LETTER TO EDITOR

Interpretation of PET scan results at varying degrees of blood glucose in diabetic patients with lymphoma: an experience of 25 cases of Non Hodgkin Lymphomas from southern India

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Dear Sir,

One of the major breakthroughs in the staging and follow-up recommendations in lymphoma is Positron emission tomography (PET). It has good sensitivity and specificity in determining response to therapy as well as to detect relapses [1]. The hypothesis of differential glucose uptake by malignant cells forms the basis of PET scanning. The increased activity of hexokinase and expression of GLUT 1 transport proteins, results in increased uptake and retention of glucose in cancer cells compared to normal cells. When these values are measured using the radiolabel FDG [F18], commonly referred to as standard uptake value (SUV) scores, malignant cells have higher values than normal cells. [2] It is known that patients with Type 1 DM exhibit reduced glucose entry through GLUT 4 transporters, owing to reduced levels of insulin, which in turn leads to higher blood glucose levels. Similarly, Type 2 DM have insulin resistance, [mainly due to decreased receptors] resulting in high blood glucose. It was hypothesized and proven in some of the studies that higher blood glucose levels

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compete with FDG, leading to decreased uptake of the radiotracer by the malignant cells, though few other studies have reported mixed results. Similarly, differences between type 1 DM and Type 2 DM were observed wherein the former group [type 1] had lower FDG uptake. The supplemental insulin leading to increased FDG by normal cells in Type 1 DM is supposed to cause lower availability for cancer cells. [3] With the widespread availability, and increasing usage of PET scan form the Indian subcontinent, high incidence of diabetes and lack of regional literature, we planned this study to evaluate the role of PET scan in diabetic patients with lymphoma. We also investigated the effect of blood glucose levels in both pre and post chemotherapy on the PET scan results as it may have influence on the future management plans. During this period, all diabetic patients diagnosed with diffuse large cell lymphoma receiving chemotherapy with CHOP [Cy-clophosphamide, Vincristine, Adreamycin and Predniso-lone]/R [rituximab] -CHOP [in cases of B cell lymphoma] were enrolled and underwent PET/CT, in addition to other standard investigations like LDH. The blood glucose levels were measured just prior to each PET scan, whenever it was performed. The other regular investigations like HbA1C, lipid levels, required for the regular management of diabetes were obtained from the case records. The procedure of PET/CT was a standardized one and Stan-dardized Uptake Value (SUV) was obtained at each visit [before treatment, 12 weeks after treatment and during follow-up] from the primary lesion sites. NCCN guidelines were followed for the management and follow-up proto-cols. [4] All the patients' details, regarding the diabetic status was obtained from the past medical records. We did not do any survival analysis, as it is a short study of 1-year duration and the end point was response rates. The student t

test was used to determine association of selected variables to initial SUV. A generalized linear model was used with backward selection to find significant independent predic-tors for initial and post treatment SUV. Twenty five patients diagnosed in the year 2008 underwent 18F-FDG PET scans as described in the methods section. The baseline characters of patients were represented in Table 1. The median number of cycles received was 6 [range 6–8]. Four patients had insulin dependent diabetes mellitus (IDDM/Type1), and 21 had Non insulin dependent diabetes mellitus (NIDDM/Type 2). Patients are stratified as having good glucose control if the random glucose samples were below 140 mg/dl. There was no difference in blood glucose in pre and post-treatment periods within the patients. Pre-treatment SUV, post treatment

SUV and percent SUV decrease by blood glucose levels are represented in Table 2. There were no differences in initial SUV between type 1 and type 2 DM, as well as those having poor serum glucose levels. Patients with B cell variant had initial higher initial SUV ($p=0.04$). Other important factor is the IPI score [in view of small sample size, individual parameter assessment was not done], where patents with score more than or equal to 3 had higher initial SUV ($p=0.03$). Metabolic control does not have any effect on either initial or final SUV scores. Clinical response rates and SUV decrease did not differ between patients having good or poor blood glucose control. Patient numbers, however, are small in each group limiting the power to detect small differences between groups. To the best of our search by MEDLAR and PUBMED, this is the first study from India examining the

Table 1 Patient Characteristics

Sex	
Male	15
Female	10
Age	Median44 (27–57)
Diabetes Mellitus	
Type 1	21
Type 2	4
International Prognostic Index	
Low risk	1
Low intermediate	6
High intermediate	8
High	10
Histology	
T cell	8
B cell	17
Glucose control	
Good	16
Poor	9

Table 2 Mean pre-treatment SUV, post-treatment SUV, percent SUV decrease and response rates

	Pre-treatment SUV	Posttreatment SUV	Percent SUV decrease	Response rates [CR+PR] (percent)
Type 2 DM	8.7±5.6	1.4±±0.6	84	81
Type 1 DM	9.3±4.2	1.5±1.1	84	75
Good glucose control	9.7±3.6	1.3±0.8	87	81
Poor glucose control	7.9±4.8	1.2±0.9	85	77
High IPI [>3]	11.7±6.6	1.4±0.7	88	78
Low IPI [<3]	9.2±5.9	1.1±0.4	88	86
T cell	7.7±6.4	1.3±0.6	83	63
B Cell	10.9±6.2	1.5±0.8	86	88

relationship between blood glucose levels and SUV uptake in diabetic patients with lymphoma. Our results, which are partially in agreement with those of Gorenberg et al. [5] suggested that the neither glucose levels nor the type of diabetes i.e. type 1 vs. type 2 influenced the SUV values, though one might expect a patient that is supplementing insulin to have a decreased SUV score in tumors by mechanism of insulin “stealing” 18F-FDG into normal cells and away from tumor cells. Another observation in our study

was high SUV values in patients having IPI score more than 3. This is quite expected as these tumors are clinically and biologically aggressive. However, it is surprising to see that B cell lymphomas had higher initial SUV compared to their T cell counterparts. Though we tried to look into the tumor biology and find some explanation, no satisfactory answer could be found. Therefore, these results suggest that further studies should be undertaken to provide mechanistic explanation. The overall response rates as well as decrease in SUV after treatment are uniform across all groups [type 1 vs. type 2, High IPI vs. low IPI, T cell vs. B cell, and good sugar levels vs. poor sugar levels]. Though these observations may be due to the small sample size, which is a major limitation of the study, the correlation of response rates and SUV decrement argues for a strong scientific basis for our results. However, we feel that the present results may be viewed just as an hypothesis for a bigger study with a larger population. Lack of insulin level estimations for individual patients, is another limiting factor, which we are thinking of rectifying in our next study. To conclude, we strongly feel that PET can be used with reasonable accuracy in patients with lymphoma irrespective of their diabetic/blood glucose status. The surprise finding of differential initial SUV of B and t cell lymphomas warrant further studies.

