dimensionality reduction (MDR) method of −786 > C, 4a/b, and 894 G > T SNPs of *eNOS* gene among CAD, T2D + CAD, and T2D cases and control groups

CAD vs control				T2D + CAD vs control				T2D vs control			
Model	Cross-validation consistency	Testing balance accuracy	*P* value[a]	Model	Cross-validation consistency	Testing balance accuracy	*P* value[a]	Model	Cross-validation consistency	Testing balance accuracy	*P* value[a]
SNP1, SNP2	5/10	0.5029	0.01*	SNP1, SNP2	10/10	0.5482	0.007*	SNP1, SNP2	10/10	0.5473	0.375
SNP1, SNP2, SNP3	10/10	0.4943	0.91	SNP1, SNP2, SNP3	10/10	0.5227	0.743	SNP1, SNP2, SNP3	10/10	0.5290	0.590

SNP1 (eNOS −786 T > C), SNP2 (eNOS 4a/b), and SNP3 (eNOS 894 G > T)

T2D type 2 diabetes, *CAD* coronary artery disease

*P value <0.05 is considered as significant

[a] P value based on 10,000 permutations

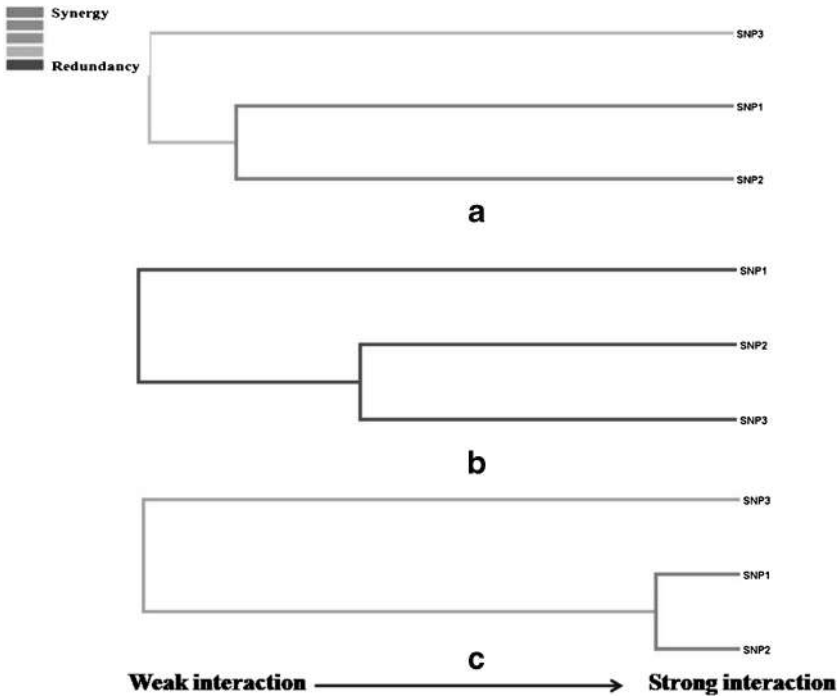

Fig. 2 The entropy-based information gain calculated for the SNP pairs **a** CAD vs control group, **b** T2D + CAD vs control group, and **c** T2D vs control group. SNP1 (eNOS −786 T > C), SNP2 (eNOS 4a/b), and SNP3 (eNOS 894 G > T). The interaction line is *red*, indicating that the interaction between SNPs provided synergistic information

C allele of the −786 T > C *eNOS* gene promoter polymorphism among Italian [29] and Han Chinese [37] population groups. Nakayama et al. [17] reported the −786 T > C genetic variation in the 5′-flanking region of the *eNOS* gene in coronary vasospasm patients. Individuals with −786 C allele had reduced activity because DNA binding protein (replication protein A1) has the ability to bind only to the −786 C allele isoform, resulting the decrease in the promoter activity by 50 %, leading to endothelial dysfunction. Similar to our findings, some other reports showed no statistical significant association

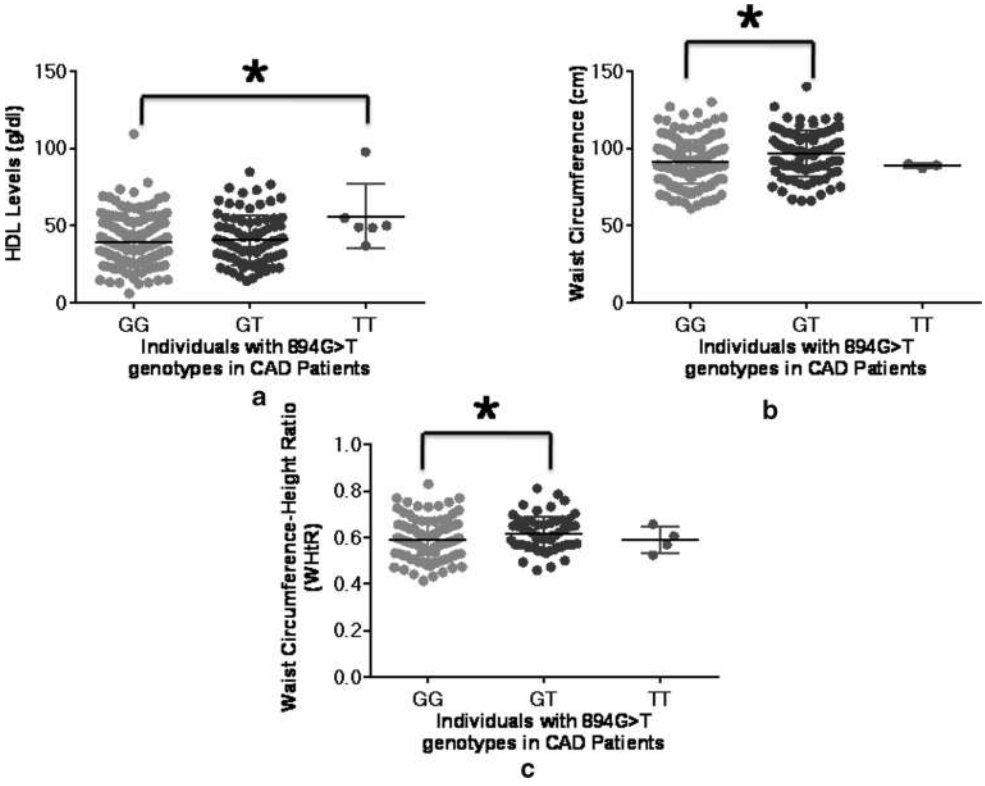

Fig. 3 Statistics (one-way ANOVA) with the help of GraphPad Prism (version 6.0). **a** HDL levels, **b** WC, and **c** waist-height ratio (WHtR) in CAD patients with different genotypes of *eNOS* 894 G > T polymorphism. The *aster sign* indicates the *p* value significance. **P* < 0.05

Table 8 Distribution of minor allele frequency (MAF) of *eNOS* polymorphisms across different population groups

eNOS −786 T > C		
Population	Minor allele frequency (%)	Reference
Punjab	17.9	Present study
Chinese	14.2	An et al. 2012
Tunisian	22.85	Ezzidi et al. 2008
Mexican American	24.0	Thameem et al. 2008
Caucasian Brazilian	35.0	Santos et al. 2011
Brazilian	35.5	Sandrim et al. 2007
eNOS VNTR 4a/b		
Population	Minor allele frequency (%)	Reference
Punjab	15.3	Present study
Japanese	7.1	Neugebauer et al. 2000
Iran	8.0	Mohseni et al. 2011
Mexican American	10.0	Thameem et al. 2008
Caucasian (UK)	13.6	Rippen et al. 2003
Polish	14.0	Buraczynska et al. 2004
Tunisian	17.2	Ezzidi et al. 2008
Caucasian Brazilian	18.0	Santos et al. 2011
Brazilian	20.7	Bellini et al. 2007
eNOS 894 G > T		
Population	Minor allele frequency (%)	Reference
Punjab	18.1	Present study
Korean	3.9	Shin Shin et al. 2004
South Indian	15.0	Angeline et al. 2011
Mexican American	20.0	Thameem et al. 2008
Caucasian Brazilian	29.0	Santos et al. 2011
Tunisian	31.9	Ezzidi et al. 2008
Brazilian	32.5	Sandrim et al. 2007
Chinese	44.6	Tang et al. 2008

of −786 T > C polymorphism with T2D in Mexican-Americans [38], with CAD among Greece population [39], and T2D + CAD among Europeans [25].

We have observed that 894 G > T polymorphism does not show any association with CAD, T2D + CAD, and T2D. A non-significant association of 894 G > T polymorphism with CAD has been observed among Chilians [40], Taiwanese [41], Caucasians [42], South Indian Tamilians [24], and North Indians [26]. Similarly, no association of this polymorphism was observed with elevated blood pressure in coronary heart disease (CHD) and T2D patients from Finish population [22]. On the other hand, studies conducted in Italy [23, 29] and South India [43] observed that the 894 G > T polymorphism in *eNOS* gene is significantly associated with the presence of CAD.

In the present study, 4a/b polymorphism does not show any significant difference in the genotype and allele frequencies between CAD and controls, which is in concordance with other studies conducted on CAD patients among German [44], Turkish [45], Caucasian [42], and South Indian [46] population groups. In South Indian population, Narne et al. [47] reported a significant association of −786 T > C polymorphism with T2D + CAD; however, no significant association was observed with 894 T > G and 4a/b polymorphisms. However, there are contradictions in the association of 4a/b polymorphism across different world populations. Studies carried out in Iranian [21], African-American, Caucasians [48], and Korean [49] population revealed that *eNOS* 4a/b polymorphism is associated with risk towards CAD. However, reports from Greece failed to show any association of 4a/b polymorphism with T2D [50]. Wang et al. [51] demonstrated that repeats of the 27-bp insert of the 4b can bind to a nuclear protein and act as a cis-acting factor of the *eNOS* promoter and regulate the transcription efficiency at a haplotype-specific fashion with the −786 T > C variant at the promoter region. Therefore, the b/b genotype probably acts both independently and in coordination with the functional SNP −786

T > C at the promoter region of *eNOS* to regulate of the expression of *eNOS* gene.

Haplotype analysis carried on these three polymorphisms revealed that T-b-G is the most common haplotype in all of the studied groups and C-b-G haplotype (C allele of −786 T > C, b allele of 4a/b, and G allele of 894 G > T) is associated with the risk towards CAD and T2D and C-a-G haplotype is providing protection towards T2D. Furthermore, TbG/CbG diplotype conferred risk towards CAD and T2D by 2-fold. Comparisons with other studies reveal variation in the association of haplotypes with T2D and CAD. A study on Korean population suggested association of C-a-G haplotype with risk of T2D in CAD patients [52]. On the other hand, a study carried on Brazilian population relate C-G-b haplotype with a lower risk of developing hypertension and enhanced NO formation in hypertensive and hypertensive–diabetic patients, while C-T-b haplotype is associated with risk of hypertension and lowers NO levels in hypertensive and hypertensive–diabetic patients [53]. A study carried on healthy white males reported that the specific *eNOS* haplotype C-b-G is associated with lower circulating NO levels; however, *eNOS* genotypes were not significantly associated with changes in the circulating NO concentration [54]. The interethnic differences in NO-mediated effects may be due to difference in the distribution of *eNOS* alleles among different population groups [55–57]. Both the LD analysis and SNP-SNP interaction analysis revealed a cumulative effect of SNP −786 T > C and 4a/b with the development of CAD and T2D + CAD. In the present study, association of haplotype, diplotype combinations, and SNP-SNP interaction with the disease state were observed but individual SNPs show lack of association. This represents the interactive role of SNPs in precipitating the disease etiology. Therefore, more functionally relevant SNPs in the *eNOS* gene should be screened to elucidate the role of interaction of polymorphisms in the disease pathophysiology.

The exact mechanism responsible for the association of the *eNOS* polymorphisms with the progression of CAD and T2D + CAD still needs to be elucidated. However, it is evident from the studies that NO is very important for the proper functioning of the cardiovascular system and maintenance of the vascular tone [10, 58] and the polymorphisms of the *eNOS* gene reduces the bioavailability of endothelial NO by reducing the mRNA expression or by altering the eNOS function [17–19]. The present study gives a baseline data for the association of *eNOS* polymorphisms with CAD, T2D, and T2D + CAD, which will help in narrowing down to causative factors and susceptibility genotypes which will help in formulating individualized drugs targets. This will help in identifying individuals at risk of developing CAD, T2D, and T2D + CAD at an early age so that preventive measure and lifestyle modifications can be initiated early to prevent the disease progression. This will greatly influence the increasing prevalence rates of the disease and also help in reducing economic burden.

Furthermore, functional analysis should be done on *eNOS* gene to understand the expression of transcribed protein and it will help to determine the risk assessment model for susceptibility gene variants and proteins that may be implicated in disease etiology in the population of Punjab. However, further studies need to be done with more SNPs to understand the detailed role of *eNOS* in disease pathophysiology.

In conclusion, our findings suggest that C allele of −786 T > C polymorphism is the risk factor for CAD. Furthermore, C-b-G haplotype and TbG/CbG diplotype conferred risk towards both CAD and T2D. Also, there is a significant interactive role of SNP −786 T > C and 4a/b in the disease etiology in the population of Punjab.

Acknowledgments The financial assistance to Kaur R by UGC is acknowledged. The financial assistance under the scheme of "Centre with Potential for Excellence in Particular Area" through grant number F.8-2/2008 (NS/PE; UGC, India) and "University with Potential for Excellence" through grant number F.14-2/2008 (NS/PE; UGC, India) to Bhanwer AJS at Guru Nanak Dev University, Amritsar, is humbly acknowledged.

Compliance with ethical standards

Competing interests It is declared that there is no conflict of interest of authors and the research work is entirely for academic purpose and no competing financial interests exist.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):298–304
DOI 10.1007/s13410-016-0504-7

ORIGINAL ARTICLE

The effect of aerobic exercise training on β-cell function and circulating levels of adipsin in community of obese women with type 2 diabetes mellitus

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Received: 13 January 2016 / Accepted: 27 May 2016 / Published online: 2 June 2016

Abstract It has been suggested that adipsin might affect insulin secretion or glucose homeostasis, and thus, there can be a missing link between β-cell function, obesity, and type 2 diabetes. However, as yet, the function of adipsin has been unknown and needs to be evaluated over a longer term. The objective of this semi-experimental study was to compare the effect of aerobic exercise training on β-cell function and circulating levels of adipsin in obese women with type 2 diabetes mellitus (T2DM). The T2DM women with fasting plasma glucose >126 mg/dl were targeted and chosen for further evaluations. Twenty-four women aged 35–50 years participated in the study. Subjects were divided in two groups of C and E standing for control and experimental groups, respectively (group C/$n = 12$, group E/$n = 12$). Anthropometric variable measurements, including fasting plasma glucose, insulin, lipid profiles, free fat acid (FFA), HOMA-IR, HOMA-β, and serum adipsin were obtained from the study samples. Following 8 weeks of aerobic training, serum adipsin levels did not alter in group E, whereas, the levels of HOMA-IR, fasting plasma glucose, weight, BMI, and percent body fat levels significantly changed in group E compared to the baseline. Other variables did not alter significantly. The study indicated that aerobic exercise training in T2DM patients had positive health effects especially on glycemic control and pancreatic β-cell function with no significant change in adipsin levels. However, adipsin is a newly identified immune regulator of metabolic diseases and more studies are needed to understand the signaling mechanisms of adipsin T2DM population.

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Keywords Adipsin · β-cell function · Aerobic exercise · Diabetes

Introduction

Declining β-cell function is a hallmark of T2DM which results in hyperglycemia and insulin resistance [1]. Besides, it is strongly established that obesity is associated with a cluster of T2DM [2]. Adipose tissue is an organ which not only stores and releases free fatty acids but also secretes the adipokines [3]. Adipsin, releasing abundant adipokine in the blood, has the metabolic role and is a cell signaling protein made by adipose tissue [4].

Previous studies have shown that the level of adipsin was lower in obese and diabetes individuals than in lean ones and hence suggesting that this protein might play a pivotal role in metabolism and stimulating insulin secretion to control blood sugar [5]. An animal model study has indicated that systemic replenishment of adipsin improves hyperglycemia in diabetic mice by boosting insulin secretion [6].

The findings suggest that adipsin produces complement 3a (C3a) that acts remotely on β-cells where reside at langerhans to stimulate insulin secretion and to eventually reduce liver glucose output [7]. However, the molecular link among adipsin with obesity and β-cell function remains to be established [7].

Under obesity where individual fat mass increases, it is first suspected that secretion of adipsin increases to stimulate pancreatic β-cells to produce more insulin [4] so that they regulate energy homestasis and metabolism [5].

No doubt, it is well established that regular physical activity improves insulin secretion and glucose homeostasis especially among T2DM patients [8, 9]. International Diabetes Federation (2013) recommended that T2DM patients should

exercise at least 150 min moderate-intensity aerobic exercise per week with 50–70 % of maximal heart rate [10].

Regular exercise would improve insulin sensitivity and pancreas would normally release a lowered-insulin secretion. There are methods enabling us to estimate these factors. The homeostatic model assessment (HOMA) method is generally used in order to quantify insulin resistance and beta-cell function. For this purpose, fasting glucose and insulin levels are needed [11].

The mechanisms improving pancreatic β-cell function increase insulin action not only linked with pancreas function but also with other major affected organs such as the liver (to decrease hepatic gluconeogenesis), skeletal muscle (to reduce insulin resistance), and anti-inflammatory cytokines secreted by both adipocytes (e.g., adipsin) and myocytes (e.g., interlukin-6) [8]. In the present study, we, therefore, intend to investigate the impact of an 8-week aerobic exercise training on β-cell function and circulating levels of adipsin in community of obese women with T2DM.

Methods

This study was approved by the Regional Scientific Ethics Committee, Faculty of Physical Education and Sport Sciences of Razi University, in accordance with the ethical standards of the 1964 Helsinki declaration. Then, participants were recruited through local Diabetes Association where the study was conducted. Twenty-four inactive and T2DM obese patients were ultimately found eligible for the study. All individuals completed a medical questionnaire to evaluate their individual lifestyles and habits such as the history of T2DM, physical activity, smoking habits, and diet. Inclusion criteria required that all participants be untrained (lack of regular physical activity during previous 6 months), normotensive, non-smokers, fasting blood glucose >126 mg/dl over the last year, glycosylated hemoglobin (HbA1C) >6.5 %, BMI >30 kg/m^2, and being menopause. Exclusion criteria were: BMI <30 kg/m^2, absence more than two consecutive sessions of aerobic exercises during the study, use of exogenous insulin, evidence of liver, renal, cardiopulmonary, neuromuscular and/or psychological disease, and any debilitating diseases that restrict physical activity [12]. All participants were under treatment with oral anti-diabetic agents, either with metformin or glibenclamide and continued their medication throughout the study. Ultimately, written informed consent was obtained from all participants.

Experimental design

In this semi-experimental study, subjects were divided into two groups: C for control and E for experimental group each comprised of 12 subjects. At the end of the study, one participant in group C was excluded from the study due to incomplete data. Group E underwent endurance exercise training three nonconsecutive days per week and lasting for 8 weeks. Every session consisted of 50–60 min at 60–80 % of maximum heart rate by 10–15 min of flexibility exercises, 30–40 min of aerobic exercises, and 5–10 min of cool-down activity. Aerobic exercises in each session included walking, running, and some Pilates program.

Chemical analysis

Fasting venous blood samples were collected from all patients in two phases before and after the 8-week treatment. Therefore, the participants fasted from 10:00 p.m. the evening before the blood sampling days (only water was allowed). The blood samples were collected from each patient centrifuged for 10 min, and plasma was stored at −20 °C for later analysis. To determine plasma adipsin concentration, colorimetric enzymatic procedure (Roche kit; Hangzhou Eastbiopharm, China) was used. Venous plasma concentrations of insulin were determined with chemiluminescence procedure (Liaison kits, Italy). Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) were measured by an enzymatic photometric analyzer (Pars Azmoun, Tehran, Iran). Additionally, plasma glucose was determined by using glucose oxidase-peroxidase/4- aminoantipyrine (GOD-PAP) method (Pars Azmoun, Tehran, Iran). Moreover, homeostasis model assessment-insulin resistance (HOMA-IR) index for insulin sensitivity and homeostasis model assessment-insulin for β-cell function (HOMA-β) were computed following these equations (when glucose and insulin are both during fasting and applied glucose unit: mg/dl) [11]:

$$\text{HOMA-IR}: \ (\text{Glucose} \times \text{Insulin}) \Big/ 405$$

$$\text{HOMA-}\beta: \ \% \Big[(360 \times \text{Insulin}) \Big/ (\text{Glucose-63}) \Big]$$

Body composition

Body weight (weight recorded to the nearest 0.1 kg) and body fat percent were measured through a standard balance beam scale (Beure model BG55 Digital beam scale, Germany) while the standing height was measured with a centimeter tape scale attached to a wall. Body mass index (BMI) was calculated as the ratio of weight (kg) to height (m^2).

Statistical analysis

All data were presented as mean ± standard deviations. Normality of data was checked by Kolmogorov-Smirnov Test (K-S test) and Levene s test for the homogeneity of

variances. Differences within and between the groups of BMI, body fat percentage, fasting plasma glucose, insulin, lipid profiles, free fat acid (FFA), HOMA-IR, HOMA-β, and serum adipsin in response to exercise during the 8-week treatment were examined by two-way analysis of variance, where groups (C and E) were used as the fixed factors. In addition, the abovementioned variables were studied as dependent factors in two time levels of study (pre- to post-test). Correlations among variables were analyzed using the Pearson's test. A p value of <0.05 was considered statistically significant. All statistical analyses were also conducted using SPSS package (Version 22).

Results

During the 8-week study, all the subjects of the two groups participated in two phases of blood sampling, except one dropout from control group who could not participate in the second phase of blood sampling at the end of the study. The subjects' characteristics and anthropometric data were summarized in Table 1.

Anthropometric status

The changes in BMI and body fat percentage following the training intervention are presented in Table 1. The measurements of BMI, in pre- to post-test, revealed that there were not any significant changes in BMI over the course of the study between the two groups, F (1, 21) = 0.087, p = 0.772, ηp^2 = 0.004. In other words, the groups did not change in BMI over the course of the study (Table 2). Although the within-subject test indicated that there was a significant time effect in BMI measurement in group E, F (1, 21) = 13.15, p = 0.002, ηp^2 = 0.385, but the interaction between time and group effect was not significant, F (1, 21) = 2.148, p = 0.158, ηp^2 = 0.093.

Body fat percentage was significantly decreased in group E compared to group C following the 8-week endurance training, F (1, 21) = 4.61, p = 0.044, ηp^2 = 0.180. The interaction between time and group was significant as well: F (1, 21) = 6.864, p = 0.016, ηp^2 = 0.246, that is, this type of exercise could affect body fat percentage of group E as compared to group C over the course of the study. There were not significant changes between the two groups following the 8-week protocol: F (1, 21) = 0.001, p = 0.977, ηp^2 = 0.009.

Fasting plasma glucose and insulin status

Following the 8-week endurance training, fasting plasma glucose and insulin concentrations did improve (p > 0.05) in group E as compared to group C (Table 2). Within-subjects tests indicated that the time effect and group × time interactions were significant on fasting plasma glucose concentration after 8 weeks of training group E: F (1, 21) = 4.743, p = 0.041, ηp^2 = 0.184 and F (1, 21) = 8.519, p = 0.008, ηp^2 = 0.289. In addition, the effects of between-subjects test were not significant over the course of the study: F (1, 21) = 0.293, p = 0.594, ηp^2 = 0.014.

Based on insulin results, the changes of time and time × group interaction were not significant as shown by within-subjects test, respectively as follows: F (1, 21) = 0.112, p = .742, ηp^2 = 0.005 and F (1, 21) = 1.70, p = 0.206, ηp^2 = 0.075. The between-subject effect was not altered significantly between C and E groups following the 8-week study: F (1, 21) = 0.046, p = 0.833, ηp^2 = 0.002.

HOMA-IR and HOMA-β

During the 8-week intervention, when data were collected from the two groups, neither HOMA-IR nor HOMA-β indicated a significant difference between the groups (Table 2). The within-subject contrasts demonstrated no differences as to the time effect and the interaction between time and group on HOMA-β, respectively as follows: F (1, 21) = 0.435, p = .517, ηp^2 = 0.020 and F (1, 21) = 0.011, p = 0.917, ηp^2 = 0.001. HOMA-β

Table 1 Subject characteristics and anthropometry

Variables	C group (n = 11)		E group (n = 12)	
	Baseline	8 weeks	Baseline	8 weeks
Age (years)	45.82 ± 6.79		46.50 ± 3.98	
Height (cm)	158.64 ± 4.24		157.33 ± 4.33	
Weight (kg)	81.73 ± 9.18	81.27 ± 9.24	79.75 ± 5.29	78.67 ± 5.97
BMI (kg/m^2)	32.46 ± 3.28	32.27 ± 3.24	32.24 ± 2.26	31.81 ± 2.49
Fat percentage (%)	38.36 ± 2.73	38.45 ± 2.65	38.83 ± 2.75	37.92 ± 3.03

Data shown as mean ± SD. C: n = 11, E: n = 12. Statistical significance was p < .05

C control group, *E* experimental group

Table 2 Biochemical measurements before and after 8 weeks of exercise training

Variables	Group	Baseline (MD ± SD)	8 weeks (MD ± SD)	Sig. (within group) time effect time × group		Sig. (between group)
FBS (mg/dl)	C	143.55 ± 43.08	150.64 ± 44.01	0.041†	0.008†	0.594
	E	163.55 ± 86.27	105.42 ± 40.01			
Insulin (IU/ml)	C	9.14 ± 4.75	10.45 ± 5.34	0.742	0.206	0.833
	E	10.95 ± 5.81	9.2 ± 3.61			
HOMA-IR	C	2.97 ± 1.14	3.64 ± 1.85	0.228	0.007†	0.228
	E	4.04 ± 2.99	2.42 ± 1.49			
HOMA-β	C	1.66 ± 1.65	1.78 ± 1.66	0.517	0.917	0.701
	E	1.84 ± 1.39	2.02 ± 0.96			
FFA (mmol/l)	C	0.31 ± 0.07	0.39 ± 0.15	0.346	0.152	0.920
	E	0.33 ± 0.09	0.31 ± 0.13			
Adipsin (ng/ml)	C	33.88 ± 5.19	30.23 ± 4.73	0.086	0.168	0.652
	E	34.46 ± 3.46	34.11 ± 3.12			
HDL-C (mg/dl)	C	39.47 ± 2.17	38.27 ± 2.51	0.990	0.177	0.022†
	E	38.17 ± 4.89	39.83 ± 4.38			
LDL-C (mg/dl)	C	122 ± 29.43	120.18 ± 43.9	0.959	0.410	0.892
	E	115.92 ± 35.24	120.33 ± 40.06			
TC (mg/dl)	C	192 ± 31.39	193.64 ± 41.91	0.675	0.838	0.777
	E	189.45 ± 38.18	191 ± 50.45			
TG (mg/dl)	C	163.82 ± 32.52	162.73 ± 39.58	0.632	0.298	0.094
	E	167.17 ± 73.92	161.33 ± 45.87			
BMI (kg/m^2)	C	32.46 ± 3.28	32.27 ± 3.24	0.002†	0.158	0.772
	E	32.24 ± 2.26	31.81 ± 2.49			
Fat Percentage (%)	C	38.36 ± 2.73	38.45 ± 2.65	0.044†	0.016†	0.977
	E	38.83 ± 2.75	37.92 ± 3.03			

Values were means ± SD. C: $n = 11$, E: $n = 12$

C control group, *E* experimental group, *FBS* fasting blood sugar, *HOMA-IR* homeostasis model assessment-insulin resistance, *HOMA-β* homeostasis model assessment-insulin for β-cell function, *FFA* free fat acid, *HDL-C* high density lipoprotein, *LDL-C* low density lipoprotein, *TC* total cholesterol, *TG* triglycerides, *BMI* body mass index

† Significant difference within or between groups from before to after 8 weeks. Statistical significance was $p < 0.05$

analysis (Table 2) did not reveal a significant difference between group effect following the 8-week study: F (1, 21) = 0.152, $p = 0.701$, ηp2 = 0.007.

The HOMA-IR response for the two groups is shown in Table 2. Although there was no time effect observed on this variable: F (1, 21) = 1.545, $p = 0.228$, $\eta p^2 = 0.069$, a significant time × group effect was observed when data were analyzed for both groups during the 8-week study: F (1, 21) = 8.984, $p = 0.007$, $\eta p^2 = 0.300$. Furthermore, no differences occurred between the two groups: F (1, 21) = 0.010, $p = 0.920$, $\eta p^2 = 0.001$.

Plasma lipids status

The analysis of TG, TC, HDL-C, LDL-C, and FFA differences did not reveal significant group × time interactions or time effects from training (Table 2). In addition, no differences were observed between the groups for these variables except for HDL-C, F (1, 21) = 6.163, $p = 0.022$, $\eta p^2 = 0.227$.

Adipsin concentrations

Following the 8-week endurance training, unlike decreased level of adipsin in group C, group E did not alter its adipsin concentration after 8-week exercise training either.

Based on the results, the changes of time and interaction between time and group were not significant within-subjects test, respectively as follows: F (1, 21) = 3.253, $p = 0.086$, $\eta p^2 = 0.134$ and F (1, 21) = 2.035, $p = 0.168$, $\eta p^2 = 0.088$. Also, the difference between group effect did not alter significantly between the two groups of C and E following the 8-week study: F (1, 21) = 0.209, $p = 0.652$, $\eta p^2 = 0.010$.

Discussion

Longitudinal follow-up studies of patients with T2DM have demonstrated that there are continuous decline in β-cell function in these patients [13]. Obesity and adipose inflammation are also known as two parameters that tightly associated with β-cell malfunction [14]. These clinical observations have

raised a potent role for adipose cells which may send important signals to the pancreatic islets [15]. Recently, a few studies have identified that adipsin, one of the most expressed adipose proteins, may link fat cells and obesity to β-cell function [16] which usually decreases in T2DM patients with β-cell failure [7]. Thus, it is important to determine the agents that improve adipsin expression. Hence, the main target of this study was to investigate whether aerobic exercise training induced probable improvement on insulin resistance and whether β-cell function can be related to circulating adipsin levels in obese T2DM women. Therefore, we examined the effect of an 8-week aerobic training program on HOMA-IR, HOMA-β, and lipids profile and also adipsin levels in obese women with T2DM. We found that an 8-week aerobic training program with 60–80 % MHR improved fasting venous glucose concentration (−0.27 %) in T2DM patients. Despite significant decrease in HOMA-IR (−0.29 %) and FBS, HOMA-β did not alter markedly following 8 weeks of our exercise training program in group E. However, post measurements of HOMA-β have shown slight tendency towards increasing in group E (0.16 %). Additionally, no effects were demonstrated on lipid status in either group. And ultimately, there was no significant difference in adipsin levels from baseline to 8 weeks of aerobic exercise in group E (0.006 %).

Most importantly, it was seen that the changes in the levels of adipsin with BMI and body fat percentage were in contrast. Indeed, regarding previous investigations, it was expected that the level of adipsin would decrease with a reduction magnitude of BMI and body fat percentage. However, the levels of adipsin during the study (from pre to post aerobic exercise) did not alter markedly. Likewise, the correlations between adipsin with BMI ($r = -0.03$, sig = 0.915) and body fat percentage ($r = -0.25$, sig = 0.433) were not significant and partially weak. Nevertheless, this result was consistent with Montague et al. [17] who have shown the lack of significant correlation of adipsin messenger RNA (mRNA) with body mass index (BMI) and absences of difference between omental and subcutaneous tissue and also between males and females. In another study, there was also found no correlation between adipsin and body weight and % body fat [18], whereas, Pomeroy showed a significant correlation between adipsin and body weight [19]. Furthermore, Muscari et al. studied 1090 middle-aged men and reported that there was an inverse relationship between physical activity and adipsin [20].The disagreement between our finding and previous studies may be attributed to participants because our participants consisted of obese women with T2DM, while those of the previous studies involved adults and adolescents. Taken together, there are very few documents on adipsin in the T2DM population; more studies may be useful.

Regarding the results, the effect of time or group was not significant for TG, TC, LDL-C, and HDL-C changes. The interaction of these two factors was not significant for these variables either.

Nor were there statistical correlations between serum adipsin levels and lipid profile including TG ($r = -0.11$, sig = 0.730), TC ($r = -0.01$, sig = 0.916), LDL-C ($r = -0.09$, sig = 0.779) and HDL-C ($r = -0.02$, sig = 0.948) following the 8-week aerobic exercise program. Additionally, there was not found any correlation between adipsin and FFA as well ($r = -0.19$, sig = 0.540). Madsen et al. also reported no effects on lipid status after 8 weeks of low volume high intensity interval training on cycle ergometer in T2DM patients and matched healthy control individuals [8]. However, there are a few studies that have shown associations of adipsin with lipids in normal healthy controls, obese, diabetic, and hyperlipidemic participants [21]. The proposed mechanism behind this correlation implies that adipsin function stimulating glucose travels through membranes and increase the synthesis of triacylglycerols in adipocytes [22]. The dissenting results on the relation between lipid profile and adipsin could be due to our small study population and insufficient power which is one of the limitations of this study. It can also be attributed to our subjects being obese and sedentary population with T2DM. Hence, it seems more time was required to bring about changes.

Although the results of this study indicated that fasting glucose secretion significantly decreased, fasting insulin levels did not alter markedly during this period. The possible reason could be that following regular exercise, pancreas normally secretes less insulin to improve insulin sensitivity. In return, under diabetes condition where pancreas is impaired and β-cell is dysfunctional, exercise augments insulin secretion concomitantly with an improvement of insulin sensitivity [23]. It has been proven that exercise is vital to prevent risk of developing T2DM [21]. Both regular moderate-intensity exercise [24] and high intensity exercise [25] have been shown to improve insulin sensitivity. Performing regular exercise lowers basal insulin levels and attenuates the insulin response to glucose load [26, 27] suggesting that peripheral insulin sensitivity is increased. However, few researches disagree on this result and demonstrate that insulin sensitivity remains unaltered following regular moderate-intensity exercise [28] and high intensity exercise [29].

HOMA-IR and HOMA-β are pivotal indices for tissue insulin resistance and pancreatic function, respectively. Following the course of the study, only HOMA-IR was significantly improved in group E in comparison to the baseline. In line with our study, there are some other studies reporting a positive correlation between aerobic exercise and HOMA-IR. In a study, following 6 months of aerobic exercise HOMA-IR was improved in T2DM patients [26]. Ho Ha et al. also reported that a 12-week supervised combined exercise

positively affected HOMA-IR, HOMA-β, insulin levels, and body fat% in Korean obese women [30]. In a Nordic study [31], 4 months of unsupervised walking did not have any impact on HOMA-IR among T2DM patients. Thus, a longer training period may be necessary to determine whether aerobic exercise training is more advantageous for improvement HOMA-IR and HOMA-β levels in an experienced population.

Different mechanisms have been proposed to explain how HOMA-IR and HOMA-β improve following exercise training in T2DM population. Meanwhile, it seems FFA plays important physiological role among the skeletal muscle, adipose tissue, liver, and pancreas. Elevated FFA concentrations are linked with the onset of peripheral and hepatic insulin resistance. FFA appears to inhibit insulin signaling, leading to a decrease in GLUT-4 translocation resulting in hyperglycaemia [24]. The nature of this relationship remains a subject for debate. Therefore, FFA represents a crucial link between insulin resistance and beta-cell dysfunction. Hence, lowered plasma FFA should be an important therapeutic target in obesity and type 2 diabetes. With regard to decreased HOMA-IR following 8 weeks of aerobic training, we expected to experience lower FFA levels in significance; while no differences were observed between the groups. It is possible that 8 weeks was not sufficient to reveal differences in the level of FFA.

Ultimately, this study hypothesized whether aerobic-related exercise improved insulin resistance and beta-cell function in line with adipsin levels in obese T2DM women. In accordance with the hypotheses, the present findings demonstrated that the correlation between percentage changes in HOMA-β with percentage changes of HOMA-IR was $r = 0.845$, $p < 0.01$. By contrast, the correlation between percentage changes in adipsin with percentage changes of HOMA-β was $r = 0.046$, $p = 0.88$ and with percentage changes of HOMA-IR was $r = -0.041$, $p = 0.89$. According to these results, it was shown that following 8 weeks of aerobic exercise, despite a strong correlation between HOMA-β and HOMA-IR related to 8 weeks of aerobic exercise, the correlations between adipsin with HOMA-β and HOMA-IR were not considerable. However, the changes in adipsin level were in line with HOMA-β and in contrast with HOMA-IR. Since this hypothesis was a new one and no research was done in this area to prove this hypothesis, a longer training period may be necessary to determine whether aerobic-related exercise improved insulin resistance and beta-cell function being in line with adipsin levels in obese T2DM women

Conclusion

The study indicated that 8 weeks of aerobic exercise training in T2DM patients had positive health effects especially on glycemic control and pancreatic β-cell function. This type of exercise resulted in improvement of fasting insulin action and fat mass. Since etiology of many of these relationships is not yet understood, more studies are needed to understand the signaling mechanisms of insulin in healthy and T2DM population.

Acknowledgments We thank Dr. Vahid Tadibi, supervisor of this study and Dr. Nasser Behpour from Razi University, Kermanshah, Iran. Moreover, we are grateful to all friends and patients who made this study possible because of their kind help.

Compliance with ethical standards

Funding/support This study was funded and supported by the faculty of physical education and sport sciences, Razi University, Kermanshah, Iran.

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):305–312
DOI 10.1007/s13410-016-0523-4

ORIGINAL ARTICLE

Differences in prevalence of diabetes mellitus type 2 and impaired fasting glucose between urban and rural areas according to PURE Poland substudy

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Received: 2 June 2016 / Accepted: 7 September 2016 / Published online: 23 September 2016

Abstract This paper presents baseline results of the Prospective Urban Rural Epidemiology (PURE) Poland substudy of differences in prevalence of diabetes and impaired fasting glucose (IFG) between urban and rural areas. It covers group of 1643 people, aged 35 to 70 years old, inhabitants of Lower Silesia. The PURE study reached 22 countries with different statuses of economic development. All participants were tested in accordance to PURE project protocol. Data was collected at family (household) level and individual level. Diabetes was found in 12.0 % of our study population, IFG in 28.1 %, and normal glucose level in 59.9 %. Diabetes and IFG were more common in rural than in urban population, and higher percentage of people with diabetes was found in men comparing to women. In the PWBEC study conducted in years 1998–2000, diabetes prevalence was 5.3 %, in the NATPOL study conducted in years 1997–2002, 5.6 %; and in the latest PONS study from Świętokrzyskie voivodeship, 8.4 %. The percentage of people with diabetes in PURE Poland substudy was as high as 12 % and was higher than projections of diabetes prevalence in Poland for year 2030 (11.6 %). Significant differences of diabetes and IFG prevalence in respect to education and place of residence may arise from different attitudes of groups of patients to many other factors connected with prevention, treatment, and rehabilitation. Our study will continue to disclose other relevant risk factors in chosen areas.

Keywords PURE study · Population study · Diabetes · IFG

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Introduction

According to the estimations of the International Diabetes Federation (IDF) in 2011, diabetes was a disease of 366 million people, what accounted for 8.3 % of world's adult population. This figure is expected to grow to 552 million people by 2030, what would be 9.9 % of world's adult population [8]. In 2010, in Poland, people with diabetes accounted for 9.6 % of population aged 20–79, while in 2030, they are expected to account for 11.6 % [3]. On top of that, around 4 million people of total Polish population are diagnosed with impaired fasting glucose (IFG). This significantly increases risk of development of diabetes de novo [15].

Factors predisposing to diabetes mellitus type 2 (DM type 2) are closely linked with lifestyle, particularly increased body mass

and low physical activity [2–4, 10]. Increased body mass is found in 80 % of patients with DM type 2 [10] and that is why the description "diabesity" used by Shafrir is becoming popularly functioning term [11]. Increase in child's obesity prevalence has been recognized as one of the main reasons of DM type 2 prevalence, which, until not long ago, had been considered to be an adults' disease [5–7]. Cardiovascular, nervous systems, eye, kidney, and many other organ damages or malfunctions are common among people with chronic hyperlipidemia [16–25]. Diabetes is a risk factor of increased morbidity and mortality; complications leading to shorten life expectancy are dominant image of people with DM type 2. In Poland, diabetes is the main cause of renal failure, blindness due to diabetic retinopathy, and non-traumatic limb amputation [26–28]. Eighty percent of deaths related to diabetes take place in low- and medium-income countries; cardiovascular diseases are accounted for majority of these mortalities (50–80 %) [9, 12, 13].

Material and methods

Prospective urban rural epidemiology (PURE) study covers 22 countries with different statuses of economic development. Poland, at the beginning of research, was one of the seven upper-middle-income countries enrolled to the study [31].

The paper presents results of the PURE Poland substudy—baseline, covering group of 1643 people aged 35 to 70 years (1044 women and 599 men), inhabitants of both urban and rural areas from Lower Silesia. All patients were tested in accordance to the PURE project protocol.

Collection of data concerning individuals was obtained at two levels [31].

1. Family/household level: demographic information, i.e., number of family/household, number of children, education, etc.; epidemiological information, i.e., morbidities, usage of tobacco products, etc.; and other important determinants, i.e., access to water, sanitation, household amenities, etc. All this information was collected with use of the Family Census Questionnaire.
2. Adult participant level, collected based on the Adult Questionnaire, included data on nine INTERHEART risk factors (lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors [stress and symptoms of depression], consumption of fruits, and vegetables, consumption of alcohol, and regular physical activity), anthropometric measures, blood pressure, spirometry, fasting blood sample, semiquantitative Food Frequency Questionnaires (FFQs), and physical activity (IPAQ).

Our paper focused on data concerning hyperglycemia. Participants have had taken their fasting blood samples and basic anthropometric measures, such as height, weight, and waist and hip circumference.

The group of people with impaired fasting glucose (IFG) was persons with fasting glucose levels between 100 and 125 mg%, the group of people with diabetes was persons with fasting glucose levels equal or greater than 126 mg% or with confirmed diabetes in the interview prior to the tests. To present population aggregated information about prevalence of IFG and diabetes, basic statistical parameters were used. Prevalence of IFG and diabetes was verified in respect to epidemiological variables known to be its significant determinates and health-related states correlated to IFG and/or diabetes. Odds ratio was calculated to present the burden of a disease- or health-related state; to assess statistical significance of the differences observed in distribution of IFG and diabetes, chi-square test was used, with significant differences at $p \leq 0.05$.

Statistical analyses were conducted with the use of Statistica 10 PL.

Results

Average fasting glucose level in studied population was 99.90 mg%. Average fasting glucose levels were higher in men than women: 101.84 vs. 98.77 mg% ($p \leq 0.05$) and were statistically different for different age groups ($p \leq 0.05$). Glucose levels were higher in older age groups (102.65 mg% in age group above 64 years, 100.87 mg% in age group between 45 and 64 years, and 93.77 mg% in age group below 45 years). Higher fasting glucose levels were found in rural population as in comparison to urban population (105.43 vs. 95.53 mg%) ($p \leq 0.05$).

It was also found that the higher the education level of the population, the lower the fasting glucose level ($p \leq 0.05$). Average results for different education levels were as follows: 105.67 mg%—primary education, 103.67 mg%—vocational education, 99.47 mg%—secondary education, 94.77 mg%—higher education, and 137.83 mg%—none or unknown. Detailed characteristics of fasting glucose levels for our population are presented in Table 1.

Population with diabetes had average fasting glucose level of 140.20 mg%. Higher fasting glucose levels were found in rural than urban populations (144.54 vs. 133.00 mg%) ($p \leq 0.05$). Fasting glucose levels of people with diabetes were significantly different in respect to education ($p \leq 0.05$) and were greater for vocational education (147.57 mg%) than secondary (140.00 mg%) and higher education vocational (132.29 mg%). No statistical differences in fasting glucose levels for diabetes were found between men and women (143.41 vs. 137.82 mg%) and also between populations of different age groups (129.16 mg% in age group below 45 years, 143.49 mg% in age group between 45 and 64 years, and 134.11 mg% in age group above 64 years).

Table 1 Population fasting glucose levels

Population		Overall			Diabetes			IFG			Normoglycemia		
		mg% (mmol/l)			People with fasting glucose levels ≥126 mg% (≥7.00 mmol/l) or with recognized diabetes prior to the study			People with fasting glucose levels 100–125 mg% (5.56–6.99 mmol/l)			People with fasting glucose below <100 mg% (<5.56 mmol/l)		
		N	Average (SD) mg%	Average mmol/l	*N*	Average (SD) mg%	Average mmol/l	*N*	Average (SD) mg%	Average mmol/l	*N*	Average (SD) mg%	Average mmol/l
Men		599	101.84 26.37	5.66	78	143.41 50.13	7.97	175	108.05 6.55	6.00	346	89.33 7.47	4.96
Women		1044	98.77 20.39	5.49	102	137.82 37.14	7.66	292	107.63 6.74	5.98	650	88.66 7.48	4.93
Age group	<45	291	93.77 13.43	5.21	12	129.16 22.44	7.18	65	106.86 6.70	5.94	214	87.80 6.90	4.88
	45–64	1094	100.87 23.35	5.60	124	143.49 42.06	7.97	329	107.64 6.39	5.98	641	89.14 7.55	4.95
	>64	258	102.65 27.27	5.70	44	134.11 49.77	7.45	73	109.27 7.65	6.07	141	89.40 7.92	4.97
Urban		920	95.53 17.12	5.31	67	133.00 28.59	7.39	212	107.81 6.76	5.99	641	87.56 7.80	4.86
Rural		723	105.43 27.46	5.86	113	144.54 49.51	8.03	255	107.77 6.61	5.99	355	91.30 6.19	5.07
Ed. level	Primary	249	105.67 26.65	5.87	51	135.27 44.24	7.52	80	108.05 6.49	6.00	118	91.26 6.72	5.07
	Vocational	273	103.67 26.59	5.76	37	147.57 48.34	8.20	89	107.53 6.35	5.97	147	90.29 6.25	5.02
	Secondary	639	99.47 20.99	5.53	64	140.00 36.38	7.78	186	108.16 6.93	6.01	389	88.65 7.88	4.93
	Higher	476	94.77 15.49	5.27	27	132.29 26.72	7.35	110	107.17 6.66	5.95	339	87.76 7.50	4.88
	None or unknown	6	137.83 106.25	7.66	1	353.00 -	19.61	2	108.50 7.78	6.03	3	85.67 10.02	4.76
Total		1643	99.90 22.80	5.55	180	140.20 43.20	7.79	467	107.80 6.70	5.99	996	88.90 7.50	4.94

Diabetes de novo was diagnosed in group of 51 people with average fasting glucose level of 139.70 mg%, and no statistical differences in diabetes de novo were found between men and women (138.00 mg% SD 11.1 vs. 141.7 mg% SD 13.90), urban and rural population (141.3 mg% SD 14.4 vs. 138.50 mg% SD 10.8), age group (135.70 mg% SD 9.0 in age group below 45 years, 139.10 mg% SD 11.9 in age group between 45 and 64 years, 147.70 mg% SD 16.9 in age group above 64 years), and in respect to education level (140.90 mg% SD 13.4—primary, 135.30 mg% SD 7.1—vocational education, 140.00 mg% SD 12.0—secondary education, 142.70 mg% SD 15.3—higher education). None of the examined factors had significant impact on diabetes de novo distribution in our study population.

In the population of people with IFG, no statistical differences were found between men and women (108.05 vs. 107.63 mg%) and urban and rural (107.81 vs. 107.77 mg%). No statistical differences in fasting glucose levels were also found between populations of different age groups or populations with different education levels. In the population with normal fasting glucose level, differences were only found between rural and urban 91.30 vs. 87.56 mg% ($p \leq 0.05$).

Diabetes was found in 12.0 % of our study population, IFG in 28.1 %, and normal glucose level in 59.9 %. Higher percentage of people with diabetes was found in men compared to women (13.9 % and 11.0 %), while normal glucose level was found in 57.2 % men vs. 61.4 % women ($p \leq 0.05$). No statistical differences were found for IFG distribution in respect to gender. Diabetes and IFG were more common in rural than urban population ($p < 0.05$). Diabetes was found more often among people living in the rural areas 16.7 % as compared to 8.4 % living in urban areas (individual odds ratio [OR] = 1.25). IFG characterizes 34.4 % of people living in rural areas vs. 22.8 % in urban areas (Fig. 1).

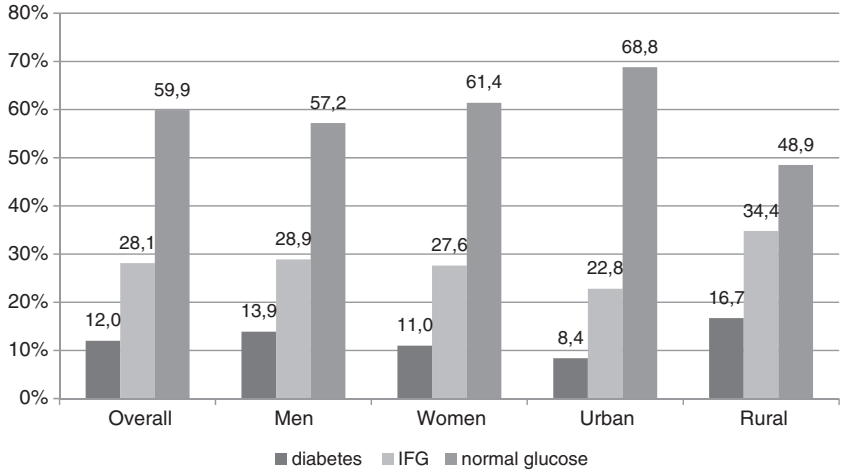

Fig. 1 Prevalence of diabetes and IFG in total study population and in respect to gender and place of residence (urban-rural)

Both for people living in urban and rural areas, diabetes was more often found in population of men than women; however, it was not statistically different. Diabetes was found in 19.4 % of men and 13.5 % of women living in rural area and normal glucose in 45.7 % of men and 51.0 % of women. Similarly, in urban areas, diabetes was a disease of 8.2 % of men and 6.8 % of women and normal glucose was present in 66.9 vs. 71.3 %, respectively (Table 2).

Diabetes de novo was diagnosed more frequently in population of men than women (27 vs. 24, $p < 0.05$) and in rural than urban (28 vs. 23, $p < 0.05$).

The prevalence of diabetes and IFG in the respective age group is presented in Fig. 2. The prevalence of diabetes increases with age of the study participants.

The prevalence of diabetes differs with education level ($p < 0.0001$). The higher the education level, the less diabetes and more normal fasting glucose levels were found in the population (Fig. 3). Population with higher education was characterized with the least share of people with diabetes (6.6 %) and highest share of people with normal fasting glucose level (70.5 %). No statistical differences were found between glucose levels and education in group with diabetes recognized de novo (11 in group with primary education, 9 in group with vocational education, 19 in group with secondary education, and 12 in group with higher education).

Correlation between prevalence of diabetes and obesity was also investigated. The mean BMI value for the study population was equaled 28.3 kg/m^2 and was significantly higher in group with IFG (29.2 kg/m^2) and diabetes (31.6 kg/m^2) (Table 3. The risk of diabetes was significantly higher for people with abdominal obesity (individual odds ratio [OR] = 1.07). The share of people with abdominal obesity, based on waist circumference and waist-hip ratio (WHR), was much higher in group with diabetes and IFG than people with normal fasting glucose level. Based on the IDF criteria, abdominal obesity is found in 76.0 % of people with IFG and 88.5 % with diabetes; based on the NCEP ATP criteria, abdominal obesity characterized 55.7 % of people with IFG and 70.5 % with diabetes. In our study, abdominal obesity (WHR 1.0 for men and 0.8 for women) concerned 61.5 % people with IFG and 71.5 % people with diabetes. Table 4 presents different methodological approaches in defining obesity and its prevalence in our study in respect to these criteria.

Table 2 Prevalence of IFG and diabetes by place of residence

Level of glucose	Men		Women		Total		*p* value
	No.	%	No.	%	No.	%	
Urban							
Normoglicemia (<100 mg%/5.56 mmol/l)	228	66.9	413	71.3	641	69.7	>0.05
IFG (100–125 mg%/ 5.56–6.99 mmol/l)	85	24.9	127	21.9	212	23.0	
Diabetes (≥126 mg%)/≥6.99 mmol/l)	28	8.2	39	6.8	67	7.3	
Rural							
Normoglicemia (<100 mg%/5.56 mmol/l)	118	45.7	237	51.0	355	49.1	>0.05
IFG (100–125 mg%/5.56–6.99 mmol/l)	90	34.9	165	35.5	255	35.3	
Diabetes (≥126 mg%)/≥6.99 mmol/l)	50	19.4	63	13.5	113	15.6	

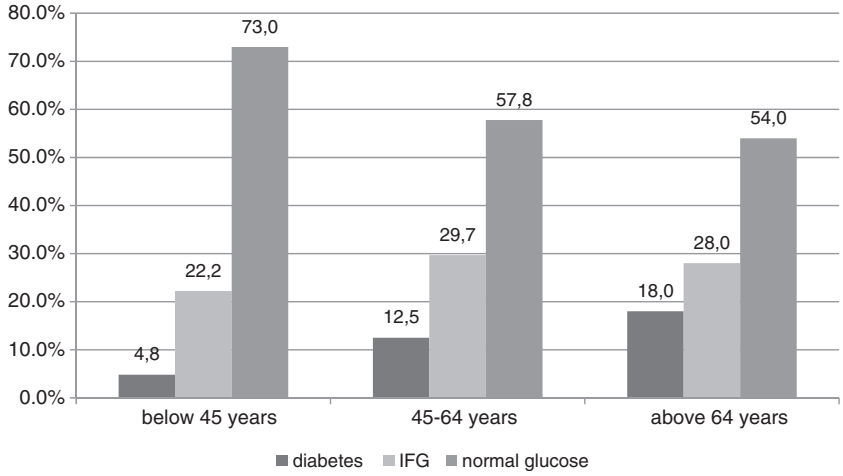

Fig. 2 Prevalence of diabetes in respect to age groups

Discussion

The PURE Poland substudy results document a significant increase of diabetes prevalence in Poland. In the PWBEC study conducted in years 1998–2000, the percentage of people with diabetes was 5.3 % [32]; in the NATPOL study conducted in years 1997–2002 (5.6 %) [33] and, in the latest study, PONS from Świętokrzyskie voivodeship, it was 8.4 % [34].

The percentage of people with diabetes in the PURE Poland substudy was as high as 12 % and was higher than projections of diabetes prevalence in Poland for the year 2030 (11.6 %) [5]. The high percentage of people with IFG is also very alerting, which rises risk of developing diabetes de novo.

Approximately 40 % of people with IFG will develop diabetes over the next 5–10 years [1, 6, 7]. The percentage of people with IFG in the PURE Poland substudy population was 28.1 %, and it was comparable with PONS study (29.0 %) [35], yet, still, it was higher than for general Polish population (10 %) [35]. The percentage of people with diabetes de novo in the PURE Poland substudy was 25.5 % of all diabetes and was lower than in PONS study (29.5 %) [35], which was very different than from Warmia and Mazury voivodeship study (1.7 %) [36].

Risk factors seem to vary in respect to place of residence. In our study, rural-urban differences were emphasized, yet there must be some underlying risk factors, sort of meta risk factors. Poland is a very homogenous society and should be expected to have general risk factors effectively disclosing the health differences. Rural-urban gradient of diabetes prevalence should be more or less constant throughout the country population, yet studies show differently. PURE rural prevalence of diabetes was over twice the size of rural prevalence of diabetes in PONS study (16.7 vs. 7.6 %), while urban diabetes prevalence in both studies was comparable (PURE—8.4 vs. PONS 8.7 %) [35]. Such findings stress necessity to look deeper and search for "more prime" risk factors behind disease prevalence differences.

Successful prevention and treatment of diabetes can reduce the burden of high cardiovascular morbidities and mortalities [14]. Treatment of diabetes and its complications bear very high proportion of healthcare expenditure. According to

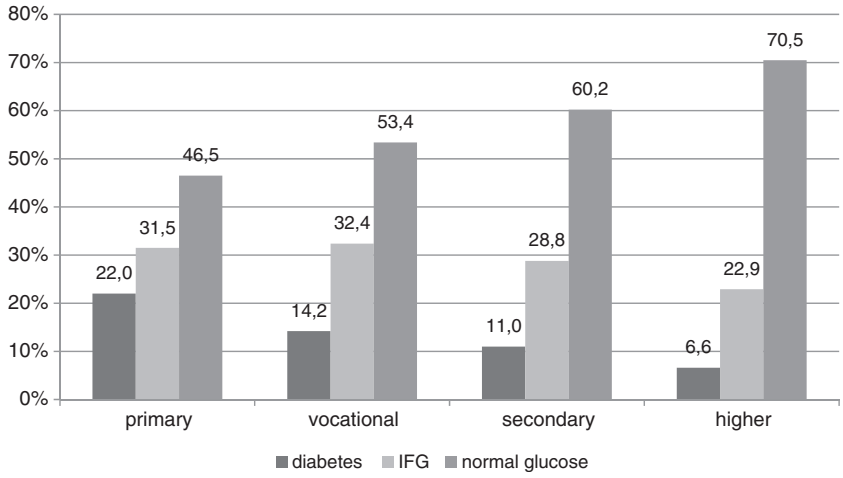

Fig. 3 Prevalence of diabetes and IFG according to education level. Education of six people was classified as "none or unknown." Information about this group of participants can be found in Table 1

Table 3 Correlation between prevalence of diabetes and BMI

Glucose level	No.	BMI (kg/m2)		*p* value
		Mean	SD	
Normal glucose (<100 mg%/<5.56 mmol/l)	996	27.2	4.7	<0.001
IFG (100–125 mg%/5.56–6.99 mmol/l)	467	29.2	5.1	
Diabetes (≥126 mg%/≥7.00 mmol/l)	180	31.6	5.9	
Overall	1643	28.3	5.2	

World Bank data, diabetes is on second position of world's social economic burden after myocardial infarction [29].

People with diabetes compared to others are much more likely to use ambulatory care; to be hospitalized; and to need an emergency care, long-term care, and complex pharmacotherapy. Costs of treatment of diabetes and its complications accounted for 12 % of total world's health care costs in 2010. In most populated countries, this expenditure accounted for 5 to 13 % and in Poland for 11 %. On average, world's per capita diabetes treatment cost is 1330 USD (it varies from 1.40 USD in North Korea to 7382.66 USD in the USA) and 593.56 USD in Poland [30].

Table 4 Correlation between prevalence of diabetes and abdominal obesity

Abdominal obesity	Normoglycemia (%) <100 mg% (<5.56 mmol/l)	IFG (%) 100–125 mg% (5.56–6.99 mmol/l)	Diabetes (%) ≥126 mg% (≥7.00 mmol/l) or with recognized diabetes prior to the study	*p* value
Waist circumference >102 (males); >88 (females)	33.0	55.7	70.5	<0.001
Waist circumference >94 (males); >80 (females)	59.5	76.0	88.5	<0.001
Waist-hip ratio ≥0.94 (males); ≥0.8 (females)	60.0	72.8	86.0	<0.001
Waist-hip ratio ≥1.0 (males); ≥0.8 (females)	49.7	61.5	71.5	<0.001

Our study results entitle to use the term "diabesity" for description of the study population. Average BMI values in our groups with diabetes and IFG were significantly higher than in group with correct fasting glucose levels: 27.2 kg/m^2 in normoglycemic group, 29.2 kg/m^2 in group with IFG, and 31.6 kg/m^2 in group with diabetes. The risk of developing diabetes for people with incorrect body mass index was 7.3 % higher than for people with correct body mass index. Also, the percentage of people with abdominal obesity, based on waist circumference and WHR, was significantly higher in group of people with diabetes or IFG than in group with correct fasting glucose levels. Comparable results were found in PONS study, in which also higher risk of developing diabetes was associated with incorrect body mass index comparing to correct body mass index. In PURE substudy, higher risk of developing diabetes or IFG was associated with abdominal obesity.

The paradigm of diabetes treatment undergoes changes. Diabetes described as an epidemic concerns growing number of people. This brings changes in many areas, i.e., advances in knowledge and process of treatment of the disease [38].

We hear physicians say that the risk of developing diabetes is associated to three factors: "knowledge, knowledge, and knowledge"; in this expression, we can see the change in approach to the disease and its treatment. Diabetes is a chronic disease with the etiology lying in social environment and personal health attitudes. This "knowledge…" can be associated to prevention—levels of prevention [37]:

- Primary prevention—health education aiming at reducing development of the disease among people who are not ill but exposed to the risk factors.
- Secondary prevention—screening people at the high-risk group before manifestation of the disease symptoms.
- Tertiary prevention—focus on disability prevention among people in later stage of the disease.

Changes in approach to treatment of diabetes are shown in the joint position of the American Diabetes Association (ADA) and European Association for the study of Diabetes, stating seven elements that should be under consideration for setting up treatment strategy and evaluating aims for the treatment [38]:

- Patient's motivation and personal involvement in the treatment process
- Risks associated with presence of hyperglycemia
- Duration of the disease
- Life expectancy
- Coexisting diseases
- Severity of vascular complications
- Support of social environment and patient's financial resources

All these elements ought to be individualized, and the physician conducting the treatment should set achievable goals for the treated individuals. Young and motivated patients will be capable of following more restrictive routine of treatment than some patients in old age and advanced stage of a disease [38].

Conclusion

Polish guidelines on the management of diabetic patients include wide scope of actions in all perspectives—prevention, treatment, and rehabilitation. Such significance differences in the diabetes distribution suggest that these guidelines are not bringing desired effect equally to all groups. Significant differences of diabetes and IFG prevalence in respect to education and place of residency may arise from different attitudes of groups of patients to many other factors connected with prevention, treatment, and rehabilitation. Our study will continue to disclose other relevant risk factors in chosen areas.

Acknowledgments Polish PURE sub-study is co-funded by Polish Ministry and Science and Higher Education (grant no.290/W-PURE/2008/0) and Wrocław Medical University. The main PURE study and its components are funded by Population Health Research Institute, the Canadian Institutes of Health Research, Heart and Stroke Foundation of Ontario and through unrestricted grants from several pharmaceutical companies [39].

Compliance with ethical standards Informed consent was obtained from all individual participants included in the study. All human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki.

Author contributions Katarzyna Zatońska wrote the manuscript, researched data, contributed to discussion, and reviewed manuscript.

Katarzyna Połtyn-Zaradna wrote the manuscript, contributed to discussion, and reviewed the manuscript.

Jakub Einhorn wrote the manuscript, contributed to the discussion, edited, and reviewed the manuscript.

Maria Wołyniec collected and researched data.

Dagmara Gaweł-Dąbrowska wrote the manuscript and contributed to discussion.

Andrzej Szuba researched the data and reviewed the manuscript.

Witold Zatoński is the article guarantor.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):313–319
DOI 10.1007/s13410-016-0538-x

ORIGINAL ARTICLE

Relationship between serum pro- and anti-inflammatory cytokine and growth factor concentrations with the age and gender adjusted prevalence of diabetes and pre-diabetes

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Received: 21 June 2016 / Accepted: 17 November 2016 / Published online: 28 November 2016

Abstract The main goal of the present study is to compare the serum pro- and anti-inflammatory cytokines and growth factor profile (IL-1α, IL-1β, IL-2, IL-6, IL-8, IFN-γ, MCP-1, TNF-α, IL-10, IL-4, VEGF, and EGF) in individuals with diabetes or pre-diabetes and control subjects matched for age and gender. This study included 270 normal control subjects, 41 pre-diabetics, and 104 diabetic subjects. Demographic and anthropometric characteristics and serum biochemistry parameters, smoking status, and blood pressure were determined. Serum cytokine and growth factor measurements were performed using a multiplex biochip array based method. After removing the effects of confounders such as age, sex, smoking, BMI, serum triglyceride and cholesterol, hypertension, and treatment with anti-diabetic drugs, serum IL-4 ($p = 0.015$), IL-1α ($p = 0.031$), and VEGF ($p = 0.012$) were significantly associated with the presence of diabetes mellitus. The presence of pre-diabetes was positively associated with serum IL-4 ($p < 0.001$), and IL-1α ($p = 0.026$). We have found a positive association between serum IL-1α, and IL-4, and a negative association of serum VEGF with the presence of diabetes, or pre-diabetes.

Keywords Diabetes · Pre-diabetes · Inflammatory cytokine · Anti-inflammatory cytokine

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Introduction

Diabetes is one of the most common chronic diseases worldwide, affecting more than 300 million people globally [1]. According to the World Health Organization (WHO) the prevalence of type 2 diabetes mellitus in 1995, 2000, and 2025 was estimated to be 5.5, 5.7, and 6.8%, respectively [2].

Diabetic complications may include retinopathy, nephropathy, neuropathy, cardiovascular disease, and peripheral vascular disease [3]. Several factors are involved in the progression of diabetes and its complications including genetics, age, diet, lifestyle, and obesity [4]. Diabetes is also associated with an inflammatory response and is often accompanied by increased levels of inflammatory cytokines [5]. Moreover, inflammatory processes are thought to be involved in the development of type 2 diabetes mellitus. While, circulating anti-inflammatory cytokines are shown to reduce the risk of diabetes mellitus [6].

The main goal of the present study was to determine the association between serum pro- and anti-inflammatory

cytokine and growth factor levels and the presence of pre-diabetes and diabetes in a population sample from Mashhad in northeastern Iran.

Materials and method

Study design

A total of 415 subjects between 30 and 70 years of age, with no known history of major systemic inflammation or infectious diseases were recruited using a population-based cluster sampling from Mashhad where in the northeast of Iran and based on IDF (International Diabetes Federation) divided into two groups: In the first group, 311 control subjects were people who accompanying the patients and hospital staffs who were interested to get involved in our study and divided into two subgroups of non-diabetic composed of 270 participates with fasting blood glucose below 100 mg/dL and the pre-diabetic subgroup composed of 41 subjects with 100 < fasting blood glucose <126 mg/dL. Our second group was comprised of 104 diabetic cases with fasting blood glucose ≥126 mg/dL who were referred to the clinic of Ghaem Hospital, Mashhad for nutrition advice. In the questionnaire, the information related to type of medications which were used in diabetic patients was recorded. Our control (non-diabetic and pre-diabetics) subjects were not taking any medications. Inclusion criteria for the type 2 diabetics were subjects who had fasting blood glucose ≥126 mg/dL. Exclusion criteria were a history of systemic diseases (such as systemic lupus erythematosus and established cardiovascular disease, heart failure, and renal failure) and people using dietary supplements. Participants fulfilled the inclusion criteria were entered into the trial. The study protocol was approved by the Ethics Committee of the Mashhad University of Medical Sciences, and all participants provided a written informed consent. Serum samples were used to measure the cytokines level in the study groups.

Blood collection and routine biochemistry

Blood samples (10 mL) were obtained in the early morning after an overnight fast. Blood samples were collected into plain Vacutainer™ tubes for lipid profile measurements, and into Vacutainer™ tubes containing fluoride-oxalate for measurement of fasting blood glucose. Samples were centrifuged for separated plasma and serum and kept at −80° C. Low density lipoprotein cholesterol, high density lipoprotein, total cholesterol, cholesterol, and glucose were measured using routine techniques using a Cobas auto analyzer system (ABX Diagnostics, Montpellier, France).

Measurement of cytokines

Cytokines measurements were performed using the Biochip Array Technology on the Randox Evidence Investigator (Randox Laboratories, Belfast, Northern Ireland). The Evidence Investigator Biochip Array Technology was used to perform simultaneous quantitative detection of multiple analytes from a single patient sample. The applied cytokine array biochip employs a sandwich chemiluminescent immunoassay for a high throughput measurement of circulating cytokines. The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a stored calibration curve. The concentration of cytokines present in the sample is calculated from the calibration curve. The Evidence Investigator Cytokine Array can simultaneously determine the concentrations of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), interferon γ (IFN-γ), epidermal growth factor (EGF), MCP-1 and TNF-α.

Statistical analysis

The Statistical Package for Social Sciences (SPSS version 16) was used for data analysis. The Kolmogorov-Smirnov test was used to assess normality of descriptive statistics (mean and standard deviation for normally and median and interquartile range for non-normally distributed variables) were determined for all variables. Baseline demographics and clinical characteristics were compared among groups using one-way ANOVA and Tukey's post-hoc test for normally distributed variables. For non-normally distributed variables, Kruskal-Wallis/post-hoc Mann-Whitney test were used. chi-squared test was used for categorical variables. Correlation between cytokines was assessed using Spearman correlation analysis. To determine the association of cytokines and diabetes, we first used univariate multinomial logistic regression. The variables with $p < 0.05$ were further analyzed using multivariate multinomial logistic regression in which covariates that were different between groups and cofounders for each cytokine were analyzed. In multivariate analysis the correlation between variables were checked which was not more than 0.3.

Results

The groups were matched for age and sex. In the diabetic and pre-diabetic groups, values for BMI were significantly higher compared with non-diabetic control group. Data presented in Table 1 shows that FBS ($p < 0.001$), TC (total cholesterol) ($p = 0.028$), TG (triglyceride) ($p = 0.004$), and hs-CRP ($p < 0.001$) were significantly higher in diabetic group compared with non-diabetic group.

Table 1 Characteristics data from all subjects in each group

		Non-diabetic (n= 270)	Pre-diabetic (n= 41)	Diabetic (n= 104)	P1	P2	P3
Age (year) (Mean ± SD)		49.09±12.54	52.00±9.87	48.60±10.81		0.279	
Sex (No. (%))	Male	117(43.3)	18(43.9)	38(36.5)		0.468	
	Female	153(56.7)	23(56.1)	66(63.5)			
BMI (kg/m2) (Mean ± SD)		28.88±5.54	30.83±5.23	30.82±4.58	0.041	<0.001	1
WC (cm) (Mean ± SD)		97.18±13.20	104.69±11.10	100.66±10.30	0.001	0.024	0.139
HC (cm) (Mean ± SD)		105.42±10.97	110.34±10.36	109.79±8.78	0.037	0.001	0.987
FBS (mg/dl) (Mean ± SD)		83.53±9.45	108.98±7.73	157.86±67.17	<0.001	<0.001	<0.001
TC (mg/dl) (Mean ± SD)		193.65±38.77	190.34±33.71	204.60±42.02	0.912	0.028	0.117
TG (mg/dl) (Median(IQR))		131.0(92.0-183.0)	155.0(100.5-221.5)	153.0(111.0-201.2)	0.073	0.004	0.940
hsCRP (mg/dl) (Median(IQR))		2.46(1.30-5.77)	5.03(1.95-6.95)	4.79(2.23-7.97)	0.010	<0.001	0.742
HDL-C (mg/dl) (Mean ± SD)		42.54±8.94	40.28±8.55	42.52±7.99		0.205	
LDL-C (mg/dl) (Mean ± SD)		119.64±31.33	119.32±26.03	128.75±41.73		0.070	
SBP (mmHg) (Mean ± SD)		123.38±23.77	127.40±27.33	116.15±33.43	0.714	0.031	0.057
DBP (mmHg) (Mean ± SD)		82.63±14.42	83.00±16.81	76.85±22.46	0.988	0.051	0.211
Smoking (No. (%))	Yes	78(32.8)	3(7.7)	26(33.8)	0.001	0.872	0.002
	No	160(67.2)	36(92.3)	51(66.2)			

Values are presented as mean ± SD, median, and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal–Wallis test. Also the post hoc test Tukey and Mann–Whitney *U* test were used for comparison between groups

BMI body mass index, *WC* waist circumference, *HC* hip Circumference, *FBS* fasting blood sugar, *TC* total cholesterol, *TG* triglyceride, *hs-CRP* high-sensitivity C-reactive protein, *IQR* interquartile range, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *P1* comparison between groups of non-diabetic and pre-diabetic, *P2* comparison between groups of non-diabetic and diabetic, *P3* comparison between groups of pre-diabetic and diabetic

Cytokines and growth factor levels in serum

The concentrations of serum IL-1α ($p = 0.002$), IL-2 ($p = 0.006$), IL-4 ($p < 0.001$), IL-10 ($p = 0.013$), VEGF ($p < 0.001$), and IFN-γ ($p = 0.001$) were significantly different between the non-diabetic and pre-diabetic groups. The diabetic group had a significantly higher serum levels of IL-2 ($p = 0.002$), IL-4 ($p = 0.016$), IFN-γ ($p = 0.022$) compared to the non-diabetic control group, while a lower level of VEGF ($p = 0.008$) and EGF ($p = 0.008$) were found. Moreover, the serum cytokine levels of diabetic group were different from pre-diabetic, as the level of IL-1α ($p = 0.017$), IL-4 ($p < 0.001$), IL-10 ($p = 0.015$) were higher, and VEGF ($p = 0.027$) was lower at pre-diabetes (Table 2).

Table 2 Comparison of serum cytokines and growth factors levels in every group

Serum Cytokines and Growth factor (pg/mL)	Non-diabetic (n= 270)	Pre-diabetic (n= 41)	Diabetic (n=104)	P(n/p)	P(n/d)	P(p/d)
Interleukin 2	2.70(2.50-3.19)	3.11(2.74-3.67)	2.99(2.60-3.61)	0.006	0.002	0.566
Interleukin 4	1.89(1.63-2.45)	2.75(2.26-3.07)	2.06(1.70-2.66)	<0.001	0.016	<0.001
Interleukin 6	1.06(0.72-1.40)	1.19(0.73-1.85)	0.88(0.62-1.62)	0.282	0.565	0.165
Interleukin 8	6.12(3.75-16.52)	6.17(3.35-11.12)	5.64(3.30-9.70)	0.582	0.106	0.693
Interleukin 10	0.81(0.70-1.10)	0.97(0.76-1.66)	0.83(0.66-1.04)	0.013	0.453	0.015
VEGF	97.52(41.58-120.21)	32.56(13.12-80.19)	59.36(21.29-134.15)	<0.001	0.008	0.027
Interferon γ	0.57(0.41-0.79)	0.82(0.57-1.58)	0.59(0.46-0.84)	0.001	0.022	0.158
TNF-α	1.79(1.30-2.19)	1.50(1.17-2.22)	1.77(1.20-2.22)	0.396	0.671	0.567
Interleukin 1α	0.56(0.50-0.63)	0.66(0.52-0.79)	0.56(0.50-0.67)	0.002	0.733	0.017
Interleukin 1β	0.58(0.44-0.70)	0.64(0.39-1.21)	0.54(0.41-0.79)	0.240	0.942	0.348
MCP-1	115.20(52.59-183.41)	73.16(49.23-185.72)	125.70(53.42-199.47)	0.517	0.462	0.385
EGF	37.60(10.31-118.30)	33.99(1.98-99.36)	23.04(5.35-57.51)	0.161	0.008	0.665

Values are expressed as median (interquartile range). Comparisons were performed by Kruskal-Wallis/post-hoc Mann-Whitney test

EGF Epidermal growth factor, *MCP1* monocyte chemoattractant protein, *TNF-α* tumor necrosis factor, *VEFG* vascular endothelial growth factor

We also determined the correlation between the serum growth factors, pro-inflammatory and anti-inflammatory cytokine concentrations in the two groups of non-diabetics and diabetic which was performed using Spearman correlation analysis (Table 3). The serum cytokine correlation analysis emphasized the negative correlation of anti-inflammatory cytokines such as VEGF and EGF with inflammatory cytokines such as IL-1α, IFN-γ in both diabetes and non-diabetes.

Confounding factors and serum levels of cytokines and growth factors

We analyzed the effects of potentially confounding factors such as age, sex, smoking, BMI, serum lipid profile, and hypertension on serum level of cytokines and growth factors. Using multivariate multinomial logistic analysis and removing the potential confounding effect, we found that serum cytokine and growth factor levels

Table 3 Correlation matrix between the growth factors, inflammatory and anti-inflammatory cytokines in two groups of non-diabetics (n = 270, Table A) and diabetic cases (n = 104, Table B) using Spearman correlation analysis

A)

		IL-2	IL-4	IL-6	IL-8	IL-10	VEGF	IFN-γ	TNF-α	IL-1α	IL-1β	MCP-1
IL-4	r	-.002										
	P	.976										
IL-6	r	.042	-.034									
	P	.514	.595									
IL-8	r	.040	-.090	.271								
	P	.529	.153	<0.001								
IL-10	r	.169	.136	.085	-.132							
	P	.007	.029	.181	.036							
VEGF	r	-.011	-.453	.178	.208	-.145						
	P	.857	<0.001	.005	.001	.019						
IFN-γ	r	.111	.132	.209	-.033	.233	-.128					
	P	.075	.031	.001	.595	<0.001	.036					
TNF-α	r	.027	-.049	.261	.163	.136	.058	.210				
	P	.674	.444	<0.001	.012	.035	.364	.001				
IL-1α	r	-.039	.265	.075	.049	.155	-.122	.126	.099			
	P	.529	<0.001	.234	.437	.012	.046	.039	.122			
IL-1β	r	.150	.117	.097	.037	.167	-.119	.078	.093	.149		
	P	.016	.058	.124	.553	.007	.051	.202	.143	.015		
MCP-1	r	.109	-.393	.063	.302	-.193	.269	-.039	.061	-.078	-.179	
	P	.089	<0.001	.331	<0.001	.002	<0.001	.538	.356	.214	.004	
EGF	r	-.024	-.115	.107	.317	-.072	.170	-.135	.273	-.087	-.018	.146
	P	.705	.063	.091	<0.001	.248	.005	.027	<0.001	.157	.772	.020

B)

		IL-2	IL-4	IL-6	IL-8	IL_10	VEGF	IFN-γ	TNΦα	IL-1α	IL-1β	MCP-1
IL-4	r	.108										
	P	.297	IL-4									
IL-6	r	-.047	-.268									
	P	.657	.013	IL-6								
IL-8	r	-.023	.154	.072								
	P	.821	.137	.500	IL-8							
IL-10	r	.184	.011	.254	.268							
	P	.067	.916	.016	.008	IL_10						
VEGF	r	-.130	-.564	.296	-.051	-.152						
	P	.192	<0.001	.004	.615	.130	VEGF					
IFN-γ	r	.078	.195	.169	.449	.353	-.172					
	P	.469	.073	.131	<0.001	.001	.104	IFN-γ				
TNΦα	r	.064	.035	.370	.370	.279	.182	.280				
	P	.556	.758	.001	<0.001	.010	.092	.014	TNΦα			
IL-1α	r	.137	.351	-.030	.301	.189	-.291	.340	.208			
	P	.168	<0.001	.772	.002	.059	.003	.001	.053	IL-1α		
IL-1β	r	.319	.124	.189	.226	.197	-.128	.118	.243	.347		
	P	.001	.230	.070	.023	.048	.196	.268	.023	<0.001	IL-1β	
MCP-1	r	.257	-.429	.088	.325	.039	.309	.188	.172	-.073	.140	
	P	.010	<0.001	.406	.001	.705	.002	.079	.116	.470	.164	MCP-1
EGF	r	-.145	.035	.084	.201	-.024	.199	-.015	.177	-.089	-.013	-.077
	P	.152	.740	.431	.048	.818	.047	.888	.110	.380	.894	.454

The unit of values is pg/mL. The results are represented with the correlation coefficient (r) and p value. The statistically significant correlations are depicted as highlight. The Spearman correlation analysis was used for calculating correlation between serum cytokines levels

EGF epidermal growth factor, *IFN-γ* Interferon γ, *IL-1α* Interleukin-1α, *IL-1β* Interleukin-1β, *IL-2* Interleukin-2, *IL-4* Interleukin-4, *IL-6* Interleukin6, *IL-8* Interleukin-8, *IL-10* Interleukin-10, *MCP-1* monocyte chemoattractant protein, *TNF-α* tumor necrosis factor-α, *VEFG* vascular endothelial growth factor

are subject to various confounding factors. The presence of diabetes and pre-diabetes was positively associated with serum concentrations of IL-4 and IL-1α while VEGF concentrations were negatively associated with the prevalence of diabetes (Table 4).

Discussion

A significant proportion of people with type 2 diabetes are overweight and obese. As observed in our study, diabetic individuals in our study had a significantly higher BMI compared to non-diabetic. Obesity and particularly the presence of activated leukocytes within the adipose tissue cause an increased secretion of inflammatory factors that are related to the development of insulin resistance and subsequently type 2 diabetes [3, 7]. There is evidence that years before the onset of diabetes serum markers of inflammation are raised [8]. We also found that in pre-diabetes the hs-CRP level, as an inflammatory marker, was already elevated. The high level of hs-CRP in diabetes has previously been reported by other studies [9–11], we also observed the same trend.

In view of serum cytokine levels, our study highlights that serum level of many cytokines and growth factors can be modulated by various confounding factors such as age, sex, smoking, BMI, and serum lipids. Correcting for the effects of these confounding effects, we found that among the cytokines and growth factors in our panel, only IL-4, IL-1α, and VEGF levels at serum can be associated with the prevalence of diabetes (Fig. 1). In individuals with pre-diabetes we found higher serum levels of inflammatory cytokines such as IL-2, IL-1α, IFN-γ, and anti-inflammatory cytokines such as IL-4, and IL-10, while they showed a significantly lower level of an anti-inflammatory VEGF cytokine in their serum. However, after removing the confounding effects, only higher levels of IL-4 and IL-1α were significantly manifested in pre-diabetic group compared with non-diabetic control group. IL-4 is known as anti-inflammatory cytokine; therefore, it may be expected to be present at a lower level in the inflammatory state associated with diabetes, while we observed the opposite. In a study, it has been shown that in diabetic

Table 4 Adjusted association of serum pro- and anti-inflammatory cytokines and growth factors level and diabetes by multinomial logistic regression

		Univariate				Multivariate[a]			
		β	CI		P	β	CI		P
IL-2	Diabetes	1.171	1.025	1.339	.021	1.126	.979	1.296	0.066
	Pre-diabetes	1.174	1.020	1.351	.025	1.142	.986	1.322	0.057
IL-4	Diabetes	1.245	.933	1.662	.137	1.784	1.120	2.842	0.015
	Pre-diabetes	1.358	1.000	1.843	.050	3.353	1.928	5.831	<0.001
IL-6	Diabetes	.948	.802	1.120	.529				
	Pre-diabetes	1.032	.953	1.117	.442				
IL-8	Diabetes	.993	.981	1.005	.246				
	Pre-diabetes	.993	.975	1.011	.457				
IL-10	Diabetes	.920	.730	1.160	.481				
	Pre-diabetes	1.111	.863	1.430	.416				
VEGF	Diabetes	.998	.995	1.001	.128	.996	.992	0.999	0.012
	Pre-diabetes	.994	.988	.999	.019	.996	.990	1.001	0.100
IFN-γ	Diabetes	1.103	.982	1.239	.099				
	Pre-diabetes	1.081	.935	1.248	.293				
TNF-α	Diabetes	.986	.827	1.177	.879				
	Pre-diabetes	1.012	.805	1.272	.919				
IL1-α	Diabetes	2.852	1.011	8.047	.048	5.149	1.157	22.909	0.031
	Pre-diabetes	3.247	1.101	9.578	.033	5.652	1.225	26.076	0.026
IL1-β	Diabetes	1.686	1.007	2.824	.047	1.376	.812	2.330	0.236
	Pre-diabetes	2.067	1.131	3.778	.018	1.790	.953	3.362	0.070
MCP-1	Diabetes	1.002	1.000	1.004	.106				
	Pre-diabetes	1.000	.997	1.004	.954				
EGF	Diabetes	.995	.992	.998	.004	.996	.993	1.000	0.052
	Pre-diabetes	.998	.995	1.002	.342	.997	.993	1.001	0.173
hsCRP	Diabetes	1.071	1.022	1.122	0.004	1.097	1.032	1.166	0.003
	Pre-diabetes	1.050	.990	1.115	0.105	1.062	.982	1.148	0.129

The predictors were analyzed as enter method in the multivariate analysis model. Reference category is control subjects
CI confidence interval, *β* regression coefficient, *p p* value

[a] In present of age, sex, smoking, BMI, TG, LDL-C, HTN, and anti-diabetic drugs

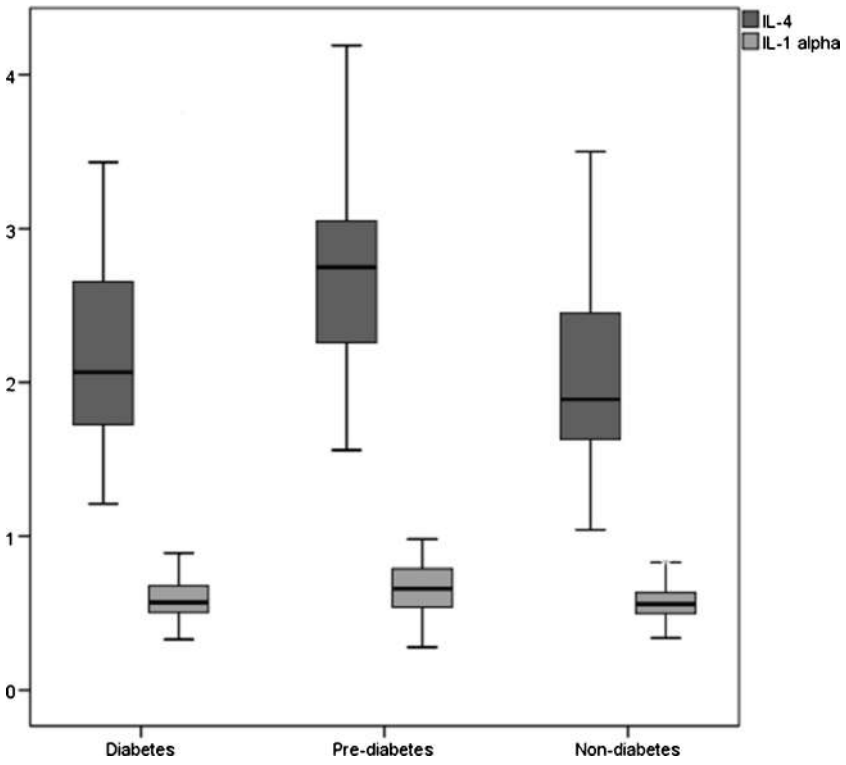

Fig. 1 Comparison of serum IL-4 and IL-1α levels in all subjects ($p < 0.01$)

mice, the IL-4 signaling is impaired. In fact, overexpression of suppressor of cytokine signaling (SOCS)-3 in diabetic animals imposes an IL-4 resistant condition [12]. Therefore, this mechanism might be an explanation for the higher level of IL-4 in diabetic and pre-diabetic groups in our study as a body compensatory response.

Previous studies reported lower concentrations of serum IL-10 and VEGF and higher amounts of inflammatory cytokine such as TNF-α, MCP-1, IL-6, IL-1β in diabetes [13–18]. A combined elevation of serum IL-1β and IL-6 were considered as risk factor for type 2 diabetes [13]. The confounding effects that we observed in our study could be a reason for discrepancies in results from different reports. Moreover, in most studies the serum cytokine level has been considered with regards to a specific diabetes related complication [14, 15], while we have excluded patients with major diabetes related complications such as heart and renal failure and any established cardiovascular disease from our study to better understand the association of inflammation with diabetes and even more important, with pre-diabetes.

As this study suggests, a serum cytokine profile may be predictive in diabetes and the changes in the level of some factors such as IL-1α, and IL-4 could be detected even before the onset of diabetes. However, there are various confounding factors such as age and sex which always should be considered to have a valid interpretation of serum cytokine profile. On the other hand, the small sample size in the pre-diabetic group is a limitation for the present study. Having a larger group may result in significant differences in the serum levels of more cytokines between pre-diabetic group and diabetic or non-diabetic control groups.

As a final point, since cytokines have synergistic and antagonistic effect on each other the cytokine network should be considered rather than isolated cytokines and growth factors.

Acknowledgements This study was conducted as a M.Sc. degree thesis with financial support provided by the Research Council at the Mashhad University of Medical Sciences, Mashhad, Iran. The authors would like to express their sincerest thanks to the patients and their family members who volunteered to participate in this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Mashhad University of Medical Sciences research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):320–322
DOI 10.1007/s13410-016-0535-0

CASE REPORT

Proliferative glomerulonephritis with monoclonal IgG deposits in a patient with diabetes mellitus

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Received: 2 August 2016 / Accepted: 17 November 2016 / Published online: 3 December 2016

Abstract Proliferative glomerulonephritis with monoclonal immunoglobulin G deposits (PGNMID) is a newly identified and rare form of glomerulonephritis which is characterized by endocapillary proliferative or membranoproliferative with monoclonal deposit stain for IgG and a single light chain. We describe the case of a 63-year-old woman with type 2 diabetes who was considered to have PGNMID.

Keywords Proliferative glomerulonephritis · Monoclonal IgG deposits · Type 2 diabetes

Diabetic nephropathy (DN) is estimated to affect one third of individuals with diabetes and it is the leading cause of end-stage renal disease (ESRD) worldwide. Nondiabetic renal disease (NDRD) can be either isolated or superimposed on DN. Differentiating NDRD from DN is of great importance for it has prognostic and therapeutic implications. Some clinical clues, such as absence of diabetic retinopathy (DR), short duration of DM, active urinary sediment, suggest the presence of NDRD [1]. However, precise diagnosis requires renal biopsy. We report a type 2 diabetes patient with nephrotic syndrome who has a rare form of glomerulonephritis—proliferative glomerulonephritis with monoclonal IgG deposits (PGNMID).

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Case report

A 63-year-old woman was admitted because of a 2-month history of anasarca and frothy urine. She had a medical history of diabetes mellitus for 10 years and hypertension for 1 year. Her medications included losartan potassium, nifedipine controlled-release tablets, metformin, and glimepiride. She was treated with diuretics for 1 month before admission. On examination, her blood pressure was 164/84 mmHg, and she had pleural effusions, ascites, and anasarca; the remainder of the physical examination was normal. Results of the urine dipstick showed protein (3+), and urine sediments contained 99 red blood cells/μL. Other laboratory tests showed the following values: serum creatinine, 1.04 mg/dL; albumin, 19.7 g/L; cholesterol, 9.00 mmol/L; and 24-h total urinary protein, 5.5 g/d. Serum immunofixation electrophoresis test results showed no monoclonal paraproteins, and the test results for anti-nuclear antibodies, serum cryoglobulins, hepatitis B surface antigen, and anti-hepatitis C virus were all negative. The kidneys measured 9.6 cm × 5.8 cm (right) and 9.9 cm × 6.0 cm (left) by ultrasound, without hydronephrosis or a mass lesion. No abnormalities were detected by bone marrow puncture. Fundus fluorescein angiogram showed proliferative DR. Thus, a renal biopsy was performed (Fig. 1). Light microscopy showed a hypercellular glomerulus with lobular accentuation of the mesangial matrix. The capillary walls were moderately and diffusely thickened with segmental double contours. Results of immunofluorescence studies showed mild intensity (+) for immunoglobulin (Ig)G, marked intensity (3+) for IgG3, moderate intensity (2+) for C3 and κ within the mesangial areas, and a granular pattern of the capillary walls. There was no significant staining observed for IgA, IgM, C4, C1q, fibrin, hemoglobin (HB)s, HBc, IgG1, IgG2, and IgG4. Findings of electron microscopy showed electron-dense immune-complex deposits in the mesangial and subendothelial areas with diffuse effacement of podocyte foot processes (not shown). The diagnosis of PGNMID was considered.

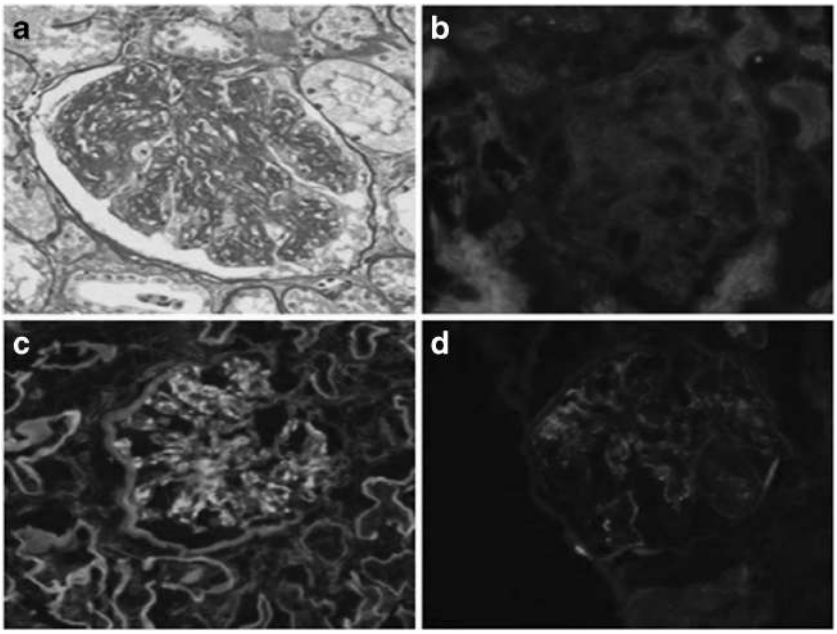

Fig. 1 Light microscopy and immunofluorescence microscopy of kidney biopsy specimen. **a** periodic acid-Schiff staining, ×400; immunofluorescence microscopy for IgG (**b**), IgG3 (**c**), and κ-light chains (**d**) (×400)

Discussion

From the traditional view, patients with type 2 diabetics with marked proteinuria and retinopathy (DR) most likely have DN. However, DN can occur in the absence of DR. In a study of 52 patients with proteinuric diabetes and no DR, 35 (69%) had DN detected by a biopsy [2]. Conversely, those with DR have non-diabetic glomerular disease (NDGD). In a retrospective cohort of 273 patients with type 2 diabetes who underwent renal biopsy, 12 of 175 with NDGD had DR [3]. Thus, DR is not a good predictor of the nature of nephropathy in type 2 diabetic patients with significant proteinuria. Therefore, we will conduct biopsy in all diabetic patients with heavy proteinuria and/or hematuria and red cell casts if conditions permit.

Biopsy characteristic of our patient is membranoproliferative lesion and monoclonal IgG and κ-light chain deposits. In such cases, diagnostic considerations should include type 1 cryoglobulinemic glomerulonephritis, immunotactoid

Table 1 Pathological characteristics of glomerular disorders with monoclonal IgG and light chain deposits

Glomerular disease	Light microscopic findings	Immunofluorescence findings	Electron microscopy findings
Type I cryoglobulinemic GN	Most membranoproliferative pattern, with intracapillary hyalin thrombi	Monotypic IgG, IgM, or IgA and light chain (κ > λ) deposits in mesangium and/or CW	Cryoglobulin deposits may be microtubular and highly organized
ITGN	Variable, most membranoproliferative pattern, less frequently membranous pattern	Monoclonal deposits for IgG (IgG1 > IgG2 > IgG3) and light chain (κ > λ) in mesangium and/or CW	Microtubules, diameter 30~90 nm, are arranged in parallel bundles
FGN	Variable, most mesangial proliferation and membranoproliferative appearance, occasionally membranous pattern	Deposits of polyclonal IgG (predominantly IgG4) and both κ and λ mesangium and/or GBM. In a few cases, monoclonal staining for IgG-κ	Non-branching fibrils, diameter 10~30 nm, are randomly arranged
PGNMID	Most membranoproliferative pattern, less frequently only mesangial proliferation	Monoclonal deposits for IgG (most IgG3) and light chain (most κ) in mesangium and/or CW	Non-organized granular deposits in mesangium and subendothelial zone, occasionally in subepithelial zone
LHCDD	Variable, most nodular mesangial appearance,	Monotypic heavy chain (most λ) and light chain deposits in TBM, GBM, and mesangium	Amorphous deposits in TBM, GBM, and mesangium

CW glomerular capillary walls, *TBM* tubular basement membrane, *GBM* glomerular basement membrane

glomerulonephritis (ITGN), fibrillary glomerulonephritis (FGN), PGNMID, and light-chain and heavy-chain deposition disease (LCHDD) (Table 1). PGNMID is a newly identified and rare form of glomerulonephritis. Its reported biopsy incidence rate ranges from 0.07% [4] to 0.17% [5]. The optimal therapeutic approach for PGNMID is uncertain. It is recommended to choose a treatment strategy based on the severity of the renal disease [6]. In patients with normal kidney function and low-degree proteinuria, conservative treatment such as angiotensin-converting enzyme inhibitors and angiotensin II blockades, and careful surveillance may be suitable. In patients with high-grade proteinuria and progressive disease, immunosuppressive therapy such as cyclophosphamide and bortezomib are the drugs of choice.

In our patient, conservative therapy, including an angiotensin II receptor blockade and diuretics, was chosen. Subsequently, her serum albumin level increased to 28 g/L, and the edema subsided. However, 1 month later, her serum creatinine level increased to 1.78 mg/dL and urinary protein level was still 7.5 g/d. Thus, cyclophosphamide (0.6 g/month) was added to her regimen. Now, she is undergoing follow-up evaluation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional. The patient signed informed consent.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):323–326
DOI 10.1007/s13410-016-0539-9

CASE REPORT

A novel genetic mutation in a Turkish family with GCK-MODY

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Received: 10 October 2016 / Accepted: 23 November 2016 / Published online: 1 December 2016

Abstract Glucokinase-maturity-onset diabetes of the young (GCK-MODY) is an autosomal dominantly inherited disease caused by heterozygous inactivating mutations in the glucokinase gene. It usually presents with mild fasting hyperglycemia. Here, we present an obese patient and her family with GCK-MODY caused by a novel heterozygous p.E51* (c.151.G>T) mutation in the GCK gene.

Keywords GCK-MODY · Obese patient · Novel mutation

Abbreviations

GCK-MODY	Glucokinase-maturity-onset diabetes of the young
GCK	Glucokinase
BMI	Body mass index
OGTT	Oral glucose tolerance test
T2D	Type 2 diabetes mellitus
FPG	Fasting plasma glucose

Electronic supplementary material The online version of this article (doi:10.1007/s13410-016-0539-9) contains supplementary material, which is available to authorized users.

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Introduction

Glucokinase-maturity-onset diabetes of the young (GCK-MODY, also known as MODY 2) accounts for 2–5% of all diabetes cases [1]. Glucokinase, a key enzyme in glycolysis, is a glucose sensor that maintains glucose homeostasis in pancreatic beta cells [2]. The GCK gene (7p15.3–p15.1) consists of 12 exons and encodes a 465-amino acid protein [3]. GCK-MODY is caused by heterozygous inactivating GCK mutations, which are usually associated with mild hyperglycemia [4]. It is treated only with diet, and complications are extremely rare [5]. Here, we report a novel heterozygous p.E51 mutation encoded by GCK exon 2, which results in the typical GCK-MODY phenotype.

Patient report

A 17-year-old girl was admitted to our department for evaluation of obesity and hyperglycemia. Despite her obesity (body mass index (BMI), 30.2 kg/m 2), her physical examination had no acanthosis nigricans or other abnormalities. Pubertal assessment revealed Tanner stage V. Blood glucose levels were repeatedly checked and showed mild fasting hyperglycemia as well as an elevated HbA1c level. Standard oral glucose tolerance test with 75 g of glucose was performed showing a fasting glucose of 131 mg/dl, 120-min glucose of 159 mg/dl and normal insulin levels. Serum autoantibodies against glutamic acid decarboxylase, islet cell antibodies, and anti-insulin autoantibodies were negative. Family history revealed that her father, grandmother, and uncle were diagnosed with diabetes (Fig. 1). Considering the clinical features and family history, mutation analysis of the GCK gene was performed. Complete sequencing of coding exons and intron-exon boundaries of

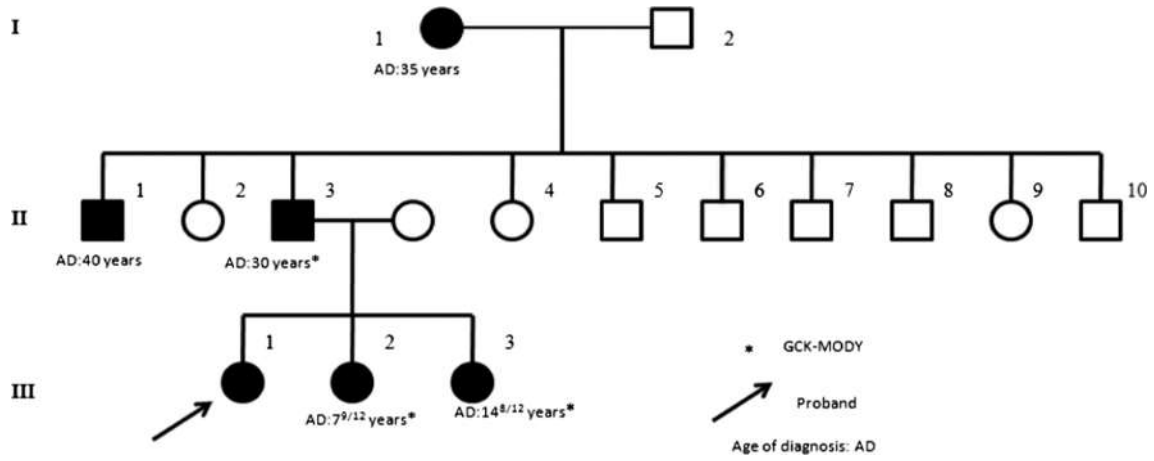

Fig. 1 Family tree of the patient

the GCK gene identified a heterozygous mutation (c.151.G>T) leading to a stop codon (p.E51*) in the GCK gene.