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Conflicts of interest None.

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Int J Diab Dev Ctries

REVIEW ARTICLE

Computerization of data in diabetes centers

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Information technology has made its in roads into clinical diabetes practice. Both hardware and software are accessi-ble and available. The reason why its full potential is not being harnessed is the bottleneck at the end-user or the practicing clinician. It is not to berate the practising physician but to say that implementation requires more than mere availability [1].

Currently computers are in widespread use in adminis-trative work: to set up appointments, for billing purposes and printing of prescriptions. They are often linked to the lab services and the pharmacy for error-free transmission of information and drug delivery. Many of the current clinical laboratory instruments have embedded microchips which interface with a computer and quality control software. IT is being used quite extensively even though many of us are not aware of the same.

What are the practical difficulties in using electronic medical records (EMR) at the point of care by the physician in the consultation room? This brings out a number of constraining factors, although the strengths of capturing data by computerization is well recognized. The constraints against the use of EMR include a relatively rigid system of data entry, difficulty in capture of non-structured data (including descriptions and line diagrams), and the need to acquire skills to communicate with the patient while feeding data to the computer system. The last requires work-flow analysis in which the physician must have a logical sequence for eliciting the information (viz diabetes-related eg duration of diabetes, treatment, associated

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conditions such as hypertension, coronary artery diseases, system wise, viz general condition, related to cardiovascu-lar, respiratory, abdominal and skeletal system, personal habits including smoking, alcohol use, physical exercise, and in women, menstrual status) [2].

The depth and breadth of information capture also depends on the purpose to which the information is sought to be used; is it to have a medium or coarse grained information on the large patient population who undoubt-edly presents to physicians? Or is it to use specific information for a specific purpose? For someone wanting focused information, that part is expanded (eg diagnosis of angina, frequency and severity of physical exercise). One must bear in mind it is a trade-off between the depth of information gathering versus the time that is

available for each patient encounter. It also depends on the kind of infrastructure that is available. One can have a physician-assistant enter the data outside the consultation room while the patient has arrived and is waiting. This can be made available to the physician before the patient presents to him/ her. Or an internet-based system may be used for the patient himself/herself to enter the information. Alternatively a touch-screen system may be arranged for patients to key in the data in the waiting space.

In addition to the infrastructure, it also depends on what kind of interaction the physician wants to maintain with the patient. By entering all the information while the patient sits across the table, it is possible to observe and assess not just what but how the information is given.

Once the ground plan for data capture is in place, it must be converted into an appropriate software. This requires close interaction with software professionals.

Such physician-specific instruments are like 'bespoke' clothes: good but expensive. Should generic software be made available, most of the outlines would be ready, but

customization is still essential for appropriate use in the particular physician's office [1, 2].

The systems can be linked among the physician, nutritionist, podiatrist, clinical psychologist, nurse educator and any other members of the health-care team. All the data would be entered, consolidated and accessed by each member. Controls can be devised to regulate what and how much can be seen, entered or edited by each member of the team. An edit-track system with electronic signatures can be generated for later review.

Such electronic medical records (EMR) can be con-structed to capture data in detail at the first encounter. Limited but relevant information can be displayed in the follow up visits (e.g. duration of disease, current age, weight, blood pressure, biochemical values, smoking status, exercise, compliance). Follow up data can be scrolled down.

The system can incorporate a logical rule based system in which the diagnoses or instructions are automatically generated in the diagnosis field, depending on the data (e.g. diagnosis depending on the age of onset, hypertension if entry for 'hypertension' field is yes, 'to stop smoking' in the prescription if the entry shows that the person smokes.

Flags can be generated to repeat the lipids at the next visit if dyslipidemia exists, or at the annual visit as a routine depending on the quality guidelines provided by national or international diabetes organizations.

Images (X ray, ultrasound, MRI, CT) can be captured and be part of medical records [3].

Rule based flagging system can be incorporated to warn against drug interactions and to suggest the use of drugs prophylactically (e.g. aspirin).

Online information can be used for differential diagnosis [4]. Similarly knowledge information systems such as Update [5] give point of care information both on the desktop and mobile devices.

It is also possible to have touch-screens where patients and their attendants can access interactive educational material about diabetes and healthy lifestyle advice, to prevent diabetes and to manage the disease. An internet based diabetes risk screening instrument can also be provided. Amalgamation of demographic, clinical and genetic information can improve the sensitivity of predict-ing the risk of metabolic complications; such additional data can be added on to the existing screening procedures [6, 7].

Interactivity offers personalized information and can also profitably use the waiting time, while optimizing the time of health care professionals who can use the resultant extra-time for more value-based activities.

The clinical performance by the diabetes-care team can be audited once the information and procedures are captured live [8]. Rather than retrospective analysis, live

capture will offer real-time feedback on performance and suggest corrective actions. This feedback can be either passive (i.e. the health-care provider will be able to access when required) or proactive, in which the information is provided even without being specifically asked (i.e. rule-based or expert-system).

In addition, questionnaires on well-being, quality of life and other parameters can be captured using touch-screens on-site or via the internet. These would provide a measure of psychological parameters, based on which clinical-care is fine-tuned. Geographical networking among different diabetes care centers located anywhere would offer a method to pool the data [9], which can be analyzed perhaps by neural-network or other artificial intelligence systems so that the performance can be further refined [10, 11].

Patients on self-home-blood glucose monitoring or continuous glucose monitors can upload their readings and send it across in advance of their visit to the diabetes centre. Visual representation of the glucose values over time and summary and descriptive statistics would improve the advice offered by the physician.

Privacy issues must be considered and ensured once the data is scattered in different physical locations and is not confined to the hard disk at the physician's office. Reliable encryptation methods are available and must be imple-mented [2]. Similarly the issues of data ownership and of ethics also arise, which must be addressed.

Images (X rays, CT, MRI, angiograms) can also be lodged on a central server(s) and information accessed at the point of care.

'Smart card' technology can be used to carry medical information with the patient, synchronizing the updated data after each clinic visit. Security issues must be addressed by incorporating either biometric (e.g. finger print) or other encryptation methods to prevent misuse of data in case of physical loss. Methods can be devised to lock the data as in case of cell phone loss now. Accessibility of medical information is important when the patient presents to a different medical care facility [12]. Both internet-based and smart-card based systems offer such data sharing methods. However it would be necessary to sort out data-sharing and privacy issues.