The father was diagnosed with diabetes at age 30 but he did not go to the doctor visits regularly. The blood glucose levels of both sisters were never tested and the sisters had impaired fasting glucose levels. The patient's father and two sisters had the same mutation. We performed a 75-g 2-h oral glucose tolerance test (OGTT) (after 12 h of fasting) to assess glycemic/insulin fluctuations in response to a glucose challenge. Clinical and laboratory findings of the patients are given in Tables 1 and 2. The grandmother refused to undergo genetic testing. Unfortunately, we could not describe other family members better as they are living outside the city for a long time.

Patients were treated with appropriate diet and exercise. All patients were screened for diabetes-related microvascular complications. We did not find any diabetes-related complications. Nephropathy was evaluated using microalbuminuria, and retinopathy was assessed by fundoscopic examination. Lipid levels and 24-h urinary albumin excretion rates were normal. None of the patients had diabetic retinopathy.

Mutational analysis

GCK gene sequence analysis was performed by using MiSeq next generation sequencing (NGS) platform, a FDA approved diagnostic system (Illumina, San Diego, CA, USA). Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All coding exons of the *GCK* gene and their flanking splice site junctions were amplified using PCR primers, designed with

Table 1 Laboratory characterization of patients with GCK-MODY

	Proband	Sister 1	Sister 2	Father
Age (year)	$16^{7/12}$	$7^{9/12}$	$14^{8/12}$	40
BMI	28.3 (>95 p)	18.1 (75–85 P)	21.1 (50–75 P)	22
Fasting Glucose (mg/dl)	130	129	135	161
Fasting Insulin (μIU/ml)	10.2	3.6	14	
C-peptide (ng/ml)	2.4	0.9	2	–
HbA1c (%)	6.6	6.4	6.4	6.9
Cholesterol (mg/dl)	165	155	145	179
LDL (mg/dl)	103	96	110	109
Triglyceride(mg/dl)	126	132	115	140
Diabetes antibodies	Negative	Negative	Negative	Negative
Urinary albumin excretion (mg/day)	10	9	6	12
Abdominal ultrasound	Normal	Normal	Normal	Normal
Fundoscopy	Normal	Normal	Normal	Normal

Table 2 Glucose and insulin concentrations during a standard oral glucose tolerance test with 75 g glucose equivalent

Time (min)	Proband		Sister 1		Sister 2	
	Glucose (mg/dl)	Insulin (μIU/ml)	Glucose (mg/dl)	Insulin (μIU/ml)	Glucose (mg/dl)	Insulin (μIU/ml)
0	130	6.9	121	3	122	11
30	153	20	187	16	139	24
60	178	20	201	28	179	51
90	176	29	170	20	159	40
120	159	27	146	18	150	31

PRIMER©—Primer Designer v.2.0 (Scientific & Educational Software programme) software. PCRs were validated by using agarose gel electrophoresis. After PCR amplification, the libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. Next-gene sequencing was carried on MiSeq (Illumina Inc.). Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.). Visualization of the data was performed with IGV 2.3 (Broad Institute) software.

In silico analysis was done with Mutation taster software, and it was predicted that variant as a disease causing variant with the probability score 1. As this variant causes a premature stop codon, it causes a truncated non-functional protein.

Discussion

In our patient, a novel heterozygous mutation of c.151.G>T leading to premature stop codon p. E51* was identified in exon 2 of the GCK gene. Glucokinase plays a crucial role in the regulation of insulin secretion and acts as a glucose sensor in pancreatic beta cells. Heterozygous inactivating mutations of GCK cause GCK-MODY characterized by mild fasting hyperglycemia, which is present at birth but often only detected later in life while screening for other reasons [5]. As the result of a change in the required glucose concentration threshold to stimulate insulin secretion, fasting plasma glucose (FPG) ranges between 100 to 153 mg/dl in a majority of patients. Significant worsening of glycemic control occurs with increasing age [6, 7]. In our case, the father's FPG and HbA1c levels were slightly higher than the children.

Our proband was obese. At the first examination, Type2 diabetes mellitus (T2D) was considered an obese patient with hyperglycemia. But she did not have acanthosis nigricans and metabolic syndrome findings also OGTT showed insulinopenia. Absence of clinical signs such as obesity and the metabolic syndrome in patients with early-onset diabetes favors a diagnosis of MODY [8]. The prevalence of obesity in patients with MODY is as same as normal population. People with GCK-MODY are usually less obese than people with T2D [9]. However, the coexistence of obesity and MODY are more frequently reported because it is likely that obesity is epidemic among teenagers and young adults [8]. The father and other siblings were not obese. We think that our patient's obesity may have been coincidental. Moreover, a study performed with pediatric-age MODY sufferers observed acanthosis nigricans in 40% of molecularly confirmed cases [10]. Patients with GCK-MODY are also less hyperglycemic than patients with young-onset T2D. The rise of blood sugar level in T2D patients during OGTT is typically above 54 mg/dl [9]. One of the characteristic features of GCK-MODY is a modest increase in the postprandial glucose levels, with a 75-g oral glucose tolerance test increment (120 min glucose minus 0 min glucose) of less than 54 mg/dl in 70% and 83 mg/dl in 95% of patients [11]. We also observed a small increase in glucose levels during OGTT in our patients.

We did not find any diabetes-related complications. Microvascular complications are very rare in individuals with GCK-MODY, because the hyperglycemia is mild, and there is no marked progression [12, 13]. A study consisting of 42 families with GCK-MODY reported that proliferative retinopathy was seen in less than 4% of the patients more than 5 years after initial diagnosis, while peripheral neuropathy and proteinuria rates were 5 and 6%, respectively. Dyslipidemia and hypertension were also reported to have low prevalence [5]. Another study reported a few cases of retinopathy and macrovascular disease [14].

MODY also should be kept in mind in obese patients with mild hyperglycemia, non-obese first degree relatives with early-onset diabetes, and especially lack of insulin resistance, and metabolic syndrome findings. Identification of GCK gene mutations is important for the correct and definitive diagnosis of GCK-MODY and helps the physician predict disease course and initiate appropriate therapy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Funding source No external funding for this manuscript.

Financial disclosure The authors have no financial relationships relevant to this article to disclose.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):327–328
DOI 10.1007/s13410-017-0552-7

LETTER TO THE EDITOR

Influx of recombinant insulin and its analogues for management of diabetes in India

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Received: 12 September 2016 / Accepted: 21 February 2017 / Published online: 9 March 2017

Diabetes is a metabolic disorder disease characterized by elevated blood glucose level due to under secretion or non-secretion of insulin from pancreas. Eighty percent of diabetes deaths occur in low-and middle-income countries. India has the largest number of surviving diabetic people followed by China and USA. In India, during the year 2000, the number of surviving diabetic people were 31.7 million and predicted to rise to 79.4 million by 2030 [1]. There are two Indian guidelines for insulin initiation and intensification. As per these guidelines, insulin therapy may be started on the basis of levels of blood sugar and HbaIc determined during fasting and or after meals. Animal insulin was being used for decades, but now, most of the companies are manufacturing biosynthetic human insulin by recombinant DNA technology. As per survey of International Diabetes Federation conducted in its member countries in 2002, the biosynthetic human insulin contributes about 70% portion of the total insulin sold. Since 2006, all insulins distributed in almost all countries are synthetic human insulin or their analogues.

In India, as per Drugs and Cosmetics Act, 1940 & 1945, Central Drugs Standard Control Organization (CDSCO) and Department of Biotechnology regulate the marketing of insulin and its analogues. National Institute of Biologicals (NIB) is a central drug testing laboratory for testing of insulin and its analogues (Table 1).

The manufacturers claim that their different insulin analogues act differently and categorized them mainly into three types: (i) fast-acting, (ii) intermediate-acting, and (iii) long-acting insulins. But, the reports released by Cochrane Collaboration in 2002, Institute for Quality and Efficiency in the Health Care in 2007 and Canadian Agency for Drugs and Technologies in Health in 2007 show that there is no clear evidence of advantages of insulin analogues over conventional insulin [2–4].

Table 1 Number of batches of insulin/analogues received from 2010 to 2015 at NIB for testing

S.No	Insulin/analogue	2010	2011	2012	2013	2014	2015
1	Lispro	51	48	55	57	41	58
2	Aspart	28	37	55	54	82	61
3	Glulisine	6	5	9	14	18	16
4	Detemir	5	5	6	8	11	2
5	Degludec	0	0	0	5	10	15
6	Glargine	30	19	21	31	51	30
7	NPH insulin	21	27	34	30	32	31
8	Soluble (regular)	60	72	73	96	75	105
9	Biphasic isophane	102	112	141	143	120	136
Total		303	325	394	438	440	454

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Funding None.

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Int J Diabetes Dev Ctries (July–September 2017) 37(3):329–375
DOI 10.1007/s13410-017-0568-z

ABSTRACTS

Abstracts of The 44th Annual Conference of Research Society for the Study of Diabetes in India (RSSDI 2016)

Prof. MMS Ahuja Symposium – Affordable Diabetes Care

Factors Governing Access to Diabetes Care in a Peri-urban Area of Goa

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Introduction: Experiences in public health have shown that for people to avail health care, the health care should be available, acceptable and affordable. Furthermore the people should be aware of such services as well as the need for such services. Given the chronic non-curable nature of Diabetes and its tendency to cause complications in the long run the spectrum of diabetes care transpasses the preventive, promotive, curative and rehabilitative diabetes care services. **Objectives**: 1. To study the availability of various diabetes care providers in a periurban area of Goa. 2. To identify the diabetes care providers chosen by the study participants and the factors governing their choice. **Material and Methods**: One hundred and fifty diabetics were identified through a house to house survey in Merces area of Tiswadi Taluka of Goa. Data was collected using a semi-structured questionnaire. Focus Group Discussions were conducted among the homogenous groups to explore the factors governing choice of a specific mode of care. **Results**: Majority of the study participants (48%) availed diabetes care from government primary health centres and subcentres citing availability of free medicines as the sole reason for it. Almost 28% availed care from local allopathic general practitioners and opined that this was more convenient and time saving. Only 12% chose the specialized care and seemed to be inetersted in quality of care. The remaining 12% chose either non allopathic mode of care or just did not care. **Conclusion**: The individual as well as the focus group discussions revealed that while the majority of the patients are visiting some health facility there exists a big doubt about the quality of care received by these patients. Lack of awareness about diabetes and its complications emerged as the single most important limiting factor as most of the discussions revealed that there was no issue about affordability or distance from a health facility if the final bet is on prevention of cardiovascular, ocular and renal complications of diabetes.

Cost Effective Make In India Fundus Imaging Device (MII Ret Cam) for Screening Diabetic Retinopathy

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Purpose: To demonstrate an inexpensive smartphone-based fundus camera device (MII Ret Cam) for the screening of diabetic retinopathy, **Methods**: A fundus camera was designed in the form of a device that has slots to fit a smartphone (built-in camera and flash) and 20-D lens. With the help of the device and an innovative imaging technique, high-quality fundus images were captured. **Results**: The MII Ret Cam and innovative imaging technique was able to capture high-quality images with a portable lightweight device. **Conclusions**: MII Ret Cam can help clinicians to monitor diseases affecting both central and peripheral retina. It can help patients understand their disease and clinicians convincing their patients regarding need of treatment at early stages of the disease. This can help bridge the unfavorable ration between Retina Specialist and population affected with Diabetic Retinopathy.

Simplicity, Safety and Convenience of Insulin Pen Use Versus Conventional Vial/Syringe Use in Patients with Diabetes Mellitus.

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Background and Aim: Injectable therapy in the treatment of diabetes mellitus needs to be simple, safe and convenient. This study was planned to objectively determine these factors in patients using pen devices versus those who use conventional syringe/vial. **Materials and Methods**: This prospective observational study was conducted after doing a small pilot study and approval by Institutional Research and Ethics committee. After an informed consent, patients were interviewed using a self made questionnaire and patients were scored based on their answers. Simplicity and safety was represented by five questions each with a possible maximum score of 15 while convenience was represented by three questions with a maximum score of nine. Higher scores represented poorer response. A total of 90 completed questionnaires (45 from each group) were obtained and analyzed. **Results and Discussion**: Baseline data was comparable except for a larger proportion of pen users being on basal insulin therapy with glargine. Mean simplicity, safety and convenience score among the pen users was 5.31, 5.4 and 4.13 respectively as compared to 9.78, 8.09 and 8.67 in syringe users respectively (P value<0.001).Pen users spent Rs1756 per month as compared to Rs590 among syringe users. Among pen users 22.2% had optimal HbA1c levels (6%-7.5%) as compared to 2.2% among syringe users (P value <0.001). **Conclusion**: Pen is simple, safe and convenient to use. It also maintains a good glycemic control. Treatment with pen device is costlier which in part may be due to higher use of basal insulin (glargine) among pen users.

Affordable Diabetes Care

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Background and Hypothesis: Postprandial blood glucose (PP BG) is the main culprit for early accelerated atherosclerosis in diabetic patients. In PP state exercise makes insulin more sensitive. It acts on the liver to stop hepatic glucose production, allows the muscle to take up more glucose even with a brief period of running in treadmill. Exercise sensitizes the muscle to push more GLUT-4 to the cell surface and their turnover is increased- making the muscle to take up more glucose **Materials and Method**: We selected 20 patents with raised PP BG for the routine OHAs. Their 2 PP and 2 ½ PP was measured. Next day the same patients 2PP normally and 2 ½ PP after making them run in treadmill for 5 minutes was measured. Female patients were able to run just for 3 to 4 minutes, male patients ran for 5 to 7 minutes. Other Parameters like Height, weight; BMI, BP, etc were all recorded. **Result and Discussion**: There was a difference of 40 to 60 mgs PP BG fall after a brief period of running in both the male and female patents on the 2nd day compared to the 1st day **Conclusion**: This is a wonderful affordable model of therapy for a

low socio economic country like India. Only thing we need educators to motivate and explain the benefits of this cost effective therapy, so that only the poor patients but also the affordable can also be really benefited.

A Study of Type 1 Diabetes Mellitus from South India

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Objective: Diabetes in children is increasing in India and resources are poor in many parts of the country. The aim of this study is to describe the clinical profile and follow up of Diabetes in children and adolescents attending the Diabetic Child Society (DCS). **Material and Methods**: The primary aim of the DCS is to support the needy children with diabetes and improve health care of diabetes in the young. A total of 220 subjects with diabetes onset below the age of 25 years are screened for glycemic control, complications and comorbidities. Subjects are educated on SMBG and insulin therapy. **Results**: Males (101) and females (119) with mean age of 17 years and mean duration of diabetes of 7 years are the subjects of the study. Majority are Type 1 diabetes. Glycemic control is seen in 16%, Ocular complications in 12%, Diabetic Kidney disease in 9% of the subjects. 35% of the ocular complications are seen in subjects with nephropathy. Ocular and renal complications are associated with long duration of diabetes and higher A1c. DKA episodes is 7% and Mortality is 1.8%. The co morbidities are: Hypothyroidism (12%), Epilepsy (2.3%), PCOS (2.3%). Associated Syndromes are DIDMOAD, Down's syndrome, Turner's syndrome and SHORT. 10% have family h/o diabetes among siblings. 58% do SMBG daily and 62% use thrice daily insulin regimen. **Conclusion** : The higher A1c, complications, frequent hospitalizations in the study are due to non-adherence to therapy due to lack of awareness of the disorder, illiteracy, lack of parental support and psychosocial support. The DCS is endeavouring to address these issues and being nascent still would take time to reach optimum goals.

Doctor- Affordable, Medicines Not Affordable

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24 years back next to Chennai we only started Exclusive Diabetic center in a rural area-Erode. Those days our consultation was 75 with 2 blood sugar tests. Whereas consultation alone 200 in metros. We could give affordable cost model to the patents in and around my district with full pledged Diabetic centre. For medicines he will be paying only Rs 2.50 for 15 tablets of chlorformin combination of chlorpropamide + phenformin a fixed dose combination in olden days.Dose just ½ od because it was a long acting powerful combination. If they get Betanase 5 mg it is – Rs 7.20 for 60 tablets. Now after 24 years also we get just Rs 150. This is still the affordable cost model what we give today. This fee with 2 blood sugar tests has just doubled. But for medicines you know very well Rs 1500 for 30 tablets of gliptins or SGLT2 I s. Diamicrom 60mg Rs 680 for 60 tablets. After 24 years with the petrol price, gold price, stitching, going up by 20 times. Effective fixed dose combination drug going up by 200 times. Next generation doctors have come, My daughter and son in law, But our fees still it Rs 150 gone up by just 1 more time, So we Doctors are Still affordable but the next generation drugs have gone up by 200 times –very much not affordable. This clearly shows us modern doctors are affordable BUT the modern medicines are not affordable by all. That too diabetic medicine has to be taken lifelong. Because of medicine price hike people blame medical community as a whole, Medicine should be made cheap or else modern doctor has to select the olden tablets for all his patents for affordability.

Prof. B. B. Tripathi Nutrition Symposium – Glycemic Index of Cooked Foods

Comparison of Glycemic Index Values of Wheat and Rice Based on Their Available Carbohydrate Content When Consumed as a Part of a North Indian Mixed Meal

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Background: Earlier studies showed that glycemic index (GI) of rice is higher than that of wheat, which has lead to preference of wheat over rice as a staple in diabetic diets. However, these studies were not based on "available" carbohydrate (CHO) content of foods and could have overestimated the values. We investigated glycemic response of North-Indian mixed meal having wheat or rice as a cereal staple supplying the same amount of available CHO, in healthy volunteers. **Methodology**: Glycemic responses of 2 mixed meals were compared with reference meal (glucose) each designed to provide a total of 50g of available CHO and administered to 10 healthy adult male and female volunteers. Test meal 1 comprised of a vegetable (ladies fingers), a pulse preparation (green gram whole) and 2 wheat chapattis. In test meal 2, wheat chapattis were replaced by cooked rice supplying an equal amount of available CHO. Capillary blood glucose estimations were done after an overnight fast of 10-14 h at 0,15, 30, 45, 60, 90 and 120 minutes after eating each test meal or glucose. GI of test meals were calculated by comparing their area under curve (AUCs) with AUC for glucose. **Results**: There were a total of 7 males and 3 females with mean age 30.9 ±5.08y. The highest mean peak blood glucose was reached for reference meal (168.5±34.96 mg/dL) at 34.5±34.9 min, followed by that for test meal 2 (133.3±10.78 mg/dL) at 40.5±7.25 min and test meal 1 (126.5±11.69 mg/dL) at 57±27.2 min. The GI of test meal 1 (85.527±11.7481) and test meal 2 (83.5673±11.4012) was not significantly different (p=0.7095). **Conclusions**: In a mixed meal, when rice is exchanged for wheat supplying same amount of available CHOs, the GI of mixed meals are similar. Only 'available' CHO content of foods should be used as basis for GI determination in foods.

Estimation of Glycemic Carbohydrate and Glycemic Index/Load of Commonly Consumed Cereals, Legumes and Mixture of Cereals and Legumes

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Abstract: Aim of the present study is to estimate glycemic carbohydrates and develop data base on glycemic index and glycemic load (GI and GL) of commonly consumed cereals and legumes by using Food and Agriculture Organization (FAO) or World Health Organization (WHO) methods. The results of glycemic carbohydrates in rice was 79.22%, wheat 63.26% and pulses in the range of 51.24% (green gram) to 56.22%, (chana dhal), mixed dhal 40.09%, wheat + chana dhal (60:40) 49.94%, wheat + chana dhal + barley (40:30:30) was 46.89% respectively. The results of GI and GL of rice were highest (GI-78.23, GL-49.38), followed by wheat chapatti (GI-65.66, GL-32.83). The pulses tested were showing lower values ranging from (GI-37.95 to 43.01 and GL- 18.97 to 21.50), mixed dhal (GI-43.64, GL-21.82), wheat + chana dhal (60: 40) (GI-32.37, GL-16.18), wheat + chana dhal + barley (40:30:30) GI-39.27, GL-19.63 respectively. The results of the study indicated that pulses have low glycemic indices and glycemic loads, hence could be safely used in the diet of diabetic patients. **Keywords:** α-amylase; Anthrone; Amyloglucosidase; Protease; Starch; Glycemic index; Glycemic load.

Research Grant Presentations

Vascular Cell Adhesion Molecule-1(vcam-1) and Insulin Resistance in Type 2 Diabetes Patients on Metformin Monotherapy for 1-4 Years Compared to Prediabetics and Newly Diagnosed Diabetics

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Background: Metformin is the primary medication prescribed to uncomplicated type 2 diabetes patients. Its role in modifying and delaying the cardiovascular risk is poorly described.This study was designed to understand the role of VCAM-1 and insulin resistance in type 2 diabetes patients on metformin therapy for 1-4 years compared to untreated Prediabetics and newly diagnosed diabetics. **Materials and Methods**: A cross- sectional study was undertaken in serum samples of 90 subjects who visited the OPD of Kasturba medical college Mangaluru with 30 subjects in each group of prediabetics, newly diagnosed diabetics and type 2 diabetics on metformin for 1-4 years. FBS, Insulin,VCAM-1 levels were estimated. HOMA- IR was calculated by HOMA calculator. Data was analysed by ANOVA followed by Tukey test and correlation of VCAM1 with HOMA-IR was done by spearman test using SPSS. **Results**: Values of FBS, Insulin and HOMA-IR were the highest and those of Quicki and beta cell mass were the lowest in the newly diagnosed group.VCAM-1 showed a stepped increase from prediabetes to newly diagnosed to treated groups.VCAM-1 was found to be correlating with HOMA-IR in prediabetes group(r=0.4; p=0.02). **Conclusion**: An alteration in insulin resistance was seen to small extent in treated group despite that cardiovascular risk prevailed in them.

SNAIL-Associated Microvascular Defects in Hyperglycemia of Pregnancy

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Background/Hypothesis: Homozygous knock out of Snail transcription factor in mice causes defects in vasculogenesis. Down regulation of SNAIL contributes to the pathology of Preeclampsia. We hypothesised that SNAIL contributes to the microvascular dysfunction represented in our cases of Gestational Diabetes Mellitus (GDM) from South India. **Material and Methods**: Immunhistochemical analysis for the Snail protein was performed on placental tissue isolated from 10 cases each of healthy and GDM cases. GDM was classified using the International Association of Diabetes in Pregnancy Study Group (IADPSG). The localization of Snail to the dilated capillaries noted in our GDM cases was particularly evaluated. **Results and Discussion:** There was an increased expression of Snail protein in capillaries per villi as compared to healthy placental capillaries ($p \leq 0.001$).. This correlated with increased angiogenic marker expression. The expression of Snail in non –vascular (epithelial and stromal) were comparable in healthy and GDM placentas. **Conclusion:** We have previously noted a striking resemblance of placental fetal vascular progenitor cells exposed to short duration hyperglycemia from GDM cases in South India that resembles adult Type 2 proliferative diabetic retinal cells. This could point to the role of the intrauterine environment influencing the development of Type 2 Diabetic microvascular disease in adult hood. Targeting newly molecules such as the Snail pathway might help our understanding of abnormal blood vessel flow in GDM placental vasculature, and in adult T2 Diabetic retinopathy.

Association of Serum Copeptin with Chronic Psychological Stress in Subjects with Glucose Intolerance

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Objective: The present study aimed to investigate the association of serum copeptin, a surrogate marker for AVP secretion with chronic psychological stress in subjects with glucose intolerance. **Methodology**: The study was conducted in 150 age and sex matched subjects consisting of three groups. Group A: NGT, group B: prediabetes (IFG±IGT) and group C: newly detected diabetes mellitus (NDDM). Anthropometry, OGTT, HbA1c, insulin, serum copeptin and salivary cortisol were measured. Assessment of chronic psychological stress was done through validated questionnaires (PSLES, PSS and SOC). Group comparisons were done by oneway ANOVA followed by post-hoc tukey's test. **Results**: Anthropometry, plasma glucose, HbA1c and HOMA-IR were found to be significantly higher in NDDM subjects compared to NGTs. Serum copeptin and salivary cortisol (8 am and 10 pm) tended to be highest in NDDM subjects followed by prediabetics and then NGTs. Salivary cortisol levels post dexamethasone suppression (PDS) and PSS score were found to significantly higher and SOC was found to be significantly lower in NDDM subjects compared to NGTs. There was a negative correlation between serum copeptin and SOC in NDDM group (r= -0.485, P=0.001) and overall (r= -0.202, P=0.02) but not in prediabetics and NGTs. Serum copeptin levels did not show any significant correlation with cortisol levels (8 am, 10 pm and PDS). Linear regression analysis showed that copeptin levels increases significantly as the SOC decreases (regression coefficient for NDDM group was -35.41, P=0.001 and for overall -14.30, P=0.02). **Conclusion**: There is a significant association of serum copeptin with SOC, a marker of stress coping in subjects with glucose intolerance.

Efficacy of Vitamin D Supplementation on Reduction of Cardio-Metabolic Risk in Patients with Type 2 Diabetes Mellitus and Dyslipidemia

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Background: Cardiovascular (CV) disease is a major cause for mortality in diabetes. Endothelial progenitor cells are biomarkers of endothelial regeneration. Previous studies demonstrate a link between Vitamin D deficiency, inflammatory cytokines and CV risk. The aim of this pilot was to evaluate the impact of Vitamin D supplementation on EPCs, inflammatory markers and HbA1c. **Methods**: Prospective randomized controlled open label study. Sixty-five patients with type 2 diabetes, dyslipidemia, HbA1c <9 %, Vitamin D deficiency (<30ng/ml) attending the outpatient between April and December 2015 were randomized to an active or control group for 6 months; active group received Vitamin D. HbA1c, hsCRP, IL-6, IL-10, TNF alpha and HOMA-IR were evaluated at baseline, 3 and 6 months; EPCs at baseline and 6 months. Data was analyzed with STATA 14. **Results**: Age, duration of diabetes, BMI, HbA1c and Vitamin D levels were 537 years, 8.45 years, 26.83 kg/m2, 7.20.8% and 145 ng/ml; 57% were men. Vitamin D supplementation increased Vitamin D levels in the active group compared to control ($p<0.01$). EPCs decreased in both groups from baseline. There was no difference in EPCs, hsCRP, IL-6, IL-10, TNF alpha, HbA1c and insulin resistance between the active and control groups at the end of the study. **Discussion and Conclusion**: Supplementation of Vitamin D did not increase EPCs, alter inflammatory markers or improve glycemic control. Further studies are needed to study the long term effects on markers of endothelial repair.

Oral Presentations

Characteristics of Preproinsulin Specific CD8+ T Cells in Subjects with Juvenile-Onset and Adult-Onset Type 1 Diabetes: A One-Year Follow-Up Study

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Type 1 diabetes (T1D) is an autoimmune disease characterized by destruction of pancreatic beta cells by infiltrating immune cells leading to insulin deficiency. Beta cell associated CD8+ T cells have been identified and characterized from peripheral blood as well as pancreatic islets in subjects with autoimmune diabetes using MHC multimers. However, there is scant data on the time course of preproinsulin (PPI)-specific CD8+ T cells during the clinical development of different forms of autoimmune diabetes. We followed the time course of PPI-specific CD8+ T cells in juvenile-onset type 1 diabetes (JOT1D) and adult-onset type 1 diabetes (AOT1D) subjects for one year, post insulin therapy, using MHC-I dextramers by flow cytometry. At follow-up, PPI-specific CD8+ T cells could be detected consistently in peripheral blood of all T1D subjects. Relative proportion of PPI-specific effector CD8+ T cells was higher in AOT1D subjects (p=0.02), whereas proportion of naïve subset was higher in JOT1D subjects (p=0.01). Proportion of effector memory subsets decreased in both groups, while central memory T (TCM) cells remained unchanged. Expression of granzyme-B and perforin in PPI-specific CD8+ T cells also remained unchanged. Our results suggest that over time, PPI-specific CD8+ T cells can be detected in T1D subjects with reliable frequency but variable pathophysiological characteristics and persistence of TCM cells poses major challenge, although as an attractive target for immunotherapy.

A Pilot Study on the Serum Levels of Mitogen Activated Protein (MAP) Kinase Phosphatases (MKP-1 and MKP-3) in Individuals with Glucose Intolerance

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Objective Insulin resistance in Type 2 diabetes is associated with low grade inflammation. Stress responsive stimuli activate Mitogen-activated protein kinases (MAPKs) and they are modulated by MAP kinase phosphatases (MKPs). So our objective is to compare the serum levels of MKP-1 and MKP-3 in healthy control, prediabetic and diabetic individuals and to find the possible correlation with insulin resistance. **Methods** In this observational cross-sectional study, 20 euglycemic, 30 prediabetic and 20 diabetic individuals were taken as per ADA criteria. Blood samples were taken for measurement of FBS, OGTT, HbA1c, lipid profile, serum insulin and HOMA IR was calculated. Venous blood serum were stored at -80*C for MKP-1, MKP-3 measurement by standard ELISA method. We excluded pregnant women, steroid use, severe renal and liver disease. **Results** The waist circumference, hip circumference, triglyceride and LDL were significantly higher and HDL was significantly lower in diabetics than prediabetic (P<0.05) and in prediabetic than controls (P <0.05). The mean± SD of MKP1 and MKP3 (ng/ml) in 3 groups were as follows: Controls 25.09 ± 7.13 & 10.26 ± 2.29, Prediabetic 15.9 ± 7.72 & 1.8 ± 0.56 , Diabetics 7.29 ± 3.19 & 1.45 ± 0.58 (p<0.05 for MKP1 and p<0.05 for MKP3 among the groups). HOMA IR was inversely correlated with MKP 1 and MKP 3 (r=0.62, p <0.05). **Conclusion** Our study results suggest that MKP 1 and MKP 3 can be used as a predictive and therapeutic marker for patients with dysglycemia. They can also be useful biomarker for insulin resistance.

Elucidation of the Role of Insulin Secretion, Insulin Sensitivity and Adiposity in the Pathogenesis of Diabetes in Asian Indians with 'Lean' Body Mass Index (BMI).

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Background and Hypothesis: Dysregulation of glucose and lipid metabolism in "Lean diabetes" in India remains sparsely researched. This study was performed to decipher the role of insulin secretion and sensitivity defects and altered fat distribution in the pathogenesis of this unique form of diabetes **Methodology**: In this prospective study a total of 50 age matched males including 10 lean diabetes(age:36 ± 4 yrs, BMI18.3 ± 0.1 kg/m2)10T2DM(age:37 ± 7.7yrs,mean,BMI: 25.8±1.1kg/m2)15 non-diabetics(age:19.1 ± 5.2yrs, BMI 19.5 ± 2.3 kg/m2)&15 T1DM subjects(age:28.6±5.9yrs,BMI:20.5±1.6 kg/m2)were included."Lean-Diabetes" subjects were negative for auto-antibodies, pancreatic calculi, pancreatic exocrine insufficiency & MODY genetic mutations. Insulin secretion was assessed by deconvolution techniques after a mixed meal challenge test. Peripheral and hepatic insulin sensitivity was analyzed through hyperinsulinemic-euglycaemic pancreatic clamp procedures.1H-Nuclear-magnetic-resonance-spectroscopy was performed to assess hepatic, intra & extra myocellular lipid distribution **Results**: Insulin secretion rate was lowest for lean diabetes(15± 3.0 pmol/ kg/ min) as compared to non-diabetics (794 ±321.3 pmol/kg/min)T2DM (231.0 ±166 pmol/kg/min) & T1DM (27.5 ± 61 pmol/kg/min) groups(p< 0.001). In the lean DM group, hepatic insulin resistance was significantly higher(p < 0.001) than non-diabetics and T1DM groups while peripheral insulin sensitivity showed no significant difference(p=0.1). Hepatic and myocellular lipid content in lean DM was significantly less than the T2DM (p=0.001) group but was similar to the T1DM & non-diabetic groups (p=0.15) **Conclusion**: These findings show that Diabetes in "lean" Asian Indian males is characterized by reduced insulin secretion, increased hepatic insulin resistance and reduced hepatic and myocellular lipid distribution. These findings can have significant implications for devising therapeutic interventions for this intriguing form of diabetes.
Keywords : Asian Indians, BMI, Lean Diabetes , Hyperinsulinemic - Euglycaemic clamp, Mixed meal challenge test, MRS.

Maturity Onset Diabetes of the Young (MODY1, MODY2, MODY3) in Indians Affected with Gestational Diabetes Mellitus- First Report from India

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Background: Maturity Onset Diabetes of the Young (MODY) is a rare monogenic form of diabetes which is caused by single gene defect and is characterized by an autosomal dominant inheritance. Gestational Diabetes Mellitus (GDM) shows impaired beta cell function, which is also a feature of MODY. In addition, women with MODY gene mutations may get misdiagnosed as GDM. There is no data available on this from India, and so we carried out this study. **Materials and Methods**: We screened around 300 consecutive patients diagnosed with GDM & attending the endocrinology clinic of Max Super Speciality Hospital, Saket, New Delhi, over a period of 1 year (Aug 2014-2015). 50 patients were clinically suspected to have GCK-MODY and thus saliva (2ml) sample was collected from all those who agreed. We used the TruSeq custom amplicon next-generation sequencing (NGS) method & sequenced the HNF4A (MODY1), GCK (MODY2) and HNF1A (MODY3) genes in 25 patients. **Results**: We observed 1 novel mutation p.Asp344Tyr at exon 9 of GCK gene and family segregation

analysis showed this mutation to be pathogenic. Its highly conserved nature and critical location suggested that it is a MODY 2 mutation. Another novel mutation, c.1501+1G>A at the junction of exon 7-intron7 of HNF1A gene was also found, which was found to be likely pathogenic, based on available database. We also observed 5 variants of unknown significance in HNF1A gene (g.121438844T>C, g.121416650A>C, g.121431225G>A, g.121435427G>A, g.121432117G>C) and 6 variants in the HNF4A gene (c.224G>A, c.505G>A, c.493-4G>A, c.42985717G>A, c.648+4A>G, c.416C>T). Further analysis is on-going to find out if there is any co-relation between these unknown variants and GDM. **Conclusion**: Since, GCK-MODY has major implications in pregnancy and data on its prevalence from India is scarce, there is an immediate need for large-scale studies to be done. This is the first report from India on GCK-MODY and GDM.

Association of HSP-70 Variants and SDF-1β Gene Polymorphisms with Diabetic Nephropathy Among South Indian Population

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Background: Diabetic Nephropathy (DN) is the leading cause of end-stage renal disease, typically characterized by progressive albuminuria and conferring additional risk of cardiovascular disease and mortality. Numerous oxidative stress related genes confirm the association of their polymorphisms with DN. Few studies have demonstrated the crucial role of heat-shock proteins (HSPs) and Stromal derived factor-1 (SDF-1) on renal function in patients with chronic kidney disease. This study aimed to investigate the impact of HSP-70 variants and SDF-1β genes on the susceptibility of type 2 diabetes mellitus (T2DM) and DN among South Indian population. **Materials and Methods**: A total of 946 subjects (549M; 397F) were recruited and divided into four groups. Among them, 256 had normal glucose tolerant (NGT), 230 individuals with normoalbuminuria, 230 subjects with microalbuminuria and 230 subjects with macroalbuminuria. Individuals with hypertension, congestive heart defects and chronic renal disease were excluded from the study. Subjects were genotyped for HSP70-2 (+1538 A/G), HSP70-hom (+2437 C/T) and SDF-1β (+801 G/A) SNPs by PCR-RFLP. **Results and Discussion**: The "G" allele of HSP70-2 (+1538 A/G) SNP showed high relative risk for normoalbuminuria, micro and macroalbuminuria subjects. The "T" allele of HSP70-hom (+2437 C/T) SNP showed significant protection against macroalbuminuria subjects whereas the "A" allele of SDF-1β (+801 G/A) SNP didn't show any significant association with DN. **Conclusion**: Our results indicate that the HSP70-2 (+1538 A/G) and HSP70-hom (+2437 C/T) SNPs, but not SDF-1β are highly associated with renal complications in T2DM among south Indian population.

Genitics Study of PPAR-Gamma Gene in Patient with Type-2 Diabetes

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Background: Type 2 diabetes mellitus is a metabolic disorder with pathological basis characterized by insulin resistance and insulin secretion defects that can be demonstrated through several alterations in carbohydrates, lipids and protein metabolism which caused by various factors such as lifestyle, environmental and genetic factors. Previous studies have shown that in genetic factors various genes are involved in type 2 diabetic such as peroxisome proliferator-activated receptors (PPARγ). PPARγ have been identified as transcription factor that stimulate protein synthesis in a wide variety of processes (carbohydrate, protein & fatty acid metabolism, proliferation, and cellular differentiation) and also regulate action of insulin. Taking this in mind the, we want to identify PPARγ involving as single nucleotide polymorphism (SNP) at the promoter region. **Method**: We had studied around 30 samples (diabetics& normal) & extracted genomic DNA by three different methods, such as salting out method, phenol chloroform isoamyl alcohol method (PCI) & modified salting out method. Next, we amplified target gene (PPARγ) by PCR. Association of PPARγ SNPs was examined by using a restriction fragment length polymorphism (RFLP) with restriction enzyme NLAIII. **Result**. Result shows that our gene of interest PPARγ -259bp by DNA extraction, PCR amplification, RFLP shows the non-mutated PPARγ gene with diabetes but we need more number of samples to be studied for further study. **Conclusions**: The present study, designed to analyse Genetic Study of PPARγ in Type 2 Diabetes Mellitus, Diabetic Patients showed that PPARγ is not associated with type 2 Diabetes Mellitus though we have analyzed the relation of PPARγ and diabetes with less amount of samples so large amount of sample is needed to find out the particular role of PPARγ in Type 2 Diabetes Mellitus.

Identification of 18O-Isotope of Breath CO2 as a Non-Invasive Marker to Distinguish Type 1 and Type 2 Diabetes

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Background: There is a pressing need to develop a new and an effective strategy for early detection of T1D and to precisely distinguish T1D from type 2 diabetes (T2D). The aim of the present study was to find out the potential link between the erythrocytes carbonic anhydrase (CA) activity and 18O-isotopic exchange of breath CO2 in T1D and T2D. **Methods**: Fasting and post-dose breath and blood samples were collected simultaneously after ingestion of 75-gm normal glucose dissolved in 150-mL water. Blood samples were analysed to measure the CA activity. The breath samples were utilised to measure the carbon dioxide isotopes (12C16O16O, 13C16O16O and 12C16O18O) by a laser based high-precision carbon dioxide isotope analyzer. **Results**: The CA activities are markedly altered during metabolism of T1D and T2D and this facilitates to oxygen-18 (18O) isotopic fractionations of breath CO2. In our observations, T1D exhibited considerable depletions of 18O-isotopes of CO2, whereas T2D manifested isotopic enrichments of 18O in breath CO2, thus unveiling a missing link of breath18O-isotopic fractionations in T1D and T2D. The optimal diagnostic cut-off points were determined to be δDOB18O‰ = 2.1‰ and ΔCA = 3.15 U/min/mL for screening T1D and T2D individuals. **Conclusions**: Our findings suggest the changes in erythrocytes CA activities may be the initial step of altered metabolism of T1D and T2D, and breath 18O-isotope regulated by the CA activity is a potential diagnostic biomarker that can selectively and precisely distinguish T1D from T2D and thus may open a potential unifying strategy for treating these diseases.

Does Insulin Resistance Contribute To Microvascular and Macrovascular Complications In Type I Diabetes Mellitus Patients?

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Background: Insulin resistance is well known to increase cardiovascular complications in patients with type 2 diabetes mellitus. However, its role in patients with type 1 diabetes mellitus is not well established. Insulin resistance in type 1 diabetes mellitus patients as assessed by estimated glucose disposal rate(eGDR) is supposed to have an impact on the incidence of microvascular and macrovascular complications. **Material and Methods**: It was a cross sectional observational study held for a period of 9 months during the year 2015-2016 in the. An initial full evaluation was done for all the patients which included detailed clinical history, clinical and neurological examination with appropriate investigations. The eGDR was computed using the standard formula. Student's t test, χ2 test and Fisher's exact test were used. **Results and Discussions**: Our study included 66 patients with type 1 diabetes mellitus (41 men and 25 women). None of the patients with eGDR levels in the second (8.16–10.44 mg/kg−1 • min −1) or third (> 10.44 mg/kg−1 • min−1) tertiles had diabetes complications. eGDR level was significantly lower in patients with diabetic retinopathy (6.01 ± 1.2 mg/kg−1 • min−1) compared with those without (9.41 ± 2.0 mg/kg−1 • min

−1, P < 0.001), and the same occurred in those with neuropathy compared with those without (5.09 ± 0.4 vs. 9.29 ± 2.0 mg/kg−1 • min−1, respectively) (P < 0.001). With regard to renal function, again significant differences were detected in insulin sensitivity, with lower eGDR levels in patients with diabetic nephropathy compared with normoalbuminuric patients (5.82 ± 1.5 vs. 9.22 ± 2.2 mg/kg −1 • min−1, respectively) (P < 0.001). **Coclusions**: Insulin resistance and low eGDR have a negative impact on both microvascular and macrovascular complications in patients with type 1 diabetes mellitus.

Nonalcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus Patients Attending JIPMER and its Association with Cardiovascular Disease

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Aims and Objectives: To identify the occurrence of NAFLD in patients with T2DM attending JIPMER hospital and to correlate the presence of NAFLD with cardiovascular disease in patients with T2DM. **Study Design**: This was a prospective observational study. Population: 300 patients with T2DM attending the Medicine and Diabetes Outpatient Clinic in JIPMER, Puducherry were included in this study from September 2014 to May 2016. **Methods**: Patients with T2DM not consuming alcohol were included in the study. Ultrasound of the liver was done in all the patients. Patients were divided into two sub- groups; NAFLD and non- NAFLD. Electrocardiogram, echocardiography, CIMT, hs-CRP, MDA and ABPI was done in 124 patients (73 in the NAFLD group and 51 in the non- NAFLD group) to assess the cardiovascular risk. **Results**: The prevalence of NAFLD in type 2 diabetes mellitus patients was 61%. Among the patients with NAFLD, 46% had mild, 42% had moderate and 12% had severe hepatic steatosis. Cardiovascular disease was present in 58 patients in the NAFLD group and 47 patients in the non-NAFLD group. Patients with NAFLD had higher mean CIMT values (0.82 mm vs. 0.64 mm) than in the non-NAFLD group(p<0.001).The median hs-CRP level was 3.1 mg/dl in the NAFLD group vs. 1.6 mg/dl (p<0.001) in the non-NAFLD group. The mean MDA level in NAFLD group was 1.54 μmol/ml vs. 1.34 μmol/ml (p<0.001) in the non- NAFLD group. **Conclusion**: This study showed a high prevalence of NAFLD in T2DM patients.There was no correlation between the presence of NAFLD with cardiovascular disease in T2DM patients. However, there was an association between cardiovascular risk factors and NAFLD in

Postprandial lipemia in Subjects with Diabetes, Pre Diabetes and Normal Glucose Tolerance

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Background: Postprandial lipemia particularly postprandial hypertriglyceredemia(pphtg) has emerged as an important cardio metabolic risk factor with an increased risk of atherosclerosis particularly in type 2 diabetes mellitus (T2DM) patients. Whether prediabetes subjects also display significant pphtg is still unclear. **Objective**: To compare postprandial hypertriglyceredemia in subjects with normal glucose tolerance, prediabetes and diabetes melitus. **Methodology**: Sixty three age and sex matched subjects were recruited in three groups (n=21 each group) on the basis of WHO criteria following a 75gm OGTT. Group A: subjects with normal glucose tolerant (NGT), Group B: prediabetes (IFG±IGT) and Group C: subjects with diabetes mellitus (DM). A Standerdized oral fat challenge test was performed after a minimum of 12 hr fasting in all the study subjects. Fasting and postprandial triglyceride levels were measured at 2, 4, 6 & 8hrs after the fat meal. Anthropometry, plasma glucose, HbA1c, and fasting serum insulin were also measured. The groups were compared by performing one way anova by SPSS 20.0. **Results**:- Postprandial triglyceride area under the curve as well as & peak postprandial triglyceride were significantly higher in group C as compared to group B (pptgauc 2165.83±965.24 vs 1779.94±914.75mgdl-12hr-1p<0.001)(peakpptg 345.36±200.15 vs 293.42±168.44 vs 173.85 ±56.16 p<0.02) and group A(pptgauc 2165.83±965.24 vs 1204.63±300.50 mgdl-12hr-1 p<0.001)(peakpptg 345.36±200.15 vs 173.85±56.16 p<0.02). pphtg & peaktg in prediabetes (group B) were also significantly higher as compared to NGT(groupA) (pptgauc 1779.94± 914.75 vs 1204.63±300.50 mgdl-12hr-1 p<0.01) (peakpptg 293.42±168.44 vs 173.85±56.16 p<0.001). When subjects were subdivided according to gender a similar trend was obtained in male but not in female subjects. There was no significant correlation found between pptg and homa IR, BMI. **Conclusions**: Results of our study shows progressively higher postprandial triglyceride levels in subjects with prediabetes and diabetes compared to NGTs.

Can N-Acetyl Cysteine - Taurine- Provide Additional Reduction in Micro Albuminuria, in Type 2 Diabetic Patients Already on Angiotensin Converting Enzyme Inhibitors(ACEI) or Angiotensin Receptor Blockers(ARB) with or Without Dual Channel Calcium Bloc

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Background and Hypothesis: To prevent the progression of micro albuminuria to macro albuminuria and DN, we use either ACEI or ARB and or dual channel calcium blocker(Cilnidepine). These drugs have reduced MA and have prevented the progression to DN but have their limitations. Animal experiments with Taurine and NAC have been very encouraging in reducing MA,. **Objectives**: To know whether the combination of NAC and Taurine would additionally reduce microalbuminuria and TGF β expression in T2 diabetics who are already on either ACEI or ARB and or DCCB, and to know the effect of this combination on HbA1C, lipid parameters and e GFR **Material and Methods**: Eighty diabetics, having microalbuminuria were recruited .50 were in the test group and 30 were in the control group. All were examined, their height, weight, BMI, WC, BP were measured initially and at the end of 3 months. The test group was given NAC+Taurine tablets, one tab daily for 3 months and placebo was given to the control group. HbA1C, Lipid profile, Serum creatinine, Micro albuminuria and TGFb, e GFR were estimated before and on completion of the study. ANNOVA and Pearson's correlation were used for statistical analysis **Results**: 41 in the test and 21 in the placebo group, completed the study. The test group did show reduction in microalbuminuria and TGFb but not statistically significant. There was no change in SC and E-GFR. The drug did not have any effect on lipids, HbA1C **Conclusion**: The combination of NAC+Taurine has additional reduction in microalbuminuria and TGF b in those on ARB or ACEI with or without DCCB. Larger studies would be benifecial in this regard
TITLE: channel calcium blockers(DCCB)? A cross sectional, comparative, placebo controlled, observational Study.(TITLE THAT HAS BEEN LEFT OUT ABOVE)

Evaluation of Organ Specific Autoimmunity in Type 1 Diabetes

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Background: T1DM is frequently associated with other autoimmune conditions such as autoimmune thyroiditis, coeliac disease and Addison's disease. There are sparse data on the prevalence of antibodies against these conditions in Indian patients with T1D. The study aims to evaluate prevalence of these T1D associated autoantibodies in Indian patients. **Materials and Methods**: Two hundred and fifty-eight patients with T1DM were recruited from VIMS & RC and BDH for the study. Participants diagnosed with diabetes before the age of 18 years, as per the ADA criteria, and who were classified as T1DM based on clinical grounds were recruited for the study. Anti-TPO and IgA tTG were estimated in all the patients. 21-hydroxylase antibody(21-OHAb) was estimated in 170 patients. All assays were done by in-house ELISA. Eighty-eight unrelated age matched healthy controls were

chosen for comparison. **Results**: The mean age of T1D patients was 15.37 years. The mean duration of diabetes was 6.8 years. Anti-TPO was positive in 43(16.6%) patients with T1D as compared to 3(3.4%) in controls. Eighteen of these 43 patients had subclinical/overt hypothyroidism. IgA tTG was positive in 12(4.68%) patients with T1D and was absent in controls. 21-OHAb was positive in 2(1.1%) patients with T1D and was absent in controls. Both the patients who had positive 21-OHab were positive for other two antibodies. 5 patients were positive for both Anti-TPO and IgA-tTG antibodies. **Discussion**: Anti-TPO antibody was the most prevalent antibody in patients with T1D. Both Anti-TPO and IgA-tTG antibodies were significantly higher than in control population. 21-OHAb was positive in two patients . In conclusion, T1D patients should be screened for autoimmune thyroid disease and coeliac disease.

Poster Presentations

Genetics of Diabetes

Genetic Association of Interleukin -6 (IL-6) – 174 G/C Polymorphism in the Promoter Region of the Gene in Type 2 Diabetes

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Background: Type 2 Diabetes mellitus (T2DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to Chronic Hyperglycemia with disturbances of metabolism. The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030. Ample evidence supports the involvement of Interleukin-6 (IL-6) in the development of T2DM. Interleukin-6 is an anti- inflammatory cytokine that might be associated with insulin resistance, and is under strong genetic control. Therefore, we investigated the relationship of the G-C variant of the IL-6 gene promoter at position-174 with insulin resistance in disease patients in south Indian population. **Material and Method**: Genomic DNA was extracted from peripheral blood mononuclear cells of 30 T2 DM and 20 non-diabetic control study subjects. Single nucleotide polymorphisms-174 (G-C) was analyzed using polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) analysis. **Results and Discussion**: Our results suggest that 50% patients were GG homozygotes (wild type), 30% were GC heterozygotes, and 20% were CC homozygotes. Increasing evidence suggests that low-grade inflammation could be one of the determinants in the pathogenesis of insulin resistance and T2DM. **Conclusion**: We conclude from this preliminary study that IL-6 gene promoter polymorphism at position -174 G-C heterozygote may serve as a genetic biomarker for early diagnosis of T2DM patients with insulin resistance. Further, our findings are consistent with a role for genetic determinants of inflammation in the development of T2DM.

Auto-Immune Hypoglycemia-A Cause Beyond Diabetes- A Case-Report

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The maintenance of blood glucose (BG) levels closely depends on coupling of insulin-insulin receptors and BG levels. Any disruption leads to BG imbalance. Auto-immune hypoglycaemia is one such cause for BG variation attributed to humoral auto-immunity,i.e. auto-antibodies against insulin or insulin receptors. Since, it is independent of exogenous insulin administration; AIH is attributed to non-diabetics. Incidence of AIH in Japan is quite high, but in India it is a prevalent condition requiring prompt diagnosis to avoid unnecessary investigation and management. **Case study**: A 62-year-old male presented to emergency with c/o altered sensorium, having no history of diabetes, but was a known hypertensive. Examinations revealed BP was 150/90mmHg and RBS was 40mg/dl. Further to that, continuous blood-glucose monitoring with CGMS-Device was done for 24–48hrs revealing persistent hypoglycaemia warranting further investigations via endoscopic USG, CT and MRI Scan to locate any insulin-secreting tumor and anti-insulin antibodies mean while. **Results**: With the presence of hypoglycaemia and BG levels as low as 25mg/dL, serum insulin–11455mU/L(3.00 – 25.00mU/L) and C-peptide–18.98ng/ml (0.81 – 3.85ng/mL), the motive was to identify the exact cause. He showed no evidence of tumor or immunity-altered disease, but anti-insulin antibodies were positive, confirming AIH requiring appropriate management. **Conclusion**: Elevated C-peptide, Serum Insulin levels and positive anti-insulin antibodies highlights the incidence of Auto-Immune Hypoglycamia. He was treated with Diazoxide 50mg and Prednisolone 20mg, later discontinuing Diazoxide and tapering the dose of Prednisolone. After 6 months,the patient was switched to Hydrocortisone 5mg. On regular follow-up, he was stable indicating positive steroid response (PPBS-119mg/dL).Thus, suspecting AIH at the right time is essential to avoid any invasive surgical procedures.

A Single Nucleotide Polymorphism in KCNQ1 Gene and its Association with Susceptibility to Diabetic Nephropathy in Subjects with Type 2 Diabetes in India

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Introduction: Diabetic nephropathy (DN) remains the most common cause for end stage renal disease (ESRD) as only 25 to 40 % of patients with T2DM develop DN irrespective of glycemic control, so there should be specific genetic background for DN. **Objective**: To find out the association of single nucleotide polymorphisms(SNPs) of rs2237897 within KCNQ1 gene with diabetic nephropathy in subjects with T2DM. **Method**: Venous blood samples of 50 cases (DN) and20 controls (T2DM without nephropathy) diagnosed by spot urine albumin creatinine ratio (ACR) was collected and PCR sequencing done to detect gene polymorphism. **Result**: Statistically significant difference found when the two groups were compared (p=0.03), with the C allele having a 2.4 fold higher risk of having Diabetic Nephropathy (RR) =1.4, 95% CI of RR = 1.1 to 1.9 Odds Ratio(OR) =2.4.Chi-square analysis showed significant difference in genotype frequency of rs2237897 ($\chi 2 = 4.63$, p=0.03) in DN subjects, compared with that of controls. **Conclusion**: Our study suggests that, in addition to KCNQ1 being an established T2DM gene, genetic variation in this gene may contribute to susceptibility to diabetic nephropathy and the C allele was the risk allele for DN, which is different from Japanese population where the T allele was risk allele.

Pathophysiology of Diabetes

Study of Serum Ferritin and Glycated Hemoglobin in Type 2 Diabetes Mellitus

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Background: Type 2 diabetes mellitus is a common metabolic disorder of multiple etiologies. Increased levels of serum ferritin have been indicated to be associated with the etiology of the diabetic process, as well as

in pathogenesis of various diabetic complications. The study aims to understand the relationship between the serum ferritin and glycated hemoglobin in type 2 diabetes mellitus. **Objectives**: To compare the levels of serum ferritin in patients with type 2 diabetes mellitus and healthy individuals. To know if any correlation exists between serum ferritin and glycated hemoglobin in type 2 diabetes mellitus **Methods**: The study was conducted at Navodaya Medical College Hospital & reseach center, Raichur from January 2015 to December 2016. A total of 50 cases of type2 diabetes mellitus of the age group 30 – 70 years were taken for the study after satisfying the inclusion and exclusion criteria. Fifty healthy volunteers in the age group 30 – 70 years during the same period were included in the study under the control group. All patients were evaluated in detail and serum ferritin level was estimated by microplate immuno enzyme metric assay and glycated hemoglobin (HbA1C) by particle enhanced immunoturbidimetric test. **Result**: Serum ferritin level was significantly high in cases compared to controls. There was moderate correlation between Serum ferritin and glycated hemoglobin. **Interpretation and Conclusion**: There was significant increase in serum ferritin levels in type 2 diabetics compared to the controls. There was a moderate correlation between serum ferritin and glycated hemoglobin. This study explores the possibility of finding serum ferritin as a marker to explain the oxidative stress process in type 2 diabetes mellitus. This valuable information would be helpful in proper medical intervention.

Factors Associated with Insulin Adherence Among Patients with Type 2 Diabetes (T2D): The MOSAIc Study

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Background: Although insulin is the most effective glucose-lowering therapy, adherence varies widely. Few studies have investigated this issue over an extended period. **Material and Method**: This analysis identified factors associated with insulin nonadherence within MOSAIc, a 2-year prospective cohort study. Patients with T2D, aged ≥18 years, and taking insulin for ≥3 months in 18 countries were included. Demographic, clinical, and self-reported data were collected at baseline and over 2 years. Insulin nonadherence was defined as missing any insulin injections within the past 7 days of a clinic visit. Multivariable logistic regression and multiple imputation were used in the analyses. **Results and Discussion**: Among 2706 patients: mean (SD) age, 62.1 (10.8); female, 50%; and nonadherent at the end of study, 608 (29.3%). These patients were younger (p<.0001), had lower diabetes knowledge test scores (p=.04), were likely to be nonadherent at baseline (p<.0001), used mixed insulin (p=.0003), injected >1 time daily (p=.001), had a worse experience with their insulin delivery system (p=.01), and had poor communication with their physicians vs adherent patients. After adjustment, age and baseline insulin nonadherence remained significantly different between the groups. **Conclusion**: Among patients with T2D utilizing insulin, younger patients with a history of poor adherence are less likely to be adherent over time. **Disclosures**: This study was supported and conducted by Eli Lilly and Company, Indianapolis, IN, USA. This is an encore of an abstract that was presented at the American Diabetes Association – 76th Annual Scientific Sessions; June 10 – 14, 2016; New Orleans, LA, USA.

Structured Care Plan Improves Metabolic Control

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Background/Hypothesis: Structured multi-disciplinary approach is needed for good diabetes management. We have comprehensive care plan which involves management of blood sugar with remote monitoring, structured visits to the clinicians and screening for all complications. We wanted to analyze if structured multi-disciplinary care is effective in improving diabetes control. **Materials and Methods**: Subjects who were taking part in comprehensive care plan for diabetes in our centre were studied. As a part of comprehensive care they received medical advice by doctors and diabetes specialist nurses, counseling by nutritionist and 24/7 support by remote monitoring team. Electronic records of all patients examined to see if there was improvement in HbA1c and other parameters. **Results and Discussion**: Data on 256 subjects [Mean age 52.7 (+/- 13.4) years & 152 (59.4%) males], who were enrolled for comprehensive study were analyzed. The Hba1c at enrolment was 9.5 (+/- 2.2) % which reduced to 8.0 (+/- 1.5) % within 3 months ($p < 0.0001$). This was maintained till 12 months. There was no difference in lipids, BMI and blood pressure during this period. **Conclusion**: Our data suggest that structured comprehensive care plan improves metabolic control without any impact on body mass.

A study of Serum Magnesium Levels in Type 2 Diabetes Mellitus and Its Complications

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Aim: To study the relationship of serum Magnesium levels in Type 2 diabetics. **Objectives**: 1) To know the relationship between Mg levels and diabetes and its association with level of control of diabetes 2) Mg levels in relation to micro and macro vascular complications of diabetes **Materials and methods**: Cross sectional study-September 2014-16 in Kasturba Hospital ,Manipal Sample size:108 Cases :Type 2 diabetic patients Controls :Non diabetics(age and sex matched) Serum Mg levels assessed by Calmagite Dye method **Results**:The mean Mg levels in cases and controls was 1.88 mg/dl and 2.1 mg/dl with p value of <0.003 which is significant. Hypomagnesemia was observed in 38.8% of our cases Our results were consistent with previous study done by AP Jain et al. which shows low Mg levels in poorly controlled diabetics compared to controlled group. More the duration of diabetes and FBS ,lower was Mg levels. In the present study there was significant association of low serum Mg levels with diabetic retinopathy and nephropathy ,while no correlation with respect to neuropathy. **Conclusion**: Mg2+ supplementation improved insulin sensitivity and metabolic control in a double-blind randomized trial done by Guerrero R F et al suggesting that Mg2+ is an important factor in etiology and management of T2 DM. So far,clinical trials that were performed on Mg levels mainly focused on general parameters like blood glucose, HbA1c.Therefore,well-designed trials studying long term effectsof Mg supplementation on pathophysiology and disease progression in T2DM are now warranted.

Postprandial Triglyceride Responses and Insulin Resistance Among Night Shift Health Care Workers

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Background: A higher cardiometabolic risk has been reported in night shift workers which could be secondary to an abnormal postprandial triglyceride metabolism in them. **Aim and Objectives**: In this study we compared the postprandial triglyceride responses to standard oral fat challenge between

health care night shift workers and non- night shift workers to ascertain if it contributes to cardiometabolic risks in them. **Methods**: 20 health care night shift workers (>=4 nights duties/month for last one year), aged 20 to 40 years with normal glucose tolerance (NGT) following a 75 gram glucose OGTT and 20 age and sex matched non-night shift workers who had not done night duty in the last one year or ever were recruited. Postprandial triglyceride responses were obtained after a standard fat meal given at 8 am, and sampling done every two hours for next 8 hours, that is at 0, 2, 4, 6 and 8 hours and compared between the two groups. **Results**: Night shift and non-night shift workers were matched for age (29.70 ± 3.92 years vs 29.70 ± 2.23 years) and sex (M:F 12:8 vs 12:8). The duration of night shift exposure was relatively short (4.2 ± 3.3 years) among cases. Postprandial triglyceride measures-PPTG area under the curve (TG AUC) and TG peak values were comparable in both the groups. PPTG response showed significant positive correlation with fasting insulin and HOMA-IR indicating insulin resistance in night shift health care workers but not in others. **Conclusions**: Post prandial triglyceride burden is significantly associated with insulin resistance in night shift workers even with a relatively short duration of night shift exposure.

The Relationship of Serum Lipids with Family History of Type 2 Diabetes, Insulin Sensitivity, Anthropometry in Normoglycemic Subjects

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Aim: This cross-sectional study analyzes relationship of serum lipids with s family history of type 2 diabetes, body mass index, waste to hip ratio, insulin sensitivity and beta cell function by multiple linear regression analysis. **Subjects and Methods**: The sample frame was the "Offspring of individuals with diabetes study" database. A total of 560 subjects, who have normal glucose tolerance (301 cases and 259 controls) were selected for the analysis. All participants underwent 75g OGTT, blood samples were collected at 0, 30, 60 and 120 minutes for insulin, C-peptide and proinsulin. Serum lipds were measured in fasting sample. **Results**: In a univariate linear regression analysis, low HDL cholesterol was related with male gender, family history of DM, high BMI, triglyceride, area under the curve (AUC) of C-peptide and AUC of proinsulin. In a multivariate regression analysis, a male gender, a positive family history of DM, high waist to hip ratio and high triglyceride levels remained significantly related to low HDL cholesterol. Even if waist to hip ratio is not included in multivariate analysis, BMI was not significant in multivariate analysis. On a multivariate regression analysis, a higher total cholesterol was related to higher age, AUC of proinsulin and triglyceride levels. On a multivariate analysis higher TG were related to, having a male sex, a high area under curve of proinsulin and glucose, high BMI, and high total cholesterol. In a multivariate analysis, a high LDL cholesterol was related to higher age, AUC of proinsulin and waist to hip ratio. **Conclusion**: The study shows the prominent effects of age, beta cell secretion, family history of diabetes, waist to hip ratio on serum lipids in comparison to BMI which only showed prominent effect only on triglyceride levels.

Correlation Between Glucagon Like Peptide 1 Levels with Beta Cell Function; Insulin Resistance; Insulin Sensitivity in Pre-Diabetes / Newly Diagnosed Diabetes Mellitus

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Background: The phenotype of the Indian diabetic is different from that of the western counterpart. The relative importance of insulin resistance insulin secretory defect and deficiency of GLP-1 in the development of diabetes in Indian patients is not clearly understood and there is a need for studies examining the relative contributions of these abnormalities in Indian patients developing type 2 diabetes. **Material and Method:** Consecutive subjects with newly diagnosed diabetes were included. All enrolled subjects underwent a standard 75g glucose OGTT. Plasma glucose, total GLP 1 levels and plasma Insulin levels were measured in both fasting and 120 min after OGTT. The homeostasis model assessment of β cell function (HOMA-β) was used to evaluate basal insulin secretion. The HOMA assessment of IR (HOMA-IR) was used to estimate IR and Matsuda index was analyzed for insulin sensitivity. **Results and Discussion:** Out of the 50 subjects enrolled, the data was completer for 41 sujects, and they were categorized in to normal glucose tolerance (NGT) (n=10), pre-diabetes (PD) (n=16) and newly detected diabetes mellitus (NDDM) (n=15). The mean F GLP 1 in NGT is 13.35±15.36 pmol/L, in PD is 23.14±15.17, in NDDM is 24.61±16.06 (P = 0.186). The mean 2h GLP 1 in NGT group is 11.77±8.32, in PD is 29.10±17.97, in NDDM is 23.70±17.23 (P = 0.035). The mean HOMA β in NGT is 68.92 ±34.89, in PD is 79.65±67.91, in NDDM is 27.50±27.84 (P = 0.015). The mean HOMA IR in NGT is 0.67±.35, in PD is 1.42±.76, in NDDM is 2.43±1.53 (P = 0.001). The mean Matsuda index in NGT is 27.25±29.41, in PD is 7.69±3.77, in NDDM is 6.69±41.3 ((P = 0.003). There is an inverse correlation between GLP 1 and HOMA β (P = 0.245), and HOMA IR (P = 0.802), and Matsuda index (P = 0.277). **Conclusion:** Insulin resistance was increased in prediabetes and diabetes groups while the insulin secretion was decreased in the diabetes group, highlighting the role of these two factors in pathophysiology of T2 diabetes. However, there was no correlation of GLP 1 levels in prediabetes or diabetes groups indicating that there could be variability in the incretin effect among different populations and this also underscores the need for larger studies to evaluate the relative contributions of these factors in the pathophysiology of diabetes.

Epidemiology of Diabetes and Its Complications

A Cross-Sectional, Multi-Centric, Epidemiological Study of Diabetic Neuropathy and Associated Co-Morbidities in Type 2 Diabetic Patients in India

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Objectives: Diabetic neuropathy, one of the most common microvascular complications affects nerves due to hyperglycemia in patients with type 2 diabetes (T2DM). This cross-sectional study was aimed to understand the clinical presentation of diabetic neuropathy; types of neuropathies; associated co-morbidities and risk factors; and treatment patterns for T2DM and diabetic neuropathy in India. **Methods**: This was a single-visit, cross-sectional, multi-centric, epidemiological study conducted at 363 centres. Adult T2DM patients with neuropathy were included. Patients with any other neurological disorder that could mimic symptoms of neuropathy; pregnant or lactating women and those with significant pain were excluded. Data collection included demographics, lifestyle habits, medical history, treatment regimens for diabetes and neuropathy, concomitant medications and laboratory investigations. **Results**: A total of 7172 patients were enrolled with mean age of 52.8 ± 8.04 years, majority being males (58%). The prevalence rates of painful and painless diabetic neuropathy were 49.1% and 50.9%, respectively. The median duration of T2DM was 6 years (range 0.1 to 35 years) and neuropathy was about 2 years (range 0.1 to 30 years). The most common types of neuropathies reported were acute sensory neuropathy (32.3%) and chronic sensorimotor neuropathy (31.4%). Reported symptoms ranged from numbness (30.7%), to paraesthesia (29.2%), and burning sensation (28.0%). Majority of the patients had uncontrolled glucose parameters (Fasting plasma glucose [> 100mg/

dL]: 90.1%, post-prandial plasma glucose [> 140 mg/dL]: 90.5%, glycosylated hemoglobin [>7%]: 69.8%) and lipid profile (low density lipoprotein cholesterol [>100 mg/dL]: 65.5% and triglycerides [>150 mg/dL]: 61%). Hypertension was the most prevalent co-morbid condition reported in 15.9%. Almost two-thirds (61.3%) were treated with metformin as monotherapy or in combination with other anti-diabetic drugs. More than half (52.3%) received mecobalamin for treatment of diabetic neuropathy. Higher proportions of patients with painful neuropathy were prescribed pregabalin as compared to painless (32.18% vs 19.79%). **Conclusion**: In conclusion, diabetic neuropathy is painful in almost half of the Indian patients with T2DM. Acute sensory neuropathy occurs in most of the patients. Onset of diabetic neuropathy could be much earlier than expected and hence, routine screening is recommended. Poor glycemic control and hypertension are the potential risk factors for diabetic neuropathy. Metformin and mecobalamin are commonly prescribed for the treatment of diabetes and diabetic neuropathy, respectively. Pregabalin is a preferred treatment option for painful diabetic neuropathy.
Keywords : type 2 diabetes mellitus (T2DM), diabetic neuropathy, metformin, mecobalamin, pregabalin

The National Diabetes Registry in India

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Objectives: Currently available data is limited in estimating/understanding the demography of diabetes patients across India. This registry was planned to create a database of diabetes across India to understand the diabetes profile, the associated complications, comorbidities, treatment paradigms and socio-economic impact aspects across India. Secondary objectives were to assess different aspects involved in the management of diabetes and glycemic indices in Indian diabetics. **Methods**: A multicenter, observational, non-interventional, 6-month follow-up registry was conducted in 26 states across India involving 2944 male and female patients with type-2 diabetes mellitus between 18-75 years of age. Detailed medical history, profile of patients, diet patterns and lifestyle methods were captured. The laboratory parameters like hemoglobin (Hb), fasting blood glucose (FBG), post-prandial blood glucose (PPBG) and glycosylated hemoglobin (HbA1C) were captured at enrolment, 3 months and 6 months. Descriptive analysis was performed on data for all patients. **Results**: Out of total 2944 patients, data of 2849 (96.77%) patients were considered for analysis. The mean age of patients with diabetes was 52.9 years with mean diabetes duration of 5.8 years. Majority (81%) of the patients were from Tamil Nadu (13.1%), Kerela (12.3%) and Maharashtra (11.6%). About one in four diabetics are hypertensive (24.05%) and majority were from the upper middle socio-economic strata (42.6%). About 15.8% patients were never advised lifestyle modifications and non-pharmacological interventions during the physician interaction. Metformin was the most commonly used oral hypoglycemic drug (58.53%) followed by glimepiride (35.87%); whereas a combination of metformin and glimepiride was used in 16.98% patients. Triple drug therapy is used in 4.86% patients and insulin in 9.21% patients. Good glycemic control (HbA1c<7%) is observed only in 20.8% and 23.4% patients at month-3 and month-6 respectively. Non-compliance to diabetic diet is found in 8% individuals. The most common cause of non-compliance is lack of motivation (5.54%), lack of information (2.28%), busy job schedules (1.94%) and financial reasons (1.56%). **Conclusions**: The one diabetes registry helps in understanding the patient flow, comorbid conditions and compliance to therapy from Indian perspective.
Keywords : type-2 diabetes mellitus national registry India.

Prevalence of Type 1 DM in Suburban Population in South India

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Study population in South India

On behalf of Diabetes Club of Tiruchirappalli

The study was done to assess the prevalence of Type 1DM among school children aged 3 to 18 years in a sub urban population. (Manikandam Panchayat union ,Covering 167 villages with a total population of one lakh, Trichy , Tamilnadu , South India) Prevalence of Type 1 DM was based on screening of all school going children in the whole region in Manikandam Panchayat union. Data was collected between 20th June to 17 September 2013. Data of 22,934 school children aged 3 to 18 year was collected by a questioner collected by investigators from children, parents and teachers, in the age group of 3 to 18 years . Doubtful cases was screened for capillary blood glucose by glucometer. Children found to have Diabetes was confirmed by venous sample for FBG, PPBG, HbA1c, C peptide assessments, and GAD antibody measurements Based upon clinical evaluation, laboratory investigations and c Peptide measurements 7 children were found to have Type 1 DM and were on treatment. 7 children were detected among 22934 children. The prevalence being 0.03% among the sample. 6 were male and 1 was female in the age group of 8 to 13yrs, all belonging to the lower or middle income group and 7 children per l lakh population. While extensive work on Type 2 DM is available in our country less work is done on Type 1 DM. There are only few reports from India. According to IDF Atlas incidence rate is around 4.2 /lakh. Few other Type 1 registry based prevalence varies from 4 to 10.5/Lakh. Our study also shows a similar prevalence of 7/lakh. Even though we have covered each and every school going child in the whole region, any child not attending school or studying elsewhere outside the locality are likely to have been missed. To conclude our study reveals a prevalence of Type 1DM in sub urban population near Trichy, South India to be 7 per lakh population in the age group of 3 to 18 years among school going children.

A Study of Microalbuminuria in Newly Diagnosed Type 2 Diabetes Mellitus

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Introduction: One of the most severe complications of Diabetes is Diabetes Nephropathy leading to end-stage renal disease (ESRD). Nearly 30% of chronic renal failures in India are due to diabetic nephropathy. Microalbuminuria, the earliest clinical evidence of nephropathy, if addressed can retard, or reverse, the progress of the disease. We aimed to study the presence of microalbuminuria in newly diagnosed Type 2 Diabetes Mellitus and to correlate it with patient's clinical profiles. **Materials and Methods**: Newly diagnosed type 2 diabetic adult patients who attended our tertiary health care center over a period of one year was included. Known diabetics for more than 6 months, younger than 20 years, primary and secondary renal disease, BP >160/100 mmHg, hypertensive patients on ACE/ARB and other confounding factors were excluded from the study. Micral Test, which is a semi-quantitative rapid dip stick test containing Monoclonal Antibodies Anti human albumin IgG labeled with colloidal gold (6μg/cm2) and fixed albumin (9.5μg/cm2), was used for estimation of microalbuminuria. **Results**: 104 patients were included in the study with 62 (59.6%) males and 42 (40.4%) females.

Mean age of patients was 49.48 + 11.9 years. 21 (20.2%) patients were found to have microalbuminuria.11(58.4%) were males and 10(47.6%) were females. 57.14% microalbuminuric patients were seen to have HbA1c level >8%. Fasting, post-prandial and random blood sugar was higher in patients with microalbuminuria (p=0.01) and so was mean blood urea , serum creatinine, mean systolic and diastolic blood pressure, BMI, serum cholesterol, LDL cholesterol and triglyceride. **Conclusion**: Screening of microalbuminuria at diagnosis and periodic evaluation of urine albumin in addition to HbA1c and other clinical profiles as mentioned in the current study may be considered in all newly diagnosed type 2 diabetic patients.

Prevalence and Predictors of Hyperglycaemia in Various Metropolitan Cities of India: A Nation-Wide Diabetes Surveillance Study

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Background: Hyperglycaemia is an important risk factor for developing micro and macro vascular complications ¬¬-and influences patient outcomes as proven by DCCT, UKPDS, and other long term trials. **Objective**: To assess the prevalence of hyperglycaemia in patients with established diabetes mellitus (DM) across India as determined by a random capillary blood glucose test (CBG). **Methods**: We undertook a cross-sectional survey to assess the prevalence of hyperglycaemia among diabetic patients. Participants across various cities of India were interviewed using a structured questionnaire for a history of diabetes and tested with random CBG. Hyperglycaemia was considered as a random CBG >180 in a person with known diabetes. **Results**: Prevalence of hyperglycaemia is presented as percentage (95% confidence intervals). Predictors for hyperglycaemia were assessed using logistic regression analysis and presented as odds ratio (OR) (95% confidence intervals). A total of 41,696 patients with DM were screened. Mean age of the subjects was 53.5 (SD 11.8) years, 78% were male. Mean CBG levels were found to be 198(SD, 81). 49.5% (n=20,619) of patients were found to have hyperglycaemia (RBG>180 mg/dL). Women (OR 0.908 (0.866-0.952)) (p<0.01), younger age (OR 0.994 (0.992-0.996)) (p<0.01), family history of diabetes (OR 1.645 (1.546-1.751)) (p<0.01) predicted hyperglycaemia. BMI (OR 0.999 (0.985-1.014)) (p 0.915) and hypertension (OR 1.300 (0.977- 1.730)) (p 0.072) did not have a significant relationship with hyperglycaemia. **Conclusion**: The study confirms that there is a high prevalence of uncontrolled hyperglycaemia in patients with established DM. These results highlight the challenges of delivering efficient diabetic care across India.

Prevalence of Diabetes in a Rural Community of North Karnataka: A Cross Sectional Study

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Background: India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". The International Diabetes Federation atlas 2015 states that globally 1 in 11 adults have diabetes and in India the prevalence of diabetes is >10%. Higher levels of diabetes have been reported in urban areas of India, but few data are available for rural regions where >70% of the population lives. **Materials and Methods**: The present cross sectional study was conducted in Vantamuri Primary Health Centre (PHC), a field practice area of Jawaharlal Nehru Medical College, Belgaum, Karnataka during December 2014. The target population consisted of all people aged 40 years and above. The health workers collected the data using a schedule which consisted of demographic information, coexisting medical conditions and family history.Urine analysis for sugar was done and interpreted by trained interns using uristix method.Individuals with a positive urine test were subjected to blood sugar examination by glucometer in the PHC. The results of glucometer were interpreted by the Medical officer. Analysis was done using SPSS 18 trial version and statistical test used was Chi-square. **Results**: Among the 2553 individuals surveyed, 851 were males and 1702 were females. 151 were diagnosed to have diabetes by blood glucose levels, out of which 62 (41.1%) were males and 89 (58.9%) were females. Hence the overall prevalence of diabetes was 5.91% (151), the prevalence in males being 7.28% and in females 5.22%. Age, BMI, hypertension and family history were found to be significantly associated with diabetes. **Conclusion**: Diabetes in rural areas needs special attention. Focus on health education related to diabetes and other NCD is the need of the hour in rural areas.

A Study to Evaluate Different Conditions Leading to Diagnosis of T2DM in a Tertiary Care Hospital

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Background: Diabetes is one of the most important public health problems. There is paucity of Indian data as to which conditions leads to diagnosis of T2DM. The present study endeavors to throw some light on the problem. **Material and Method**: A cross-sectional and retrospective study, performed at a tertiary care hospital. Pooled Chi-square/Fischer's exact test used to explore association between study variables. **Results and Discussion**: A total of 321 patients (male: female – 2.61: 1) diagnosed with diabetes over a period of 24 months were included. Mean age at diagnosis being 45.54 ±10.8 years respectively. Out of these, only 23.05 % presented with osmotic and other symptoms (foot ulcer, weight loss etc.) suggestive of diabetes. A substantial 43.52% of total patients were diagnosed during general healthcare check-up (35.2% in OPD and 8.32% during perioperative evaluation). Remaining patients diagnosed with diabetes included 8.28% patients admitted with acute coronary syndrome, 13.4% with infections of different types and 11.75% with other non-related diseases. Among diagnosed diabetics, 50.47% had HTN and 41.12% had IHD. Diabetic Males have higher prevalence of IHD (48.28%) than females (22.47%), p<0.001. Prevalence of HTN is higher among diabetic females (48.28%) than males (22.47%), p<0.046. The incidence/prevalence of HTN and IHD steadily increased with the increasing age, p<0.001. Conclusion: It is prudent to evaluate adult patients coming in contact with healthcare system for diabetes by simple inexpensive test.

Prevalence of Metabolic Syndrome in Type 2 Diabetes Mellitus in Rural Areas of Western Uttar Pradesh

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Bckground: Metabolic syndrome describes a clustering of factors including dyslipidemia, glucose intolerance and hypertension with central obesity. The metabolic syndrome has a marked impact on the prevalence of cardiovascular disease and type 2 diabetes worldwide. Aim of study: The aim of study was to determine the prevalence of metabolic syndrome in people with type 2 diabetes mellitus using National Cholesterol Education Program (NCEP) ATP III Criteria, International Diabetes Federation (IDF) and the World Health Organization (WHO) definitions. **Methods**: This Population-based cross-sectional study involved 630 type 2 diabetic subjects from the rural areas of Etawah and neighbouring regions of western UP. Subjects in the age group of 26-65 yrs were included in the study. Type I diabetics, pregnant ladies and those with chronic viral and bacterial infections and serious metabolic disorders were excluded from the study.Fasting blood glucose, Blood lipids (T-

cholesterol, triglyceride, HDL-cholesterol) were assessed and anthropometry and blood pressure were measured from all the subjects using standardized methods **Results**: The Prevalence of metabolic syndrome was found to be 54.3 %, 46.03% and 57.8 % following Modified NCEP-ATP III Criteria, IDF and WHO definitions, respectively. Using all the three definitions the prevalence was higher in women. Highest prevalence was observed following WHO definition. **Conclusions**: This study reveals a high prevalence of metabolic syndrome amongst the type 2 DM patients in rural western UP. Maximum prevalence of Metabolic syndrome was recorded when WHO criteria was followed. There are very few studies measuring the prevalence of metabolic syndrome in rural diabetic population This provides opportunity for evaluating the epidemiology and formulating preventive strategies for preventing the clustering of cardiovascular risk factors.

Baseline Characteristics of Patients Initiating Insulin Treatment for Type 2 Diabetes in the Western Pacific: Evidence from the Verifying Insulin Strategy and Initial Health Outcomes Analysis (VISION) Study

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Background: VISION is an 18-month, 9-country, prospective observational study of patients initiating insulin therapy as part of routine practice. This ongoing study assesses treatment approaches/decisions, clinical effectiveness of insulin therapy, cost/resource use, treatment patterns, and patient demographic/clinical characteristics. This report describes the baseline characteristics, including patient-reported outcomes, of patients from the Western Pacific. **Material and Method**: The Western Pacific region included 1025 patients from Thailand, Malaysia, Philippines, Taiwan, and Hong Kong. Assessed baseline variables included HbA1c, questionnaires (satisfaction with medication, Expectations about Insulin Therapy Questionnaire [EITQ]), and initial insulin regimen. **Results and Discussion**: Mean HbA1c at initiation was 9.69% (Thailand), 9.86% (Malaysia), 9.87% (Philippines), 9.93% (Taiwan), and 10.57% (Hong Kong). The proportion of patients dissatisfied with their medication varied: 7.5% (Thailand), 16.2% (Malaysia), 25.6% (Taiwan), 27.5% (Hong Kong), and 43.9% (Philippines). Mean EITQ scores were 44.0 (Taiwan), 45.5 (Hong Kong), 48.6 (Philippines), 48.7 (Thailand), and 49.2 (Malaysia). Initial regimen varied; premixed insulin was more commonly prescribed in Malaysia, Philippines, and Thailand. Basal insulin was more commonly prescribed in Taiwan and Hong Kong. No patients were prescribed basal-bolus insulin at initiation. Few patients in Malaysia and Philippines were prescribed basal+prandial insulin at initiation. **Conclusion**: There were several differences by country in baseline characteristics that may affect treatment requirements/clinical outcomes. **Disclosures:** This study was supported and conducted by Eli Lilly and Company, Indianapolis, IN, USA. This is an encore of an abstract that will be presented at the International Diabetes Federation – 11th Western Pacific Region Congress; October 27 – 30, 2016; Taipei, Taiwan.

Analysis of Morbidity Among Diabetics Attending Outpatient Department in an ESI Hospital in State Capital of Telangana, India

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Background: Diabetes is a major threat in modern India. It increases the risk of different type of infection including Respiratory Tract Infection (RTI). Poor glycaemic control increases the morbidity. **Material and Method**: A retrospective analysis of outpatient record of diabetics was conducted between Jan-June 2016. The data was analysed for HbA1C level, peripheral neuropathy status & presenting illness. **Result and Discussion**: A total of 414 known diabetic patients attended the ESI clinic for their illness & routine visit during six months of data accumulation. In the cohort, 52.2% were female; according to age distribution 45.7%, 26.1% & 28.3% were in <50, 50-59 & 60< years-range respectively (Mean=50.9 years) and 71.7% were suffering from peripheral neuropathy. Among the group, 65% attended with current HbA1C report (mean=7.85, Range: 6.3-11.1). 41.3% of the attended diabetics presented with chief complaint of different infections (32.6% with RTI including pneumonia, 6.5% with UTI & 2.2% with other infections). Both HbA1C level & Age had shown significant ($p<0.01$) positive correlation with the infections observed in the group (Pearson Correlation coefficient of 0.717 & 0.557 respectively). **Conclusion**: Peripheral neuropathy is one of the major long term challenges in diabetic patients. Respiratory Tract Infection & UTI are common illness among the diabetics to seek medical advice. Prevailing HbA1C level & age are directly proportional to their morbidity. **Acknowledgement:** Authors would like to thank Dr Partha De & Dr Sudhanshu Pandey for their contribution in data analysis & manuscript development.

One in 25 People in Shopping Mall have Undiagnosed Hyperglycaemia

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Background/Hypothesis: The number of people with diabetes in India is increasing due to population growth and aging, urbanization, increasing prevalence of obesity and physical inactivity. Early diagnosis can prevent complications but there is no screening programme in India. As a part of World Diabetes Day, we screened people who visited shopping mall to know the prevalence of diabetes and impaired glucose tolerance. **Materials and Methods**: Subjects were invited to attend screening for blood glucose within the store where they were shopping. Diabetes (DM) was defined as random blood glucose of 200mg% or more. Impaired glucose (IG) was defined as those subjects with random blood glucose of 140 mg% or more. **Results and Discussion**: 41457 subjects were screened with capillary blood glucose at 50 Landmark Stores across 6 cities in India over a 10 day period. 3822 had diabetes and were excluded. Of the remaining 37642 subjects, 390 (1.04%) had DM and 1100 (2.92%) had IG. Of the 12247 subjects below the age of 25 screened, newly diagnosed DM & IG were present in 24 (0.2%) & 93 (0.76%) subjects respectively. **Conclusion**: There is a need for structured screening programme for diabetes in India in order to diagnose this condition early and prevent its chronic complications. There is high prevalence of diabetes in younger population so screening at the site of their preference such as shopping mall should be considered.

Prevalence of Chronic Periodontitis in Type II Diabetes Mellitus

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Background: Diabetes mellitus is a metabolic disorder with several complications affecting both the quality and length of life. Periodontal disease is a chronic inflammatory condition that elicits considerable impact on systemic disease. One such systemic condition of global importance is diabetes mellitus of which type II diabetes has more prevalent. Though

India is considered as Diabetic Capital, there is inadequate data in this regard from our country. Hence our objective was to study the prevalence and severity of periodontal disease in type II diabetes mellitus patients. **Materials and Methods**: 302 type II diabetic patients belonging to the age group of 35-75 years were included in the study. The study group was divided based on Glycated hemoglobin level into well, moderate and poorly controlled Diabetes mellitus. Information regarding oral hygiene and personal habits was obtained. Plaque index(PI) and Community periodontal index (CPI) was assessed to evaluate oral hygiene and periodontal status. The results were statistically evaluated. **Results**: The mean CPI score and the number of missing and mobile teeth were statistically significant ($p<0.05$), indicating that prevalence and extent of periodontal disease were more severe in diabetic patients. There is a positive correlation with Glycated hemoglobin, duration of diabetes, oral hygiene habits with periodontal destruction. **Conclusion**: This study has made an attempt to determine the association between type II diabetes mellitus and periodontal disease. It was found that type II diabetes mellitus subjects manifested relatively higher prevalence (70.5%) and severity of periodontal disease.

Largest Global Real World Settings study Discovering Treatment Reality of Type 2 Diabetes (DISCOVER)

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Aims and Objective: The aim of this multinational study is to describe the disease management patterns and clinical evolution over three years in type 2 diabetes mellitus (T2DM) patients initiating a second line anti-diabetic treatment, either as add-on, or switching from one monotherapy to another. **Methodology**: The study has enrolled over 15 000 patients in 38 countries across six continents, and will provide a comprehensive and contemporary picture of treatment patterns and outcomes in patients with type 2 diabetes worldwide. India has completed patient recruitment with approx. 3150 patients and has become No.1 in term of patient recruitment across the globe. Patients with T2DM initiating their second line anti-diabetic therapy after first line diabetic therapy was the target subject population. Data collected during follow up is expected to provide the details of Patient characteristics; demographics, Vital signs and lab tests, Medical history of T2DM, including presence of risk factors, Co-morbidities and co-medications, Changes in diabetes treatments during follow-up and reasons, Number of major hypoglycemic events, occurrence of minor hypoglycemic events, Microvascular complications (nephropathy, retinopathy, neuropathy and amputation) and macrovascular complications, Patient reported outcomes. **Results and Conclusions**: In contrast to clinical trials, the DISCOVER study evaluates treatment in the everyday clinical practice. This study aims to provide data on real world second and further line anti-diabetic therapy use among T2DM patients in different geographical regions. The association of these therapies with achieving disease control and in preventing and controlling diabetes complications will be documented. The DISCOVER program will be the largest global study of this kind ever performed.

Prevalence of Prediabetes and Diabetes in an Urban Area in East Delhi

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Objective: To find out the prevalence of prediabetes and diabetes in an urban area in East Delhi. **Methodology**: The present study was conducted in Dilshad Garden which is an urban area in East Delhi, among all the individuals older than 20 years of age and residing in study area for more than 6 months and included both sexes. Multistage cluster sampling design was followed and OGTT was done in 360 randomized non-diabetic subjects to find out the prevalence of prediabetes and newly detected diabetes mellitus (NDDM), who were then classified as NGT, Prediabetes (IFG±IGT) and NDDM, on the basis of WHO criteria. Also, data from the same study population was collected to find out the prevalence of known diabetes. **Results**: Thirty nine percent (39.1%) of the subjects were found to have glucose intolerance. Prevalence of prediabetes and diabetes was found to be 20.8% and 18.3% respectively. Among the diabetic subjects, prevalence of known diabetes was 10.80% and prevalence of NDDM was 7.5%. The mean age, BMI and waist circumference for subjects with prediabetes were 47.58 ±14.56 yrs, 25.87±8.55 kg/m2 and 95.05±10.69 cm. The mean age, BMI and waist circumference for subjects with diabetes were 56.14±12.04 yrs, 23.78±9.18 kg/m2 and 94.37±9.88 cm respectively. Among the subjects with prediabetes, 84.9% were centrally obese and 84% were overweight/obese (BMI≥23 kg/m2). The proportion of subjects with central obesity was 77.3% and overweight/obesity was 70.8% among those with diabetes. The household prevalence of known diabetes was found to be 34.01%. **Conclusion**: High prevalence of glucose intolerance was found in East Delhi with nearly forty percent of the population being either diabetic or prediabetic and every third household having at least one family member with known diabetes.

Prevalence of Subclinical Hypothyroidism and Iodine Deficiency Among Type 2 Diabetes Patients in a Tertiary Care Hospital in Eastern India

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Objective: Type 2 diabetes and thyroid dysfunction are two major health problem but exact prevalence of thyroid dysfunction in T2 DM is not known. Again Iodine status in diabetic individuals is not known. So our objective is to find the prevalence of thyroid dysfunction and iodine deficiency in type 2 diabetes patients attending a tertiary care center at Eastern India. **Methods:** In this observational cross-sectional study, consecutive 100 patients with diabetes attending our OPD were screened for TSH, FT4, Anti TPO antibody using chemiluminescence assay and urinary iodine by Sandell-Kolthoff method. Complications of diabetes were screened according to standard protocol. We excluded pregnant women or patients taking drugs that can alter thyroid function. Subclinical hypothyroid and overt hypothyroidism were diagnosed as per standard definitions. Urinary iodine < 100 μg/L suggested iodine deficiency. **Results**: Out of 99 patients (1 excluded as he was taking amiodarone) 50 (50.5%) were male. The number of patients suffering from comorbidities were as follows: hypertension 55 (55.5%), dyslipidemia 91 (91.9%), retinopathy 10 (10.1%), neuropathy 19 (19.2%) (as screened by monofilament test), moderately increased albuminuria 34 (34.3%) and severely increased albuminuria 8 (8.1%). The prevalence of subclinical hypothyroidism and overt hypothyroidism were 23/99 (23.2%) and 3/99 (3.03%) respectively. Thyroid auto-antibody was positive in 13 (13.1%) patients. None of the patients were found Iodine deficient. **Conclusion**: Our study results suggest high prevalence of thyroid dysfunction in type 2 diabetes patients and thus routine screening should be performed. We also found salt iodination programme is a huge success at this part of country.

Diagnosis of Diabetes and Impact on Lifestyle: the Health-Belief Story of Rural India

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Background: Diabetes Mellitus type I, the silent killer is one of the biggest public health concern, both in developed and developing world. In rural India the problem is even higher owing to poor infrastructure and inadequate access to testing and care. Even after diagnosis owing to poor awareness, a large proportion of these rural patients continue to have uncontrolled disease. To ensure appropriate control, necessary changes in the lifestyle is critical. Unfortunately there remains a dearth of information regarding the impact of the diagnosis on the lifestyle among rural residents of India, more so in the eastern part. **Methods:** In a cross-sectional study between October 2014 and March 2015, 1449 randomly-selected consenting adults (Male=774, 51.63%) from a population-based cohort were interviewed, and tested for fasting capillary-blood glucose (CBG). Subjects with previous diagnosis or fasting CBG≥126mg/dl were termed as diabetics. SAS 9.4.3 was used to analyze the data to determine the association between diagnosis of diabetes and lifestyle impact. **Results:** Among 1499 rural residents, 175 (11.7%) were diabetic. Diabetics were more likely [for non-diabetic, adjusted odds ratio:AOR=0.21(95% confidence interval:95%CI=0.12-0.38)] to get their blood sugar checked regularly, compared to their non-diabetic counterparts. They were also much more involved in regular physical exercise [AOR=2.36(1.36-4.11)] compared to normal subjects. Interestingly enough these rural diabetic patients had better knowledge [AOR=1.82(1.04-3.17)] regarding their disease compared to those whose blood sugar levels were always normal. As expected dietary modifications were much more common among diabetics [AOR=6.78(3.30-13.94)] compared to non-diabetics. **Conclusion:** Diagnosis of diabetes seemed to exert a strong influence through health-belief perception on the motivation for lifestyle modification and related knowledge among rural patients. Thus appropriate counselling for behavioral modification are likely to benefit pre-diabetics as well as these patients, to ensure better diabetes control even in the poor resource settings.

Beta Cell Function Index Web-Calculator Using Fasting and Two-Hour Post 75 GM Glucose Blood Glucose Values

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Objectives: To create a computer-based public domain application to calculate the Beta Cell Function Index. **Materials and Methods**: This calculator is based on the data collected by administering 'oral glucose tolerance test(OGTT) with insulin levels' to six hundred adults. The data was analyzed using the 'ogttplus' web-calculator at 'http://www.ogttplus.com'. Using this Insulin Resistance Calculator, these subjects were grouped according to their respective OGTT category. Various disposition indexes(DI), which are the known surrogates of beta cell function(BCF) and which have been validated in past, were calculated. To begin with, we used one such DI, i.e. "Insulin Secretion Sensitivity Index-2(ISSI-2)" for our calculations. When this ISSI-2 data averages were plotted against the fourteen categories from ogttplus calculator, a curvi-linear relationship from 'NoDM' to 'DM' category emerged. We felt that while calculating the Matsuda composite insulin sensitivity index, the formula repeats the fasting glucose and insulin values twice. Hence, we recalculated the ISSI-2 by omitting one pair of these values from the Matsuda index and called this as 'ISSI-3' index, which showed an augmented curvi-linear relationship. We further used this ISSI-3 and the averages of fasting and two-hour post-glucose blood glucose values to develop two nomograms, one each for fasting and post-glucose pathway ogttplus categories. Using these nomograms, trend-lines were added and arrays were developed using polynomial equations of sixth order and the R^2 value nearing 0.98. An application was developed which selected the final ISSI-3 value selecting either fasting or post-glucose array corresponding to the average of fasting and post-glucose values. This value is further converted as a percent value and is presented as the final BCF index value. **Conclusions**: This novel web calculator will be useful for risk stratification of not previously known as well as in the clinical management of known diabetic patients & can be accessed at this link, "http://www.ogttplus.com".

Status of Micronutrients and Connected Biochemical Pathways in Diabetic Nephropathy

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Background: Multiple factors are likely to be involved in predisposing diabetic subjects to the microvascular and macrovascular complications. Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease. The role of micronutrients in the development of diabetic complications including DN is not explored. Hence, we have evaluated the micronutrient status and its influence on biochemical mechanisms in DN. **Methods**: A case-control hospital-based study of Type-2 diabetic subjects with nephropathy (DN); Type 2 diabetes with no complications (DNC) and healthy controls (C), with 150 subjects in each group was conducted. Blood and urine samples were collected for the estimation of vitamins, minerals, and clinical parameters. Aldose reductase (ALR2) activity, advanced glycation end products (AGE) index and homocysteine were analyzed as these biochemical pathways are known to be influenced by vitamins B1, B6, and B12, respectively. **Results**: Estimated glomerular filtration rate was significantly low, and plasma creatinine and urinary albumin to creatinine ratio were significantly high in the DN group. Plasma levels of zinc were low whereas that of copper were found to be higher in the DN group compared to the other two groups. In all the groups, the levels of Vitamin B1 were sufficient whereas the levels of Vitamin B2 were significantly deficient. Vitamin B6 levels were significantly low in the DN group. Folate, total B12, and active B12 levels were higher in the DN group. ALR2 activity, sorbitol levels, and AGE index were higher in the diabetic groups and were further higher in the DN group. **Conclusion**: This study focuses on the status of micronutrients in

DN and emphasizes the role of these biochemical pathways in the development of DN.

An Observational Study of Vitamin B 12 Levels in Patient with Type 2 Diabetes Mellitus on Metformin and Its Effects

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Objective: An Observational Study Of Vitamin B 12 Levels In Patient With Type 2 Diabetes Mellitus On Metformin And Its Effects. **Study design**: Descriptive cross sectional study at Institute of Diabetology, Madras Medical College between March 2016 to August 2016. **Methodology**: 50 number of Type 2 Diabetes patients attending the Diabetic out patient department with any duration of diabetes were selected for the study with male:female selection ratio of 1 : 1. Inclusion Criteria Both male and female type 2 diabetes patients aged >/= 30 yrs Irrespective of duration of diabetes. Exclusion Criteria Type 1 Diabetes mellitus,Post operative gasterectomy ,Gestational Diabetes mellitus ,Diabetic kidney disease,Intensive insulin therapy patients, Patients on Vitamin b12 therapy/ supplementation. **Results**: This study shows that vitamin B12 deficiency was found to be 24% of those with T2DM on metformin (i.e severe in 7% moderate in 12% and borderline in 4%). CBC and peripheral smear results showed 7% anemia which includes 4% of hypochromic microcytic anemia and 3% of hyperchromic macrocytic anemia. Biothesiometry revealed that 11% of those with T2DM on metformin (were affected) with peripheral neuropathy presented. **Conclusions**: Metformin therapy is associated with a higher prevalence of biochemical B12 deficiency more common longer duration(>15-20 yrs) of diabetes and associated with anemia and peripheral neuropathy.

Time Trends, Predictors and Outcomes in Patients Attending a Diabetes Care Centre in Mumbai A Tale of Two Population Sub Groups

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Introduction: The burden of diabetes is substantially increasing with almost one person dying because of diabetes in every 6 seconds. We evaluated the T2DM patients across 1 year in our diabetes specialised clinic with the factor of time trends of glycaemic control in adult subjects with type 2 diabetes from 2014-2015 graded into two sub groups based on the control as estimated by the HbA1c levels **Methods**: We retrospectively graded the patients into two sub groups based on the follow up with HbA1c with atleast one visit of follow up in last 6 months. The patients were graded with HbA1c upto 7% as the well-controlled group and the other with HbA1c > 7% as the poorly controlled **Results**: The analysis revealed approximately 31% patients showed HbA1c < 7% with mix of regular and poor follow up and a predominant affordable patient base. A total of 60% patients had HbA1c between 7-10 %, of which 30% were in the range of 7-8%. **Conclusions**: The status of control follows global patterns but focusing on patients in the 7-8 % HbA1c range may improve the percentage of well controlled patients to around 50%, thus reducing the over all burden of complications. The data from the single centre experience also demonstrates that despite the availability and accessibility of the latest resources, the glycemic control is always challenging. Technology, Techniques and Tools (3Ts) for patient empowerment and motivation could be an effective driver for the trend change with more patients achieving a glycemic control. A customized graded approach is necessary to deliver improvised diabetes care.

Complications of Diabetes

Frequency and Determinants of Diabetic Gastroenteropathy in Adults with Type 2 Diabetes Mellitus

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Background: Diabetic gastroenteropathy (DGP) is a major contributor of increased morbidity but their pathogenesis are still poorly understood.Thus, the study aimed to explore the frequency and determinants in type 2 diabetes. **Methods**: A cross-sectional observational study was conducted on type 2 diabetes subjects with duration of diabetes ≥10 years. A group of type 2 diabetes patients attending the out-patient department of diabetes care hospital were distributed with a self-reporting questionnaire (Bengali adaptation of Rome III diagnostic questionnaire for the adult) and 301 respondents were included in the study.A subgroup of 91 subjects were studied for glycemic status, liver function, kidney function, fasting blood glucose, postprandial blood glucose, SGPT lipids, insulin secretion&sensitivity and serum C peptide. **Result and Discussion**: Out of 301, 187 (90.7%) subjects had one or more of GI disorders. About 41% (123) subjects had single followed by double disorders (29.60%). Unspecified functional bowel disorder (UFBD) was the most frequent (76.10%) one. Male (57.50%) were found to sufferer more from GIDs compared to female [(42.50%), p<.05]. Urban dwellers (60.8%) had a more GIDs compared their rural counter parts (39.2%). No significant association was found with glycemic and insulinemic status, but gender and urban residence remained as significant predictors of GID. Functional fecal incontinence seems to be associated with c-peptide, age & sex. CVS seems to have an association with fasting triglyceride and insulin secretory capacity in type 2 DM. Sex appears to be a predictor of UFBD. **Conclusion**: Male and urban dwellers had more DGP in T2DM.

Patterns of Persistence with Antihypertensive Medications Among Newly Treated Type 2 Diabetic and Nondiabetic Patients

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Background and Objective: Hypertension is a major risk factor for vascular disease and the risk is further increased by the presence of diabetes. Persistence and adherence to effective treatment are essential to treatment success. This study has investigated the antihypertensive drug persistence in hypertensive diabetic patients.. **Materials and Methods**: Patients above the age of 20 years with stage 1 hypertension, who have received the first prescription for hypertension were included in the study. Patients were followed up for 1 year. During each follow up the parameters noted includes systolic and diastolic blood pressure; changes in the prescription by the treating physician; number of days patient missed the medication and the probable reason for missing the dose; total doses of anti-hypertensive medications received. Persistence with first-line single treatment were

categorized as continuers, combiners, switchers, discontinuers. **Results and Discussion**: A total of 77 patients was included in the study, among them 51(66.2%) are males and 26 (33.8%) were females. Among these patients, 67 (87.1%) showed 100% adherence to the medication. Amlodipine was the most common antihypertensive used followed by atenolol and then losartan. There were no significant differences among the types of antihypertensives used among males and females as well as among diabetics and nondiabetics. The target blood pressure goal (<140/90mmHg) was reached in 70 (90.9%) patients at the end of 1 year. **Conclusion**: Majority of patients were on monotherapy. Adherence to antihypertensive therapy was good in a tertiary care setting. Around 18% of patients needed addition of a second drug.