Point-of care access to evidence-based information prevents errors in prescriptions, supply of drugs and in warning against possible drug interactions. This requires transport of systems to hand held devices such as smart phones or dedicated equivalent portable systems.

Technology exists to describe, model and improve delivery of medical care [13] National societies such as Research Society for Study of Diabetes in India (RSSDI) are in a position to take a lead in translating these concepts to reality in the management of diabetes.

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Frequency of prediabetes and influence of various risk factors on the development of prediabetes: a tertiary care hospital experience

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Abstract The objective of study was to determine the frequency of prediabetes and observe the influence of various risk factors on the development of prediabetes at Liaquat University Hospital, Hyderabad. Descriptive case series. Medical outpatient department Liaquat University Hospital, Hyderabad. From 01-03-2007 to 31-03-2008. Total 500 subjects with BMI>25 and aged either 45 year and above or BMI>25, with an additional risk factor were enrolled for the study.All diagnosed patients of diabetes were excluded from study. A well designed proforma included demographic information, history regarding first degree relative with diabetes, gestational diabetes, delivery of large baby and related investigations. Fasting blood glucose (FBG)≥100 mg/dl but <126 mg/dl and/ or OGTT level ≥140 mg/dl but <200 mg/dl was considered predia-betes. The collected data was analyzed on SPSS version 16.0. This study comprised of 500 subjects of which 306 (61.2%) were male and 194 (38.8%) female. The mean age of the cases were 49.42±7.73 years. Prediabetes was found in 147/500(29.4%) cases of which 109 were female and 38 were male. A strong correlation was found between prediabetes and BMI, persons with h/o diabetes in first degree relative, h/o gestational diabetes, h/o delivery of high birth weight child, HDL <35 mg/dl. Considering the high frequency of prediabetes in the region, provision of vast educational program to prevent the disease is essential and as well screening for prediabetes using FBG and OGTT, especially for obese subjects and those with other risk factors of diabetes.

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Keywords Prediabetes • BMI • WHR • IGT • IFG • Triglyceride

Introduction

The incidence and prevalence of type 2 diabetes mellitus (T2DM) have reached epidemic proportions, with further increases appearing inevitable [1]. Unless appropriate action is taken, this will place an impossible economic burden on health systems and on individuals. As a result, major prevention campaigns are needed worldwide. These can be of two types: the population approach, in which an entire population is advised on the benefits of healthy living (i.e. increased physical activity and weight control), or the targeting of high-risk individuals [2, 3]. Often the two approaches are combined. In recent years, targeting of high-risk individuals has focused on the identification of those with prediabetes [4].

In 1979, the term impaired glucose tolerance (IGT) was coined by the World Health Organization (WHO) and the National Diabetes Data Group [5] to replace an older term of borderline, chemical or asymptomatic DM coined in 1965. In 1997, an expert committee from the American Diabetes Association (ADA) recommended the following criteria for PDM; a fasting blood glucose of 110 to

<126 mg/dl and/or a postprandial blood glucose of 140 to <200 mg/dl 2 h after a 75-g oral glucose challenge [6].

The number of prediabetic patients is ever increasing worldwide. In 2003, an estimated 314 million people developed prediabetes. By the year 2025, the number is expected to increase to 472 million (9% of all adult population) worldwide. However, it differs from region to region. Prevalence was reported at 13.2% of all adult population in the south-east Asian region, 10.5% in the European region and 5.7% of adults in the western Pacific region [7].

Persons with prediabetes can have mortality 40% greater than the normal population [8]. Coronary heart disease is 1.33 times higher than the normal population [9]. Recent data have also shown that both lifestyle and pharmacologic therapy can alter the progression of prediabetes to overt diabetes [10, 11].

The objective of the study was to determine the frequency of pre-diabetes at Liaquat University Hospital Hyderabad and to observe the influence of various risk factors such as sex, BMI, H/O gestational diabetes, first degree relative with diabetes and H/O delivery of large baby with the occurrence of prediabetes.

Subjects and methods

This descriptive case series study included 500 consecutive cases attending the medical outpatient department of Liaquat University Hospital Hyderabad from 01-03-2007 to 31-03-2008.

The study included all persons 45 years or older age with BMI 25 kg/m^2 or less than 45 years with BMI 25 kg/m^2 or more, if having another risk factor [6]:

i. Blood pressure over 140/90 mmHg
ii. HDL cholesterol 35 mg/dl or less.
iii. Triglyceride level 250 mg/dl or more.
iv. History of first degree relative with diabetes.
v. History of gestational diabetes.
vi. History of delivery of large baby (weighting more than 9 lbs.).

Subjects with known diabetes were excluded from initial enrollment

All the patients who met above inclusion criteria included in the study after taking informed consent. A thorough medical history regarding first degree relative with diabetes were taken, gestational diabetes, delivery of large baby and physical examination including of blood pressure, BMI by measuring height in meters (m) and weight in kilogram (kg),waist circumference in centimeters (cm) and waist-to-hip ratio (WHR) was carried out and entered into proforma. After an initial blood sample was drawn for FPG testing after overnight or eight hour fasting,

participants were asked to drink a calibrated dose of 75 g glucose. Two hours later, second plasma sample was drawn and tested for post load glucose concentrations. These samples were collected in test tubes containing no preservative and were transported within half hour to Liaquat University Hospital Laboratory; the method used was “PAP” enzymatic calorimetric test. IFG was defined as having FPG >100 mg/dl but <126 mg/dl. IGT was defined as having2h glucose >140 mg/dl but <200 mg/dl. Pre-diabetes was defined as having IFG and/or IGT [6].

Another twelve hour fasting blood samples were taken for HDL cholesterol and triglyceride level and sent to the same laboratory. All this information was enrolled in well-designed proforma. Patients with any of variables such as no h/o first degree relative with diabetes, no h/o gestational diabetes, no h/o delivery of large baby, BMI <25 kg/m^2, HDL Cholesterol >35 mg/dl and triglycerides <250 mg/dl were grouped as one. Patients with any of variables such as h/o first degree relative with diabetes, h/o gestational diabetes, h/o delivery of large baby, BMI >25 up to 30 or >30

kg/m^2, HDL Cholesterol <35 mg/dl and triglycerides >250 mg/dl were grouped as two.

Data analysis

Quantitative variables such as age, height, weight, waist circumferences, waist to hip ratio (WHR), systolic blood pressure, diastolic blood pressure, fasting plasma glucose level and oral glucose tolerance test level were expressed as Mean and Standard deviation. Qualitative variables such as sex, subjects with history of first degree relative with diabetes, with history of gestational diabetes and with history of delivery of large baby, BMI, HDL, triglycerides, prediabetes were expressed as frequency & percent. The qualitative variables such as sex, h/o of first degree relative with diabetes, h/o gestational diabetes h/o delivery of large baby, BMI, HDL, triglycerides were compared with the prediabetes by Chi-square test. Statistical analysis was performed by SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). P value of 0.05 was considered statistically Significant.

Results

This case series study included 500 consecutive subjects with BMI≥25 of which 306 (61.2%) were male and 194 (38.8%) were female. The mean age were 49.42± 7.73 years, height 1.70±0.06 m, weight 82.32±8.37, fasting plasma glucose 97.56±19.2 mg/dl, oral glucose tolerance test 154.74±35.88 mg/dl, HDL cholesterol 42.60±4.54, and triglycerides 184.4±14.7 mg/dl. h/o diabetes in first degree relatives was present in 157/500

Table 1 Baseline characteristics of the cases studied

Quantitative Variables	Number	Mean	SD±
Age (yrs)	500	49.42	7.73
Weight (kgs.)	500	82.32	8.37
Height (m)	500	1.70	0.06
FBG (mg/dl)	500	97.56	19.2
Oral glucose tolerance test (mg/dl)	500	154.74	35.88
HDL cholesterol (mg/dl)	500	42.6	4.54
Triglycerides (mg/dl)	500	184.4	14.7
Qualitative Variables	Number	Frequency	Percentage
Sex-Male	500	306	61.2%
Female		194	38.8%
H/O gestational diabetes		32	6.4%
H/O delivery of large baby		32	6.4%
H/O diabetes in 1st degree relatives	500	157	31.4%
Prediabetes	500	147	29.4%
BMI (kg/m^2)	500		
<25		118	23.6%
25 to30		281	56.2%
>30		101	20.2%

(31.4%), h/o gestational diabetes in 32(6.4%) and h/o delivery of large baby in 32(6.4%) cases. Prediabetes was present in 147/500 (29.4%) of which 95 were male and 52 female. Table 1 explains the baseline character-istics of the cases studied.

Among the 306 male 95 had prediabetes and amongst 194 females 52 had prediabetes (p=0.316).BMI<25 was present 118 cases of which 18 had prediabetes, >25 up to 30 was present in 281 persons of which 70 had prediabetes and 101 persons had BMI>30 kg/m^2 of which 60 had

prediabetes (p=001). In 62/147 persons with pre diabetes had h/o diabetes in first degree relative (p=0.001), 11/32 with h/o gestational diabetes had prediabetes (p=0.549),

19/32 female with h/o delivery of high birth weight child had prediabetes (p=0.001). 18/34 persons with HDL <35 mg/dl had prediabetes (p=0.003) and 6/26 with triglyceride >250 mg/dl had prediabetes (p=0.659). Table 2 explains the relation of various risk factors with the development of prediabetes.

Discussion

In this study, frequency of prediabetes was 29.4% which is higher as compared to that reported by the National Diabetes Prevalence survey of Pakistan which showed that

Table 2 Relation of various risk factors with the development of prediabetes

Variables	Number	Total N:	Prediabetes	P value
Sex - Male	306	500	95	0.316
Female	194		52	
BMI -				
<25	118	500	17	0.001
>25–30	281		70	
>30	101		60	
h/o Diabetes in first degree relative	156	500	86	0.001
h/o gestational diabetes	32	500	11	0.549
h/o delivery of high birth weight child	32	500	19	0.001
HDL <35 mg/dl	34	500	18	0.003
Triglyceride >250 mg/dl	26	500	06	0.659

over 10% of people in the age group 25 years and above are suffering from prediabetes and further that prevalence of prediabetes among adult population in Sindh was 11.1% [12]. It is because we have enrolled high risk subjects while National Diabetes Prevalence survey of Pakistan enrolled all the adults above 25 years irrespective of any risk factor. While secondary analysis report of New castle Heart Project showed that prevalence of prediabetes is 23.4% among South Asians [13]. Also a study conducted at primary health care clinics of Israel to assess the glucose tolerance state in healthy, over-weight Arabs aged above 40 years and showed that 42% had prediabetes [14]. NHANES-III showed that among the overweight adults aged above 45 years, 45.9% had abnormal glucose metabolism, out of these 12.5% had self-reported diabetes, 10.8% had undiagnosed diabetes 22.6% had prediabetes [15]. NHANES-III shows the severity of problem in developed country where people are much more conscious of their health and take regular medical checkups as compared to our setup where there is no such trend and people seek medical help when problems like complica-tions arise. It is also estimated that major part of increase in diabetes prevalence is occurring in developing countries with the rate of 170% increase and by the year 2025 approximately 75% of all persons with diabetes will be living in developing countries [12].

In this study, the frequency of prediabetes is higher in males than in females, NHANES-III also showed that higher proportion of male than female had prediabetes (55.4% versus 44.6%) [16].

In this study history of first degree relative with diabetes has shown a strong association with the prediabetes. In NHANES-III prevalence of prediabetes was high (44.3%) in person with positive family history of diabetes [16]. Type 2 diabetes appears to have strong genetic associations. Studies in twins have demonstrated that the concordance rate of type 2 diabetes in monozygotic twins range between 34% and 83% [17]. The broad range of observed correlation suggests both a complex genetic predisposition and an interaction between environ-mental and genetic factors in the pathogenesis of type 2 diabetes. People who have one first degree relative suffering from prediabetes have a 40% risk of having this disease. If diabetes is seen in both parents, the risk is doubled [18].

In our study h/o gestational diabetes and history of delivery of large baby has shown a non-significant association with prediabetes. This is in contrast with the study that showed that 36% of women who were diagnosed with gestational diabetes mellitus had persistent abnormal glucose tolerance [19]. The reason seems to be enrollment of small number of patients in our study.

A positive association between high BMI with prediabetes is seen in this study. Mean weight, BMI, Waist circumference and waist to hip ratio (WHR) were all higher in prediabetes as

compared to normal non-diabetics. Several longitudinal cohort studies have demonstrated the association between obesity and glucose intolerance [20]. Data from NHANES-II show that 67% of those with type 2 diabetes have BMI that meets the criteria for being overweight, and almost half have BMI that meets the definition of obesity [21].