Prediabetes in Acute Coronary Syndrome

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Aims and Objectives: To estimate the relationship of prediabetes with Acute Coronary Syndrome (ACS). Objectives were to identify prediabetics among patients with ACS, to reclassify them at 1 month follow up as stress hyperglycemia is well known in ACS and analyze the relationship of glycemia with coronary angiographic severity. **Material and Method**: A cross sectional study was conducted in Kasturba medical college, Manipal from September 2014 to June 2016 to identify Impaired Fasting Glucose (IFG) as defined by ADA in patients with ACS. Diabetics, old IHD patients and those with deranged renal and liver parameters were excluded from the study. A total of 140 patients were included in the study, they underwent detailed assessment including Coronary Angiogram. They were reclassified at one month follow up based on their FBS and 2hr PP after a 75 gm OGTT into IFG, IGT and diabetes. The degree of glycemia was then correlated with coronary angiographic severity (Gensini Scoring). **Results and Discussion**: Of the 140 patients(IFG) included in the study, 38 patients became normoglycemic(i.e., 27%) at follow up which accounted for stress hyperglycemia. The median Gensini scores of those with IFG,IGT and GlyHb(prediabetic range) were 34.5, 66 and 34. There was a moderate positive correlation of Gensini score with 2hr PP(0.41) than with FBS(0.1) and GlyHb (0.187). Multiple linear regression performed after adjusting for confounding variables showed only 2 hr PP to have significant correlation with Gensini (1.006) compared to FBS and GlyHb. **Conclusion**- In our study, percentage of Stress hyperglycemia was 27%. Significant Positive correlation was found between 2hr PP and Gensini score which was statistically significant, thereby highlighting the importance of assessing Post prandial glucose in predicting macrovascular complications like ACS.

"Hyperbaric Oxygen Therapy" (HBOT) - as a Limb Saving Option in Diabetic Foot Ulcers / Infections & Non Healing Wounds

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Hyperbaric oxygen therapy (HBOT) has been used as an effective adjunctive treatment for diabetic foot wounds/ infections. HBOT improves wound tissue hypoxia, improves micro vascular circulation, reduces edema, promotes fibroblast proliferation, collagen production, and angiogenesis makes it a useful adjunct in the management of "problem wounds" such as diabetic foot ulcers. It is a hospital based observational study during Sep 2104- Dec 2015. Wounds response to HBOT is assessed based on Pressure Ulcer Scale for Healing criteria. Total number patients are 210 (N- 210). Majority of ulcers were due to diabetic foot ulcers (43.8%) followed by osteomyelitis (19.5%). One HBOT session is for 90 minutes /day. Average number of HBOT sessions needed was 23.14.Majority of wounds belongs to Wagner stage 3 & 4 (39.02%, 36.58%). Complete healing of wounds was observed in 72.38% patients. Number of patients who underwent major amputation following HBOT was 2/210 (0.95%). Recurrence of ulcers has noticed in 6.66% patients in the one year follow-up. No adverse events are noticed in any of the patients who underwent HBOT. Our study highlights the significance of HBOT as a limb saving option in diabetic foot ulcers/ infections were the standard modalities of wound treatment does not showing any improvement.

Evaluation of Efficacy of Amit Jains Grading System for Debridement in Diabetic Foot Wounds in a Day Care Setting

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Background: To evaluate the efficacy of new Amit Jains grading system for debridement in diabetic lower limb wounds in a day care setting. **Methods and Material**: A retrospective analytical study was conducted at Gwalior diabetes and foot care center for day care, Gwalior, India. The study duration was from February 2015 to January 2016 **Results**: A total of 136 diabetic foot patient underwent surgical debridement during this period. Majority patients with type1 diabetic foot complications (51.5%), underwent debridement followed by (57.5%), cases with type2 diabetic foot complications. Right lower limb was involved in 47.8% patients. Abscess (40%) was the predominant type1 diabetic foot complication. In (54.4%) cases debridement was the sole procedure done without any amputation. Grade 2 debridement was attempted in patients. Among 3.7% cases that underwent major amputations below knee amputation was commonest procedure done. The mortality following debridement (1.57%) **Conclusion**: Surgical debridement serves as a vital adjunct to promote faster and improved healing of diabetic lower limb wound. It also reduces the risk of delayed foot complication such as major amputations .In this series that studies grading of debridment using amit jains classification we found majortly patients were debrided once and there were very few major amputations with significant lower mortality. **Keywords:** Diabetic lower limb wounds, debridement, amit jains, grading.

Hypoglycemia: Prevalence and Risk Factors Among Odia Diabetic Subjects

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Background and Aims: Maintaining stringent glucose level is crucial in preventing diabetic complications. Hypoglycemia has been a major hurdle in achieving glycemic goals in diabetes as it restricts the patient as well as the physician from intensifying the treatment regimen. We investigated the prevalence of hypoglycemia among subjects presenting to a single centre in Odisha. **Methodology**: After consenting, 450 subjects (M/F: 245/205) consulting at Sevayan Diabetes Centre (01.03.2016 to 31.05.2016) underwent a validated questionnaire (Edinburgh Hypoglycemia Scale). To avoid subjective bias the questionnaire was administered by a single social-activist. The mean age and duration of diabetes were 49±12 years and 3.6±1.9 years respectively. **Results**: The overall incidence of hypoglycemia was 11.6%. Incidence of hypoglycemia was highest among those taking insulin alone (31.8%) compared to insulin + OADs (11.5%) or OADs only (10.3%). Seventy

percent of the subjects taking OADs were on sulfonylureas. Sulfonylurea use in monotherapy or in various combinations was associated with more hypoglycemic events. No significant difference in hypoglycemia attacks were noticed across gender and age, still older adults had severe hypoglycemia. Diabetes duration directly influenced hypoglycemia events. Among those experiencing hypoglycemia 27% had an HbA1c <8%. HbA1c in 8-10% range seemed to be a comfort zone for the patients (6.7% hypoglycemia). **Conclusion**: Insulin monotherapy, sulfonylurea use and low HbA1c were associated with higher incidence of hypoglycemia. Severe hypoglycemia was common among older adults. There was no impact of gender on the incidence of hypoglycemia.

Relationship of Neutrophil Lymphocyte Ratio and Blood Glucose Regulation in Patients with Type 2 Diabetes Mellitus

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Introduction: Leukocytosis is thought to be directly associated with the pathogenesis of atherosclerosis and metabolic syndrome. Type 2 diabetes mellitus is one component of metabolic syndrome. Increased white blood cell (WBC) count is related to cardiovascular disease in patients with type 2 diabetes mellitus. Raised neutrophil lymphocyte ratio (NLR) is an essential marker of systemic inflammation and an indicator of increased risk for cardiovascular events in patients with metabolic syndrome. There is little information, however, concerning a correlation between glycosylated hemoglobin (HbA1c) and NLR. **Aim**: To investigate the relationship between NLR and blood glucose regulation. **Methods**: This study was conducted in patients with type 2 diabetes mellitus, divided into two groups according to HbA1c levels: group 1, HbA1c levels <7%; group 2, HbA1c levels>7%. Venous WBC, neutrophil and lymphocyte counts were determined. **Results**: Of 120 patients included, fasting serum glucose, neutrophil and WBC counts were significantly higher in group 2 compared with group 1. NLR had a positive correlation withHbA1c. **Conclusion**: There is a significant relationship between NLR and blood glucose regulation. Increased NLR may be associated with elevated HbA1c in patients with type 2 diabetes mellitus.

Neutrophil-Lymphocyte Ratio As a Reliable Marker for Early Stage Diabetic Nephropathy

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Introduction: Diabetic nephropathy (DN) is a common complication in diabetics. Urinary albumin excretion is used to diagnose diabetic nephropathy. White blood cells(WBC) count is more economical, readily available and sensitive indicator of inflammatory status. Neutrophil- lymphocyte ratio (NLR) affects the development and progression of diabetic complication. But not many studies have been done to evaluate relationship between NLR and diabetic nephropathy. **Aim**: To evaluate the relationship between DN and NLR. **Methods**: The study included 253 patients with type 2 diabetes mellitus, 115 of whom have early stage DN. The control group was composed of healthy age and sex matched subjects. **Results**: The NLR values of the patients with diabetes were significantly higher than those of the healthy controls ($P < 0{\cdot}001$), and the NLR values of the patients with early stage DN were higher than those of the patients without DN ($P < 0{\cdot}001$). Logistic regression analysis showed that the risk predictors of DN include NLR, creatinine, total cholesterol, systolic blood pressure, HbA1c and insulin resistance. NLR levels positively correlated with DN. The DN odds ratio increased by a factor of 2·088 (95% CI, 1·271–3·429) for every one-unit increase in NLR. **Conclusions**: Increased NLR was significantly associated with DN, and high NLR values may be a reliable predictive marker of early stage DN.

Characteristics and Correlates of Lipohypertrophy in Insulin Injecting Patients of Diabetes Mellitus as Detected by Clinical Examination and Ultrasonography and Its Impact on Glycaemic Control

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Background and Hypothesis: Lipohypertrophy is the commonest local complication significantly affecting glycemic control in patients of Diabetes Mellitus on treatment with insulin.Our study aimed at assessing the clinical and ultrasonographic characteristics and risk factors for lipohypertrophy at the abdomen in a cohort of insulin-injecting Indian Diabetes patients. **Materials**: 88 consecutive patients with Type 1(15/88) or Type 2 Diabetes Mellitus(73/88)were included in this cross-sectional study over a period of six months.The prevalence of lipohypertrophy and associated risk factors was assessed by clinical examination.A novel ultrasonographic characterisation of lipohypertrophy (LH) using a predetermined grading system was performed by two sonologists who were blinded to the clinical findings.Kappa statistics was used to calculate the agreement between the clinical and Ultrasound methods of detection of Lipohypertrophy. **Results and Discussion**: The prevalence of Lipohypertrophy was 68.2% on clinical examination and 89.8% on ultrasonography with moderate kappa agreement(60%).The commonest patterns on clinical and ultrasonographic assessment were Grade 2(palpable and visible-43%)and Nodular hyperechoic subcutaneous dystrophy(33%)respectively.Duration of insulin use,incorrect site rotation and repeated needle reuse($p < 0.01$)were the most important risk factors.Hypoglycemic episodes,total daily dose of insulin and Hba1c(8.8%)were significantly higher in those with clinically detected Lipohyeprtrophy($p<0.001$).Needle length,caliber,mode of delivery or regimen of insulin used did not significantly impact development of lipohypertrophy($p=0.15$). **Conclusion**: A thorough clinical examination of insulin injection sites is paramount to detect Lipohyperyrophy. Adequate control of risk factors can significantly impact insulin requirements and glycemic control while Ultrasound can be an effective adjunct to characterization of lipohypertrophy.

Evaluation of Efficacy and Safety of Topical Honey in Comparison with Povidone Iodine for the Treatment of Diabetic Foot Ulcer: a Randomized Controlled Clinical Trial

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Background/ Hypothesis: Honey has been used to treat wounds since ancient times, but medical evidence supporting this is limited. Review of literature showed that there is insufficient good quality data to conclude on efficacy of honey. Hence this study was planned to compare the efficacy and safety of topical honey in comparison with povidone iodine for the treatment of diabetic foot ulcer. **Materials and Method**: This study was a randomized controlled clinical trial with 2 arms; Topical honey and Povidone Iodine. Diabetic patients with Wagner ulcer grade of I & II for at least 2 weeks were included. After informed consent, patients were randomized to either group and followed up for 6 weeks. Reduction in ulcer size, depth, exudates, slough, and granulation tissue were observed and scored in each visit. Time taken for complete microbiological clearance was also noted. Blood sugar monitoring was done in each visit. Any adverse effect during

study was noted down. **Results**: Median reduction in ulcer size was 725(1875,500)sq.mm and 700(1087,475)sq.mm for topical honey and povidone iodine respectively and median reduction in ulcer depth was 5(10,0)mm and 5(22.5,1)mm respectively for topical honey and povidone iodine. Mean time taken for microbiological clearance in honey group was 3.5±1.3 weeks and in povidone iodine 3.4±1.1 weeks. Both the groups were comparable; there was no significant difference between two groups. There were only two mild adverse effect noted in both groups. **Conclusion**: A comparable efficacy and the absence of adverse effect make honey a suitable alternative to povidone iodine for the treatment of diabetic foot ulcer

Correlation of Increased Levels of Glycated Hemoglobin with Red Blood Cell Parameters in T2DM

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Introduction: Hyperglycaemia has multiple effects on the red blood cell (RBC), including glycation of haemoglobin, reduced deformability and reduced lifespan. Red cell distribution width (RDW) is a measure of erythrocyte variability and heterogeneity. **Aim**: To explore the relationships between HbA1c and red blood cell parameters in patients of diabetes mellitus. **Methods**: This cross-sectional study was conducted on 204 diabetic patients. HbA1c, FBG, HbA1c, Hb, MCV, MCH, MCHC and RDW were measured in these patients. A Pearson product-moment correlation coefficient was computed to assess the relationship between HbA1c and red blood cell parameters. **Results**: RDW significantly correlated inversely with HbA1c. There was no significant correlation between HbA1c and other red blood cell parameters. **Conclusion**: In contrast to the observations of previous studies, this study showed that HbA1c was inversely correlated with RDW and there was no significant correlation between RDW and MCV, MCH and MCHC. There was no significant relationship between RDW and fasting plasma glucose. Further studies with large sample size are required to explain relationship between red cell parameters and HbA1c.

Mean Platelet Volume in Type 2 Diabetes Mellitus

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Background: To Study platelet volume indices in type 2 diabetes, to determine if the MPV in the diabetic patients is higher compared to the non-diabetics , to study correlation of MPV with control of sugars.and to see if there is a difference in MPV in diabetics with and without micro vascular complications. **Materials and Methods**: A case control study. Platelet counts and MPV were measured in 100 Type 2 diabetic patients and 100 non -diabetic subjects attending either outpatient.or inpatient of department of Kasturba Medical College, Manipal.The blood glucose levels and HbA1c levels were also measured. Statistical evaluation was performed by SPSS using Student's t test and Pearson correlation tests. **Results**: The mean platelet counts and MPV were higher in diabetics compared to the nondiabetic subjects [277.46 ± 81 X 109/l vs. 269.79 ± 78 X 109/l (P= 0.256)], 9.20 ± 0.97 f. versus 7.43 ±0.36 f. (P= 0.001), respectively. MPV showed a strong positive correlation with fasting blood glucose, postprandial glucose and HbA1C levels (P=0.001). **Conclusion**: MPV is higher in diabetics than non-diabetic, Large correlation with fasting , post prandial sugars and with Glycosylated haemoglobin. and MPV was significantly higher in diabetics with microvascular complications compared to those without microvascular complications.

Diabetic Retinopathy Awareness and Multiple Associations Across the Comorbidities Data from Diamond Study

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Background: To study Diabetic Retinopathy Awareness and Multiple Associations across the Comorbidities **Material and Method**: Diabetic retinopathy (DR) is the gateway to diabetic complications. 6000 Type 2 Diabetes Mellitus patients attending a tertiary care hospital over 2 years were evaluated retrospectively to analyze awareness , prevalence, risk factors, glycemic control status and comorbidities associated with DR. DR was graded by modified Airlie House classification. Pearson Chi-Square was performed for statistical analysis. **Results and Discussion**: 63% (n=3780) were unaware of DR, with only 32% (n=1920) were aware that DR can be treated. The prevalence of DR was 64.9% (n=3894). Stratified analysis performed in 5 age tertile describes a pattern with an increased prevalence of DR during the economically productive age group.Propensity score analysis reflect a strong epidemiologic trends and association of risk factors and comorbidities with rising prevalence with higher grades of DR, across non proliferative DR, proliferative DR and diabetic macular edema ($p< 0.00001$).Independent risk factors included male sex, smoking, duration,glycemic control.higher systolic BP,insulin use and Triglycerides. Presence of diabetic retinopathy is associated with increased risk of other diabetic complications such as diabetic nephropathy and cardiovascular diseases. **Conclusion**: The results of our study underline the need for regular dilated fundus eye examination to detect and prevent asymptomatic vision threatening diabetic retinopathy.Diabetic retinopathy is an integral component of the vascular syndrome with aberrant angiogenesis involving both micro and macro vessels,even one micro aneurysm is not innocent.

Prevalence of Microvascular Compliactions in Type 2 Diabetes After First Decade of Diagnosis

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Objective: To study prevalence of microvascular complications in Type 2 diabetes after 10years of Diagnosis **Materials and Methods**: Inclusion criteria - Type 2 Diabetes 10years after diagnosis. Exclusion criteria - Type 1 Diabetes, type 2 diabetes less than 10yrs, secondary diabetes ,smoking, alcohol ,hypertension. Sample size – 120. Study period - 1year. Micro vascular complications - Retinopathy, Nephropathy , Neuropathy were assessed in each patient, as per the proforma **Results**: Total 120 patients with duration of diabetes more than 10yrs. Age distribution with maximum number of patients in 61-70 age group. 11% of patients were in 71-80 age group. Sex distribution - 65.5% were males and 34.5% were females. Positive family history in 40% of patients. Mean FBS - 180mg/dl (highest was 320 and lowest was 60). Mean PPBS - 250 (highest was 480 and lowest was 156). Mean HbA1C was 9 (lowest value was 6.6 and highest was 16.4).majory in between 9-12. 62% had evidence of retinopathy, 66% had nephroptahy and 59% had neuropathy. 11% of patients had cataract and fundus examination could not be done in those patients. **Conclusion**: Microvascular complications and their severity increased with increased duration of uncontrolled diabetes. Strict Glycemic control can prevent Microvascular complications of Diabetes

Microalbuminuria as a Marker for Vascular Complications in Type 2 Diabetes Mellitus

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Aim: To study the micro and macrovascular complications in type 2 diabetes mellitus in relation to microalbuminuria **Material and methods**: Prospective

study with sample size of 100 CASES - 50 diabetic patients tested positive for microalbuminuria CONTROLS - 50 diabetic patients tested negative for microalbuminuria Inclusion criteria - Diabetic patients with IHD proven by ECG/TMT/ECHO/ coronary angiography, diabetic patients with CVA proven by Computed Tomography scan of brain and diabetic patients with peripheral vascular disease proven by arterial doppler were included with age more than 30 years and duration of diabetes more than 5 years Exclusion criteria - Type 1 Diabetes mellitus, duration of diabetes less than 5 years, Smokers, alcoholics, patients with systemic hypertension, dyslipidemia. To evaluate and compare the variables like body Mass Index(BMI), glycemic control, Ankle Brachial Index(ABI), Peripheral Vascular Disease(PVD), Cerebro vascular Accident (CVA) , Diabetic Retinopathy, peripheral neuropathy between cases and controls. Study Period - 1 year. Sample size – 100 **Results and Discussion**: IHD was found to be in 24% in caes and 16% in controls. PVD in 52 % of cases and 20% in controls. CVA in 8% of cases and 0 in controls. Retinopathy in 12 % of cases and 16% in controls. Neuropathy in 40% of cases and 32% in controls **Conclusion**: On comparision of vascular complications of micralbuminuria and normoalbuminuria in diabetic patients, peripheral vascular Disease prevalance was found to be higher in cases in the abscence of other risk factors like smoking , dyslipidemia. Incidence of IHD, peripheral neuropathy, diabetic retinopathy and CVA in cases was found to be higher but a significant positive correlation could not be established. Early treatment of microalbuminuria may be beneficial in patients with type 2 Diabetes mellitus in preventing the vascular complications

Risk Factors for Diabetic Foot Problem in Urban Indian Population

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Background/Hypothesis: Foot screening is one of the most neglected field due to the lack of education both of patients and health care providers. We developed a concept of mobile screening van, which can be taken to sub-urban areas for screening. We wanted to analyse various risk factors present for diabetic foot. **Materials and Methods**: We used 12 mobile vans equipped with screening for all complications of diabetes including retinal camera, point of care pathology test, ECG and detailed foot assessment tools and drove to primary care practices of urban and sub-urban India across 8 cities in India. Foot screening was performed by trained nurses using standard procedure. **Results and Discussion**: Foot data on 969 subjects [mean age of 54.1 (+/- 11.5) years & 58.4% males] showed that 34 had a history of foot ulcers and 16 had amputations. 55 (5.7%) subjects needed active treatment for active ulcers of painful corn. Neuropathy in the form of absent monofilament sensation was present in 149 (15.4%) subjects. 34 had absent DP and 35 absent PT pulses. Foot deformity was present in 38 subjects. Fissures were present in 452 (46.6%) of subjects and painful neuropathy in 434 (44.8%). **Conclusion**: Neuropathy was common possibly due to younger age and longer duration of diabetes in this population Foot deformity was less common possibly due to use of open shoes. There is a need to increase awareness of diabetic foot problem in India.

The Relationship Between Diabetic Retinopathy and Cognitive Impairment in Elderly Type 2 Diabetes Mellitus Patients in a Tertiary Care Hospital

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Objective:To study the association between severity of diabetic retinopathy and cognitive impairment in elderly diabetic individuals. **Materials and Methods**: It is a hospital based observational study. A total of 100 cases of type 2 diabetes satisfying the inclusion and exclusion criteria are included in the study. Careful history and examination is done according to proforma. Fundoscopy and Mini Mental State Examination [MMSE] were done to all subjects. Data is analysed by logistic regression analysis **Results and Discussion**: Type 2 diabetes is associated with an increased risk of age-related cognitive impairment and decline in addition to higher incidences of stroke and dementia. Typical retinopathic changes associated with diabetes are associated with white matter lesions in the brain, magnetic resonance imaging (MRI)-defined cerebral infarcts, and incident stroke. 0ut of 100 type 2 diabetic patients with cognitive impairment, 44% of patients have retinopathy changes and 56% have no diabetic retinopathy. 30 cases[68.18 %] showed mild to moderate Non Proliferative Diabetic Retinopathy [NPDR] changes. 14 cases [31.8%] showed severe NPDR with cognitive impairment.Logistic regression analysis showed after adjusting for compounding variables, cognitive impairment is not related to diabetic retinopathy [p=0.032]. **Conclusion**: There is no association between cognitive impairment and retinopathy changes.Hence the diabetic retinopathy changes cannot be taken as indicator for severity of cognitive impairment.

Association Between Subclinical Hypothyroidism and Diabetic Retinopathy in Type 2 Diabetic Patients

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Objective: To determine the association between subclinical hypothyroidism and diabetic retinopathy in type 2 diabetic patients. **Materials and Methods**: This is a hospital based case control study. A total of 300 subjects of type 2 diabetes were enrolled,150 subjects with diabetic retinopathy[DR] and 150 without diabetic retinopathy (satisfying the inclusion and exclusion criteria) are included in the study. Careful history and examination is done according to proforma. Fundoscopy and thyroid function tests were done to all subjects. Data is analysed by logistic regression analysis. **Results and Discussion**: Subclinical hypothyroidism (SCH) is defined as an asymptomatic state characterized by a normal serum free thyroxine level and elevated serum concentration of thyrotropin [>4.0 μIU/ml]. Of 300 diabetics,67 subjects [22.5%] were diagnosed as subclinical hypothyroidism. Prevalence of subclinical hypothyroidism in diabetic retinopathy subjects [41/150, 27.3%] is higher than in subjects without diabetic retinopathy [26/150, 17.4%]. Logistic regression analysis showed after adjusting for compounding variables, subclinical hypothyroidism is independently related with diabetic retinopathy [p=0.032]. **Conclusion**: These results indicate that type 2 diabetic patients with retinopathy are at increased risk of subclinical hypothyroidism. A routine screening for thyroid function is advised for patients with diabetic retinopathy. This may be helpful in implicating new strategies for preventing and treating diabetic retinopathy in clinical practice.

Risk Factors for Early Renal Impairment in Type 2 Diabetes

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Background: Diabetes has been increasing worldwide and hence the diabetic nephropathy. Risk factors for the early onset of nephropathy in diabetes are not well known. Early renal involvement is eGFr less than 60mg/min/1.73m2 or proteinuria more than 300mg with ten years of onset of diabetes. In our study, we try to identify the risk factors for early renal involvement with a special reference to insulin resistance **Material and Methods**: It is a case control study, conducted at KMC,Mangalore in which 45 controls and 34 cases. CASES are patients with early nephropathy. CONTROLS are patients without nephropathy. In our study, various risk factors like family history of chronic renal disease, Body mass index, hypertension,lipid profile, glycemic control and insulin resistance are compared and statistically analysed by multiple logistic regression analysis. **Results**:- Mean age of controls is 57.1 years and of cases is 55.8 years. 64%of male population in control group and 61.7% of males among cases. Results shows insulin (p 0.001), insulin resistance (p 0.05), TC/

HDl (p 0.043), family history of CKD(p 0.014) are significant with early nephropathy. Proteinuria was significantly associated with insulin (0.006),IR(p-0.002),HbA1c (p-0.012) and reduced eGFR correlates negatively with insulin(p 0.010),insulin resistance(p 0.000). **Conclusion**: Patients with family history of nephropathy, TC/HDL more than 4.2, poor glycemic control, and hyperinsulinemia and high insulin resistance are at a higher risk of developing nephropathy at an early course of diabetes. Thus, these patients can be managed aggressively to prevent need of renal replacement therapy and thus morbidity and mortality at the early stage of the disease.

Non Invasive Predictors of Esophageal Varices in Diabetic Patients with Non Alcoholic Fatty Liver Disease (NAFLD)

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Background: To study and identify the laboratory and radiological parameters which correlate with the presence of esophageal varices on upper gastrointestinal(UGI) endoscopy in diabetic patients with NAFLD and to compare various laboratory and radiological parameters in those patients, with and without esophageal varices. **Materials and Methods**: This observational study included 59 diabetic patients with NAFLD, fulfilling the inclusion and exclusion criteria. All patients underwent basic blood tests and Ultrasonography(USG) of abdomen to assess Radiological data. UGI Endoscopy was performed to look for the presence of Esophageal Varices.The laboratory and radiological data were compared between patients with and without varices. All parameters were compared between the varices and non varices population, statistical analysis was done and results were tabulated. **Inclusion Criteria**: ⎩ Age >18 years ⎩ Diabetic patients ⎩ USG evidence of fatty liver or cirrhosis **Exclusion Criteria**: ♣ Significant alcohol consumption ♣ Other etiologies of cirrhosis ♣ Patients with active UGI bleeding ♣ Patients previously diagnosed or have undergone endoscopic intervention for management of esophageal varices. **Results**: The laboratory parameters with statistically significant difference between patients with and without varices were: lower hemoglobin (9.3gm% vs 11.4gm%) lower platelet count (1.32 lakh vs 1.81 lakh) larger spleen (13.8cm vs 12.02 cm) larger portal vein diameter (13.41mm vs 12.27mm) presence of ascites. The ratio of Platelet count(PC) by Spleen diameter(SD) showed a significantly lower value in patients with varices, with a majority of patients with varices having a PC/SD ratio <1000, contrary to those in the non varices group. **Conclusion**: Thus,non invasive parameters are very useful in predicting the presence of esophageal varices in diabetic patients with NAFLD and aids in avoiding unwarranted endoscopic procedures.

Estimation of High Sensitivity C-Reactive Protein Levels as a Early Marker of Diabetic Nephropathy

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Introduction and Objectives of the Study: Diabetic nephropathy (DN) is a progressive kidney disease caused by angiopathy of capillaries in glomeruli and Is secondary to longstanding diabetes and is the major cause of morbidity and mortality in patients with Type 2 DM Early interventions in patients with Type 2 DM reduce the risk of diabetic nephropathy.CRP has a long half life, affordability of estimation, and stability of its levels with no circadian variation, and therefore is one of the best markers of vascular inflammation. **Objectives of the Study**: To study the correlation between serum levels of hs-CRP in diabetics with microalbuminuria which progress to diabetic nephropathy. **Materials and Methods: Source of Data**: This study done in the Department of General Medicine at R L Jalappa Hospital, Kolar over a period of 6 months, both inpatients and outpatients was taken into study.Type of study :Institutional based case-control study :Study period:6 Months **Study Design Group 1 [Cases]**: Forty patients fulfilling the inclusion criteria were included in the study after obtaining a written informed consent.**Group 2 [Controls]**: Forty diabetic patients without microalbuminuria were taken as controls whose age, gender and diabetic status are matched. **Results** :Total 40 cases of which 31 were male patients and 9 were female. Both case and controls matched.Both case and control matched. Maximum number of patient was seen in age group between 56-70years(21). Followed by 35-55 years (10) and >70 years (9).Mean HbA1c level is more In cases(7.8) than controls (6.95) Controls had good glycemic control than cases. mean Hs CRP levels was more in cases than controls. **Conclusion**: In type 2 diabetic patients, microalbuminuria is accompanied by elevated HS-CRP, suggesting activation of inflammatory pathways in progression of renal disease. As it is a easier and cheaper test for assessment of diabetic nephropathy.So hs – CRP can be used as early marker for detection of diabetic nephropathy.

Prevalence of Thyroid Dysfunction in Type 2 Diabetes

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Thyroid disease and Diabetes are two common endocrinopathies found in the general population. Thyroid disease is a pathological state which can adversely affect Diabetes control and contribute to negative patient outcomes. Hyperthyroidism contributes to hyperglycemia while hypothyroidism contributes to episodes of hypoglycemia. However, uncontrolled diabetes on the other hand has been shown to impair TSH response to TRH which normalizes with improvement in glycemic control **Objectives**: To determine the prevalence and patterns of thyroid dysfunction in patients with Type 2 Diabetes Mellitus. **Methodology**: This was a cross-sectional descriptive survey of participants who were over the age of 30 years selected from patients with type 2 Diabetes attending outpatient diabetes clinics. A sample size of 180 was obtained. Venous blood samples were collected for assessment of, i.e. TSH & fT4. **Results**: In this study, majority of the patients were female (62.4%), with a mean age of 59 years and had a mean duration of 9.5 years with diabetes mellitus. Those with a previous diagnosis of thyroid dysfunction were about 10.6% and 22.7% had a positive family history of thyroid dysfunction. The prevalence of thyroid dysfunction in patients with type 2 Diabetes was found to be 61%, of which subclinical hypothyroidism was the most predominant type at 58%. No patient was found to have evidence of overt hyperthyroidism. **Conclusion**: The prevalence of thyroid dysfunction among patients with type 2 Diabetes is high, particularly sub clinical hypothyroidism. The clinical significance of this thyroid status on metabolic control and outcomes need further evaluation.

A Case of Chorea - Non Neurological Etiology

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Background: CHOREA- Greek word meaning DANCE refers to rapid, quasipurposeful, graceful, dance like, non-patterned movements involving distal or proximal muscle groups. Etiology being associated with inherited disorders, systemic diseases,drug induced,rheumatic,stroke etc.. **Methods**: A 65 year old female presented with c/o involuntary movements involving all four limbs since 3 days,insidious onset first involving right side of the body and then involved even left side of the body. Patient is aware of it and she cannot suppress them, not affected by environment,temperature or

posture,the movements persist in sleep. Patient is a known Diabetic since 10 years but was not taking regular medications since 2 months. No similar complaints in the past. No similar complaints in family. No drug history i.e Phenytoin,neuroleptics,Dopamine agonists. No H/O RHD,HTN,CVA.. CNS examination revealed no focal neurological deficit and no cranial nerve palsy. **Results**: Normal- Haemogram, peripheral smear& ESR. MRI brain revealed age related cortical atrophy, Thyroid & ANA profiles are normal. Blood glucose-512mg/dl, urine sugar-positive, urine ketones-negative,blood urea-20mg/dl, serum creatinine-1mg/dl. Sugar levels were controlled by Insulin & subsequently she improved clinically and she was advised glimiperide on discharge. **Conclusion**: A case of Chorea due to hyperosmolar non ketotic hyperglycemia

Diabetic Macular Edema Associations with Multiple Comorbidities: Data from the DIAMOND-ME Study

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Background and Aims: Chronic hyperglycemia results in both Microvascular and macrovascular complications with an overlapping pathophysiology. The initial changes indicating diabetic microangiopathy are detectable immediately after the setting of hyperglycaemia and in the long term results in diabetic macular edema (DME) **Materials and Methods**: We conducted a retrospective analysis across 1308 patients with DME, evaluated by using optical coherence tomography angiography. This was part of the study in Indian patients to assess awareness of diabetic retinopathy and measure comorbidities across 6000 Type 2 Diabetes Mellitus patients attending a tertiary care hospital over 2 years who were evaluated retrospectively to analyse for prevalence, risk factors, glycaemic control status and comorbidities associated with Diabetic Retinopathy. Pearson Chi-Square was used for statistical analysis. **Results**: The prevalence of DME was 21.8 % (1308/6000). Stratified analysis performed in 5 age tertile describes a pattern with increased prevalence of DME in economically productive age group of age <70 years, associated hypertension and dyslipidaemia. Propensity score analysis reflect strong epidemiologic trends, association of risk factors and comorbidities with rising prevalence of DME ($p< 0.00001$). Pioglitazone use of daily dose > 15 mg for more than 5 years was significantly ($p< 0.00001$). associated with the risk of DME. Presence of other diabetic complications such as diabetic nephropathy including dialysis is significantly associated with the risk of DME ($p< 0.00001$). **Conclusions**: DME may be the presenting sign and therefore may already be established at diagnosis. Screening of diabetic persons for early signs of retinopathy, which are treatable, with lasers in the initial stage and improves the visual prognosis, options for the delayed treatment are often ineffective and expensive with anti VEGF agents with wide economic ramifications.The control of established risk factors for the development and progression of DME, including hyperglycemia, hyperlipidemia and hypertension remains the cornerstone of therapy which serves to prevent blindness.

Prevalence of Diabetic Retinopathy in a Diabetes Centre of South Gujarat

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Introduction: Diabetes is a global health problem. Recent studies have shown prevalence of diabetes and its associated complications is increasing due to increased life expectancy because of modern treatment modalities.Diabetic Retinopathy (DR) is one of the dreaded preventable complication of diabetes. Data regarding its prevalence in different region of the countries are sparse and in South Gujarat region are not available.We screened T1DM and T2DM patients attending our diabetes centre for Diabetic Retinopathy. **Aims and Objectives**: To know the prevalence of Diabetic Retinopathy in T1DM and T2DM patients. To correlate prevalence of Diabetic Retinopathy with HbA1c, other micro and macro vascular complications. **Materials and Methods**: We screened a total of 149 diabetic patients out of which 109 patients wereof T2DM and 40 were of T1DM. Using 3 nethra classic version 3.0 fundus camera, the patients were screened in the period between September 2015 to January 2016. Fundus photograph was reported by an expert via electronic communication. Fundus photography was performed on undilated pupils. Patients baseline data regarding anthropometry,glycemic control and other micro and macro vascular complications were collected from computerized data record system. All data were analyzed using SPSS software version 20. **Results**: We screened a total of 149 patients for presence of Retinopathy using fundus camera. Out of the total number of patients screened, 109 were having T2DM and remaining 40 were of T1DM. Mean age of T2DM patients was 48.3 ±12.2 years.Out of total T2DM patients, 67 (61.46%) were male and 42 (38.54%) were female.Theirmean duration of diabetes was 6.02±5.33years,mean BMI was 27.8±5.63 kg/m2.Mean HbA1c was 9.04±2.78% out of which 10.9 % were under target HbA1c of <7 while remaining (89.09%) were above the target value.Out of the screened T2DM patients, 55(50.45%) had other micro vascular complications and 22(14.7%) had macro vascular complications. • Mean age of T1DM patients was 19.3±9.21years. Out of total T1DM patients, 18 (45%) were male while

Prevalence of Peripheral Arterial disease in Type 2 Diabetes Patient in a Diabetes Centre in South Gujarat

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Introduction: Diabetes is a heterogeneous disorder. Its prevalence is increasing world wide and more so in our country. Precise data regarding prevalence of the disease and its complications in the different parts of the country are lacking. Ours is a tertiary diabetes care centre where we looked into the prevalence of Peripheral arterial disease in lower limb in type 2 diabetic patients. Peripheral arterial disease is one of the serious complications leading to lower limb amputation. PAD is uncommonly screened. **Aims and Objective**: To know the prevalence of peripheral arterial disease in lower limbs in type 2 diabetes. To correlate it with the duration of diabetes, level of glycemic control, presence of micro vascular and other macro vascular complication and tobacco abuse. **Material and Methods**: Type 2 diabetes patients presenting to our centre between June 2013 to June 2015 who gave informed consents were screened for PAD and other diabetes related complications using appropriate screening test. All patients were screened for lower limb arterial occlusion using ankle brachial pressure index (ABPI) by Hadeco Doppler smart drop 45, segmental pressure records and pulse wave velocity. Based on ABPI, patients were classified as normal, mild, moderate and severe occlusion with ABPI >0.9, 0.7-0.9, 0.69-0.5, <0.5 respectively. ABPI >1.3 was considered as non compressible artery due to heavy calcification in arterial wall. All data are collected from computerized data record system., and were analyzed using SPSS software, version 20. **Results**: We screened total of 709 patients attending diabetic clinic between the period of 2013 to 2015 out of which 440 (62.1%) were male and 269 (37.9%) were female. Mean age of the screened patients

Combination of Clinical Assessment with Questionnaires for early Diagnosis and Staging of Diabetic Neuropathy in Coimbatore - A Regional Survey

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Background: There is strong evidence that patients with diabetes have difficulty adhering to their recommended regimens resulting in less than optimal control of A1c causing medical and psychosocial complications and reducing patients' quality of life. One such complication of diabetes is peripheral-neuropathy which if detected early can reduce the risk of amputation. This study aims to evaluate the presence and staging of Diabetic Peripheral Neuropathy(DPN) **Research Design**: About 200 Type-II diabetic patients were physically examined using 10g mono-filament followed by tuning fork test and via integrated questionnaire framed by the Endocrine Department of a Tertiary care Hospital, Coimbatore which included United-Kingdom Screening Test and Michigan Diabetic-Neuropathy Score to assess the presence and severity of DPN. **Results and Discussions**: The subjects were over-looked for any signs and symptoms of neuropathy and screened via physical assessment and questionnaires. Results were analysed subsequently. The distribution of DPN was as follows: 16%(32)- Mild Neuropathy 22.5%(45)- Mild-Moderate Neuropathy 19.5%(39)- Moderate Neuropathy 4.5%(9)- Moderate-Severe Neuropathy 14%(28)- Severe Neuropathy 23.5%(47)- No Neuropathy. All the patients with positive neuropathy showed symptoms like burning sensation, pain, swelling or numbness of feet, with 4% showing false-positive DPN. This indicates incidence and extent of symptomatic peripheral neuropathy is more common in diabetic patients irrespective of the duration. **Conclusions**: This study illustrated relatively higher prevalence (76.5%) of diabetic peripheral neuropathy compared to other studies conducted, which may be attributed to the tertiary care setting of the study site. Thus, timely screening with earlier detection and intervention would be useful in preventing its progression and unnecessary surgical intervention.

The Ratio of Red Cell Distribution Width to Mean Corpuscular Volume in Patients with Diabetic Ketoacidosis.

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Background: The relationship between erythrocyte parameters and diabetic ketoacidosis (DKA) remains uncertain. This study aimed to investigate the potential role of erythrocyte indices in diabetes patients with DKA. **Methods**: This study included 48 patients with diabetes, 26 patients with DKA, and 30 age- and gender-matched controls. Erythrocyte parameters were measured and evaluated at the time of admission and after treatment. **Results**: Data were analyzed by One-Way ANOVA, SPSS software. The DKA patients had higher levels of plasma glucose (28.87 +/- 9.01 mmol/L), HbA1c (13.08 +/- 3.10%), red cell distribution width (RDW, 41.24 +/- 3.08 fL), and the RDW to mean corpuscular volume (MCV) ratio (47.50 +/- 3.70%) compared to non-DKA cases and controls (all $p < 0.05$). Pearson's correlation test showed that osmolality was positively correlated with plasma glucose ($r = 0.699$, $p < 0.001$) and negatively correlated with mean corpuscular hemoglobin concentration (MCHC) ($r = -0.409$, $p = 0.049$). A logistic regression revealed that the RDW/MCV ratio can act as a robust risk marker for the presence of DKA (OR = 1.548, $p = 0.0360$, 95% CI: 1.029 - 2.330). The RDW returned to normal, and plasma glucose levels and metabolic acidosis were well controlled following treatment. **Conclusions**: The RDW and the RDW/MCV ratio were significantly correlated with DKA. The RDW/MCV ratio can act as a robust biomarker that is more sensitive than RDW in reflecting the presence of DKA.

Association Between Diabetic Retinopathy and Cognitive Impairment in Elderly Individuals with Type 2 Diabetes, in a Tertiary Care Hospital, Kolar

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Objective: Type 2 diabetes is associated with an increased risk of age-related cognitive impairment and decline .Retinal and cerebral small vessels share similar embryological origin, size, structure, and physiological characteristics. Previous studies have found a significant association between the presence of microaneurysms and type 1 diabetes and between retinopathy and risk of cognitive impairment but present knowledge about the relationship between Diabetic Retinopathy (DR) and cognition in older people with type 2 diabetes has not been examined extensively, therefore association between diabetic retinopathy and cognitive decline was examined in older people with type 2 diabetes. **Research Design and Methods**: A total of 70 cases of elderly diabetics above age of 60 years with diabetic retinopathy assessed by fundoscopy examination were taken and cognitive function was assessed using MMSE scale. It was an observational study design.The association between cognitive impairment and diabetic retinopathy was studied. **Results**: Severity of DR demonstrated an inverse relationship with cognitive impairmentThe no/mild DR group had lower cognitive impairment scores on MMSE (adjusted mean +- SE +- 1.9) compared with the PDR group (82.5 +-2.2, $P < 0.001$). The MMSE cutoff scores showed that 12% of the no/mild DR group ($n = 31$) had positive screening results for dementia or significant cognitive impairment compared with 5% in the PDR group ($n = 6$). **Conclusion**: Patients with advanced DR demonstrated less cognitive impairment or normal MMSE scores .Therefore, the increased prevalence of cognitive impairment in diabetes may be associated with factors other than evident retinal microvascular disease.

Effect of Age, BMI, Duration of Diabetes and Glycemic Control on Severity of Erectile Dysfunction in Type 2 Diabetes

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Aim: To determine the effect of Age, BMI, duration of diabetes and glycemic control in severity of erectile dysfunction in type 2 diabetic males. **Materials and Methods**: **Study Design**: A retrospective cross sectional study SAMPLE SIZE- 100. Inclusion criteria- 1. type 2 diabetic male patients 2. patients with symptoms of erectile dysfunction.3. Age 30-65 years. Exclusion criteria: 1. Patients on known erectile dysfunction causing drugs (beta blockers, diuretics, Hydralazine, TCAs, MAO inhibitors, digoxin) 100 patients with complaints of erectile dysfunction were selected for the study. The previous records were screened for the age, duration of diabetes, BMI and glycaemic control of the patients. Their IIEF-5 scores were studied to estimate the severity of erectile dysfunction. Lab tests recorded for analysis included the serum testosterone, prolactin, LH and FSH levels and the HbA1c level for the glycaemic control. We used available TMT reports to look for presence of subclinical CAD. **Results**: The incidence of erectile dysfunction increases with age of the patient, increased duration of diabetes and raised HbA1c. Low testosterone levels were associated with overweight and obese diabetics. **Conclusion**: Erectile dysfunction is an important clinical finding in light of decreasing future CVD risk. Severity of erectile dysfunction is related to increased age, increased duration of diabetes and was associated with an inadequate glycaemic control. Obesity is known to be a risk factor for endothelial dysfunction which has been implicated in the pathogenesis of erectile dysfunction.

Hypoglycemia in Diabetes: Not Always Drug Induced

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Hypoglycemia is a common medical emergency. It is the most frequent complication induced by anti-diabetic treatment. However, it can be observed in other conditions unrelated to diabetes such as insulinoma, autoimmune disorders, and neoplasia. Herein, we report the case of a rare cause of severe and recurrent hypoglycemia in a 83 year old diabetic and hypertensive lady who was subsequently diagnosed with adrenal malignancy and hypoglycemia was a paraneoplastic manifestation due to excess of IGF2 by the tumor.

Obstructive Airway Disease in Type 2 Diabetes Mellitus

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Aims and Objectives: Lung has been hypothesized as one of the target organs in Type 2 Diabetes Mellitus (Type 2 DM) Aim of this retrospective study was to compare obstructive airway disease between patients with Type 2 DM and nondiabetic controls. We assessed the correlation of pulmoary functions test (PFT) parameters with HbA1c and duration of diabetes **Materials and Methods**: Sixty five individuals with Type 2 DM and 45 individuals without DM who underwent PFT from 15/7/2016 - 15/8/2016 (all outpatients) were recruited. Patients with history of COAD/bronchial asthma, NYHA grade III or above that dyspnoea, recent major surgery (< 6 months), BP>160/100 mm of Hg, smoking, BMI >35 kg/m2, TSH > 6 uIU/ ml, female individuals with pregnancy, age <18 years and not willing to take part in the study were excluded from the study. Fourty patients with Type 2 DM and 28 controls were included in the study. Their basline parameters along with duration of diabetes, HbA1c, FEV 1, FVC, FEV1/FVC %, FEF25%-75% were noted. Above pulmonary function parameters were compared in two groups. The effect of glycemic control(HbA1c) and duration of diabetes were on above parameters were compared. **Results**: When Mean and SD of PFTs of Diabetic and control groups were compared using unpaired t test, it showed a significant difference in FVC (p-0.000), FEV1 (p-0.000), FEV1/FVC (p-0.000) and FEF 25%-75% (p-0.000). When PFTs parameters were compared among the groups according to HBA1c level, it showed a significant difference in FVC (p-0.056) but did not show a significant difference in rest of the parameters. When PFTs parameters were compared among the groups according to the duration of diabetes, it did not show a significant difference in any of the parameters. **Conclusion**: There was significant difference in PFT parameters between diabetes and control group. Poor glycemic control(risingHbA1c) showed significant changes only in FVC, irrespective of duration of disease. We did not find significant change with duration of diabetes.

Acute Transient Mono-Ocular Loss of Vision in a Diabetic Patient –A Rare Medical Condition

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We report a case of acute bacterial subretinal abscess in a diabetic patient, presenting with history of right-sided headache and eventually developed loss of vision of her right eye. Aggressive management with intravitreal antibiotic treatment, resulted in a successful visual outcome

Background: A 43 year old lady with a background history of diabetes, presented with complains of right-sided headache of 15 days duration.CT scan of the brain was normal. Post admission she reported sudden total loss of vision in the right eye. Fundoscopy revealed subretinal abscess in the right eye. The patient was commenced on intravitreal injection of Amoxicillin-clavulanate. She was followed up for the next 3 months with total recovery of vision **Material/ Methods**:Clinical data including medical history and findings on physical examination were collected. Ophthalmological examination including visual acuity, fundus photographs, fluorescent angiography, were captured. **Results and Discussion**: Early aggressive intervention with intravitreal antibiotics showed regression of the abscess.The improvement of vision was complete over next three months. **Conclusion**: Acute onset of monocular visual loss due to a subretinal abscess is a rare but devastating condition. In this case, a high degree of suspicion and prompt intervention with intravitreal antibiotic therapy resulted in unprecedented visual recovery.

Comparative Study of Microalbuminuria and HbA1c Levels in Type 2 Diabetes Mellitus Patients in Tertiary Care Hospital of Western Up

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Background: Diabetes mellitus is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action or both. Microalbuminuria is a potential risk factor for developing hypertension, neuropathy and cardiovascular diseases. **Aim and Objectives**: The present study was aimed to assess the association of presence of microalbuminuria with complications associated with type II Diabetes mellitus. **Material amd Methods**: The study was carried out in department of General Medicine, SIMS included 50 patients with type 2 DM, among them 25 patients with complications like neuropathy, hypertension and 25 patients without complications. **Result and Conclusion**: the study revealed that microalbumin levels and HbA1c levels were found to be higher range with complications associated to type II diabetes.

A Multi Centric Study on Assessing the High Risk Foot and Its Associated Complications in India- Preliminary Data from the Diabetic Foot Research India

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Aim: The aim of this ongoing study was to determine the prevalence of foot complications such as neuropathy, peripheral vascular disease (PVD), amputations and the associated diabetic complications and practice of foot care among people with Diabetes from diabetic centres from different regions of the country **Subjects and Methods**: A total of 235 type 2 diabetic patients, were selected from four different centres across India. The centres were Prof M. Viswanathan Diabetes Research Centre (DRC), Chennai, Sun Valley Hospital Guwahati ,Sushruta Diabetes Care Centre, Salem, Harshita hospital, Tirupathi. Details were collected regarding foot problems and associated complications. The data was collected by four members belonging to Diabetic Foot Research India from their centres respectively. **Results**: The prevalence of neuropathy was 27%; PVD was 16%, Calcified vessels in ABI 21%. Nearly 3% of subjects had undergone a minor or major amputation. **Conclusion**: This study found that the, prevalence of amputation was 3%. Peripheral neuropathy which was present in 27% of the patients was found to be an important risk factor for diabetic foot infections. Effective foot care advice should be propagated to reduce the burden imposed by diabetic foot complication particularly in developing countries like India.

A study on Profile of Diabetes Patients Admitted with Ambulant Ketosis in a Government Tertiary Hospital

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Background: Diabetes patients often presents with ketosis without developing acidosis. The aim is to study the profile of diabetes patients admitted with ambulant ketosis and to identify the various causes leading to ambulant ketosis **Materials and Method**: Descriptive study done at Institute of Diabetology, Madras Medical college Diabetes patients both type1 and type2 (n=160) admitted with hypeglycemia and ambulant ketosis(urine ketones—positive) were selected .Patients were subjected to history taking, clinical examination and blood investigations like fasting plasma glucose post prandial plasma glucose, urine ketones, renal function test, liver function test, complete blood count ,lipid profile, urine analysis, USG abdomen, ECG, urine culture and sensitivity and chest x-ray **Results**: The mean age of type1 and type2 diabetes are 24.4±11.1and 46.5 ±10.9 years respectively. The mean duration of type 1 and type 2 diabetes are 8.1±6.5 and 7.4±6.9 respectively. The mean fasting plasma glucose is 265±14.6.In both type1 and type2 patients infection(78% and 61.4%) is the most common cause leading to ketosis. In Type2 patients 34.4% have neuropathy,24.1% retinopathy and 20.6% have nephropathy. About 48% of type2 paients have underlying cardiovascular risk or disease. **Conclusion**: Diabetes patients with hyperglycemia should be screened for urine ketones and if positive the underying cause should be identified. Most of the Ambulant ketosis patients in this study have microvascular and macrovascular complications. **Keywords :** Ambulant ketosis, infection, microvascular complications, cardiovascular risk.