In this study, systolic and diastolic blood pressure increased from normal to prediabetes to undiagnosed diabetes. Masoumeh Sadeghi et al. [22] and A. Basit [23] also show same association of blood pressure to prediabetes and diabetes. Epidemiological studies report at least 2 fold incidence of high blood pressure in diabetes [16]. NHANES-III shows 56.5% hypertensive had prediabetes [13]. In the NHANES II study, the prevalence of hyperten-sion, defined as blood pressure >160/95 mmHg among individual aged 65 to 74 year, increased with decreasing glucose tolerance [24]. Approximately 60% of subjects with diabetes, 50.7% of those with IGT, and 38.3% of those with normal glucose were affected [25].

In this study, HDL cholesterol level decreased from normal to prediabetes to undiagnosed diabetes while the triglyceride level increased. Type 2 diabetics often have elevated triglyceride and depressed HDL cholesterol. It develops concomitantly with the failure of insulin activity, which in turn leads to the release of fatty acids from adipose tissue, increased delivery of free fatty acids to the liver, and increased hepatic synthesis of very low density lipoprotein NHANES-III shows high prevalence of predi-abetes 94.9% in dyslipidimics [16]. Dyslipidemia is associated with markedly increased cardio vascular risk among diabetic patients [26].

Conclusion

This study showed a meaningful relationship between obesity, dyslipidemia, history of first degree relative with prediabetes. Therefore, identifying and mitigating these factors are of great importance to the health of the general population. The high frequency of prediabetes in this region makes it necessary to generalise the screening methods of the disease for the population with above risk factors and with early detection of prediabetes and its management with life style modification can prevent or delay the onset of type 2 diabetes in a cost effective way. In addition, promoting the level of general knowledge on the risk factors of diabetes or its symptoms and complications can play an effective role in the prevention and control of the disease. This can be done through the mass media or distribution of educational pamphlets or books written in simple language. More studies are needed to improve the correlation between gestational diabetes or delivery of large baby and prediabetes.

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ORIGINAL ARTICLE

Islet cell autoantibodies in patients younger than 20 years of age with recently diagnosed diabetes in Northwest of Iran

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Abstract: The presence or absence of islet cell autoanti-bodies is one of the most direct ways to distinguish between type 1 and 2 diabetic patients. The objective of the present study was to assess the prevalence of β-cell autoantibodies such as glutamic acid decarboxylase-65 antibodies (GADAs) and islet cell antibodies (ICA) among patients younger than 20 years of age with recently diagnosed diabetes in northwest of Iran. From 2006 to 2008, 163 patients were enrolled in this study. They were clinically classified into two groups: 136 with type 1 diabetes (T1D) and 27 with type 2 diabetes (T2D). Serum levels of GADAs, ICA and C-peptide were determined with enzyme

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linked immunosorbent assay (ELISA) kits. Fasting blood glucose and HbA1c levels were also determined. Chi-square test, independent t test and one-way analysis of variance were used for data analysis. The prevalence of GADAs in T1D patients was 33.1%, slightly lower than that of ICA 35.3%. Forty-eight patients (35.3%) with T1D were positive for ICA compared to only one (3.7%) in T2D patients. The overall occurrence of any autoantibody in T1D patients (60.3%) was significantly higher than that of T2D patients (18.5%) ($P<0.001$). There was a statistically different association with family history of diabetes among the autoantibody positive versus autoantibody negative patients with T1D ($P<0.01$). Our results confirmed the presence of GADAs and ICA in T1D patients in Iran, though roughly at a lower prevalence than that reported for Caucasian T1D patients, but very similar to other non-Caucasian ethnic populations.

Keywords Type 1 diabetes mellitus • Anti-GAD65 autoantibodies • Anti-ICA • C-peptide

Introduction

Type 1 diabetes mellitus (T1D) is a heterogeneous disorder, resulting in most cases from an autoimmune mediated destruction of pancreatic β-cell, which leads to an absolute insulin deficiency [1]. The rate of destruction is quite variable, being rapid in some individuals and slow in others [2]. It is well known that genetic as well as environmental factors contribute to the pathogenesis. Glutamic acid decarboxylase (GAD) is one of the

autoantigens that trigger β-cell specific autoimmunity. However, its precise role in T1D pathogenesis is not known [3]. Other antigens are also involved, such as tyrosine phosphatase, carboxypeptidase-H, heat shock protein, insulin, pro-insulin, chymotrypsinogen-related 30-kD pancreatic antigen, DNA topoisomerase, glima 38, and GLUT 2 [4]. Autoantibodies against some of these antigens have been used in clinical practice as important tools to elucidate difficult cases, when the classification of diabetes cannot be done solely based on the clinical presentation. Four of them are considered to be the most useful: islet cell autoantibodies (ICA),

insulin autoanti-bodies (IAA), glutamic acid decarboxylase-65 autoanti-bodies (GADAs), and tyrosine phosphatase autoantibodies (IA-2A). One or more of these markers are present in 85– 90% of individuals with a recent diagnosis of T1D [4– 7]. Studies in Caucasians have shown that about 80–90% of patients with T1D have at least one of these β-cell autoantibodies detected [8]. On the other hand, in Caucasians about 10–15% of patients clinically diagnosed with T2D also have islet cell autoantibodies, especially GADAs [9]. On the other hand, studies indicate that as many as 10% of adult patients, initially diagnosed as type 2, eventually become insulin dependent. The term latent autoimmune diabetes of adults (LADA) was introduced to describe autoimmune late onset type 1 diabetes. Like classical type 1, late onset type 1 diabetes results from the autoimmune destruction of the β-cells [10]. Because populations with Type 1 diabetes in Asia and Africa exhibit less than 50% prevalence of diabetes related autoantibodies, the American diabetes association (ADA) [5] and the World Health Organization [11] have sub-classified Type 1 diabetes into Types 1a and 1b, where Type 1a is autoimmune mediated, and Type 1b is non-autoimmune mediated, or idiopathic Type 1. There appear to be no significant clinical differences between patients with or without circulating autoantibodies [12–15].

ICA has been utilized to diagnose type 1 diabetes because this antibody circulates in most patients before and at the onset of the disease, and frequently declines following diagnosis of disease; but GADAs appear to remain positive for long periods of time [16]. Although some investigators have shown a higher prevalence of GADAs in newly diagnosed patients than in individuals with a longer duration of diabetes, persistent positive results are found in many cases [17].

Identification of the population at risk for developing T1D has many important clinical implications, especially for the implementation of preventive measures at the optimum time, if available. Prediction and prevention of T1D is based on defining the genetic risk of individuals at an early stage and subsequently following up those at genetic risk by additional investigations such as determination of disease-related autoantibodies and first-phase insulin release [18]. There are relatively few studies regarding autoantibody prevalence and its clinical usefulness in diabetic patients in Iran. The aim of the present study was to elucidate the prevalence of β-cell autoantibodies in patients younger than 20 years of age with recently diagnosed diabetes in northwest of Iran and to compare these results with those reported in other studies from different countries.

Subjects and methods

From 2006 to 2008 163 patients, aged<20 years, with newly diagnosed diabetes were recruited at Endocrinology and Diabetes Clinic, Sina teaching hospital, Tabriz University of Medical Sciences and outpatient clinic of Tabriz University of Medical Sciences. All of the patients were evaluated within 3 weeks of diagnosis, which was based on the report of the Expert Committee held in 2003[5]. Patients with any evidence of chronic complications at diagnosis, pancreatic exocrine diseases, end-stage renal disease, immunosuppressive diseases, or using immuno-suppressive drugs were excluded. Before the beginning of the study, informed consent was obtained from the patients or their parents. The Ethics Committee of Tabriz University of Medical Sciences approved the study. A structured interview schedule was used to collect infor-mation regarding age at diagnosis, duration of disease, gender, family history of diabetes, BMI (Body Mass Index) and blood pressure.

All patients were given a thorough physical examina-tion. They were classified as Type 1 or Type 2 according to the clinical criteria recommended by the American Diabetes Association [5] and the World Health Organiza-tion [11]. Classifications were based on age at diagnosis, duration of disease, BMI, blood pressure, fasting glucose, HbA1c and ketoacidosis. A classification of Type 1 diabetes was defined by the following criteria: presenta-tion of acute classical symptoms and requirement for insulin therapy to control hyperglycaemia. Young diabetic patients categorised as Type 2 when they had constellation of two or more of findings such as obesity, signs of insulin resistance (Acanthosis Nigricans or Polycystic ovary syndrome), strong family history of Type 2 diabetes, and documented hyperglycaemia long before presentation without insulin requirement. Patients that did not clearly exhibit the clinical features of either Type were excluded from the study.

A 5–10 ml morning blood was sampled for GADAs, ICA, C-peptide and for other examinations related to this study. Samples were centrifuged and the serum was separated, aliquoted and immediately stored at −20°C.

The serum glucose levels were determined using an Autoanalyser (Clinical System, Sweden). Hyperglycaemia was defined as the serum glucose level exceeding 126 mg/ dL. HbA1c was measured using a commercially available chromatographic-spectrophotometric ion exchange assay kit (Biosystems S.A, Spain).

Serum GADA levels were determined using a commer-cially available Enzyme linked immunosorbent assay kit (Medizym® anti-GAD; manufactured by Medipan GMBH, Dahlewitz/Berlin, Germany). Briefly, 25 µl of undiluted patient's sera were incubated with human recombinant GAD65 in microtiter plates at room temperature. After washing off unbound serum materials, bound antibodies were detected with GAD65-Biotin/Streptavidin peroxidise/ colori-metric substrate (TMB) complex. The optical density (OD) for each well was read at 450 nm. The GADA concentration in each sample was determined by comparison with a calibration curve plotted from levels of standard sera. Sera were considered to be positive for GADAs if antibody levels were ≥5.0 IU/ml. The inter- and intra-assay coefficients of variation were <5% and 4%, respectively. The sensitivity and specificity of the assay were 92.3% and 98.6%, respectively, regarding with newly onset T1D. All samples were tested in duplicate, including positive and negative control sera.

Serum ICA levels were determined using a commercially available Enzyme linked immunosorbent assay kit (Isletest®-ICA; manufactured by Biomerica, INC, California, USA). In short, 100 µl of diluted patient's sera were incubated with a purified mixture of pancreatic antigens that is immobilized onto microwells at room temperature. After washing off unbound serum materials, alkaline phosphatase labelled goat antibody, specific to human IgG, was added to the antigen-antibody complex. After another thorough wash-ing, a substrate (PNPP) was added and the colour generated was measured at 405 nm. Sera were considered to be positive for ICA if antibody levels were > cut-off value X (X=2.5×N; where N is the reading of the negative control data). The specificity of antigen coated Isletest microwell strips was established by Western blot analysis using confirmed positive samples for IgG to Islet Cell Antigens. All samples were tested in duplicate, including positive and negative control sera.

Serum C-peptide levels were determined using a commercially available Enzyme linked immunosorbent assay kit (IBL® C-peptide; manufactured by IBL, Hamburg, Germany) that is based on the principle of competitive binding. Briefly, 100 µl of diluted patient's sera were incubated with 50 µl monoclonal mouse anti C-peptide antibody and 100 µl biotinylated C-peptide in microtitre wells coated with anti-mouse antibodies at room temperature. After washing off unbound materials, horseradish peroxidise was added to the wells. In order to visualize the amount of bound enzyme, a colorimetric substrate (TMB) was added and the colour generated was measured at 450 nm. The C-peptide concentration in each sample was determined by comparison with a calibration curve plotted from levels of standard sera. Sera were considered to be abnormal for C-peptide, if the levels were <0.5 ng/ml. The minimum detection limit was 0.04 ng/ml with inter- and intra-assay coefficients of variation of <6% and <4%, respectively. All samples were tested in duplicate, including positive and negative control sera.

Statistical analysis

All analyses were performed with the statistical package SPSS 16.0 (SPSS Inc., Chicago, IL, USA). For bivariate analysis, the association between categorical variables was assessed using Chi-square test and continuous values were compared between or among groups using independent t test and one-way analysis of variance. Considering some skewness present in HbA1c distribution, robustness of t test was confirmed applying Mann–Whitney U test which produced similar results; so results of the t test are reported in this article. To model the possible predictors of type of diabetes and control for confounders, logistic regression analysis was used. Statistical significance level was set to be 0.05.