Hypoglycemic Encephalopathy in a Young Female Without Hypoglycemic Unawareness, not on Insulin with a Single Episode of Hypoglycaemia

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A 38 year old female diagnosed as type 2 DM four years back was brought in emergency with decreased responsiveness for last 6 hours. Past history reveals one episode of frothing from mouth with the simultaneous inability to move the limbs and inability to talk. Ongoing treatment of diabetes with the combination of glimepiride and metformin, with no history of skipped meals in last 12 hours.In the hospital Emergency intubation was done. **Investigations** Random Blood Sugars: Random blood sugars in the emergency ward revealed blood glucose values of 60 mg/dl. MRI and MRA: The MRI and MRA (Magnetic Resonance Angiography revealed symmetrical area of restricted diffusion in the pulvinar nuclei of thalamus, frontoparietal and temporal cortices including the insula and hippocampi, deep cerebral white matter and splenium of the corpus callousum. Common carotid artery, internal carotid artery, middle cerebral artery, anterior cerebral artery were normal in calibre with no narrowing. Posterior cerebral artery, vertebral arteries were well opacified with no significant focal stenosis. These findings were consistent with hypoglycaemic encephalopathy. EEG: Findings were suggestive of diffuse encephalophathy and no sub clinical seizures Serum electrolytes, LFT, RFT were all within normal range. CT brain: suggestive of no evidence of acute haemorrhage or thrombotic attack, mild diffuse cerebral oedema, no herniation or hydrocephalus 2D echo: revealed Ejection Fraction – 55% with no pericardial effusion and no clots. **Diagnosis hypoglycaemic Encephalopathy Course**: The hypoglycaemia was immediately corrected by administration of 25% Dextrose. Patient became euglycaemic 10 minutes later. Marked cognitive and motor impairment was noted to persist several days into her admission. Since, there were no evidence any thrombotic or haemorrhagic cause, the supportive treatment was administrated. Ventilation support was gradually tapered and rehabilitation started **Conclusion**: This was a rare case of hypoglycaemic encephalopathy in a young female who was not on insulin, patient was wellwith

Diagnosis of Peripheral Arterial Disease: Is a Hand Held Doppler Good Enough?

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Background: Peripheral arterial disease(PAD)is diagnosed using the ankle brachial index (ABI). The automated ABI instruments are expensive and unavailable in rural areas. An alternative less time consuming, inexpensive test is the calculation of ABI using a hand held doppler. Studies comparing the two methods are limited. The aim of this study was to evaluate the sensitivity and specificity of a hand held doppler in diagnosis of PAD, in asymptomatic patients with diabetes. **Methods**: This was a cross-sectional study of 309 patients with diabetes and one risk factor for PAD: age>50 years, diabetes duration>10 years, hypertension, dyslipidemia, BMI>23 kg/m^2or smoking. Patients with claudication, known PAD were excluded. ABI was measured with the Kody's automated ABI instrument and a Hadeco hand held doppler with an Omron BP cuff in all patients. An ABI ≤0.9 and >1.3 was defined as abnormal. Mean(95% CI) for continuous variables, proportions for categorical variables, p values at 5 % level of significance were calculated using STATA 14. **Results**: Mean age, duration of diabetes, HbA1c and BMIwas 59.65(58.73-60.56), 13.05(12.25-13.85), 8.4(8.21-8.6) and 26.55(26.12-26.98). Sixty-five percent were men. Hypertension, dyslipidemia, smoking was present in 79.61%, 93.85% and 21.68%. There was a significant difference ($p<0.01$) in the diagnosis of PAD using the two methods. ABI calculated by the hand held Doppler had a sensitivity of 48%, specificity of 96% with the automated ABI instrument as the reference. The AUC with the hand held Doppler was 0.72(0.68-0.76). The positive and negative predictive value with the hand held doppler was 80% and 85%. **Discussion and Conclusion**: The hand held doppler has a low sensitivity to diagnose PAD in asymptomatic patients with diabetes. This cannot be used as a reliable test in limited care settings. **Acute Uncomplicated/Mildly Complicated Hyperglycemia**: Acute Hyperglycemia(without overt DKA/HHS) is a common condition encountered at the Diabetes Care Centre outpatient Department of the Princess Marina Hospital Gaborone. This is also a very common condition in other Primary care facilities in advanced countries as well. Standard treatment protocols exist for the treatment of DKA/HONK/HHS, but no standard protocols exist for the treatment of Acute Hyperglycemia which may/may not develop into established HHS/DKA. Further it is not clear as to which of these patients with Acute Hyperglycemia progress to the established complications. There are very few papers published with different regimens for the treatment of this condition but have not been standardised yet. This protocol was developed at the outpatient Diabetes Clinic of the Princess Marina Hospital with the sole objective of minimizing the admissions of DKA/HHS to the overcrowded A&E unit of the main Hospital. Since DKA and HHS can develop at blood sugar ranges between 13.5 mmol/l and 33.3mmol/l and above. Patients presenting to the OPD with Blood Sugars between the above range were enrolled for treatment on a weight and Blood sugar based administration of short acting Insulin along with adequate hydration to see if the blood sugars drop down to safer ranges (16.2mmol/l).(13.2 in the above range refers to Fasting Blood sugar). The criteria for choosing these blood sugar ranges are explained in the main paper. A total of 130 patients were treated so far with the following regime. 1.0.18 units/kg body weight for FBS between 11.3 and 16.7 mmol/l. 2.0.2U/kg body weight for FBS >16.7mmol/l. 3.0.18U/kg for RBS between 22.2 and 26 mmol/l. 4.0.2 U/kg for RBS above 26 to 35 mmol/l. 5.Patients who presented with Blood sugars higher than 37 mmol/l were administered a single dose of .2U kg and referred to the emergency department, despite being uncomplicated at the time ofm examination since they automatically fail the the protocol. The idea is to reduce the blood sugar levels to <16.2 mmol in 4 hrs. (The average OPD time). All the patients treated with above protocol achieved the target blood sugar levels of <16.2 mmol/ in less than 3hrs, and were

returned for follow up /dose adjustments of their medications within 48/72 hrs, with being treatment being continued until they return. There were no complications reported until the present date. And the protocol was successful I all the patients treated so far. **Conclusion**: Results of the above study suggest that Acute uncomplicated/mildly complicated Hyperglycemia can be managed in the outpatient department with a standardised protocol like the above thus preventing their progression to overt DKA/HHS thereby largely reducing the admissions to the emergency units for treatment of the overt DKA/HHS.

Diabetic Micro- and Macro-Vascular Complications are more in RA Patients: Is Inflammation a Link?

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Introduction: The micro- and macro-vascular complications may be attributable to inflammation in diabetes. RA is an inflammatory condition with high inflammatory burden. When DM is associated with RA with prevailing high inflammation, vascular complications are expected to be higher. We assessed incidence of the vascular complications between the groups. In addition, clinical findings were documented and cytokine and CRP levels as a marker of inflammation were determined. **Methods**: Subjects recruited at 2 centers: BDC (DM only-90) and CRICR (RA+DM-89) between Feb to Sept 2016. Patients screened for traditional vascular risk factors like BP, smoking, alcohol consumption, sugar levels. Blood levels of IL-6, IL-10 and TNF-a were determined in all patients on 1st visit. All patients were screened for the presence of neuropathy, nephropathy, retinopathy and coronary artery disease both by recollection and clinical examination. **Results:** The groups matched by age, smoking and hypertension. However, females were predominant in RA group (89%). The inflammatory parameters like IL-6, TNF alpha and ESR were higher among RA group (p-value < 0.0001). Whereas CRP was high among DM only group (p-value< 0.0001). HBA1c and microalbuminuria was significantly higher among RA, whereas eGFR was significantly lower. There were 14 with neuropathy, 3 with retinopathy, 5 with CAD in the RA group. None of these complications were seen in DM only group. **Conclusion:** Micro- and macro-vascular complications were higher among RA+DM group. It had a variable association with inflammatory parameters. Higher state of inflammation in RA might be the reason for increased vascular complications. This is an interim analysis. Further recruitment is required for elaboration of results. More studies are needed on the subject.

Co-Relation Between Inflammatory Markers and Ankle Brachial Index in Subjects with Type 2 Diabetes.

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Objective: An increasing body of evidence supports the concept that inflammation plays a major role in the development and progression of atherosclerosis which in lower extremities leads to peripheral vascular disease. We hypothesized that inflammatory markers, WBCs, neutrophils, IL-6 and TNF-α would be associated with ABI, a hallmark of atherosclerotic burden. **Research designs and Methods**: Among 60 type 2 diabetic patients, 35 were diagnosed for PVD (ABI ≤0.9), mean age 51.58±11.38 yrs, and 25 were without PVD (ABI >0.9), mean age 54.66±6.52 yrs. Serum concentrations of IL-6 and TNF-α were determined by enzyme linked immuno-sorbent assay. **Results**: ABI was lower in PVD patients then without PVD (0.677 ±0.13 v/s 1.27±0.41, p<0.001, respectively). By linear regression analysis, TNF-α and IL-6 were significantly co-related with ABI in an inverse manner (β= -0.006, p= 0.030, β= -0.005, p= 0.018) respectively. There was no significant co-relation of ABI with WBCs and neutrophils. **Conclusion**: ABI was strongly associated with PVD in type 2 diabetic patients. This study demonstrated that ABI was associated with IL-6 and TNF-α concentration with an inverse pattern making them prominent markers of inflammatory cascade involved in atherosclerosis.

Effects of Chronic Unpredictable Environmental Stress on Glucose Metabolism, Antioxidants Profile and Impaired Tissue Physiology: A Key Link in the Pathogenesis of Diabetic Complications.

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Background: Little evidence showed chronic unpredictable environmental stress (CUES) may induce predisposition to diabetes mellitus. The present study investigates the role of CUES on carbohydrate metabolism, lipid profile, antioxidants, stress-induced DNA damage and tissue anatomy. **Materials and Methods**: Mice were randomly divided into stress (n=20) and control group (n=20). Stressed group (n=20) were exposed to CUES with stressors for 16 weeks. Weekly body weight, feed consumption, fasting blood glucose were monitored in both groups. Plasma HbA1c, serum lipids, antioxidants and carbohydrate metabolizing enzymes activity were assessed along with DNA damage and histopathological examination of peripheral tissues that includes liver, kidney, pancreas, spleen and skeletal muscles. **Results and Discussion**: Fasting blood glucose levels (116.10±10.62 mg/dL) & HbA1c (7.1±0.13 %) in the stressed were significantly higher compared to control (93.18±2.37 mg/dL and 6.15±0.31 % respectively; p<0.001). Serum lipids were found in significantly higher in stressed mice compared to control group (p<0.001). Body weights of the stressed mice (23.63±1.06 gms) were significantly lower than control mice (26.35±2.821 gms; p<0.001). Feed consumption in the stressed group was not different. Significant changes were observed in antioxidants level, carbohydrate metabolizing enzymes activity (p<0.001). Histopathology of the peripheral tissue showed abnormal changes due to CUES. DNA integrity showed impairment in the comet assay. **Conclusions:** In conclusion, exposure to chronic unpredictable environmental stress leads to an alteration in carbohydrate metabolism, antioxidants profile and tissue homeostasis due to initiation of reactive oxygen species (ROS) that may play an important key link in the development of pre-diabetes state with complications.

Pharmacological Therapy of Diabetes

Saroglitazar: Six Month Safety and Effectiveness in Patients with Diabetic Dyslipidemia

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Saroglitazar is a dual PPAR α/γ agonist currently approved in India for the treatment of hypertriglyceridemia in type 2 diabetes not controlled with statins. A retrospective analysis was conducted to evaluate six month safety and effectiveness of Saroglitazar 4mg once daily in Indian diabetic dyslipidemia patients. In this analysis, the eligible patients meeting the criteria of diabetic dyslipidemia (type 2 diabetes and baseline triglycerides above 150 mg/dL) and prescribed Saroglitazar 4mg once daily over and above the ongoing antidiabetic and statin therapy were selected. A total number of 43 patients were identified by above criteria and were included in this analysis in whom safety and effectiveness (lipid and glycemic parameters) were

evaluated at 3 and 6 months. The mean age of study population was 54 years with 58.14% participants being male. The mean baseline triglycerides (TG) and Non-HDL cholesterol were 437.26 mg/dl and 150.72 mg/dl respectively. At 6 months, the TG was significantly reduced from 437.26 mg/dL to 226.86 mg/dL (p=0.019) and non HDL-C level was significantly reduced from 150.72 mg/dL to 123.21 mg/dL (p=0.022). Mean HbA1c was also significantly reduced from 8.59% at baseline to 7.83% after 6 months (p<0.0001). Saroglitazar treatment was found to be weight neutral. No major adverse event had occurred during 6 months of treatment. Hence, the analysis concluded that Saroglitazar is a potential add on therapeutic option for the treatment of hypertriglyceridemia in type 2 diabetes not controlled with statins along with additional significant improvement in glycemic parameters.

Can N-Acetyl Cysteine - Taurine- Provide Additional Reduction in Micro Albuminuria, in Type 2 Diabetic Patients Already on Angiotensin Converting Enzyme Inhibitors(ACEI) or Angiotensin Receptor Blockers(ARB) with or Without Dual Channel Calcium Bloc

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Background and Hypothesis: To prevent the progression of micro albuminuria to macro albuminuria and DN, we use either ACEI or ARB and or dual channel calcium blocker(Cilnidepine). These drugs have reduced MA and have prevented the progression to DN but have their limitations. Animal experiments with Taurine and NAC have been very encouraging in reducing MA, .**Objectives**: To know whether the combination of NAC and Taurine would additionally reduce microalbuminuria and TGF β expression in T2 diabetics who are already on either ACEI or ARB and or DCCB, and to know the effect of this combination on HbA1C, lipid parameters and e GFR **Material and Methods**: Eighty diabetics, having microalbuminuria were recruited .50 were in the test group and 30 were in the control group. All were examined, their height, weight, BMI, WC, BP were measured initially and at the end of 3 months. The test group was given NAC+Taurine tablets, one tab daily for 3 months and placebo was given to the control group. HbA1C, Lipid profile, Serum creatinine, Micro albuminuria and TGFb, e GFR were estimated before and on completion of the study. ANNOVA and Pearson's correlation were used for statistical analysis **Results**: 41 in the test and 21 in the placebo group, completed the study. The test group did show reduction in microalbuminuria and TGFb but not statistically significant. There was no change in SC and E-GFR. The drug did not have any effect on lipids, HbA1C **Conclusion**: The combination of NAC+Taurine has additional reduction in microalbuminuria and TGF b in those on ARB or ACEI with or without DCCB. Larger studies would be benifecial in this regard
TITLE: channel calcium blockers(DCCB)? A cross sectional, comparative, placebo controlled, observational Study.(TITLE THAT HAS BEEN LEFT OUT ABOVE).

Efficacy and Safety of the Dipeptidyl Peptidase-4 Inhibitor Gemigliptin Compared with Sitagliptin as an Add-On to Metformin in Patients with Type 2 Diabetes Inadequately Controlled on Metformin Alone: India Subgroup

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Background: Gemigliptin is a potent, selective, competitive, long-acting DPP-4 inhibitor. This study assessed the efficacy and safety of gemigliptin versus sitagliptin in patients with type 2 diabetes (T2D). **Material and Method**: This double-blind, randomized, active-controlled, phase III trial in 425 Asian patients (Korea, 28 sites and India, 10 sites) with T2D uncontrolled with metformin alone (≥1000 mg), was conducted during December 2009–June 2011. Eligible patients were randomized into three groups: 50 mg gemigliptin OD, 25 mg gemigliptin BID, and 100 mg sitagliptin OD for 24 weeks. Change in HbA1c from baseline to study end at 24 weeks, was noted. There was an additional extension phase of 28 weeks. **Results and Discussion**: We report results of 129 randomized patients from India. The demographic and baseline characteristics of the Indian patients were similar to those of Korean patients. The mean±SD change in HbA1c from baseline to week-24 was: -0.6%±0.95% by sitagliptin, -0.82%±0.85% by gemigliptin 25 mg BID, and -0.83%±0.96% by gemigliptin 50 mg QD. Gemigliptin was well-tolerated; there were no SAEs and no reports of pancreatic or cardiac clinical disorders with gemigliptin during the main 24-week treatment period and the extension period of 28 weeks. Being a subgroup analysis, no inferential statistics was performed. **Conclusion**: In Indian patients with T2D uncontrolled on metformin alone, gemigliptin as an add-on therapy is clinically effective in reducing HbA1c and has a favorable safety profile. The efficacy and safety of gemigliptin seen in Indian patients is similar to that of the overall study population.

SGLT2 Inhibitors- a Ray of Hope for Unmet Needs in Diabetes Mellitus (A Single Centre Study) N

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Background: Typical features of Type-II Diabetes Mellitus are insulin resistance of various organs and required glucose stimulated insulin secretion. Correcting insulin resistance and substituting insulin, currently is regarded as the gold standard of diabetes therapy. Only 50% of patients reach glycemic control with currently available therapies. SGLT2 inhibitors are a new class of drugs with an insulin independent mechanism of action. **Material and Methods**: This study was done to see the effectiveness of SGLT2 Inhibitors for glycemic control and outcome in Type-II Diabetes Mellitus. The study focuses on canagliflozin. The baseline glycemic parameters, ongoing therapy, impact on glycemic control & outcome were analyzed, through the records of 75 patients (43 males and 32 females) over a period of 6 months. **Results and Discussion**: Out of 75 patients, 20 with basal Insulin+ metformin+glimipride showed significant reduction in HbA1C, FBS, body weight & systolic B.P., along with reduction in insulin requirement. 30 patients on dual therapy & 25 patients were on triple drug & they all showed improvement in all the parameters with a small increase in HDL-C and LDL-c & reached HbA1c of <7% at the end. The incidence of hypoglycemia was low across all the groups with zero incidences of urinary tract & genital tract infections. **Conclusion**: SGLT2 inhibitors have a remarkable advantage compared with already established anti-diabetics, increasing urinary glucose excretion without inducing hypoglycemia, there by promoting weight loss. They have a favourable effect on HbA1c, systolic B.P. & HDL-C. However they are reported to be associated with significantly increased incidence of urinary tract and genital tract infection.

The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Controlled Evaluation (CREDENCE) Study Design and Rationale: An Indian Perspective

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Background: Diabetic nephropathy is the commonest cause of chronic kidney disease (CKD) in India. Canagliflozin (CANA) is an SGLT2

inhibitor, lowering plasma glucose by reducing uptake of filtered glucose in the kidney tubule leading to increased urinary glucose excretion. SGLT2 inhibitors have been shown to improve HbA1c, blood pressure, albuminuria, and cardiovascular (CV) outcomes. However their effects on kidney and CV outcomes in people with established kidney disease has not been studied. The CREDENCE trial aims to determine the effectiveness of CANA, compared with placebo, at preventing clinically important kidney and CV outcomes in people with established diabetic kidney disease. **Methodology**: The CREDENCE design is a 1:1 randomized, double-blind, event-driven, placebo-controlled multicenter trial with a projected duration of ~5 years. The CREDENCE trial is set in ~900 sites on 6 continents. It will recruit 4,200 adult participants with type 2 diabetes, eGFR ≤30 to <90 ml/min, and albuminuria treated with standard of care including a maximum labelled or tolerated dose of an ACE inhibitor or angiotensin receptor blocker. Participants are randomly assigned to CANA 100 mg daily or matching placebo. The primary outcome is the composite of end stage kidney disease, doubling of serum creatinine, and renal or CV death. Secondary and exploratory outcomes include an ordered hierarchy of CV and kidney disease endpoints. Nearly 200 patients will be recruited across 20 centers from India. **Conclusion**: The CREDENCE study will provide evidence on the role of SGLT2 inhibitors for the treatment of people with established diabetic kidney disease.

Initial Combination Therapy with Dapagliflozin (DAPA) + Metformin ExtendedRelease (MET XR) Impacts Quality Measures Relevant to Diabetes Care

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Diabetes measures are designed to improve the quality, safety, and affordability of healthcare for patients with type 2 diabetes mellitus (T2DM). In 2 phase 3 trials in treatmentnaive patients with T2DM, DAPA 5 or 10 mg as initial combination therapy with MET XR significantly reduced HbA1c from baseline at 24 weeks vs. DAPA or MET alone. A pooled analysis of data from these studies (N=814) was performed to determine the effect of DAPA 5 or 10 mg + MET XR vs. PBO + MET XR treatment on U.S. diabetes quality measures. Among all measures, the most stringent HbA1c, systolic/diastolic blood pressure (SBP/DBP) and lowdensity lipoprotein cholesterol (LDLC) thresholds were: HbA1c <7%, SBP/DBP <130/80 mmHg, and LDLC< 100 mg/dL. The proportion of patients with baseline body mass index >25 kg/m who lost ≥4.5 kg was also assessed. Outcomes showed significantly more patients in the DAPA 5 or 10 mg groups achieved HbA1c <7% vs. PBO (Table, P<0.02 for each dose). The difference from PBO in the proportion of patients with SBP/DBP <130/80 mmHg was statistically significant with DAPA 5 mg, although not with 10 mg. A similar proportion of patients had LDLC <100 mg/dL among groups. Significantly more patients lost ≥4.5 kg with DAPA vs. PBO. These data suggest that initial combination therapy with DAPA 5 or 10 mg + MET XR improves quality measures relevant to clinical outcomes and diabetes care

Teneligliptin Monotherapy in the Treatment of Type 2 Diabetes: An Analysis of Analyses

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Background/Hypothesis: Teneligliptin has been widely used in the treatment of type 2 diabetes mellitus(T2DM) in India. There limited clinical trial are available on teneligliptin Monotherapy. The present analysis is aimed to assess the therapeutic efficacy of Teneligliptin in the treatment of T2DM as monotherapy from different published clinical trials. **Material and Method**: We searched the databases like Medline, Embase, Google scholar and PubMed from inception until June 2016, and identified clinical trial where teneligliptin was used as monotherapy for treatment of T2DM. Weighted mean difference(WMD) was calculated for efficacy analysis. **Results and Discussion**: Seven clinical trials were included for this analysis. Total, 749 patients were included in these studies for mean duration of 18.3 weeks. Teneligliptin was associated with a significant reduction in HbA1c(WMD), - 0.96 % (range:−0.63 to−1.96, 6 study). Weighted percentage of patients who achieved target of HbA1c<7% were 56.25%. Fasting plasma glucose decreased significantly with Teneligliptin by -25.33 mg/dl (WMD; range: −14.1 to−44.0, 6 study), while Post-prandial plasma glucose reduced by- 47.94 mg/dl(WMD; range: −43.7 to −49.4, 3 study). Moreover, HOMA-B improved with teneligliptin by 11.94 % (WMD; range 8.1- 17.61, 4 studies). There was no difference in incidence of hypoglycaemia or serious adverse events in teneligliptin compared to control. **Conclusions**: Teneligliptin provided a clinically meaningful reduction in HbA1c and other glycemic parameters & no difference in serious adverse effects compared to controlled group in treatment of T2DM when used as monotherapy; it was thus found to be efficacious and well tolerated.

Management of Type 2 Diabetes Mellitus: Insights into Prescribing Trends

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Background: Recently, management of diabetes has changed with advent of novel agents like DPP4i, SGLT2i and GLP-1 agonist. Of these, DPP4i have emerged as promising agents for improved glycemic control and as an add-on to metformin (Met). This survey was planned to explore current prescribing trends of physicians of India for the management of diabetes mellitus. **Methods**: A survey questionnaire consisting of 10 questions related to management of diabetes in real-world clinical settings was prepared. The questionnaire was later validated in a small group of physicians and then administered to physicians and endocrinologists. **Results**: Responses from 502 physicians were received. 59% physicians prefer DPP4i as first add-on to Met followed by sulfonylurea (SU) (30%). Amongst DPP4i, Vildagliptin and Sitagliptin are preferred by 48% and 28% physicians respectively as first add-on to Met. For patients uncontrolled on met + SU therapy, 54 % physicians prefer DPP4i as second add-on. Vildagliptin is perceived to have the highest efficacy and safety data, as suggested by 40% and 43% physicians respectively. 48% physicians were hesitant to prescribe teneligliptin due to insufficient data. SGLT2 inhibitors are preferred as second/ third add-on by 36% and 44% physicians respectively. **Conclusion**: DPP4i are being increasingly preferred by physician, as an add-on to metformin. Further research should continue to document changes in diabetes management trends, especially given the increasing number of medications available.

Efficacy of Canagliflozin (CANA) Versus Dipeptidyl Peptidase-4 Inhibitors (DPP-4i) in Patients With Type 2 Diabetes Mellitus (T2DM): Results From Randomized Controlled Trials (RCTs) and a Real-World (RW) Study

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In RCTs, CANA was shown to be more effective than the DPP-4i sitagliptin (SITA) in lowering glucose. RCT and RW results tend to differ as RW studies may include a broader set of patients with more advanced conditions; thus it is important to assess the effects of agents in clinical practice. We compared the A1C-lowering efficacy of CANA 100 and 300 mg versus SITA 100 mg in 3 RCTs of patients with T2DM, and the effectiveness of CANA (pooled data for all doses) in a retrospective RW matched control-cohort study using US integrated claims and laboratory data from a large population of insured patients with T2DM (65%

and 34% of patients received CANA 100 or 300 mg, respectively [1% other]). Patients in the CANA cohort were matched 1:1 to patients in the DPP-4i cohort using propensity score matching that incorporated demographics and baseline characteristics. In RCTs with baseline A1C ~8.0%, CANA 100 mg provided similar and CANA 300 mg provided greater A1C reductions versus SITA 100 mg. In the RW study with baseline A1C ~9.0%, greater A1C reductions were seen with CANA (−1.07%) versus DPP-4i (–0.79%). In summary, the relative magnitude of A1C reduction with CANA and SITA was similar in the RCT and RW studies; CANA consistently lowered A1C versus DPP-4i in patients with T2DM.

Real-Life Baseline Characteristics of Drug Naïve Asian T2DM Patients in the Multinational Initial Study

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Background/Hypothesis: A sharp increase in type 2 diabetes mellitus (T2DM) prevalence has been observed in South-East Asia region and is expected to further rise. Early therapeutic intervention is clinically important considering the multifactorial impact of diabetes on cardiovascular risk. Real-life evidence assessing value of initial DPP-4 inhibitor combination therapy in drug naïve patients is limited. **Methods**: The INITIAL study is a 24-week non-interventional, prospective study in adult drug naïve T2DM patients prescribed vildagliptin/metformin initial combination therapy within 4 weeks of study entry according to local label, with documented HbA1c >7.5% (>8% in India). Primary end-point is change in HbA1c from baseline to study end. Patient baseline characteristics are presented here. **Results**: A total of 522 patients across Asia (India, n=197; Bangladesh, n=154; Philippines, n=127; South Korea, n=44) were analyzed. Overall, study population was relatively young (mean age: 49.6±11.35 years; >65 years: 9.6%), with 55.6% men, mean diabetes duration of 0.9±2.49 years and diabetes family history in ~28%. Mean baseline HbA1c was high (9.3±1.58%, range: 7.5-15.7%; HbA1c>10%: 25.1%). Mean body weight was 70±12.5 kg, with 17.6% obese (≥30 kg/m2) (mean BMI: 26.8±4.5 kg/m2); ~30% patients reported either medical history of hypertension or dyslipidemia. Approximately 71% patients received twice-daily dose of vildagliptin/metformin and ~26% once-daily. **Conclusion**: Patients enrolled in the INITIAL study are relatively young, present with high baseline HbA1c (possibly due to delayed diagnosis) and often associated with multiple cardiovascular risk factors at diagnosis/therapy initiation. The study would provide valuable evidence to guide clinical treatment decisions in this real-life setting.

Glycemic Outcomes in Timely and Delayed Insulin-Initiated Patients with Type 2 Diabetes (T2D)

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Background: To compare timely vs delayed insulin-initiated patients with T2D by 2- and 5-year glycemic outcomes in general practice in the UK. **Material and Method**: This retrospective cohort study used the UK Clinical Practice Research Datalink to assess the glycemic control of patients initiating insulin during 2005-2012. Timely/delayed initiation cohorts were defined by elapsed time between the recommended insulin initiation point (persistent HbA1c ≥7.5% with ≥2 noninsulin agents) and actual initiation (≤1 or >1 year, respectively). We used matched samples by propensity scores adjusting for baseline characteristics for comparing glycemic outcomes. **Results and Discussion**: In total, 12367 patients had sufficient HbA1c/noninsulin agent data (timely, N=2702; delayed, N=9665). The algorithm produced 2293 comparable patients (each cohort). Time to achieve HbA1c ≤7%, 7.5%, or 8% was significantly shorter in the timely cohort, but no significant difference was found in time needed to reach a 1% reduction from baseline. Mean HbA1c during the second and fifth years postinitiation were similar. Mean HbA1c postinitiation was significantly lower in the timely cohort. **Conclusion**: In UK clinical practice, delaying insulin initiation >1 year was associated with longer time to achieve targets and poorer glycemic control postinitiation. Glycemic control remained suboptimal (both cohorts), with mean HbA1c values above recommended targets, indicating insulin titration inertia. **Disclosures:** This study was supported and conducted by Eli Lilly and Company, Indianapolis, IN, USA. This is an encore of an abstract that was presented at the International Diabetes Federation – 23rd World Diabetes Congress; November 30 – December 4, 2015; Vancouver, Canada.

Consistency of Treatment Effect Across the Range of Baseline HbA1c in Patients with Type 2 Diabetes Mellitus (T2DM) Treated with Once-Weekly Dulaglutide or Comparators in AWARD-1, -5, and -6

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Background: To characterize the effects of once-weekly dulaglutide (DU) and active comparators on HbA1c change across the continuous range of baseline HbA1c values. **Material and Method**: Adults were randomized to DU 1.5 mg, DU 0.75 mg, or exenatide 10 mcg BID (AWARD-1; N=835); DU 1.5 mg, DU 0.75 mg, or sitagliptin 100 mg QD (AWARD-5; N=921); or DU 1.5 mg or liraglutide 1.8 mg QD (AWARD-6; N=599) with metformin (AWARD-5, -6)/metformin+pioglitazone (AWARD-1). HbA1c changes were evaluated at primary endpoint (AWARD-1, -6: 26 weeks; AWARD-5: 52 weeks) and analyzed by study using LOCF ANCOVA with treatment-by-baseline HbA1c interaction terms. **Results and Discussion**: All treatments reduced HbA1c from baseline. DU 1.5 mg showed greater (exenatide, sitagliptin)/similar (liraglutide) mean HbA1c reductions vs the comparator. In AWARD-1 and -6, there was no indication of a differential treatment effect of baseline HbA1c. In AWARD-5, a differential treatment effect of baseline HbA1c was observed, driven by the sitagliptin group, with more pronounced between-treatment differences favoring DU as baseline HbA1c increased. Results were similar with DU 0.75 mg in AWARD-1 and -5. **Conclusions**: HbA1c improved with all treatments. The relative effects on HbA1c of DU 1.5 mg vs active comparators were consistent across the range (exenatide, liraglutide) or the difference between treatments increased as baseline HbA1c increased (sitagliptin). **Disclosures :** This study was supported and conducted by Eli Lilly and Company, Indianapolis, IN, USA. This is an encore of an abstract that was presented at the Diabetes UK Annual Professional Conference; March 2 – 4, 2016; Glasgow, UK.

Real-World 12-Month Outcomes of Patients with Type 2 Diabetes Mellitus (T2DM) Treated with Canagliflozin in a US Managed Care Setting

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Canagliflozin (CANA), the first approved agent that inhibits sodium glucose co-transporter 2, improves glycemic control through an insulin-independent mechanism. This study evaluates glycemic control pre- and post-CANA over a 12 month period. This retrospective cohort study used data from a large US health plan for adult commercial and Medicare Advantage enrollees with T2DM filling CANA between April 2013 - August 2014 who had A1C results pre and post the first observed CANA prescription and a pre-CANA A1C ≥7.0%. Of identified patients (n=2,269), 61% had CANA 100mg on the first observed fill, 41% were female, and mean age was 56 years. Pre-CANA mean A1C was 8.93% ±1.56%. Patients, on average, used 2.4 ±1.1 unique antihyperglycemic agents (AHAs) in the pre-CANA period, inclusive of injectables. Based on the last A1C result ≥30 days following the first observed CANA claim in the 12-month post-CANA period, patients had a mean reduction of 0.96% ±1.56%, with an average time to post-CANA A1C of 262 days. The proportion of patients achieving A1C <7.0% and <8.0% were approximately 25% and 59% post-CANA. CANA was prescribed to patients with T2DM who were often uncontrolled (mean pre-CANA A1C of 8.93%) despite prior treatment with multiple AHAs. Improvements in A1C consistent to those found in clinical trials were observed in the 12 months following the first CANA prescription

Pooled Analysis of Four Randomized Studies with Insulin Glargine 100 U/mL (Gla-100) versus NPH Insulin in Adults with T1DM Using a Basal Plus Meal-time Insulin Regimen

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Background/Hypothesis: We examined clinical outcomes in adults with T1DM treated with Gla-100 or NPH insulin (NPH) in a basal plus meal-time regimen. **Materials and Methods**: Standardized patient-level data were pooled from four RCTs of 28 weeks duration comparing Gla-100 od at bedtime and NPH (55% od at bedtime, 45% bd), combined with human insulin (HI) or insulin lispro (lispro) at meal-times. Baseline and Week 28 HbA1c, FPG, weight, insulin dose and hypoglycemia were analyzed overall and by meal-time insulin. **Results and Discussion**: Of 1,526 participants, 756 used Gla-100 (694 HI, 62 lispro) and 770 NPH (707 HI, 63 lispro). Baseline characteristics were similar across treatment arms. Overall, there was no significant difference between Gla-100 and NPH in HbA1c reduction (-0.16 versus -0.14 %, respectively). In the lispro-treated group, those treated with Gla-100 had significantly greater HbA1c reductions compared with NPH (-1.01 versus -0.55 %; P=0.018). FPG reduction was significantly greater with Gla-100 (-36 versus -21 mg/dL; P=0.0003) with significantly lower basal insulin doses at Week 28 for Gla-100 overall (0.31 versus 0.35 U/kg; P<0.0001). Body weight increased in all groups, though no significant differences were observed. Confirmed nocturnal and severe nocturnal hypoglycemia event rates were significantly lower with Gla-100 versus NPH (6.5 versus 8.0 events/person-year [P=0.006] and 0.19 versus 0.33 events/person-year [P=0.048], respectively). **Conclusion**: In this pooled analysis of adults with T1DM, FPG, insulin dose and nocturnal hypoglycemia rates were lower with Gla-100 than NPH. When Gla-100 was combined with lispro, HbA1c and FPG appeared lower versus those on NPH.

Patient Characteristics and Clinical Outcomes Associated With Hypoglycemia Frequency During Titration of Insulin Glargine 100 U/mL (Gla-100) in People With T2DM

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Background/Hypothesis: Hypoglycemia during insulin initiation and intensification can be a barrier to dose optimization and achievement of glycemic control targets. **Materials and Methods**: This post-hoc subject-level analysis examined standardized data from 16 RCTs (fasting plasma glucose [FPG] target ≤100 mg/dL, ≥24 weeks duration) adding Gla-100 to OADs in insulin-naive people with T2DM. The impact was studied of overall hypoglycemia frequency (confirmed PG <70 mg/dL or assistance required, stratified according to 0, 1−3, ≥4 events during titration from Weeks 0−8) on glycemic outcomes and insulin dose at Week 24. **Results and Discussion**: Data from 3,549 participants were analyzed. Group size declined as hypoglycemia frequency increased but mean age was similar (58 years) across all groups. Those with ≥4 hypoglycemic events during titration had the lowest baseline body weight (77.6 versus 87.7 and 83.3 kg), and HbA1c (8.5 versus 8.8 and 8.6 % for 0 and 1-3 events, respectively). In contrast, those experiencing less hypoglycemia (≤3 events) had higher FPG (194, 187 and 185 mg/dL for 0, 1-3 and ≥4 events, respectively) at onset. These patients also had a greater change in insulin dose from baseline to Week 24 (0.31 versus 0.20 and 0.10 U/kg, for 0, 1-3 and ≥4 events, respectively). In all groups, change in HbA1c from baseline to Week 24 was -1.5 %. **Conclusion**: Lower hypoglycemia incidence occurs during insulin titration in people with T2DM with a greater insulin resistance (higher insulin dose requirement), in contrast to people experiencing more hypoglycemia during titration.

Electronic Survey on Teneligliptin (ESOT) - Insights on Efficacy of Teneligliptin in Indian Setting

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Background: Dipeptidyl Peptidase-4 inhibitor (DPP-4i) is a promising class of drug to manage type 2 diabetes mellitus (T2DM). Teneligliptin, is a DPP-4i with various advantages like reduced risk of hypoglycemia, beta cell preservation, cardiac safety and reduced risk of weight gain compared to other anti-diabetic drugs. However, not much is known about its efficacy in Indian setting. Hence we conducted a survey to understand real life experience of Indian doctors regarding the efficacy of teneligliptin. **Material and Method**: An online electronic survey was conducted to gain insights on efficacy of teneligliptin in India. A survey questionnaire containing 9 questions was open online for two months. Doctors practicing in India and

prescribing the molecule teneligliptin to T2DM patients were eligible to participate in the survey. R-software was used for data analysis. **Results and discussion**: A total of 683 doctors from all over India (18 States and Union Territories) participated in this survey. Efficacy of teneligliptin was rated as excellent by 34% and good by 61% of doctors. It was prescribed as first add-on therapy by 34% of doctors. All doctors considered teneligliptin in T2DM with hypertension and dyslipidaemia. Also, 62% prescribed it in T2DM with mild hepatic impairment. 77% of doctors agreed and 14% of the doctors strongly agreed that no dose adjustment was required in mild, moderate and severe renal failure for teneligliptin. When compared with other DPP-4i, it was rated as good by 42% and excellent by 14% doctors. **Conclusion**: Teneligliptin was widely prescribed and considered as first add-on therapy with 20mg being the preferred dose by Indian doctors. Overall efficacy of teneligliptin was found to be good in T2DM patients with comorbid hepatic conditions, renal conditions and also when compared to other DPP-4i. Therefore this low cost gliptin is a good alternative to other costly gliptins in developing countries like India.

Possible Regression of Chronic Kidney Disease in Type 2 Diabetes Associated with Linagliptin-based Therapy: A Series of 3 Cases

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Background: In diabetic patients predisposed to CKD, appropriate therapy selection merits essential consideration. Evidence from trials suggests superior renal safety associated with linagliptin therapy, and a clinically meaningful early reduction in proteinuria. Real-world evidence may contribute to the existing knowledge in this aspect. **Material and Method**: A case-series based on retrospective analysis of patient-records, from a single centre. **Results and Discussion**: Case 1: Long-standing diabetic male receiving insulin-based regimen, presented with uncontrolled glycemia, early renal impairment (CKD stage 2 and microalbuminuria), and frequent episodes of hypoglycemia. Following modification to a linagliptin-based regimen, the glycemia control improved, hypoglycemia was mitigated, and sustained reduction in albuminuria was observed. Serum creatinine reduced significantly over 42 weeks. Subsequently, on introduction of an SGLT2-inhibitor, serum creatinine increased as expected. The patient was maintained off-insulin, with good glycemia control over 7 months. Case 2: Elderly female presented with long-standing diabetes, uncontrolled glycemia, diabetic neuropathy, and advanced CKD (stage 4). Following introduction of linagliptin-based regimen, macroalbuminuria regressed to microalbuminuria, over subsequent 4 months. Case 3: Elderly male presented with long-standing diabetes, hypertension, dyslipidemia, microvascular complications, normoalbuminuria, and serum creatinine of 2.9 mg/dL (CKD stage 4). Following introduction of linagliptin-based regimen, glycemia control improved, and serum creatinine reduced to 1.6 mg/dL indicating regression to CKD stage 3, over 3 months. **Conclusion**: This real-world evidence suggests the possibility of improvement in renal function at various stages of chronic kidney disease, associated with linagliptin therapy. Further confirmatory evidence in this regard, will be available from the currently ongoing CARMELINA trial.

Efficacy and Safety of Linagliptin in Type 2 Diabetes Patients with Liver Disease: A Retrospective Analysis

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Objective: Evaluation of efficacy and safety of linagliptin in type 2 diabetes mellitus patients with liver impairment. **Material and Methods**: In this retrospective study, patients with type 2 diabetes mellitus associated with liver impairment were treated with linagliptin 5 mg once daily. Efficacy of linagliptin on glycemic control was evaluated by examining change in glycosylated haemoglobin. The effect of linagliptin on liver dysfunction was assessed by abdominal ultrasound after 3-4 months therapy. **Results**: A total of 20 type 2 diabetes patients (55% male and 45% female) with mean age 63.35±8.93 years were enrolled. Glycosylated haemoglobin (HbA1C) reduced from 7.74 (0.18) to 7.12 (0.14) % [mean (SEM)] (p=0.0004). No significant effect on underlying liver pathology was observed with repeat ultrasound. **Conclusion**: Linagliptin use in type 2 diabetes patients with liver impairment is safe and effective in reducing glycosylated hemoglobin. Linagliptin does not cause deterioration of liver pathology in these patients.

Teneligliptin Real World Efficacy Assessment of Type 2 Diabetes Mellitus Patients in India (TREAT-INDIA Study)

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Background/Hypothesis: Teneligliptin was introduced in India in May 2015. It has gained popularity and is already widely prescribed in Type 2 diabetes mellitus (T2DM). This 'real life' data collection was conducted to assess the efficacy of teneligliptin in Indian T2DM patients. **Material and Method**: Predesigned structured proforma was used to collect information from the prescribing physicians regarding the efficacy of teneligliptin when prescribed as monotherapy as well as combination therapy with other antidiabetic drugs in T2DM patients. Information on the glycemic parameters at baseline prior to starting teneligliptin and at the end of 3 months therapy was collected. The efficacy was assessed by analysing the mean change in 3 month values of glycosylated haemoglobin (HbA1c), fasting plasma glucose (FPG) and post-prandial plasma glucose (PPG). **Results and Discussion**: Data of 4305 patients was available for analysis. There was statistically significant improvement in mean HbA1c, FPG and PPG with teneligliptin therapy. Means changes in HbA1c, FPG and PPG was -1.37±1.15%, 51.29±35.41mg/dl and 80.89±54.27mg/dl respectively. Subgroup analysis revealed that HbA1c(%) reduction with teneligliptin when used as monotherapy, add-on to metformin or add-on to metformin plus Sulphonylureas(SUs) combination, add-on to metformin plus alpha glucosidase inhibitor (AGIs) combination or add-on to insulin was 0.98±0.53, 1.07 ±0.83, 1.46±1.33, 1.43±0.80, 1.55±1.05 respectively. **Conclusion**: Real-world data suggests that teneligliptin significantly improves glycaemic control in Indian patients with T2DM when prescribed either as monotherapy or as an add-on to one or more other commonly prescribed antidiabetic drugs.

Adding Once-Daily Lixisenatide for Type 2 Diabetes Inadequately Controlled by Established Basal Insulin (GetGoal-L): India Subgroup

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Background: Lixisenatide, a novel GLP-1RA, has demonstrated significant improvements in glycemic control, low rates of hypoglycemia, and a beneficial effect on weight. This study examined the efficacy and safety of adding lixisenatide to established basal insulin therapy alone or together with metformin, in Indian patients with type 2 diabetes (T2D). **Material and Method**: A randomized, double-blind, placebo-controlled, 2-arm parallel-group study in 496 patients (111 centers in 15 countries), with a 24-week main treatment period and an extension phase. Patients were randomized to lixisenatide 20 mg or placebo, given subcutaneously within 1 h before the morning meal. **Results and Discussion**: We report the results of the Indian subset (N=50; at

baseline mean±SD age: 51.1±9.7 years; BMI: 27.09±4.06 kg/m2). As add-on to basal insulin, the mean change in HbA1c% from baseline to week 24 was -0.9 with lixisenatide and -0.5 with placebo (-0.4 placebo corrected; 95% CI -1.272 to 0.473). The LS Mean change in 2hr PPG values was 49.5 mg/dL and 18.72 mg/dL with lixisenatide and placebo, respectively (-30.6 mg/dL placebo corrected; CI -83.52 to 22.14). Additionally, mean±SD change in body weight was -1.1+2.41 kg and -0.1+1.56 kg in the lixisenatide and placebo arms, respectively. Rates of hypoglycemia were similar in both groups. Overall, lixisenatide was well tolerated during the study period. Being a subgroup analysis, no inferential statistics was performed. **Conclusion**: By improving HbA1c and postprandial hyperglycemia without weight gain in T2D with inadequate glycemic control despite stable basal insulin, lixisenatide may provide an alternative to rapid-acting insulin or other treatment options. Indian subgroup results were similar to worldwide results in demonstrating superiority of lixisenatide versus placebo in glycemic control.

Patient Characteristics and Clinical Outcomes Associated With Hypoglycemia Frequency During Titration of Insulin Glargine 100 units/mL (Gla-100) in People With Type 2 Diabetes (T2D)

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Background / Introduction: Hypoglycemia during insulin initiation and intensification can be a barrier to dose optimization and achievement of glycemic control targets. **Methods**: This post-hoc subject-level analysis examined standardized data from 16 RCTs (FPG target ≤ 100 mg/dL, ≥ 24 weeks duration) adding Gla-100 to OADs in insulin-naive people with T2D. The impact was studied of overall hypoglycemia frequency (confirmed PG < 70 mg/dL or assistance required, stratified according to 0, 1−3, 4−6, or > 6 events during titration from Weeks 0−8) on glycemic outcomes and insulin dose at Week 24. **Results**: Data from 3,549 participants were analyzed. Group size declined as hypoglycemia frequency increased but mean age was similar (58 years) across all groups. Those with > 4 hypoglycemic events during titration had the lowest baseline body weight, FPG, and HbA1c, and longer diabetes duration. In contrast, those experiencing less hypoglycemia (≤ 3 events) had higher BMI, FPG and HbA1c at onset with a greater change in insulin dose from baseline to Week 24. **Conclusion**: Lower hypoglycemia incidence occurs during insulin titration in people with T2D with a greater insulin resistance (higher insulin dose requirement and smaller HbA1c reduction), in contrast to people experiencing more hypoglycemia during titration with greater HbA1c reduction.

Pooled Analysis of Four Randomized Studies with Insulin Glargine 100 U/ml vs NPH Insulin in Adults with T1DM Using a Basal Plus Meal-Time Insulin Regimen

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Objectives: To examine the efficacy and safety outcomes in people with T1DM treated with insulin glargine 100 units/ml (Gla-100) or NPH insulin in a basal plus meal-time regimen. **Methods**: Standardized patient-level data were pooled from four RCTs of 28 weeks duration comparing once daily Gla-100 at bedtime and NPH insulin (55% QD at bedtime, 45% BID), in combination with either human insulin (HI) or insulin lispro (lispro) at meal-times. HbA1c, fasting plasma glucose (FPG), weight, insulin dose and confirmed hypoglycemia were analyzed from baseline to week 28 by meal insulin type and overall. **Results**: of 1526 partcipants, 756 used Gla-100 (699 HI, 62 lispro) and 770 NPH insulin (707 HI, 63 lispro). HbA1c reductions were comparable between Gla-100 and NPH insulin overall, but greater with Gla-100 and meal-time lispro. FPG decrement was significantly greater with Gla-100 vs NPH insulin (P=0.0003) with a significantly lower basal insulin dose at week 28 for Gla-100 overall (P<0.0001). Event rates of confirmed nocturnal and severe nocturnal hypoglycemia were significantly lower with Gla-100 vs NPH insulin; rate ratios 0.80 and 0.57. **Conclusions**: In this pooled analysis of adults with T1DM, FPG, insulin dose and nocturnal hypoglycemia rates were lower with Gla-100 than NPH insulin therapy. When Gla-100 was combined with meal-time insulin lispro, HbA1c and FPG appeared lower vs those on NPH insulin

Efficacy of Canagliflozin (CANA) in Combination with Metformin (MET) in Patients with Type 2 Diabetes Mellitus (T2DM): Results from 3 Studies

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Objective: In the AACE clinical practice guidelines, SGLT2 inhibitors, such as CANA, are the first oral medication recommended for patients inadequately controlled on MET. This analysis assessed the efficacy of CANA in patients with T2DM in combination with MET in 3 studies. **Methods**: CANA 100 and 300mg were assessed vs placebo (PBO) at Week 26 and sitagliptin 100 mg (SITA) at Week 52 in Study 1, and vs glimepiride (GLIM) at Weeks 52 and 104 in Study 2. In Study 3, drug-naïve T2DM patients received initial combination therapy with MET+CANA 100mg (CANA100/MET) or MET+CANA 300mg (CANA300/MET) vs MET alone for 26 weeks. **Results**: In Study 1, CANA 100 and 300mg significantly lowered A1C vs PBO at Week 26; CANA 100mg demonstrated noninferiority and CANA 300mg demonstrated superiority vs SITA at Week 52. In Study 2, CANA 100mg demonstrated noninferiority and CANA 300mg demonstrated superiority in A1C lowering vs GLIM at Week 52; reductions were –0.65%, –0.74%, and –0.55% at Week 104. In Study 3, CANA100/MET and CANA300/MET significantly lowered A1C vs MET at Week 26. Significant BW reductions were seen in Study 1 with CANA 100 and 300mg vs PBO at Week 26 and vs SITA at Week 52. In Study 2, CANA 100 and 300mg significantly lowered BW vs GLIM at Week 52; BW changes were sustained at Week 104. In Study 3, significantly greater weight loss was seen with CANA100/MET and CANA300/MET vs MET at Week 26.. CANA was generally well tolerated in each study, with increased incidence of adverse events related to SGLT2 inhibition (eg, genital mycotic infections) and low rates of hypoglycemia. **Conclusion**: In 3 studies, CANA in combination with MET improved A1C, BW, and SBP, suggesting that a fixed-dose combination of CANA+MET may be beneficial in patients with T2DM.