Results

Table 1 shows clinical and metabolic characteristics of subjects in the current study. One hundred and sixty three patients were enrolled; 100(61.3%) were women. This

Table 1 Clinical and metabolic characteristics of patients in the current study

[a] Pearson χ^2 test; [b] 2-tailed t test; [c] Fisher's Exact Test

*P <0.05; **P <0.01; ***P < 0.001

[d] Normal C-peptide levels: fasting

Variables	Type 1 D	Type 2 D
Total number n (%)	136 (83.4)	27 (16.6)
Female[a] n (%)	83 (61.0)	17 (63.0)
Family history of diabetes[b] n (%)	61(44.9)	12(44.4)
BMI[b] (mean ± SD) kg/m^2	19.17±2.43	27.57±1.26***
HbA_{1c}[b] (mean ± SD) (%)	9.14±1.36	7.86±0.62***
Normal C-peptide levels[c,d] n (%)	8 (5.9)	18(66.7)***

>0.5 ng/ml

Table 2 Prevalence of Anti-GAD65 and Anti-ICA in Type 1 and Type 2 diabetes

[a] Pearson χ2 test; [b] 2-tailed t test; [c] Fisher's Exact Test

*P <0.05; **P <0.01; ***P < 0.001

Antibody status	T1D (N=136) n (%)	T2D (N=27) n (%)
Both anti-GAD65 and Anti-ICA positive[a]	11(8.1)	0***
Anti-GAD65 positive only[a]	34(25.0)	4(14.8)
Anti-ICA positive only[a]	37(27.2)	1(3.7) ***
Antibody negative[a]	54(39.7)	22(81.5) ***
Any antibody positive[a]	82(60.3)	5(18.5) ***

population consisted of 27 (16.6%) patients with T2D and 136 (83.4%) patients with T1D. The sex distribution in the two groups was not different: 17 (63.0%) of patients with T2D and 83 (61.0%) with T1D were women. Compared to subjects with T2D, subjects with T1D were younger, had acute onset of disease and significantly lower BMI (P< 0.001). In addition, a significantly (P<0.001) higher proportion of patients with T2D (66.7%) had normal C-peptide levels compared to patients with T1D (5.9%). Patients with T1D had also significantly higher levels of mean HbA1c (9.14±1.36) compared to that observed in patients with T2D (7.86±0.3) (P<0.001).

Table 2 shows the prevalence of autoantibodies in Type 1 and 2 diabetic patients. Of the 163 patients, 49(30.1%) had positive GADAs levels (≥5.0 IU/ml): 45 (27.6%) with T1D and 4 (2.5%) with T2D. 49 of the 163 (30.1%) patients had positive ICA levels: 48 (29.5%) with T1D and only 1 (0.6%) with T2D. In the patients with T1D the frequencies of positive GADAs (33.1%) and ICA (35.3%) were not significantly different. However, the detection of only ICA and both GADAs and ICA was significantly more frequent among patients with T1D than in patients with T2D (P< 0.001). Eighty- two (60.3%) patients with T1D versus 5 (18.5%) patients with T2D had at least one autoantibody positive titre, and 11(8.1%) patients with T1D versus no patients with T2D tested positive for both GADAs and ICA. The detection of autoantibodies was not significantly influenced by gender in either class of diabetes.

Table 3 compares clinical and metabolic characteristics of antibody-positive and antibody-negative patients with T1D. HbA1c and BMI were not significantly different between patients with T1D that were autoantibody positive or autoantibody negative. There was, however, a significantly

higher proportion of patients with a family history of diabetes among those who were autoantibody positive

compared with those who were autoantibody negative (P<0.01).

Consistent with bivariate analysis results, logistic regres-sion results showed that abnormal C-peptide, higher HbA1c levels and also having any autoantibody positive titre could independently predict type of diabetes but the confidence interval for C-peptide was quite wide.

Discussion

The distinction between type 1 and 2 diabetes is very important but it is not always straightforward. The presence or absence of islet cell autoantibodies is one of the most direct ways to distinguish between type 1 and 2 diabetic patients. It is now believed that among the non-insulin requiring diabetic subjects at diagnosis, a significant minority are islet cell antibody-positive [9, 19]. These patients who clinically are difficult to distin-guish from type 2 diabetic subjects test positive for those markers that characterize patients with Type 1 diabetes and are defined as LADA [10]. Although T1D is considered predictable on the basis of immune markers, no single autoantibody is thought to be adequate to predict this disease. Recently, combinations of GADAs, ICA and IA-2A, have been reported to be useful screening tests for T1D [6, 7].

Earlier reports had found that islet cell autoantibodies to be rare among Asians, Malaysians, Arabic and African origins [12– 15, 20– 26] compared to Caucasians [8, 27]. In this study, we investigated the prevalence of GADAs and ICA in patients with recently diagnosed diabetes in northwest of

Table 3 Comparison of anti-body positive vs. antibody neg-ative patients with T1D and their clinical and metabolic characteristics

[a] Pearson χ^2 test; [b] 2-tailed t test; [c] Fisher's Exact Test

*P<0.05; **P<0.01; ***P<0.001

[d] Normal C-peptide levels: fasting >0.5 ng/ml

Variables		Antibody status	
		Any antibody positive	Any antibody negative
Total number n (%)		82(60.3)	54(39.7)
Female[a] n (%)		49(59.8)	34(63.0)
Positive family history of diabetes[a] n (%)		45(54.9)	16(29.6) **
BMI (mean ± SD)[b] kg/m^2		19.42±2.33	18.79±2.56
HbA1c[b] (mean ± SD) (%)		9.18±1.48	9.09±1.16
Normal c-peptide levels[c,d]	n(%)	3(3.7)	5(9.3)

Iran. The main finding from this study was a significantly higher prevalence of detecting any autoantibody (60.3%) among patients with T1D compared to the prevalence (18.5%) among patients with T2D. 11 (8.1%) patients with T1D and none patients with T2D had both GADAs and ICA. Our findings suggest that around 60% of patients with T1D have diabetes due to an autoimmune process. The prevalence rate of islet cell autoantibodies among Caucasians T1D patients (70–80%) [8, 27] is higher than Iranian diabetic patients. The prevalence rate of only GADAs and ICA in our T1D patients (25.0% and 27.2%, respectively) are markedly lower than those observed among Caucasians (70% and 80%, respectively) [27]. In summary, these results confirm the presence of GADAs and ICA in T1D patients in Iran, though roughly at a lower prevalence than that reported for Caucasian T1D patients, but very similar to other non-Caucasian ethnic populations. Such prevalence is of value in developing early intervention strategies, correct classification of diabetes and for public health purposes.