Real-World Evaluation Of Weight Loss In Patients With Type 2 Diabetes Mellitus (T2DM) Treated With Canagliflozin (Cana) - An Electronic Health-Record (EHR)-Based Study

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Objectives: Canagliflozin (CANA) has been shown to improve glycemic control and body weight (BW) in T2DM patients. This study leveraged EHR data to evaluate BW over time among patients with T2DM receiving CANA in a real-world setting. **Methods**: Adult patients with ≥1 T2DM diagnosis and ≥12 months of clinical activity (baseline) before first CANA prescription (index) were identified in the Cegedim Strategic Data US EHR dataset. Paired t-tests were used to compare baseline BW to BWat 3 and 12 months post-index. **Results**: A total of 16,163 CANA users were identified (35% CANA 300 mg users, 48% female, mean age: 59 years,). Mean exposure to CANA was 155.6 days. Among patients evaluated at 3 months (N=6,811; mean baseline BW=102.9 kg), BW decreased from baseline by 1.8 kg (P<0.001) and 13.3% of patients had a weight loss ≥5%. At 12 months (N=1,288; mean baseline BW=103.8 kg), BW decreased from baseline by 2.6 kg (P<0.001) and 25.8% of patients had a weight loss ≥5%. Among patients with a baseline BMI≥30 kg/m2, at 3 months (N=5,155; mean baseline BW=110.3 kg) BW decreased by 2.1 kg (P<0.001) and 13.6% of patients had a weight loss ≥5%; at 12 months (N=995; mean baseline BW=110.8 kg), BW decreased by 3.0 kg (P<0.001) and 27.5% of patients had a weight loss ≥5%. **Conclusions**: Patients with T2DM treated with CANA in a real-world setting experienced statistically significant weight loss over time, in both the overall population and in patients with BMI≥30 kg/m2.

TREAT INDIA – Subgroup Analysis Based on Baseline Glycosylated Hemoglobin (TREAT INDIA - Teneligliptin Real World Efficacy Assessment of Type 2 Diabetes Mellitus Patients in India)

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Background: Baseline glycated hemoglobin (HbA1c) is critical in assessing anti-diabetic agent efficacy. Teneligliptin showed significant efficacy when used as monotherapy or as add on to anti-diabetic drugs during the retrospective real life analysis – TREAT INDIA. Current subgroup analysis done to see the efficacy of teneligliptin when patients categorized based on baseline HbA1c. **Material and Methods**: All 4305 patients from TREAT INDIA study categorized in three subgroups based on baseline HBA1c i.e. <7.5% (Group A), ≥ 7.5 - ≤ 9% (Group B) and > 9% (Group C). Change in HbA1c, Fasting and postprandial glycemic (FPG & PPG) parameters from baseline at the end of three months was assessed in three subgroups. **Results**: Most of the patients (n=2826; 65.65%) belonged to Group B; followed by 21.25% (n=915) in Group C and 13.10% (n=564) in Group A. The mean baseline HbA1c in Group A, B and C was 7.15±0.21%, 8.23±0.44% and 10.35±1.37% respectively. The reduction in HbA1c was directly proportional to baseline HbA1c with maximum mean reduction of 2.76±1.68% at the three months seen in Group C followed by 1.07±0.48% in Group B and 0.57±0.31% in Group A. The similar corresponding reduction in FPG and PPG was seen in all three subgroups. However the maximum percentage of patients achieving the HbA1c target of < 7% was seen in Group A. **Conclusion**: The reduction in glycemic parameters with teneligliptin strongly correlated with baseline glycemic values i.e. more the HbA1c at baseline; more was the reduction at the end of 3 months.

Real Life Experience of Efficacy and Safety of Teneligliptin

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Background: Teneligliptin is an affordable gliptin available in India since June 2015. This study planned to evaluate the efficacy and safety of teneligliptin in T2DM patients as add on to current ongoing therapy. **Material and Method**: Clinical records of patients at Apollo Sugar Clinic, Banashankari, Bangalore; who were prescribed with teneligliptin and having atleast one follow up visit after three months has been analyzed. **Results**: Available clinical data along with demographic features of total 21 patients was analyzed. Baseline demographics were (mean): age 48.62 yr, weight 67.06 Kg, BMI 25.621, duration of diabetes 6.07 yr with 15 males and 6 females. Teneligliptin found to be used most commonly as combination therapy with three or more anti-diabetic drugs. Class of anti-diabetic drugs used with teneligliptin were – Biguanide (100%), sulfonylurea (80.95%), alpha glucosidase inhibitor (42.86%), SGLT2 inhibitors (38.10%), thiazolidinediones (14.29%), Insulin (14.29%) and hydroxychloroquine (4.76%). Total of 5 patients (23.81%) were switched from sitagliptin to teneligliptin because of affordability and one patient was drug naïve. At 3 months follow up, changes in HbA1c, FPG and PPG from baseline were statistically significant with mean reduction of 2.83% (P Value=0.0001), 78.91 mg/dl (P Value=0.0003) and 113.57 mg/dl (P Value=0.0001) respectively. No significant change in body weight was seen. Almost 52% of patients achieved ADA target of HbA1c <7%. There was no adverse event reported during three months follow up after teneligliptin prescription. **Conclusion**: In this single center real life experience; teneligliptin is effective for T2DM management as add on to other anti-diabetic drugs and generally well tolerated.

Efficacy and Safety of Dapagliflozin in Patients with Type 2 Diabetes (T2D): Outcomes by Body Mass Index (BMI)

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Using data pooled from 10, 24-week placebo (PBO)-controlled studies of dapagliflozin (DAPA) as monotherapy or add-on therapy to other antidiabetes drugs in patients with T2D, we conducted a posthoc analysis assessing the efficacy and safety of DAPA 5 and 10 mg/d in subgroups by baseline BMI category. At baseline, mean age (54-60 y), T2D duration (6.0-10.2 y), and A1c (8.12%-8.33%) were similar across treatment and BMI groups. Mean fasting C-peptide values ranged from 2.1 to 4.9 ng/mL, increasing with increasing BMI. At week 24, DAPA 5 and 10 mg/d significantly reduced A1c and body weight from baseline vs. PBO across all BMI subgroups (Table). In addition, substantially more overweight (11% and 13% vs. 5%) and obese (18%-27% and 20%-29% vs. 9%-13%) patients shifted 1 BMI category lower with DAPA 5 and 10 mg/d vs. PBO, respectively. Genital (0-13.6% vs. 0.1%-1.1%) and urinary tract (2.6%-9.6% vs. 2.2%-6.4%) infections were more frequent with DAPA vs. PBO across subgroups and appeared to be more frequent with higher BMI. Hypoglycemia (excluding data after rescue) rates were similar or higher with DAPA (7.0%-16.5%) vs. PBO (8.5%-11.9%) and did not appear to differ by BMI; major hypoglycemia was reported for 4 patients (BMI 18.5-<25, DAPA 5 mg/d; 25-<30, PBO; 30-<35, DAPA 10 mg/d [n=2]). These data support DAPA as an effective and well-tolerated treatment option for patients with T2D and BMI ranging from 18.5 to ≥40 kg/m2.

Safety and Efficacy of Dapagliflozin in Lean vs. Overweight Asian Patients with Type 2 Diabetes Mellitus

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Asian patients develop type 2 diabetes mellitus (T2DM) at a relatively lower body mass index (BMI) than Western patients. Dapagliflozin (DAPA) is a sodium-glucose co-transporter 2 inhibitor, reducing hyperglycemia by increasing glucosuria, which also results in caloric loss. This

pooled analysis assessed the safety and efficacy of DAPA in Asian patients with T2DM and baseline BMI <25 kg/m2 (BMILO) vs. ≥25 kg/m2 (BMIHI). Data were analyzed from 8 Phase 2b/3 studies of ≤24 weeks in which Asian patients with T2DM received DAPA (5 or 10 mg) (N=956) or placebo (PBO; N=497) ± background glucose-lowering therapy. Baseline characteristics were balanced across treatment groups; BMIHI patients had a slightly shorter duration of diabetes, and higher systolic blood pressure and triglycerides. DAPA was well tolerated, and baseline BMI category had no clear impact on adverse event rates, including hypoglycemia, genital and urinary tract infections, volume depletion and fractures (Table). After 24 weeks of treatment, changes in HbA1c from baseline were comparable in the BMI subgroups. As compared with BMIHI, absolute weight reductions in BMILO seemed slightly less pronounced. In conclusion, DAPA was effective and well tolerated in both lean and overweight Asian patients with T2DM.

Dapagliflozin Efficacy Is Unaffected by Baseline Albuminuria Level in Patients with Type 2 Diabetes

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SGLT2 inhibitor dapagliflozin (DAPA) blocks glucose reabsorption in the proximal renal tubules to increase glucosuria. At clinically employed doses, the DAPA plasma protein bound fraction is stable at ≈91%, leaving a free fraction of ≈9% available to inhibit SGLT2. As albuminuria is a marker for diabetic nephropathy, we sought to examine clinically important questions of whether albuminuria (AU) affects DAPA efficacy or whether DAPA shifts AU levels over time. For efficacy analysis, change in HbA1c by baseline (BL) AU, pooled data were analyzed from 10 randomized, placebo-controlled, double-blind clinical trials of 24 weeks duration in patients with type 2 diabetes (T2D), which included DAPA 10-mg (N=2224) and placebo (N=2153) groups. For AU shifts analysis, 3 further studies were available (N=2360 and N=2295 in the DAPA 10-mg and placebo groups, respectively). HbA1c decreased to a similar extent at 24 weeks with DAPA 10 mg, regardless of BL AU level (Figure). The patient percentage shifting by ≥1 AU level (macro-AU ≥300 mg/g or micro-AU 30 to <300 mg/g) at BL to a lower level (micro-AU 30 to <300 mg/g or normo-AU <30 mg/g) at 24 weeks was 9.0% with DAPA 10 mg and 7.9% with placebo. Percentages shifting to a higher level were 6.1% and 6.9%, respectively. Treatment with DAPA in patients with T2D and proteinuria was efficacious, and more patients shifted to a lower than a higher AU level by 24 weeks.
Note: AstraZeneca India Medical team will be presenting this data on authors behalf.

A Non-Interventional, Multicentre, ProspecTive, ObservAtional Study to UndeRstand Usage and Effect of SaxaGliptin as First Add-On After METformin in Indian Type 2 Diabetes Patients. (ONTARGET-India)

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Aims and Objective: The aim of this multicentre observational study is to understand the usage and effect of saxagliptin as first add on after metformin in Indian patients. Also to assess effect on HbA1c reduction, side effects, hypoglycaemia and changes in quality of life scores. **Methodology**: This multicenter, observational, prospective study is expected to enrol approx. 1500 patients from 50 centres and each patient will be followed up for 3 months. Patients with T2DM those are not controlled on metformin alone and saxagliptin is added in past 15 days will be the target subject population. Data collected during follow up is expected to provide the details of Patient characteristics; demographics, Vital signs and lab tests, Medical history of T2DM, including presence of risk factors, Co-morbidities and co-medications, Changes in diabetes treatments during follow-up and reasons, hypoglycaemic events and Patient reported outcomes. Study is currently recruiting participants and approx 1100 patients have been enrolled so far. Study results will be available at the time of RSSDI conference. **Results and Conclusions**: In contrast to clinical trials, this study evaluates treatment in the everyday clinical practice. This study aims to provide data on real world to understand the usage and effect of saxagliptin as first add on after metformin in Indian patients. The study will be the largest national study of this kind ever performed.

Usage of Dapagliflozin - A Sodium Glucose Co-Transporter Inhibitor, in the Management of Type-2 Diabetes Mellitus: A Real World Evidence Study in Indian Patients

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Aims and Objective: The aim of this multicentre observational study is to understand the usage and effect of Dapagliflozin in patients with inadequately controlled diabetes (HbA1c>7%) with existing anti-diabetic medications, prior to initiation of dapagliflozin treatment. **Methodology**: This study is a non-interventional, multicentre, prospective, observational study to be conducted at 50 sites in India. The study targets to enrol 2000 patients with 40 patients per site. The study would enrol T2DM patients who are/were inadequately controlled (HbA1c >7%) with existing anti-diabetic medications and who have been prescribed dapagliflozin within past 3 months. No study medication will be prescribed or administered as a part of study procedure. Patients, who have been treated as per Investigators' routine clinical practice and prescribed dapagliflozin within last 3 months, will be screened for enrolment in study. Dosage of dapagliflozin and other medications should be as per the routine clinical practice and prescribing information. After the patients are found to be eligible, they will undergo physical examination on baseline visit and demographic information, medical & surgical history with relevant lab reports, HBA1c data, and current medication data would also be collected. At visit 2 and visit 3 which would be after 3 months and 6 months respectively of baseline visit, demographic information, physical examination, HBA1c data, and any AEs would be collected. The study would not interfere with the current or ongoing treatment of patients **Results and Conclusions**: In contrast to clinical trials, this study evaluates treatment in the everyday clinical practice. This study aims to provide data on real world to understand the usage and effect of Dapagliflozin in Indian patients. The study will be the largest national study of this kind ever performed.

The Relationship of Glycemic Control with Concordance of Therapy in Patients with Type- 2 Diabetes Mellitus

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Background: Even though diabetes mellitus is recognized as a major chronic illness, adherence to antidiabetic medicines has often been found to be unsatisfactory. This study was conducted to assess adherence to medications and to identify factors that are associated with non-adherence in type 2 diabetes mellitus (T2DM) patients at the Endocrinology Department of Max Healthcare, Saket, New Delhi India. **Materials and Methods**: This prospective cross-sectional study was conducted at Max Super Speciality Hospital, Saket, New Delhi over a period of 10 months (December 2014-Oct 2015). Different scales were used like Morisky Medication Adherence Scale, Diabetes Knowledge Test, Diabetes Self Care Activities and Culig Scale of Adherence for assessing the reasons of non-adherence. Medical records were reviewed for recent hemoglobin A1C (HbA1C) levels (within 3 months of the inclusion), fasting Blood sugar levels (FBS) & other clinical parameters. **Results and Discussion**: Out of the 1200 patients screened, 231 patients were recruited as per inclusion criteria, 38.5% patients were found to have high adherence, 47.6 % patients were found with medium adherence while 13.9 % patients were found to have low adherence towards their anti-diabetic medications. We observed a statistically significant co-relation of Fasting blood sugar with Diabetes Knowledge Test Score (p-value=0.010) and Morisky medication adherence scale (p-value=0.000). The reason for being non-adherent towards medication or treatment was majorly just forgetting to take the medicine, as given by 47% of the patients in our study population. **Conclusion**: Clinicians should educate diabetic patients on the use of their medications and the importance of medication adherence. Such services will bring the healthcare system a step closer to achieving better clinical outcomes in this group of patients.

Maximizing Beta Cell Potential Through Multi-Modal Approach at a Tertiary Clinic Setting

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Background: Diabetes has become an uncontrollable epidemic in India. A multi modal approach including lifestyle and therapeutic modification play a vital role in control of sugar levels and complications. There is a need for rationalizing drugs in order to optimize therapy and patient comfort. The multi-modal approach at this clinic consisted of lifestyle modification counseling, co morbidity screening and management and diet counseling. **Methods**: It is a retrospective study in patients with type 2 diabetes mellitus. Patients who were on insulin and oral therapy at baseline were followed-up for a mean follow-up period of 90 days from the database. The Effectiveness of multi-modal approach was analyzed from Mean insulin units (IU) (Rapid, short, intermediate and long acting), FBG (Fasting blood glucose) and PPG (Post-postprandial blood glucose) of patients at baseline and follow-up. Patients were on routine standard of care as per physician's choice. **Results**: At baseline and follow-up, the mean age of patients enrolled (n=36) in the study was found to be 43.6 (5.3) years. Males (20) were more in number than females (16). There was a significant reduction ($P<0.01$) in the prescription of insulin to the patients. At baseline mean IU were 24.2 (2.3) and 18.3 (1.6) at follow-up. There was a significant reduction ($P < 0.01$) in FBG and PPG respectively. **Conclusion**: The present study found a significant improvement in the number of insulin units prescribed to patients along with clinical significance. This shows that a multi-modal approach in terms of diet, exercise and other lifestyle modifications leads to a notable reduction in blood sugar levels.

Safety and Efficacy of Dapagliflozin (DAPA) in Combination with Potassium (K)-sparing Agents

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K-sparing agents are commonly used in patients with heart failure (HF) and hypertension (HTN). SGLT2 inhibitors (SGLT2i) have recently been shown to reduce cardiovascular (CV) mortality and HF events in patients with type 2 diabetes and established CV disease. It is therefore likely that SGLT2i and K-sparing agents will be co-administered - including in patients with HTN and HF. While there are theoretical benefits to co-administration of K-sparing agents and SGLT2i (sodium loss, reduced blood pressure [BP] without increases in heart rate [HR], complimentary effects on neurohormonal axis), it is unclear if such a combination increases hyperkalemia risk. We examined the effects of DAPA 10 mg vs. placebo (PBO) in patients treated with K-sparing agents, using pooled data from 14 phase 2b/3 trials over 24 weeks (DAPA N=108; PBO N=119). Demographics and baseline characteristics were balanced between the groups (mean age 62 yrs, BMI 35 kg/m2, eGFR ~69 mL/min/1.73m2, in both groups). DAPA lowered HbA1c, body weight and SBP vs. PBO (Table); the rate of serious adverse events was similar in both groups. No increase in serum K was seen with DAPA; the proportion of patients with K ≥6 mEq/L during follow up was lower with DAPA vs. PBO. When co-administered with K-sparing agents, DAPA resulted in significantly lower HbA1c, weight and SBP, with no evidence of increase in serum K, and lower rate of significant hyperkalemia compared with PBO.
Note: AstraZeneca India Medical Affairs team will be presenting this poster on behalf of authors.

Insulin Usage & Barriers in Type 2 Diabetes: The SCOPE-i Physician Survey

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Background: Timely insulin initiation and intensification is critical for achieving durable glycaemic control and can potentially delay risk of complications in type 2 diabetes patients. This survey was conducted among clinicians to understand current insulin usage and barriers, and preference for non-invasive insulin formulations. **Material and Methods**: A multiple-choice questionnaire encompassing above study objectives was administered to clinicians across India. A descriptive analysis of the data was performed. Results were expressed in terms of percentages based on the number of responses obtained for each question. **Results**: The survey was completed by 390 clinicians; >60% had a post graduate degree in medicine (MD) and 75% had a private practice or belonged to a corporate hospital. Up to 30% type 2 diabetes patients were treated with insulin, either alone (11%) or in combination with oral drugs (21.52%). More than half the clinicians reported insulin initiation 5-10 years after diagnosis. Needle phobia (80%), insulin myths (61.52%) and social pressure (53.4%) were the key barriers to insulin initiation. The type of insulin was chosen mainly based on major hyperglycaemic pattern, risk of hypoglycaemia and number of injections required. Most common choices for insulin initiation were premix human insulin (52.07%) followed by basal insulin analogues (28.10%). Shifting to premix insulin (61.48%) was preferred over adding prandial insulins (38.52%) for intensification from basal insulin, mainly due to lesser number of injections. Almost all (89.48%) clinicians were in favour of a non-invasive route for insulin administration. Majority opined that inhaled insulin would overcome following barriers: needle phobia (92%), viewing insulin therapy as the last resort (49.87%) and flexibility in meal schedule vs. premix insulin (44.53%). **Conclusion**: Currently, insulin usage appears to be suboptimal and delayed due to multiple reasons. A non-invasive insulin formulation (e.g. inhaled insulin) can help reduce the barriers and thus potentially result in increased and early adoption of insulin therapy.

Real-World Evidence of Albuminuria Reduction with Linagliptin, Compared to Glimepiride, as an Add-On Therapy in Uncontrolled Type-2 Diabetes Mellitus

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Background/Hypothesis: Albuminuria is a surrogate of vascular dysfunction in type-2 diabetes. Linagliptin does not require any dose adjustment in renal impairment. Moreover, the tissue-effects of linagliptin have been hypothesized to protect the kidneys, beyond glycemia control. This real-world analysis is aimed to assess the effect of linagliptin on albuminuria reduction, as compared to glimepiride, as an add-on therapy in type 2 diabetes. **Material and Methods**: A retrospective cohort analysis of patient-records from a single centre, involving patients with uncontrolled type-2 diabetes and albuminuria, prescribed either linagliptin or glimepiride as an add-on agent. Patients having measurements of HbA1c and UACR at baseline, and at 12 months, were included. Patients receiving any other incretin therapy, or having other co-morbid conditions, were excluded. HbA1c, BP and Geometric mean change in UACR over 12-months, were assessed. **Results and Discussion**:107 patients were included in the analysis (54 in linagliptin group and 53 in glimepiride group). All the patients were receiving RAAS blockers in the background. At baseline, mean HbA1c was 8.32% in linagliptin group, and 8.15% in glimepiride group. Over 12 months, similar Glycemia control and BP reductions were observed in both the study groups (P >0.05). Geometric mean reduction in UACR was more prominent in the Linagliptin group (-239.1 mg/dL) compared to the Glimepiride group (-155.3 mg/dL); p <0.001. **Conclusion**: Linagliptin demonstrated superior albuminuria reduction as compared to glimepiride, despite similar control of glycemia and blood-pressure. This could suggest possible renal protection with linagliptin-based regimen, in the real-world setting.

Findings from the Evidence Based Perspectives for Linagliptin: A Scoping Review of ADA 2016 (FREEDOM)

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Introduction: Substantial evidence for the efficacy and safety of linagliptin has been published in last one decade. We explored the contemporary evidence based perspectives of the research on linagliptin presented at the most scientific meeting in diabetes, 76th Annual Scientific Sessions of Annual American Diabetes Association's meeting held on June 10-14, 2016 **Methods**: We conducted a scoping review for the recent research findings of linagliptin to explore the emerging evidences for linagliptin, across various levels and strengths of evidence **Results**: The emerging research perspectives demonstrate that 30 studies had utilised linagliptin as an exploratory or a comparator arm, of which 14 were human studies. Cumulatively, 3723 patients have been evaluated for linagliptin across various profiles categorised into absence of co-morbidity (diabetes per se) or presence of comorbidity (cardiovascular and renal) including patients with moderate to severe renal impairment for 52 weeks. The patient population was represented across the globe from US, Europe, China, Korea and many other countries. The first multi-center, prospective, controlled, open, and randomized three arms parallel study on linagliptin from Japan demonstrated the improvement on Endothelial Function Assessed by Flow Mediated Dilation in addition to glycaemic efficacy. The results from the late breaking MARLINA-T2D Trial (Lina n=182) demonstrated improved glycaemic control without a significant effect on Urinary Albumin Creatinine Ratio in 6 months. The SAFEGUARD trial demonstrated that linagliptin did not induce any significant negative change in any of the studied cardiovascular parameters while showing some improvement in renal function. Linagliptin monotherapy had a stronger glucose-lowering effect than voglibose (L-STEP) **Conclusions**: Reflections of research presented at the ADA 2016 highlight that linagliptin is emerging as a 'global benchmark' gliptin, compared to all other antidiabetic therapies, with higher level of evidence for its consistent benefits on efficacy and safety in presence of comorbid conditions like diabetic kidney disease.

Non-Pharmacological Therapies

Yogic Approach on Diabetes

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Introduction: Diabetes now emerged as a major health problem globally.Sedentary life style,stress and uncontrolled diet are represents the major risk factors for pathogeneisis of diabetic paradigm. Yogic intervention remains the cornerstone of the approach in controlling these risk factors. **Objective**: Evaluate the value of lifestyle management particularly yogic approach on diabetes control. Study.for the study 86 type 2 diabetes individuals were taken, among them 42 in Group 1 trained with yogic procedure. Group 2 of 44 were with normal management. Group 1 were trained with regular practice of easy exercises prepared from 'Padanjalis fundamental's of Yoga with Meditation' for 45mts per day. In the beginning base line data's were collected for both groups. After 9 months all parameters were repeated & compared. During that period treatment regime were unaltered and 6drop outs in group 1 and 4 in group 2. **Result**: The study shows evidence of better benefits by yogic approach. Fasting glycaemic levels of gr. 1 vs. gr.2 shows mean value of 140.97+-11.10 to 122.16+-09.8 vs.138.25+-10.90 to 130.36+-9.7. Post postprandial value shows 236.70+-24.30 to 204.50+-21.4 vs 230.26+-22.0 to 214.41+-19.82. Regarding hypertension mean systolic pressure 142.67+-6.62 to 130.61+-7.60 vs 142.47+-8.62 to 136.56+-6.40.and diastolic pressure 82.04+-5.63 to 73.49+-4.71 to 82.56+-6.22 to 79.60+-5.68. The value of HbA1c shows significant reduction in gr.1 than 2 shows 08.95+-1.76 to 8.03+-01.8 vs 8.78+-01.60 to 8.57+-1.72. **Discussion**: The study shows reduce caloric by diet restriction, energy expenses by physical exercise, stress control by meditation were highly beneficial to bring down glycemic level and hypertension.Conclusion:Yogic procedure shows better benefits in diabetes control so, emphasis proper implementation for better benefits.

Relationship Between Dairy Consumption, HbA1c and Serum MCP-1 Levels in Individuals with Type 2 Diabetes – A Pilot Study

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Background/Hypothesis: The effect of dairy products on glycemic control in diabetic patients has provided varying results. In a country like India, where dairy is a staple part of the diet, knowledge of the effects of dairy on glycemic control and the levels of inflammatory markers in the serum of diabetic patients would help in better management of the disease and help improve the quality of life. **Materials/Methods**: 52 participants were administered a semi-quantitative proforma to obtain details about their dairy consumption. The study included patients with T2DM who didn't have any complications due to diabetes and history of infections or antibiotic use in the last 2 months. HbA1c values were obtained from preexisting laboratory reports. Serum was collected and Monocyte

Chemoattractant Protein – 1 (MCP-1) was assayed using sandwich ELISA (standards – 1000pg/ml to 15.625pg/ml). **Results and Discussion**: In this study, we observed no relationship between HbA1c and serum MCP-1 levels. We did observe an increase in MCP-1 with an increase in BMI and waist size of the patients. Interestingly, MCP-1 levels increased with curd consumption and remained unchanged with milk or buttermilk consumption. **Conclusion**: Since increased MCP-1 levels have been shown to be associated with increased risk of microvascular complications in diabetes, curd consumption may not be beneficial to diabetic patients.

21-Day Kickstart: Translating Plant-based Research into Practical Applications

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Obesity and type 2 diabetes have reached epidemic proportions worldwide over the last two decades. Compelling research supports a plant-based eating pattern for the prevention and treatment of type 2 diabetes. Based on our research findings, including a NIH-funded diabetes study and a landmark workplace wellness study, we have implemented an innovative online nutrition education program, the 21-Day Kickstart which serves as an effective therapeutic model for the prevention and treatment of these diseases. Running since 2009 with over 360,000 participants, the 21-Day Kickstart is a free, three-week program that runs the first of every month. The program includes community support, recipes and menus, nutrition education, and cooking instruction videos. Participants report that they experience more energy and weight loss. Many return month after month to repeat the program to support their long-term health goals. This program, offered in four different languages demonstrates the wide-spread potential of online nutrition education tools as diet interventions for preventing and reversing diabetes and obesity.

A Randomized Controlled Pilot Study on the Dietary Intervention for Chronic Diabetic Neuropathy Pain

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Objectives: Diabetic peripheral neuropathy is associated with significant morbidity, including amputations. Available treatments are limited. We investigated the effect of plant-based diet in reducing painful symptoms of diabetic neuropathy. **Methods**:In this pilot study, individuals with painful diabetic neuropathy were randomly assigned to 2 groups. The intervention group was asked to follow a low-fat, plant-based diet, with weekly classes for support, and to take a daily vitamin B12 supplement. The control group was asked to take the same vitamin B12 supplement, but received no other intervention. At baseline, 20 weeks, and 1 year, the following data were collected: weight, blood pressure, blood glucose, HbA1c, blood lipid concentrations, electrochemical skin conductance on hands and feet. Questionnaires included 2-day dietary records, an analog "worst pain" scale, Michigan Neuropathy Screening Instrument, global impression scale, Short Form McGill Pain Questionnaire, Neuropathy Total Symptom Score, a weekly pain diary, and Norfolk Quality of Life Questionnaire. **Results**: Thirty-five patients were enrolled into the study, with a mean age of 57 years. After 20 weeks, in the intervention group: body weight change was -6.4 kg (95% CI -9.4 to -3.4, $p<0.001$); HbA1c decreased by 0.8 percentage point; Electrochemical skin conductance in the foot improved by an average of 12.4 microseimens (95% CI 1.2 to 23.6, $p = 0.03$). The between-group difference in change in pain, as measured by the McGill pain questionnaire, was -8.2 points (95% CI -16.1 to -0.3, $p = 0.04$). Michigan neuropathy screening instrument patient questionnaire score change was -1.6 points (95% CI -3.0 to -0.2, $p = 0.03$). The 1-year findings were similarly promising. **Conclusion**: This was the first randomized controlled study of diet and diabetic nerve pain, and it suggests the potential of a dietary approach for treating diabetic neuropathy.

Therapeutic Effect of Various Herbs on the Blood Glucose Level in Subjects of Type 2 Diabetes Mellitus.

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Objective: The present study was conducted to analyses the effect of various herbs on blood glucose levels in patients of Type 2 Diabetes Mellitus. **Subjects**: Total 56 subjects were enrolled, age ranging from 35-65 years. **Method**: The method used for study was questionnaire cum interview method. Blood glucose levels was assessed before and after intervention period by using one touch horizon glucometer. Everyday herbal powder (1.5-2.5 gm. according to blood glucose level of the subjects) was used to intervene the subjects in two divided doses. **Result**: Among 56 respondent 40% (22) were Male, 60% (34) were Female. The fasting blood glucose level of before intervention was 173±73.43mg/dl whereas, after the intervention was 122 ±33.92mg/dl. The post-prandial blood glucose level before intervention was 231±81.76mg/dl whereas, after the intervention it was 162±32.56mg/dl. The Hb1Ac level of before intervention was 9.3±2.1 whereas, after the intervention it was 7.5±1.17. **Conclusion**: Herbal powder of these herbs can decrease fasting and post prandial blood glucose. These can be used as adjunct for treatment of Type 2 Diabetes Mellitus. Herbs include-1. Bitter gourd (Momordica charantia) 2. Neem (Azadiracta Indica) 3. Gurmar (Gymnema Sylvestre) 4. Tumba (Citrullus colocynthis) 5. Rasaunt (Berberis aristata)

Adoption and effectiveness of a scalable digital intervention for lifestyle modification in patients with Type-2 Diabetes in India - Results from a real world pilot.

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Background: This study was aimed at testing the adoption and clinical effectiveness of a culturally relevant scalable digital intervention for lifestyle modification in Indians with Type-2 diabetes. **Materials and Methods**: Forty-two patients were enrolled in this prospective observational study. The intervention consisted of a 120-day structured coaching program delivered by health coaches using pre-scripted interactive digital media tools through a smartphone chat application. Intervention focussed on building skills relating to healthy eating, being physically active, self-monitoring, medication adherence, problem-solving, and healthy coping in a culturally relevant context. Patients were coached to share information on their meals, SMBG values, and weight with the Health Coach, who provided personalized feedback for each interaction. In addition, health coaches also provided weekly and monthly summaries to patients on their performance. HbA1c test was done before and after completion of intervention. Thirty-two of 42 patients completed the program. One completer to was lost to follow-up. **Results and Discussion**: Seventy-six percent patients (32/42) completed the intervention with a mean reduction in HBA1c of 0.59% (CI:0.23-0.95, n=31, $p<0.01$). Sixty-Eight percent (21/31) of completers documented reduction in HbA1c with a mean of 1.04% (CI:0.63-1.44, n=21, $p<0.01$). **Conclusion**: Our results provide early evidence on successful adoption of a culturally relevant and scalable digital coaching intervention by patients with Type-2 Diabetes in India and prove its effectiveness in improving blood sugar control with a demonstrable reduction in HbA1c.

Health Economics in Diabetes – an Emerging Concept in India

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Background and Objective: Diabeto-economics in India is still an emerging concept. Health economics is pretty much relevant in resource-limited country like India where patient have to shell out the expenses from their own pocket. Good amount of data on the cost-effectiveness of different anti-diabetic therapies is available in western countries but India-specific data is limited. The varied local practices not only differ from region to region but also are a hurdle in conducting health economic study. **Methods**: Articles on health economics published between 2000 and 2016 were identified by limited literature search on Pubmed, MedLine and Google Scholar. The key words used for search were "India [ti] economics diabetes, India [ti] health economics diabetes, India [ti] pharmacoeconomics diabetes, India [ti] diabeto-economics". **Summary of the Results**: The search resulted in total of 48 articles (including studies/review articles). The data available on health economic studies in India is limited; there were only few studies (n=5) which used some kind of model/statistical tools for evaluation of economic parameters. **Conclusions**: Considering the magnitude of problem, multidisciplinary clinical-economic analysis of diabetes care in India is the need of the hour. Various simulation models (e.g. UKPDS model, ECHO-T2DM) are available to simulate lifetime health outcomes of patients with type 2 diabetes mellitus. The outcomes from the studies, both at macroeconomic and microeconomic levels, will be very useful to policy makers in allocating proper resources/ budget for management of diabetes and thus save overall costs in long run.

Alerts with Remote Monitoring Improves Diabetes Control and Increases Confidence

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Background/Hypothesis: Monitoring of blood glucose with smart meter which can transfer data to a centrally monitored station could be intrusive on one hand but could also give increased confidence in managing diabetes. At Diabetacare, smart blood glucose has SIM enabled technology, which transmits blood glucose reading securely to a central database. This is monitored 24/7 and sends appropriate alerts for high or low blood glucose levels. We wanted to know how patients perceived this technology. **Material and Method**: We conducted a semi-structured interview by face to face interview or by telephone. They were selected when they came to visit the centre or when contacted by telephone. **Results and Discussion**: Randomly selected 14 subjects with type 2 diabetes [Mean age 50.2 (+/- 13.9) years; 7 Males] who were being managed with remote monitoring took part in this study. Their HbA1c reduced significantly ($p = 0.1$) from 10.0 (+/- 2.5) % to 8.0 (+/-1.9) % during follow up. All felt that their knowledge in diabetes had improved and 92.9% felt more inclined to manage their diabetes. Majority (92.9%) felt their control was good or excellent. Most (85.7%) felt that alerts were useful. Majority (85.7%) felt confident to manage their diabetes with the remote monitoring support. **Conclusion**: Our data suggest that people become more confident in managing their diabetes when they have assurance that someone is available to alert them if things goes wrong. This translates into improved diabetes control.

High Rates of Diabetes Reversal in Newly Diagnosed Asian Indian Young Adults with Type 2 Diabetes Mellitus with Intensive Lifestyle Therapy

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Aims: There are variable reports on the reversibility of type 2 diabetes mellitus (type 2 DM) with higher rates among younger patients with short duration of diabetes. Hence, we studied the reversibility of diabetes among young adults with newly diagnosed type 2 DM. **Methods**: This prospective study included 32 patients with newly diagnosed type 2 DM. All type 2 DM patients were initially treated with intensive lifestyle therapy (ILT) (low-calorie diet [1500 kcal/day] and brisk walking for 1 h/day]). Four patients who with HbA1C <9.0% were treated with ILT alone. Except for three patients with concomitant infections who were treated with insulin, remaining 25 patients with HbA1C ≥9.0% were treated with metformin (1000–2000 g) in addition to ILT. When fasting plasma glucose was <126 mg/dl or HbA1C was <6.5% antidiabetic drug dose was reduced or stopped. The patients were followed for a minimum period of 2 years. **Results**: Reversal/ remission rates at 3 months, 1 year, and 2 years were 24 (75%), 24 (75%), and 22 (68.75%), respectively. Seventeen (53.1%) patients achieved complete reversal and seven (21.9%) patients achieved partial reversal at 3 months. Rates of complete and partial remission at 1 year were 50% and 25% and at 2 years were 46.9% and 21.9%, respectively. **Conclusion**: Young adults with newly diagnosed type 2 DM have high rates of diabetes reversal and should receive ILT to achieve reversal of diabetes.

Addressing the Ground Reality in 100 Diabetics at KMC, Manipal.

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Background/Hypothesis: We Indians, due to evolutionary lifestyle changes have become vulnerable to Diabetes Mellitus than our ancestors to the level being called as Diabetic capital. India has 70 million diabetics. But awareness about the disease which plays a crucial role in successful management, remains very less. This study analyses the prevalence of Diabetic awareness among Diabetics attending KMC-Manipal, a major institution in South-Karnataka. **Material and Method**: This is a cross sectional study conducted at KMC-Manipal. 100 diabetic patients were interviewed by means of a simple questionnaire covering basic knowledge about diabetes. They were also given basic education at the end of questionnaire. Prevalence of awareness was calculated using bar diagrams. **Results and Discussion**: In our study, the following level of awareness was obtained for each: Food and exercise importance -67%, complications-55%, footcare-53%, hypoglycaemia-73%, periodic cardiac, eye, renal evaluation – 30%. The awareness for hypoglycaemia is convincing. Least awareness was for the need for periodical cardiac, eye and renal evaluation. Awareness was more when the patient had already suffered the complication like an amputated person was better aware of taking care of the other foot. **Conclusion**: This study portrays lack of the simplest, most cost effective, bridging tool between patient and treatment of diabetes which is education about diabetes. Patient education has shown to increase compliance to treatment and decrease incidence of complications in various studies. Only when patient knows what to do and why it has to be done, a physician can achieve ground reality from ivory towers.

Self-Monitoring of Blood Glucose: Are Indian Diabetic Patient optimizing its use?

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Background: By 2030 it is estimated that every 5th diabetic person will be an Indian highlighting subsequent increase in related complications. To add further woes, many patients are not completely aware of potential

benefits of Self-Monitoring of Blood Glucose (SMBG) for glycemic control. This article aim to review use of SMBG in monitoring glycemic range and to understand the barriers in their use if any. **Method**: Extensive web search for period between 2000 and 2016 for SMBG using various keywords. **Result**: From review of available data and guidelines, treatment when augmented with SMBG can prevent early and chronic complications related to abnormal glycemic episodes and to some extend may help lessen the growing epidemic burden of diabetes in India. However, Indian data shows that SMBG is less followed practice in India. Reasons cited in the literature were lack of awareness, knowledge about accuracy, low inclination towards using meters and may be socio-economic factors. The need to develop meter that may help to break these barriers is realized. Identifying this gap, today meters with much technical advancement are available in market, for example: Color Range Indicator (CRI) and other such technical features may help patients read their glucose values. **Conclusion**: Awareness and usage of SMBG is very low in India. Technical features of meters may help patient to be compliant with SMBG. Extensive survey is required to capture real time scenario on use of SMBG in Indians.

Ameliorative Effect of Wheat Grass in Streptozotocin Induced Diabetic Rats

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Background: Diabetes mellitus; a metabolic disorder is associated with relative or absolute deficiency or insufficient release of insulin from islets of langerhans resulting in large number of lipid abnormalities. Alterations in lipid metabolism along with free radicals leads to oxidative stress causing detrimental effects at cellular and tissue levels. **Methodology**: The present study was undertaken to evaluate the efficacy of wheat grass on hyperlipemia, diabetes and oxidative stress induced by dietary and pharmacological means. The dry powder (50/kg b.w); aqueous extract (100ml/kg b.w) and juice of wheat grass (50ml/kg b.w) was investigated for its antioxidative potential in male albino wistar strain. Rats were rendered hyperlipidemic by feeding high fat high cholesterol diet (HFHC) and diabetic by single intraperitoneal injection of freshly prepared streptozotocin (STZ) (45mg /kg b.w.). Glibenclamide (5mg/Kg b.w.) was used as a standard reference drug. The experimental diets were supplemented for a period of 45 days. **Results**: High fat-high cholesterol feeding and STZ induced diabetes resulted in significant increase in oxidative stress levels of blood and hepatic tissues of rats. Wheat grass treated groups significantly restored the physiological parameters (lipip-lipoprotein and oxidative stress markers) to near normal. The effect of wheat grass was better than glibenclamide. **Conclusion**: Thus wheat grass can be used as a prophylactic agent for prevention and progression of hypercholesterolemia and diabetes mellitus.

Evaluation of Antioxidant Activity of Two Indian Seaweeds – Comparative Study

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Seaweeds; large marine benthic algae, are generally consumed in the Oriental countries. Besides being a source of various nutrients seaweeds contains various bioactive compounds. However, the antioxidative activity and their efficacy need to be explored. The study was planned to evaluate and compare the polyphenol content and antioxidant capacity of Indian Seaweeds i.e., Sargassum fusiforme (Pheophyta) and Ulva lactuca (Chlorophyta) from Gujarat. The seaweeds were washed thoroughly to remove dust and epiphytes, freeze dried and ground to a fine powder. The extracts were prepared in organic (acetone, ethanol, methanol); inorganic (petroleum ether, chloroform) and water and were stored at -200C for phyotochemical, and antioxidative assay in vitro using standard protocols. Results showed that antioxidant activity of Sargassum fusiforme was significantly ($p<0.05$) higher than Ulva lactuca probably due to high polyphenol content. Among the various extracts, the methanol extract was found to have the highest total antioxidant capacity. Seaweeds like Sargassum fusiforme and Ulva lactuca being rich source of bioactive compounds can be exploited for treatment of chronic diseases like diabetes. Thus they could be used in nutraceutical and functional food applications thus opening new frontiers as antioxidant therapies for humans.

Clinical and Humanistic Outcomes of Diabetes Management by Involving Clinical Pharmacist in the Multidisciplinary Team

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Psychosocial care and other patient specific characters also need to be considered in addition to provision of diabetes knowledge to people with diabetes. Involving a clinical pharmacist in the design of a multidisciplinary team approach for effective management of diabetes might achieve this objective of improving patient knowledge of diabetes. To compare clinical and humanistic outcomes at pre and post intervention in subjects receiving diabetes care from multidisciplinary team and pharmacist.Methods: Design: Pre-post intervention design. Setting: Osmania hospital, Govt. tertiary care hospital. Subjects: Subjects with type 2 diabetes who were not at goals for fasting glucose levels as recommended by ADA. Subjects who were noncompliant to treatment. Study duration: One year. Intervention: Pharmacist reinforced multidisciplinary team lifestyle modifications and diabetes care program. Outcome measured: Clinical outcome - fasting blood glucose levels and humanistic outcome- subject's diabetes knowledge. Results: Fasting blood glucose level goal was achieved by37.5 % (n=36) of subjects when compared to 18 % (n=75) of subjects at baseline .Diabetes knowledge scores improved significantly from pretest and posttest. Paired samples test and ANOVA was utilized for statistical analysis. Additional psychosocial support and patient education provided by involving clinical pharmacist in the multidisciplinary diabetes management team has improved subject's diabetes knowledge and glycemic control.

Gabapentin Topical: Efficacy and Safety evaluation in Diabetic Peripheral Neuropathy

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Aim: We evaluated the efficacy and safety of the topical preparation containing predominantly gabapentin (8% w/w) in combination with ketoprofen (5% w/w), capsaicin (0.035 % w/w), methyl salicylate (5% w/w) in people with diabetes with peripheral neuropathic pain **Methods**: The patients included were either drug naïve to treatment for neuropathic pain or were on the existing oral therapy with either gabapentin, pregabalin, amitriptyline or duloxetine with a minimum duration of 2 weeks. **Results**: 20 patients (12 males, 8 females), mean age 53.7 years. The pain characteristics which were rated high on the Pain Quality Assessment Scale were unpleasant (9.5), sharp (9.25), hot (9.2), intense (8,8). The neuropathy pain scores consistently

improved over the weekly follow up, reduced by 52% (Day 0 – 64.35 to 30.88 at the end of 4 weeks) with similar reductions in sub scores- NPS 8 (53%) and NPS 4 (57.4%) ($p<0.0001$). The reductions in the pain scores across NPS, NPS 8 and NPS 4 were comparable ($p=0.2593$ (NS). The % reductions (pre and post treatment score) in individual pain characteristics were; intense pain 53% (8.8, 4.13), sharp 55 % (9.25, 4.19), hot 55% (9.2, 4.13), dull 59% (5.5, 2.25), sensitive 55% (7.85, 4.94), unpleasant 55% (9.5, 4.3), surface pain 65 % (2.45. 1.67), deep pain 60% (6.85, 2.73). The change in the individual pain characteristics significant ($p<0.013$). The patients did not report of any significant side effects **Conclusions**: The change in the pain scores demonstrates that the mechanistic action of topical gabapentin to inhibit peripheral sensitisation translates into meaningful clinical benefits.

HbA1c Point of Care Device with Disposable Strips using a Novel Electrochemical Technology

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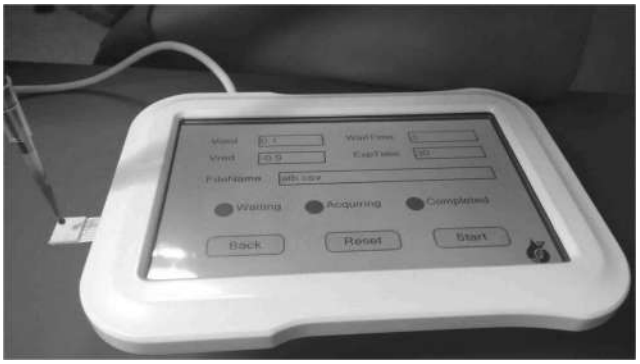

Figure 1 PoC device with disposable strip

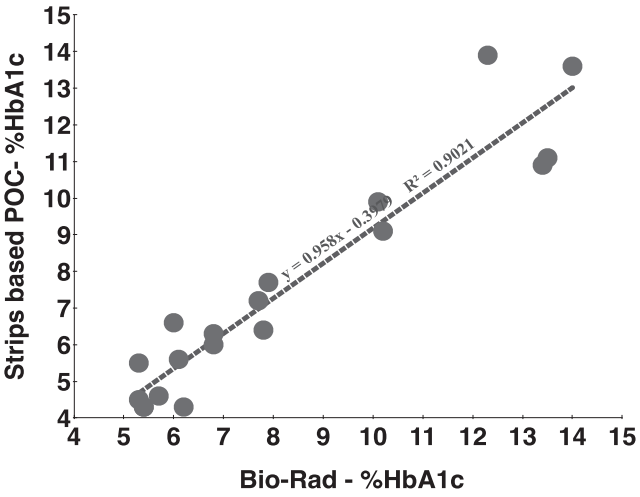

Figure 2 Correlation between Bio-Rad and PoC device

HbA1c has emerged as the gold standard for diabetes management, as it gives average blood glucose in the body over 2 to 3 months period. Three major studies for diabetes, the Diabetes Control and Complications Trial (DCCT), Epidemiology of Diabetes Interventions and Complications (EDIC) and The United Kingdom Prospective Diabetes Study (UKPDS) have established the direct relation between an increase in % HbA1c level with increased risk of diabetes complications. A recent survey conducted by the Association of Physicians of India (API) showed that 90 percent of people surveyed with uncontrolled diabetes [HbA1c>7%] in India continue to believe that they have control over their glucose levels, despite facts suggesting otherwise. HbA1c tests are very expensive and time consuming. Besides, HbA1c test is not routinely available in several Primary Health Centres. Hence access to HbA1c test is a burden for economically disadvantaged patients, especially in rural areas. Some table top devices, based on immunoassay, introduced in the recent past are still not robust enough for point of care (PoC) setting. These devices involve reagent handling and mixing, necessitating skilled operators and stringent storage conditions. Hence there is an urgent need for a robust HbA1c PoC device. Herein, we demonstrate the first of its kind point of care (PoC) device based on disposable strips for the accurate measurement of %HbA1c level in whole blood samples without any sample preparation steps. With a finger prick and minimum sample volume (75 μL), even an untrained operator can get the %HbA1c value in 15 seconds. We use electrochemical sensing technique using a novel Aza-heterocyclic receptor in conjunction with boronate affinity principle. Figure 1 shows the PoC device along with disposable strips and Figure 2 shows the correlation of POC device against Bio-Rad laboratory gold standard.Bio-Rad and PoC device

Effects of Diabetic Education on Body Mass Index, Fasting Blood Sugar and Knowledge Gained by Diabetic Patients in Central Hospital Nampula

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Background: Mozambique, has 274,700 diabetic patients and 9716 deaths due to diabetes, according to a report of 2015 (IDF 2015). There is a poor knowledge of non-pharmacological treatment of diabetes mellitus among the diabetic population. **Methods**: This is Interventional study, 648 of the participants of diabetes mellitus in out-patient diabetic clinic in hospital central Nampula, taken into the study according to inclusion and exclusion criteria. The education sessions were conducted on regular patients of the out-patient department of diabetes. The participants signed the consent form, completed the pre-test at baseline and post-test after the second session of education. The participants attended a baseline, first follow-up and second follow-up session of education where their body mass index and fasting blood sugar were recorded. Education commenced with instruction in groups of each session followed by individual advice sessions for each patient with different specialists. **Results**: The present study found that educational intervention of diabetes was highly effective to gain knowledge of diabetes compare pre-test and Post-test score (P <. 001). The Fasting blood sugar also significantly decreased from baseline in the second follow up (P <. 001). Age was significantly correlated with body mass index and fasting blood sugar (P<. 001,) Posttest with body mass index and fasting blood sugar was significantly correlated (P<. 01), A post hoc Turkey test showed that body mass index when compared with the dependent variable of fasting blood sugar found significant (P=. 05) at baseline, at first follow up (P=. 005) and at second follow up (P=. 005). **Conclusion**: The present study found that educational intervention was highly effective in controlling body mass index, fasting blood sugar and

improves knowledge of diabetes among participants of diabetes mellitus.

Pregnancy & Diabetes

Role of PPAR Gamma Polymorphism in the Risk of Gestational Diabetes Mellitus – A Case Control Study

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Introduction: Recent studies have shown an increase in the prevalence of Gestational Diabetes Mellitus (GDM) word over and more so in developing countries. This study was undertaken to study the association of Pro◦ Ala polymorphism in GDM and its role in birth weight and fetal outcomes. **Methodology**: This is a hospital based case control study. All consenting pregnant women were screened by an obstetrician to fulfill the inclusion criteria and included in the study. 5 ml of blood was used to extract genomic DNA which was amplified and RFLP was performed. **Results**: Of 100 cases and 100 controls, the mean age of the women was 27 years. The Pro◦ Ala SNP was seen among 14% of the cases and 13% of the controls. There was a positive family history in 25% of the study population. The birth weight was less than 2.5 kg in 26% in the GDM group and 23% of the control group. A chi square analysis showed no significant association between PPAR gamma SNP and the occurrence of GDM ($p>0.05$) and also birth weight ($p>0.05$) There was no significant association of birth weight and GDM status. **Discussion**: Our study showed no association between PPAR gamma polymorphism and GDM, birth weight and adverse fetal outcomes. We need more studies which would explore the functional mechanism of the PPAR gamma action in the etiology of GDM.

Does Initiation of Metformin in First Trimester of Pregnancy Affect Maternal and Fetal Outcomes in Asian Indian Women with Gestational Diabetes Mellitus(GDM)?

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Background and Hypothesis: Metformin has emerged as an oral antidiabetic agent which is as effective and as safe as insulin in the treatment of Gestational Diabetes Mellitus(GDM).However,it is not yet recommended for use in the first trimester in Gestational Diabetes Mellitus(GDM).Our study aimed to evaluate the maternal and fetal outcomes in women with GDM initiated on metformin within the first trimester. **Materials**: In this retrospective study,540 women with GDM were included of which 186 had been initiated on metformin alone in first trimester(Group A), 203 had been initiated on metformin alone after the first trimester(Group B) and 151 had been initiated on insulin alone during any trimester of their pregnancy(Group C). Women with pre-gestational diabetes and hypertension were excluded. The incidence of primary(composite of neonatal hypoglycemia,respiratory distress,need for phototherapy,birth trauma,5-minute APGAR score less than 7 and prematurity)and secondary(composite of neonatal anthropometric measurements,maternal glycemic control,maternal hypertensive complications,postpartum-glucose tolerance and acceptability of treatment)outcomes were compared between the groups. **Results**: A total of 184(47.30 %)subjects taking metformin required supplemental insulin of which 99(53.22 %)were in Group A and 85(41.87 %)in Group B. Although not statistically significant, a higher fasting plasma glucose level at the time of diagnosis was seen in Group A(120.67± 29.56 mg %)compared to Group B(116.10± 44.49 mg %).Among primary outcome variables, premature birth was numerically higher (9.9%) in Group A compared to Group B (6.9%) patients($p=0.54$) and Group C (9.3%)patients($p= 0.537$).No other individual primary or secondary outcome variables showed statistically significant difference. The composite of primary and secondary outcomes in group A showed no significant difference from Group B($p=0.33$)or Group C($p=0.56$). **Conclusion**: Metformin when initiated in the first trimester in women with GDM has no significant adverse fetal or maternal outcomes when compared to those initiated on metformin after first trimester or those on insulin during pregnancy.

An Observational Study of Vitamind3 Status in Gestational Diabetes Mellitusin South Indian Population.

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Objective: Estimation of the vitamin D3 status in gestational diabetes mellitus women reporting at Rajiv Gandhi General Government hospital,Chennai and to study the prevalence of both vitamin D3 deficiency and insufficiency in gestational diabetes mellitus individual **Study Design**: Open labeled , Non probability, Purposive sampling based Descriptive study was conducted in the Institute of Diabetology ,RGGGH and Madras Medical College between June 2016 to November 2016. **Methodology**: 100 individuals were selected and detailed relevant history was taken from them including duration of pregnancy and complete physical examination was done. blood sample was collected from all participants for assessment of Vitamin d3,haemoglobulin and Thyroid profile. Pregestational diabetes mellitus individuals were excluded from the study. **Results**: The mean age was 28 .16yrs. The study population was grouped agewise for data analysisv(<20,21-25,26-30 and >30 years).Subnormal levels of vitaminD3 were recorded 4%,14%,50% and 22% in the age group cohort respectively. Overall prevalence of vitamin D3 in the study population was 22%,50%,14%and 4% in insuficency,mild, modrrate and severe deficiency.22% with bad obstetric history have mild to moderate vitamin D3 deficiency. **Conclusion**: Mild vitamin D3 deficiency was more common in gestational diabetes mellitus women and recurrent gestational diabetes mellitus has been associated with mild to moderate vitamin D3 deficiency which may be prevented for which studies are required.

Diabetes in Special Groups

Relative Gene Expression Analysis of Pancreatic Transcription Factors Pdx-1, Ngn-3, Isl-1, Pax-4, Pax-6 and Nkx-6.1 in Trans-Differentiated Human Hepatic Progenitors: A Potential Source for Treatment of Type-1 Diabetes Mellitus

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Aims/Introduction: Diabetes is a major health concern throughout the world because of its increasing prevalence in epidemic proportions. b-Cell deterioration in the pancreas is a crucial factor for the progression of diabetes mellitus. Therefore, the restoration of b-cell mass and its function is of vital importance for the development of effective therapeutic strategies and most accessible cell sources for the treatment of diabetes

mellitus. **Materials and Methods**: Human fetuses (12–20 weeks gestation age) were used to isolate human hepatic progenitor cells (hHPCs) from fetal liver using a two-step collagenase digestion method. Epithelial cell adhesion molecule-positive (EpCAM+ve)-enriched hHPCs were cultured in vitro and induced with 5–30 mmol/L concentration of glucose for 0–32 h. Pdx-1 expression and insulin secretion was analyzed using immunophenotypic and chemifluorescence assays, respectively. Relative gene expression was quantified in induced hHPCs, and compared with uninduced and pancreatic cells to identify the activated transcription factors (Pdx-1, Ngn-3, Isl-1, Pax-4, Pax-6 and Nkx-6.1) involved in b-cell production. **Results**: EpCAM+ve cells derived from human fetal liver showed high in vitro trans-differentiation potential towards the b-cell phenotype with 23 mmol/L glucose induction after 24 h. The transcription factors showed eminent expression in induced cells. The expression level of transcription factors was found significantly high in 23 mmol/L-induced hHPCs as compared with the uninduced cells. **Conclusions**: The present study has shown an exciting new insight into b-cell development from hHPCs trans differentiation. Relative quantification of gene expression in trans-differentiated cells offers vast possibility for the production of a maximum number of functionally active pancreatic b-cells for a future cure of diabetes.

Study of Thyroid Dysfunction in Patients with Type 2 Diabetes Mellitus

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Background / Hypothesis: Diabetes mellitus (DM) and thyroid disorders are among the most common endocrinal diseases. There is a high prevalence of thyroid dysfunction in patients with DM, but the prevalence varies with different studies. The objective of the study is to estimate the prevalence of thyroid dysfunction in patients with type 2 DM (T2DM) and the effect of thyroid dysfunction on DM and its complications. **Material and Method**: A sample of 120 cases of T2DM, who were admitted in our hospital, were interviewed, clinically examined and investigations like FBS, PPBS, HbA1c, thyroid profile, lipid profile and target organ evaluation for diabetes were done. **Results and Discussion**: Thyroid dysfunction was found in 40.83%. FBS, PPBS and HbA1c are higher in the hyperthyroid group, while they are lower in the hypothyroid group. Macrovascular complications were present in 20.04% (CAD 14.28%) of cases with thyroid dysfunction compared to 11.26% (CAD 2.81%) cases without thyroid dysfunction. T2DM patients with thyroid dysfunction had higher levels of total cholesterol, LDL-C, triglycerides (except for a lower level of triglycerides in hypothyroid patients) and lower levels of HDL-C and VLDL-C. **Conclusion**: Prevalence of thyroid dysfunction is high among patients with T2DM. So, cases with T2DM should be routinely screened for thyroid dysfunction. Diabetics with hyperthyroidism have uncontrolled sugars and those with hypothyroidism are prone to hypoglycaemic episodes. These complications can be overcome by proper management of thyroid disorders in diabetic patients. T2DM patients with thyroid dysfunction have hyperlipidemia and are also at increased risk of CAD. Hence, these patients should be on good lipid control measures.

Genetically Confirmed Neonatal Diabetes: A Single Centre Experience

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Neonatal Diabetes Mellitus (NDM) also called "early-onset" diabetes is a rare form of monogenic disorder diagnosed with onset usually within 6-9 months of age. It can be either Permanent Neonatal Diabetes Mellitus or Transient Neonatal Diabetes Mellitus. There is scant data describing the genotypic and phenotypic characterization of NDM from Indian subcontinent. We describe here the spectrum of genotypic and phenotypic characteristics of genetically confirmed NDM patients from a single tertiary care centre from Western India. ABCC8 mutation was most common, with varied age of onset of diabetes. Genetic testing has a crucial therapeutic implication in the management of NDM.

A Rare Case of Fibrocalcific Pancreatitis Presenting in Children – Case Analysis

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Case 1: A 14-year old girl child presented with complaints of tiredness, dehydration, amenorrhea since puberty, mental retardation, the absence of fifth metacarpal. The RBS was found to be 246 mg/dL. Urinary Ketones, Serum ketones, anion gap, bicarbonate and pH of blood were all indicative of DKA. USG abdomen revealed chronic calcific pancreatitis. Necessary IV fluids were initiated and insulin therapy was provided **Case 2**: An 11-year old boy presented with complaints of polyuria, polydipsia, polyphagia, abdominal pain, weight loss, difficulty in eating and increase bowel movements. The RBS was found to be 622mg/dL. Urinary Ketones, Serum ketones, anion gap, bicarbonate and pH of blood were all in the normal range. Thus indicative of Hyperglycemic Hyperosmolar Syndrome. USG abdomen and MRI revealed dilated duct showing calcific pancreatitis. Necessary IV fluids were initiated and insulin therapy was provided. **Comparison Study**: Both cases had calcific pancreatitis, consanguinity, poor diabetic control in common. What was unusual between them was, one presented with DKA and other had No DKA. Mental retardation, facial features, absent metacarpal, and amenorrhea were presented only in case 1, thus the associated syndromes must be taken into account. **Conclusion**:The clinical presentation of fibrocalcific pancreatitis is rare in children and associated with the SPINK 1 gene. Conservative glycemic control, frequent blood glucose monitoring, and correction of the micro and macronutrient deficiencies are very fundamental in the management of patients with FPD and should be strongly emphasized. Treatment responses will be variable in both cases and the other associated conditions of hormonal abnormalities need to be corrected for a better quality of life for the children. Such cases of calcific pancreatitis in children are rare and should be thoroughly investigated.

Nesidioblastosis a Rare Onset in Adults – Case Report

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Nesidioblastosis is a medical term which describes the pathological hyperplasia of primary islet cell, which refers mainly to beta cell pancreatic dysfunction and causes hyperinsulinemic hypoglycemia. Nesidioblastosis has been commonly associated with children and its incidence being mostly sporadic in adults. **Case Study**: A 39-year-old male patient was admitted with complaints of tiredness, giddiness, hunger & sweating. He was a known case of seizure disorder. On outpatient examination his BP- 130/80mmHg and RBS – 48mg/dL. His blood glucose levels were suggestive of hypoglycemia indicating further investigation. **Results**: The patient was admitted and a continuous glucose monitoring with Freestyle Libre was performed which indicated a serious

decrease in blood sugars as low as 26mg/dL. Urine screening for Sulphonylureas was negative. The serum levels of insulin – 56.26mU/L (3.00 – 25.00) and C-peptide – 3.79ng/mL (0.81 – 3.85) were as follows. Anti-Insulin antibodies were negative. He was further thoroughly investigated including endoscopic USG to locate insulin secreting tumor, in the meantime, DOTA EXENDIN PET suggested a diffusively increased Ga68 uptake in the pancreas, is of concern for diffuse nesidioblastosis. **Conclusion**: Radiological DOTA PET studies aided in differentiating Noninsulinomic pancreatogenous hypoglycemia from Insulinoma. The majority of hypoglycemic spells are caused by insulinoma and 5% are caused by noninsulinomic hypoglycemic syndrome which is also referred to as nesidioblastosis. The mutations associated with GLUD1, GCK, SLC16A1 are commonly associated with children and some of the milder mutations of ABCC8 and KCNJ11 may escape recognition in infancy and first be discovered to have hypoglycemia as adults. Thus the pancreatic cells undergo morphological changes resulting in the formation of a set of new cells that intervene with the adjacent acinar parenchyma. This case highlights the evolving incidence of Adult Onset Nesidioblastosis.

A Study to Evaluate Association of Celiac Disease with Insulin Dependent Diabetes and Hypothyroidism in North-West Rajasthan

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Objective: To study association of Celiac Disease with Insulin Dependent Diabetes and Hypothyroidism in young adults in North-Western Rajasthan. **Method** : Total eighty-seven newly diagnosed adult patients of celiac disease were included. Sera of all patients were tested for presence of IgA tissue transglutaminase (tTG) antibody by ELISA using commercially available kits. Three to four intestinal mucosal biopsies were obtained with GI endoscopy from the second part of duodenum in patients with presence of tTG antibodies. Fasting blood glucose (FBG) was measured after overnight fasting (8 hours of fasting overnight). Serum TSH testing was performed on automated immunoassay platforms employing advanced IMA technology. **Results**: In our study, out of total 87 patients, 46(52.87%) were from serum TTG group 50-200 and 41(47.12%) from serum TTG group >200. Out of total 87 patients, 14 patients had their TSH >4.2 and out of them 12 and 2 patients were from serum TTG group 50-200 and >200 respectively and the difference was statistically significant ($p<0.01$).15 patients were found with impaired glucose tolerance and out of them 11 and 4 were from serum TTG group 50-200 and >200 respectively. Only 10 patients had their fasting blood sugar >125 and out of them 9 and 1 were from serum TTG group 50-200 and >200 respectively and the difference was found significant ($p<0.01$). **Conclusion**: Our study concludes that there is significant association of CD with thyroid dysfunction and impaired glucose tolerance. All CD patients should be screened for thyroid dysfunction and impaired glucose tolerance. Early recognition of CD and hence early appropriate management may help in reducing severity of various autoimmune disorders, improving quality of life in these patients.
Keywords : Celiac Disease, Diabetes, Hypothyroidism

Prevention of Diabetes

Pterostilbene Inhibits Cytokine-Stimulated Pancreatic β-Cell Apoptosis by Up Regulating Nrf2 Signaling Cascade

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Background: Nuclear factor erythroid 2-related factor 2 (Nrf2) is a central transcription factor that regulates the antioxidant defense system. We reported that Pterostilbene (PTS), a dimethylated derivative of resveratrol, as a potent Nrf2 activator. In this study, we aimed to investigate the protective effect of PTS against cytokine induced β-cell apoptosis through Nrf2 signaling cascade. **Materials and Methods**: MIN6, a pancreatic β-cell line, the effects of PTS administration on cytokine-mediated cell death and abolition of insulin secretion were evaluated by a viability assay, cell cycle analysis, and insulin assay. In addition, the expression of downstream targets and apoptotic proteins were measured by immunoblot, qPCR and reporter assays. **Results and Discussion**: PTS showed protection of MIN6 against cytokine-induced cell death as assessed by MTT assay. The Nrf2 activation potential of PTS was evaluated by nuclear translocation of Nrf2 and its downstream targets using ARE luciferase reporter system. PTS increased the expression of Nrf2 downstream genes, such as hemeoxygenase-1, superoxide dismutase, catalase and glutathione peroxidase. Further cell cycle analysis by FACS revealed the reduction in the percentage of sub-G1 population by PTS treatment in cytokine exposed cells. The antiapoptotic property of PTS was confirmed by Annexin V labeling assay using FACS and the expression of apoptotic markers BAX, Bcl2 and Caspase-3. PTS prevented cytokine-induced NO production, iNOS expression, p-AKT, NF-κB activation and inhibition of glucose-stimulated insulin secretion (GSIS). **Conclusions**: The results suggest that PTS can be used for the prevention of functional β-cell damage and preventing the progression of Type 1 diabetes mellitus (T1DM).

Awareness About Diabetes Mellitus Amongst Diabetics in a Secondary Care Hospital in Bangladesh

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Background: Diabetes Mellitus (DM) is a chronic, non-communicable disease caused by inherited and /or acquired deficiency in production of insulin by the pancreas or in its effects. Prevalence of type 2 diabetes mellitus (T2DM) is now increasing rapidly around the world and emerging as a global health problem that is expected to reach pandemic levels with 439 million people by 2030. This increase will be noticeable in developing countries where the number of people with T2DM is expected to increase from 84 million to 228 million people. About 7.1 million people are affected with Diabetes in Bangladesh. There are limited reports available on the level of awareness about Diabetes Mellitus amongst Diabetics in Bangladesh. The objective of this study was therefore, to determine awareness of DM among Diabetics in Bangladesh. The information from this study will be useful in educating the communities on risks factors and possible interventions and control measures against DM. **Material and Method**: This cross-sectional study was conducted at outpatient clinic of Sadar Hospital, Feni, Bangladesh. The study was carried out over a period of six months (March, 2015 to August, 2015). Diabetic patients visiting the hospital for consultation were included. Patients suffering from either type 1 or type 2 Diabetes Mellitus, between the ages of 16-80 years were included in the study. Demographic data and awareness about Diabetes Mellitus of participants was recorded using structured questionnaire after obtaining informed written consent. Student t-test and chi-square test was applied. $p< 0.05$ taken as statistically significant. **Results and Discussion**: A total of 100 participants were included in this study. 62% were males and 38% were females. Mean age of the respondent was 43.84 ± 10.80 years. Most (46%) respondents were in the age group 30-40 years. A significant proportion of study participants (37%) attributed excessive intake of sweets for the causes of diabetes. Role of physical activity and exercise was acknowledged by 36% patients. 17% participants had no idea about any complications related to diabetes but 43% had awareness about kidney complications. 31% respondents had awareness about hypoglycaemia and only 27% were aware about foot care. Low cholesterol diet was reported to be protective for heart by 14%. **Conclusion**: The awareness about Diabetes Mellitus in majority of Diabetic patients was inadequate. Public education about DM should be emphasized. Health education on causes, risk factors and management of DM should be promoted and people should be encouraged to educate others about Diabetes.

Can N-Acetyl Cysteine - Taurine- Provide Additional Reduction in Micro Albuminuria, in Type 2 Diabetic Patients Already on Angiotensin Converting Enzyme Inhibitors(ACEI) or Angiotensin Receptor Blockers (ARB) with or Without Dual Channel Calcium Bloc

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Background and Hypothesis: To prevent the progression of micro albuminuria to macro albuminuria and DN, we use either ACEI or ARB and or dual channel calcium blocker(Cilnidepine). These drugs have reduced MA and have prevented the progression to DN but have their limitations. Animal experiments with Taurine and NAC have been very encouraging in reducing MA, .**Objectives**: To know whether the combination of NAC and Taurine would additionally reduce microalbuminuria and TGF β expression in T2 diabetics who are already on either ACEI or ARB and or DCCB, and to know the effect of this combination on HbA1C, lipid parameters and e GFR **Material and Methods**: Eighty diabetics, having microalbuminuria were recruited .50 were in the test group and 30 were in the control group. All were examined, their height, weight, BMI, WC, BP were measured initially and at the end of 3 months. The test group was given NAC+Taurine tablets, one tab daily for 3 months and placebo was given to the control group. HbA1C, Lipid profile, Serum creatinine, Micro albuminuria and TGFb, e GFR were estimated before and on completion of the study. ANNOVA and Pearson's correlation were used for statistical analysis **Results**: 41 in the test and 21 in the placebo group, completed the study. The test group did show reduction in microalbuminuria and TGFb but not statistically significant. There was no change in SC and E-GFR. The drug did not have any effect on lipids, HbA1C **Conclusion**: The combination of NAC+Taurine has additional reduction in microalbuminuria and TGF b in those on ARB or ACEI with or without DCCB. Larger studies would be benifecial in this regard
TITLE: channel calcium blockers(DCCB)? A cross sectional, comparative, placebo controlled, observational Study.(TITLE THAT HAS BEEN LEFT OUT ABOVE)

A Model-Based Breath Analysis Method for Monitoring Blood Glucose Profile to Diagnose Diabetes Mellitus

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Background: The blood glucose measurement is necessary for diagnosis and treatment of diabetes mellitus. Current methods are invasive. Obtaining blood samples by the invasive methods are not only painful but also inconvenient. The present invention provides a system by utilizing human breath analysis for quantitative non-invasive estimations of blood glucose levels and diabetic conditions in subjects. **Materials and Methods**: The system based model comprised of following steps a) administration of a test meal containing suitable amount of 13C-labelled glucose to the subject b) measuring the pre-dose (basal) and post-dose exhaled breath 12CO2/13CO2 stable isotope ratios by the system. c) obtaining different physical parameters of the subject d) generating model equations as the functions of breath CO2 isotopic compositions and physical parameters of the subject and storing the model equations in a central computer e) estimations of the post-dose blood glucose levels with time using a custom written suitable computer programme. **Results and Discussion**: The final equation of the model is C4 (t) = F+ C3 (t) *(1000/50), where C4 and C3 are the two parameters measured in the model. The model can determine the blood glucose profile of an individual for long time from the exhaled breath analysis. The model input parameters are height, sex, exhaled breath carbon-13 isotope in exhaled breath. **Conclusions**: The present study shows a new approach for real time estimation of the blood glucose concentrations. Thus it may be applicable as an alternative diagnostic method for diabetes mellitus.

The Relationship Between Serum 25 (OH) Vitamin D and Insulin Resistance in Prediabetes

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Background and Objectives: Vitamin D supplementation has been found to decrease the insulin resistance in patients with type 2 diabetes mellitus. However similar observations among individuals with prediabetes are not well documented. The aim of this study was to find out the relation between serum 25 (OH) vitamin D and insulin resistance in prediabetes. **Methods**: A total of 80 prediabetes individuals, in the age group of 20-50 years, were included in the study based on oral glucose tolerance test results. An equal number of normal healthy adults were taken as controls. Family members and attendants of patients attending the diabetic clinic underwent 75 gm Oral Glucose Tolerance Test. Individuals with fasting blood glucose between 100-125 mg/dl and/or 2-hour post glucose of 140-199 mg/dl after ingesting 75 gm of glucose were recruited for this study after applying inclusion and exclusion criteria. **Results**: The presence of vitamin D deficiency was 83 % in prediabetes group and 95 % in normal healthy controls. Severe vitamin D deficiency (< 10 ng/ml) was seen in 37.5 % of individuals with prediabetes and 61 % individuals with normal glucose tolerance. Serum 25(OH) Vitamin D levels were 13.30 ± 9.85 ng/ml in cases and 9.80 ± 5.86 ng/ml in controls. There was statistically significant difference in the 25 (OH) vitamin D levels among the two groups with prediabetes group having higher vitamin D levels than normal healthy controls. The correlation between serum 25 (OH) vitamin D levels and HOMA-IR in prediabetic individuals was significant (p value 0.041). **Interpretation and Conclusion**: Overall, both the groups were vitamin D deficient irrespective of their glycaemic status. Serum 25 (OH) vitamin D levels were inversely related to insulin resistance in prediabetes.

Oral Microbiota in Type 2 Diabetes Mellitus and Impact of Serum Monocyte MCP-1 Levels Following Neem Stick Usage

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Introduction: Oral microbiome impacts health and disease. Neem (Azadiracta indica) has antibacterial activity against oral microbiota.**Objectives**: To characterize oral microbiota (OMB) in saliva samples of T2DM patients by Next generation sequencing. To analyze MCP-1 levels among the T2DM patients before and after a month of neem stick usage as a toothbrush. **Materials and Methods**: Blood and saliva samples were collected from adult T2DM patients attending out patient with HbA1c level more than 8% without any dental complications. Metagenomic sequencing was performed on saliva samples targeting V6 region of 16s rRNA. Twelve patients were provided with Neem stick (5thinai organics, Chennai) and instructed to use neem stick as a tooth brush everyday for one month and report for follow up.DNA extraction was performed in saliva samples according to

manufacturers instruction (Qiagen) and amplified using Qiagen Multiplex PCR kit (Qiagen, Germany). All the PCR products were subjected to Metagenomic sequencing using Ion torrent PGM . Serum MCP-1 levels were determined using a quantitative sandwich Human MCP-1 standard ABTS development kit (Peprotech, USA). **Results**: The profile of oral microbiota of T2DM patients (n=24) consists of Streptococcus (95.8%) counts ranging from 2644 to 27214, Veillonella (72.2%), Neisseria (87.5%), Rothia (63.6%), Actinomycetes (25%), Fusobacterium (21%), and Pigmentiphaga (12.5%). Oral microbiota in healthy controls (n=10), consists of Streptococcus (26.1%), Veillonella (21.9%), Neisseria (16.9%), Haemophilus (10.7%), Actinomycetes (2.6%), Rothia (3.1%).After the use of neem stick in 8 patients who reported for follow up.there was drastic reduction in the the load of bacteria which was statistically significant.After neem stick usage significant reduction on bacterial loads and MCP-1 levels were recorded.

HbA1c Point of Care Device with Disposable Strips using a Novel Electrochemical Technology

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HbA1c has emerged as the gold standard for diabetes management, as it gives average blood glucose in the body over 2 to 3 months period. Three major studies for diabetes, the Diabetes Control and Complications Trial (DCCT), Epidemiology of Diabetes Interventions and Complications (EDIC) and The United Kingdom Prospective Diabetes Study (UKPDS) have established the direct relation between an increase in % HbA1c level with increased risk of diabetes complications. A recent survey conducted by the Association of Physicians of India (API) showed that 90 percent of people surveyed with uncontrolled diabetes [HbA1c>7%] in India continue to believe that they have control over their glucose levels, despite facts suggesting otherwise. HbA1c tests are very expensive and time consuming. Besides, HbA1c test is not routinely available in several Primary Health Centres. Hence access to HbA1c test is a burden for economically disadvantaged patients, especially in rural areas. Some table top devices, based on immunoassay, introduced in the recent past are still not robust enough for point of care (PoC) setting. These devices involve reagent handling and mixing, necessitating skilled operators and stringent storage conditions. Hence there is an urgent need for a robust HbA1c PoC device. Herein, we demonstrate the first of its kind point of care (PoC) device based on disposable strips for the accurate measurement of %HbA1c level in whole blood samples without any sample preparation steps. With a finger prick and minimum sample volume (75 μL), even an untrained operator can get the %HbA1c value in 15 seconds. We use electrochemical sensing technique using a novel Aza-heterocyclic receptor in conjunction with boronate affinity principle. Figure 1 shows the PoC device along with disposable strips and Figure 2 shows the correlation of POC device against Bio-Rad laboratory gold standard.

Piramal | Swasthya - WDF Denmark Project

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Background: Create a scalable and replicable rate form for type 2 diabetes prevention, screening and management for the rural poor in Assam **Objectives**: λ Primary prevention λ Perform stages 1 symptomatic screening λ Monitor and manage people with type 2 diabetes. λ Build a strong referral network Target groups:λ 6,500 people in Assam λ 1,200people at risk or above 40 years λ 38,000 rural people living below the poverty line **Material and Methods**: Early diagnosis and management of DM in younger age group which increases the disease burden is a priority to minimize the disabling complications of this disease. • Training of health care professionals & workers • Collaborators and stakeholders • Project activities and developments are to be regulated by a project team **Observation and Achievements**: Prevalence of diabetes - 2.02 for 1000 population per year.• Low Prevalence of obesity - BMI on average is 3 to 5 units 3) • 30 to 69 % women likely to developed diabetes in next pregnancy • 24% Neonatal hypoglycemia from GDM mothers • Macrosomia in GDM - 14% • GDM in Assam 0.54% of total population • Positive effect on the general turnout of people at the Service Points, which jumped by a quantum. **Conclusion**: In rural Assam, diabetes is increasing in BPL group because of Change of life style Diet - they are all under nourished. Hence challenge to treat diabetes in rural set up compared to urban population.

Comparison of Fasting Plasma Glucose, Oral Glucose Tolerance Test and Haemoglobin A1c for Diagnosis of Diabetes Mellitus in High Risk Subjects

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Background: Performance of HbA1c among Indian subjects in diagnosis of diabetes and prediabetes has not been evaluated adequately, when compared with that of glucose based tests. There is no consistency in the results of previous studies which had compared FPG, 2-hPG and HbA1c. **Objective**: To assess the diagnostic performance of HbA1c with that of FPG and 2-hPG for diagnosis of diabetes and prediabetes and to study the variability of FPG, 2-hPG and HbA1c over a period of at least 2 weeks. **Materials and Methods**: This was a cross sectional study done at JIPMER, Pondicherry. Subjects at high risk of developing diabetes underwent testing twice (with a minimum gap of 2 weeks) after an overnight fast. At each visit, FPG, 2hPG post-75g glucose challenge and HbA1c were done. Intra class correlation coefficient was used to assess the reliability on repeated measurements of FPG, 2-hPG and HbA1c.The final diagnosis in subjects based on individual tests and combination of tests was categorized as normoglycemia, prediabetes and diabetes. The final diagnosis for the subject was arrived at based on a combination of test results of FPG, 2-hPG and HbA1c, as per American Diabetes Association guidelines. The inter observer agreement between individual tests and the final diagnosis made was assessed using Cohen's kappa statistic. ROC curve with AUC was used to arrive at cut-off values for HbA1c to diagnose diabetes and prediabetes. **Results and Discussion**: A total of 424 individuals were screened. 92 individuals were excluded because they had creatinine >2mg/dl, haemoglobin <10g/dl or could not undergo testing twice. 2-hour PG had the highest sensitivity of 98.9% to detect diabetes. HbA1c and FPG had a sensitivity of 97.8% and 87.9% respectively for diagnosis of diabetes. HbA1c of ≥6.5% [mean of values at two visits] had a sensitivity of 100%, specificity of 94.8%, PPV of 88.3% and NPV of 100% for diabetes diagnosis. A HbA1c value of <5.6% was associated with 100% NPV for diabetes/ prediabetes diagnosis. HbA1c had the least variability followed by 2-hour PG and FPG. **Conclusion**: HbA1c is a convenient alternative to glucose based tests to diagnose diabetes among high risk Indian subjects. A HbA1c cut-off of 5.6% can be used to exclude prediabetes/ diabetes.

Assessment of Rural Public Health Facility's Capacity to Manage Diabetes and Hypertension in India

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Background: India is currently facing a twin epidemic of diabetes and hypertension. The public health system needs to be strengthened to address the epidemic effectively. We assessed the capacity of Primary Health Centres (PHCs) and Sub Centres (SCs) to manage diabetes and hypertension by comparing the availability of manpower, investigations, instruments and drugs as mandated by the Indian Public Health Standards (IPHS). **Methods**: Study was carried out in the PHCs and SCs of culturally distinct rural areas of Sonipat and Visakhapatnam. IPHS guidelines were used to develop checklist. Data were collected using interviewer administered questionnaire. **Results**: Three PHCs, 17 SCs in Sonipat and 3 PHCs, 19 SCs in Visakhapatnam were surveyed in 2014. Eighteen SCs of Visakhapatnam did not have adequate manpower. Except for paramedical staff, all the other posts in both PHCs were not filled according to the standards. Capillary blood sugar and urine albumin tests were conducted in all PHCs. In addition, one PHC in Sonipat carried out venous blood sugar test. Almost all the facilities reported availability of instruments like adult weighing scale, height measuring scale, glucometers and blood pressure apparatus. All the recommended anti-diabetic and anti-hypertensive medications were available at PHCs in both sites except for captopril, enalapril and insulin in PHCs of Visakhapatnam. No health facility maintained national guidelines for diagnosis and management of diabetes or cardiovascular diseases. **Conclusion**: The public health facilities need to be strengthened in terms of manpower, investigations and medications in order to provide the quality care for diabetes and hypertension.

Psychosocial Issues

A Study on Prevalence of Cyberchondriasis Among Patients with Metabolic Syndrome and Its Impact of Their Psychological Health

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Introduction: Since there are plenty of health related websites, it is becoming common practice to search internet before going for a medical consultation. **Objective**: The main objective of this study is to determine the impact of cyberchondriasis among patients with metabolic syndrome. The instrument used is a survey questionnaire containing both close-and-open ended questions. Kessler 10 Psychological stress instrument (K10) was used to measure the levels of stress. **Results**: The study was conducted among 529 (M:F 275:254) subjects. 1. Higher percentage (65.2%) of people preferred information from internet in the first place. 2. Mostly internet was searched for details regarding their treatment, side effects of prescribed medicines, availability of alternative treatment, dietary guidelines and other people's experience on the similar problem. As observed in the study, the main reason for searching internet was instantaneous availability of all the required information at minimal cost. 3. About 350 subjects made self-diagnosis, out of which a large majority of them (84.5%) was ruled out by the doctor. However they sought second opinion when their doctor differed from internet-based diagnosis. 4. Around 31% of people had adjusted their medication according to online suggestion. About 78.8% tried free of cost alternative therapies. It is noticed that everyone tried different dietary suggestions available on internet. 5. About 74% recorded that after searching for health information they were anxious and 21% said that the details only confused them further, which had a significant impact on their psychological health (p value = 0.000). **Conclusion**: Though searching online gives more information on health care, it also increases the anxiety of patients and deviates them from proper management.

Myths and Barriers to Insulin Use in Uncontrolled Diabetes Mellitus

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The present study aimed to elicit the myths and barriers to insulin use in uncontrolled diabetes mellitus. A prospective study was conducted in department of endocrinology, Sri Ramachandra University, containing 142 patients who are taking insulin. Patients medication adherence was assessed using MMAS-8 (Morisky Medication Adherence Scale). Patients myths and barriers were analyzed using modified QUID (Questionnaire for Use of Insulin in Diabetes). One-way ANOVA, Wilcoxon test and Pearson chi square test were performed to derive statistical significance between parameters. Out of 142 patients, 53 patients were highly adherent (37.3%) to insulin therapy and 45 patients were moderately adherent (31.6%) and after counselling 77 patients were highly adherent (54.2), 47 patients were moderately adherent (33%). The low adherence rate dropped from 30.0% to 12.6%. It is observed that the majority of the patients, 61.9% were comfortable with the use of insulin, because of the knowledge about their disease, complications and education received by the patients during their follow up visits. HbA1c was found to have significant relationship (p = 0.05) with the adherence. The people who were highly adherent to insulin therapy was having the mean HbA1C of 8.5 and the mean HbA1C for the people who are low adherent were 10.5. About 91.5% patients reported increase in Quality of Life with the use of insulin. Major factor for insulin non adherence is forgetfulness followed by too busy, travelling etc. which are almost patient related and can be easily overcome by the selection of proper regimen, diet etc.

Obesity & Metabolism

A Comparative Study of Prevalence of Impaired Glucose Tolerance Test in Non Alcholic Fatty Liver Disease Patients and Normal Controls

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Background: Nonalcoholic fatty liver disease (NAFLD), which develops in the absence of alcohol abuse, has been recognized as a major health burden. The clinical implications of NAFLD are derived mostly from its common occurrence in the general population and its potential to progress to cirrhosis and liver failure. Estimates suggest that about 20% to 30% of adults in developed countries have excess fat accumulation in the liver, 50% among people with diabetes, and about 80% in the obese and morbidly obese. **Methods**: The study shall be RETROSEPECTIVE OBSERVATIONAL STUDY, The cases for the study were selected retrospectively who were diagnosed as fatty liver by ultrasound imaging who attended the Department of General medicine and Department of Gastroenterology, Gandhi Hospital, and sex matched controls were selected randomly fallowing which the data will be enumerated who fulfills the inclusion criteria. This study was conducted between March 2015-March 2016 1. A fasting plasma glucose of >126 mg/dl (after no caloric intake for at least 8 hours) or, 2. A casual plasma glucose >200 mg/dl (taken at any time of day without regard to time of last meal) with classic diabetes symptoms: increased urination, increased thirst and unexplained

weight loss or, 3. An oral glucose tolerance test (OGTT) (75 gram dose) of >200 mg/dl for the two hour sample. **Results**: According to the OGTT results, 19 out of 50 (38%) patients were diagnosed as having IGT and 18 out of 50 (36%) have IFG and 2 out of 50(4%) patients were diagnosed as having diabetes.

High Sensitivity C-reactive Protein (hs CRP) in Metabolic Syndrome and Its Components

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Background/Hypothesis: To determine if hs CRP level, an inflammatory marker is raised in metabolic syndrome (MS) and if this level is related to number and types of components of MS. **Material and Method**: 100 patients with ≥ 3 components of MS were studied at St. Stephen's Hospital, Delhi for hs CRP level and its correlates. They comprised 62 females, 38 males. Mean age was 53.9±11.2; range 30-85 years). Statistical analysis was performed using SPSS version 17.0. **Results and Discussion**: Mean hs CRP of 100 patients was high (5.5±5.4 mg/dl; normal <1 mg/dl) and individually, 86% of them had raised hs CRP. Mean hs CRP was 2.8, 5.0 and 10 mg/dl with 3, 4 and 5 MS components, p values being 0.016, <0.001 and <0.001 for 3 vs 4, 3 vs. 5 and 4 vs 5 components respectively. Mean hs CRP was significantly higher with components of high WC and triglycerides (TG) vs normal WC and TG (p <0.001in both cases) but not with components of low HDL and hypertension (p 0.076 and 0.328). Raised FBG, the 5th component was present in all. Patient's age, sex, serum cholesterol, LDL and underlying disorder had no effect on mean hs CRP level. **Conclusion**: Raised hs CRP is common in metabolic syndrome. Mean hs CRP level showed significant rising trend with increasing number of MS components. High WC and TG but not hypertension or low HDL components of MS were associated with raised mean hs CRP. Age, sex, cholesterol, LDL and underlying disorder had no relation with hs CRP levels.

Metabolic Surgery in Indian Diabetic Patients: How Close are We to Adopting Guidelines?

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Background: Post Diabetes Surgery Summit (DSS II;2015) and Joint Statement by International Diabetes Organizations(June;2016), Metabolic Surgery (MS) today is recognized as an effective treatment for type 2 diabetes mellitus (T2DM) and reflects in the treatment algorithm for T2DM. However, it is interesting to understand the adaptation of this guideline in Indian scenario with respect to different procedures, patient class and follow-up data. **Method**: PubMed search with keywords such as "Indians", "MS/BS", T2DM and others were searched with no period restrictions **Result**: Comprehensive review of Indian studies show that BMI of patients undergoing bariatric surgery range from 58 kg/m2 - 28.9 kg/m2. Data for diabetes resolution for these patients is available from month 1 to 6 years. Roux-en-Y gastric bypass showed 80% resolution at month 1 and Mini Gastric Bypass (MGB) showed 93.2% at 6 years. A latest study by Kular with seven years data showed good long-term control of T2DM in patients with class I obesity with MGB. Mean BMI of patients were 33.4± 3.3 kg/m2. These results highlight paucity of comparative analysis with different MS procedures in line with latest recommendations. Understanding of efficacy & safety of different types of MS procedures for different class of T2DM diabetic patients and subsequent role of these procedures in evaluating diabetes parameters like glycemic control, c-peptide and others. **Conclusion**: There is a need for more published clinical evidence to associate appropriate MS procedure to help better adopt the guidelines.

Obesity Significantly Predicts Abnormal Blood Glucose: Results of Pan India surveillance campaign Conducted by Apollo Sugar Clinics, India

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Background: From past two decades there is a significant increasing trend observed for obesity in developing countries including India. Evidence clearly showed that obesity is a significant risk factor for developing diabetes mellitus, metabolic syndrome and other chronic diseases. There is a need for community screening to identify subjects who are at high risk of diabetes mellitus or metabolic syndrome. **Objective**: Present study assessed the role of high body mass index in predicting abnormal blood sugar in subjects screened in a mass community surveillance campaign. **Methods**: It is a cross sectional study where participants from community were interviewed and tested for RBG across various cities of India. Subjects were interviewed using a structured questionnaire where various demographics and disease details were collected if applicable. Study subjects were classified based on BMI according to the guidelines laid down by Indian Council of Medical Research, India. BMI of <23, 23-25 and >25 Kg/m2 as normal, overweight and obese respectively. Abnormal blood sugar is considered as a person having random blood glucose >180 mg/dL at the time of screening. **Results**: A total of 27,056 subjects were recruited during community surveillance campaign. Mean age, BMI and RBG were found to be 45.4 years, 25.6 Kg/m2 and 132.2 mg/dL. Prevalence of overweight and obesity were found to be 21.4% and 53.9%. 13.3% (n = 3,595) subjects were found to have abnormal blood glucose (RBG >180 mg/dL). Prevalence of abnormal RBG is highest in obese subjects (14.9%) followed by overweight (13.4%) and normal and underweight subjects (9.8%). Binary logistic regression analysis revealed that higher BMI as a significant (P <0.01) predictor of abnormal RBG (Odds ratio, 1.05 (95% CI, 1.04-1.06)). **Conclusion**: The present Pan India surveillance campaign study revealed high burden of obesity in India and also found obesity as a significant predictor of abnormal sugar levels. This study reiterates the fact that obesity and uncontrolled blood sugar as co-existing and need to be managed using multi-dimensional treatment approach.

Truncal Obesity Indicies as Predictive Factors in Obstructive Sleep Disorders

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Background: Obstructive sleep apnea (OSA) is a disease characterized by collapse of pharyngeal airways which leads to oxygen desaturations, sleep fragmentation and daytime sleepiness. Obesity is the major risk factor for OSA. **Aim**: To Optimize the use of polysomnography in the diagnosis of obstructive sleep apnea (OSA). To assess the predictive value of truncal obesity measurements in the diagnosis of OSA. **Methods and Procedures**: One sixty one patients, who were suspected to have OSA underwent an overnight polysomnography study and their truncal obesity measurements were obtained. **Results**: Correlation between truncal obesity indices like waist hip ratio, neck circumference, BMI and Sleep indices such as apnea hypopnea index was observed using Pearson's correlation

coefficient. According to our study, there was a positive correlation between the truncal obesity indices and OSA. However only correlation of waist hip ratio and OSA was observed to be statistically significant. **Discussion**: Obtaining simple measurements may help prioritize the use of Polysomnography in patients with risk of OSA.

To Study the Association of Cerebrovascular Accident (CVA) and Coronary Artery Disease (CAD) with Metabolic Syndrome.

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Background/Hypothesis: Whereas the previous epidemic of coronary heart disease between 1910 and the 1960s was largely attributed to increased intake of saturated fat, it is quite plausible that the current epidemic of obesity and metabolic syndrome will lead the new epidemic of coronary heart disease, throughout the world. Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. Metabolic syndrome is also known as metabolic syndrome X, syndrome X, insulin resistance syndrome, Reaven's syndrome, and CHAOS (Australia). **Material and Method**: To study the association of Metabolic syndrome (MS) with Cerebrovascular accident & Cardiovascular disease. This is a case control study. **Result and Discussion**: In the present study, the overall prevalence of metabolic syndrome was found 71% in patient groups. The prevalence was 69.23% in cerebrovascular accident group and was 72.91% in coronary artery disease group. The data shows that there is a positive correlation of cerebrovascular accident & coronary artery disease with metabolic syndrome. In the present study, among the male patients of metabolic syndrome, frequency of hypertension was highest and that for waist circumference was lowest among the different components of metabolic syndrome. **Conclusion**- Among the female patients maximum were suffering from raised fasting blood sugar values 86.95%(n=40) followed by raised waist circumference 82.60%(n=38) and least with low HDL values 73.91% (n=34) ,high TGs values 73.91%(n=34) and hypertension 73.91%(n=34). In the frequency distribution of various components of metabolic syndrome, among the male patients maximum were suffering from hypertension 79.16%(n=76) followed by high TGs values 77.08%(n=74), raised fasting blood sugar 77.08%(n=74), low HDL values 77.08% (n=74) and least with raised waist circumference 56.25%(n=54).

rs2306283 (p.N130D, c.388A>G) Polymorphism in SLCO1B1 Gene is Associated with Altered Efficacy of Atorvastatin in Patients with Metabolic Syndrome

Sukhpreet Singh
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Introduction: Atorvastatin is actively transported via Organic Anion Transporter Polypeptide 1B1 (OATP1B1) into the liver and acts by inhibiting HMG CoA reductase enzyme in the liver. **Aim**: To study the frequency and impact of allelic variant of SLCO1B1 rs2306283 (p.N130D, c.388A>G) polymorphism encoding OATP1B1, with respect to pharmacodynamic effects of atorvastatin in North Indian Population. **Method**: This was an analytical study. Newly diagnosed patients with Metabolic Syndrome (MetS) as per IDF guidelines, of either sex, aged between 18- 60 years were enrolled in the study. Lipid Profile, Cardivascular Risk Ratios, such as Non High Density Lipoprotein Cholesterol (Non HDL), Atherogenic Coeffecient (AC), Cardiac Risk Ratio (CRR), Atherogenic Index of Plasma (AIP), liver function test and renal function test were done at 0 week and after 8 weeks of atorvastatin 20 mg per day, administration. Gene amplification and polymorphism analysis was done by PCR-RFLP method. Final sample size was 80. Statistical analysis was done using ANOVA followed by post hoc Tukey Test. Correlation analysis was also done, p value <0.05 was considered as significant **Results**: The genotype frequency was be 41.25%, 33.75% and 25% for GG, AG and AA genotypes, respectively. The allelic frequency for G allele and A allele was 58.1% and 42.9%, respectively. The patients with homozygous G allele, achieved 10.72% greater increase in HDL levels, 32.93% greater reduction in LDL, 16.53% greater reduction in non-HDL, 21.15% greater reduction in AC when compared to those who were homozygous for A allele ($p < 0.05$). Patients having G allele had negative relationship with the values of the predicting markers for future cardiovascular diseases. **Conclusion**: Patients having G allele demonstrated much favorable efficacy and a greater reduction in risk of future cardiovascular diseases after 8 weeks of atorvastatin administration

RSSDI Research Grants

- For providing research grants, RSSDI invites proposals from Indian scientists, interested in conducting original research in the field of diabetes mellitus. Furthermore, limited grants are also available for the students of medical colleges for smaller projects.
- There is no deadline for submission of the proposals, which can be sent throughout the year. These proposals may fall into one of the following three categories:

1. Projects involving funding up to Rs 40,000 per project (preference will be given to young scientists <40 years).
2. Projects involving funding up to 10 lakhs.
3. We also invite proposals for more than 10 Lakhs as major projects but these have to be preferably multicentric.

- The detailed proposals should include the following:
  - ◊ Title, names of principal and co-investigators, summary, introduction/background, review of literature, aims, methodology, study design, and detailed plan of work and bibliography. Brief biodata of principal investigator and other co-investigators
  - ◊ Importance of work in the context of national priorities. Detailed budget sought along with full justification/ proposed utilization, of funding sought from RSSDI
  - ◊ Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.
  - ◊ Ethical committee clearance of the institution or other bonafide body.

Travel grants for young diabetes researchers to attend International Conferences

Criteria's for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, Full time, residential "Advanced Certificate Course in Diabetology". This two years course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 16 centres across India at present and more centers are being inspected for accredition. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

List of RSSDI Accredited Centres

S.N.	Institute Name	Institute Location
1.	Diacon Hospital	Bengaluru, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Totall Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	G D Hospitals and Diabetes Institute	Kolkata, West Bengal
10.	Aditya Diagnostics & Hospital	Dibrugarh, Assam
11.	Sunil's Diabetes Care N' Research Centre Pvt Ltd.	Nagpur, Maharashtra
12.	Marwari Hospital and Research Centre	Guwahati, Assam
13.	Down Town Hospital	Guwahati, Assam
14.	St.Theresa's Hospital	Hyderabad, Telangana
15.	Aegle Clinic	Pune, Maharashtra
16.	Tulip Hospital	Sonipat, Haryana

COURSE DETAILS

Name of the Course: Advanced Certiicate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The

result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given preference.

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

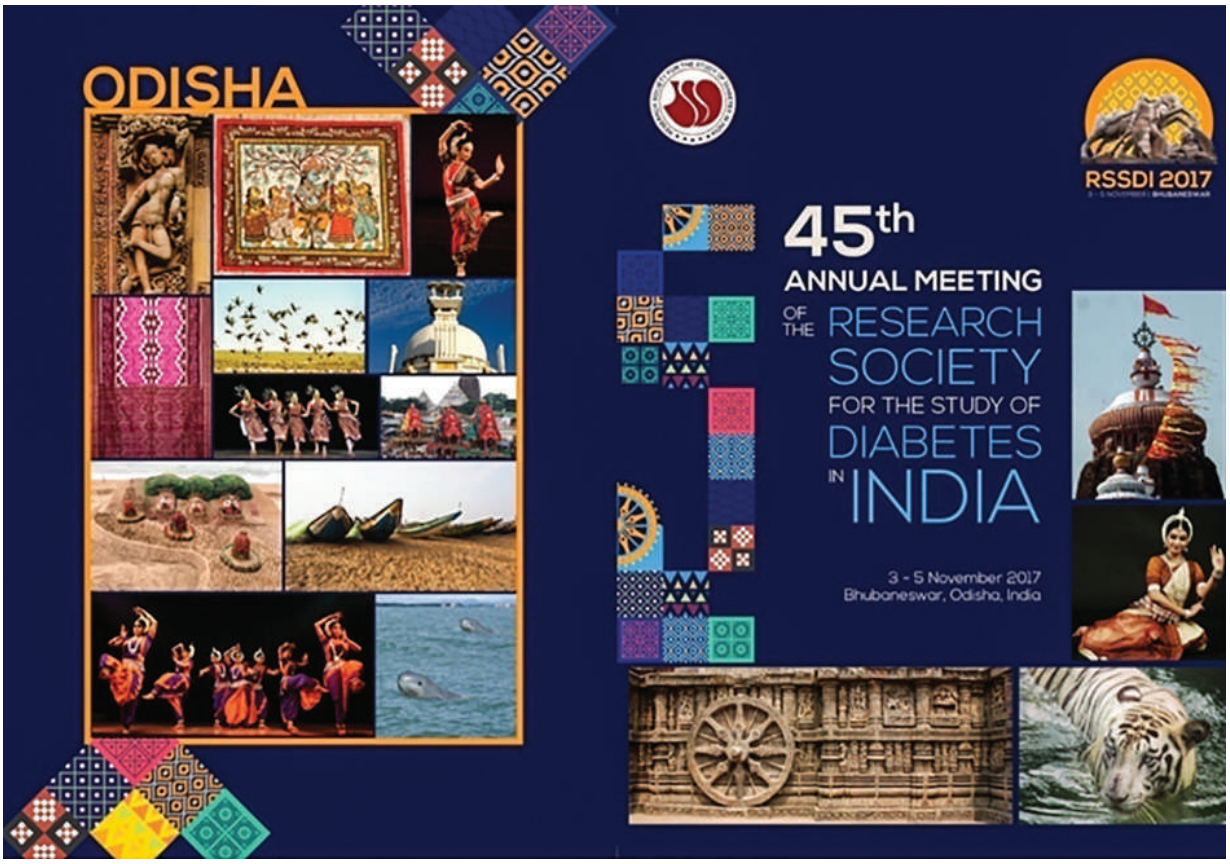
Session: 1st January & 1st July

Announcements

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

Invitation to the RSSDI,2017 Conference



Dear Friends,

We have great pleasure in inviting you to the 45th Annual Conference of Research Society for the Study of Diabetes in India (RSSDI) to be held in the smart city of Bhubaneswar from 3rd to 5th November 2017.

Bhubaneswar is the capital city of scenic Odisha. the soul of India which is naturally beautiful with forests. wildlife, sea beaches, heritage temples and many historical monuments. It is also known for its flavor of hospitality and varieties of delicious foods. We assure your convenient travel and comfortable stay with a relaxing time and a complete updated academic exposure.

The organizing committee will leave no stone unturned to make the event memorable for you and your family. please block your dates and register early to be a part of the event.

Wishing you a very happy. bright and prosperous new year ahead.

Team RSSDI 2017
Bhubaneswar, Odisha

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International Journal of Diabetes in Developing Countries

Volume 37 | Issue 3 | July–September 2017

Springer