Present study showed that the prevalence of T2D in our study population is around 16.6%, which confirms the notion that the incidence of T2D is increasing in children and adolescents [28, 29]. We also detected

autoantibodies in 18.5% of T2D patients, a value that lies within the percentage range of 10–20% reported for the world population [9, 19]. It also supports the idea that the presence of autoantibodies could be a useful predictive marker for development of insulin dependency in T2D.

To our knowledge, this is the first study to evaluate combined GADAs and ICA measurements among Iranian patients with diabetes. As reported in other studies, we found that measuring more than one type of diabetes-related autoantibody increased the likelihood of detecting Type 1 diabetes autoimmunity in a population [6, 7].

Our findings suggest that around 60% of our patients with T1D have the autoimmune mediated form of the disease (Type 1a diabetes, Table 2). These results support the existence of unknown disease mechanisms that operate in the other 40% of our population with T1D. We may, however, speculate that future studies in Iran will likely to uncover novel autoantibodies against β-cell components, demonstrating that the pathogenesis of Type 1b diabetes is autoimmune mediated as well.

Significantly more patients with T1D that were autoan-tibody positive had a positive family history of diabetes compared with those who were autoantibody negative; this is in accordance with the well-known notion that autoim-mune diabetes has a genetic basis [30– 32] and more than 20 quantitative trait loci (QTL) for T1D have been characterized so far [33]. Therefore, future association studies in T1D patients in Iran could reveal the contribution of gene variants like PTPN22 and CTLA-4 in the pathogenesis of this autoimmune disease.

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Conflicting Interest None declared

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Echocardiographic evaluation of cardiac function in asymptomatic type 2 diabetes mellitus

P. K. Jabbar, P. H. Salim, T. M. Anoop, Anil P. John

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Abstract Diabetic patients are at increased risk of cardio-vascular disease. We do not have a definite data regarding the echocardiographic findings in asymptomatic type 2 diabetics in our population. The present study conducted an echocardiographic evaluation of asymptomatic type 2 diabetic patients of Kottayam district in central part of Kerala state. Hundred totally asymptomatic known type 2 diabetic patients were included in the study. Trans-thoracic echocardiography was performed in these patients and parameters were compared with age and sex matched controls. Diabetic patients were divided into three groups depending on the age, and duration of diabetes mellitus and each group was compared for E/A ratio(early diastolic flow velocity / late diastolic flow velocity), EDT(deceleration time), IVRT(Isovolumic relaxation time), EF(ejection frac-tion),LVmass(Left ventricular mass).The same parameters were also compared depending on the modes of therapy. Mean EDT (Early deceleration time), IVRT(Isovolumic relaxation time) , LV Mass (Left ventricular mass) ,IV septal thickness and left atrial diameter were significantly increased in the diabetic as compared with the control group. The mean E/A ratio was significantly lower in diabetic than control. There was no significant difference in ejection fraction ,LVIDD (left ventricular internal diastolic diameter), LVIDS(left ventricular internal systolic diameter) between diabetics and controls. There was a progressive increase in EDT and IVRT as the age and duration of diabetes increased. No significant differ-ence was noted with mode of control of diabetes. Left ventricular diastolic dysfunction is much more common than previously reported in subjects with well controlled asymptomatic type 2 diabetes and clinically undetectable heart disease.

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Keywords Diabetes mellitus • Echocardiography • Diastolic dysfunction

Introduction

Diabetes is one of the biggest and most difficult challenges facing in the twenty first century worldwide. Diabetes has two to four fold increased risk of cardiovascular disease and 75% of the deaths in diabetic patients may be attributed to Coronary Artery Disease (CAD) [1, 2]. In general, CAD in diabetic patients is detected in an advanced stage, whereas the disease in its premature, asymptomatic stages remains unfortunately undetected [3]. In view of these worrisome figures it

is imperative for us to shift our focus from treatment to early detection, prediction and prevention of CVD (Cardio Vascular Disease). We do not have a definite data regarding the echocardiography findings in asymp-tomatic type 2 diabetics in our population of central Kerala. .Hence this study was planned to do echocardio-

graphic evaluation of asymptomatic type 2 diabetic patients and to compare the data with age and sex matched controls.

Materials and methods

One hundred totally asymptomatic known type 2 diabetic patients were included in the study. Patients with overt cardiac disease, liver, pulmonary, renal and neurological diseases were excluded. Also other diseases which can affect the heart like hypertension or myocardial, valvular heart disease were excluded from the study. Echocardio-graphic indices were obtained according to the recommen-dations of the American Society of Echocardiography. Trans thoracic echocardiography was performed in these patients and compared with age and sex matched controls. LV(Left Ventricle), LA(Left atrial) dimensions, E/A ratio, EDT, IVRT and LV mass were measured and compared with controls. Diabetic patients were divided into three groups depending on the duration of diabetes ie.—less than 5 year, 5 to 10 year and more than 10 year and each group was compared for E/A ratio, EDT, IVRT, EF,LVMASS. Patients were divided into three groups depending on age, less than 40 years, 40 to 50 years and more than 50 years and above parameters were compared. The same parameters were also compared depending on the modes of therapy in study patients who were controlled by drugs or insulin.

Statistical analysis

A commercially available statistical programme (SPSS 10.1 and 11.1) was used. All of the results which were measured using echocardiography were expressed as the mean±SD.

The data were tabulated and categorical variables were compared by chi-square test. A probability value of less than 0.05 was considered significant.

Results

Total 100 asymptomatic type2 diabetics were enrolled in the present study. One hundred age and sex matched controls were also selected. Out of 100 patients 56(56%) were male and 44(44%) were females. Table 1 shows the clinical characteristics of the study population. There was no difference in the mean age between males and females (50.84 vz 51.3).Family history of diabetes was not significant between males and females (57.1% vz 47.4%). Family history of coronary artery disease was not different between sexes(19.6% vz 13.65%). 75% of the patients were controlled on oral hypoglycemic agents, 24% on insulin and 1% by diet control. In the study group significantly more males were controlled on oral hypoglycemic drugs (83.93% vz 63.64%).whereas ,control by insulin was significantly more in females (34.1% vz 16.2) (Table 1).

The whole group was divided into three depending on the duration less than 5 years,5 to 10 years and more than 10 years.47% were of less than 5 year duration.27% were of between 5 and 10 year duration. Twenty-six percent of the patients were more than 10 year duration of diabetes. There were no significant sex differences in each group (Table 1).

Echocardiographic data

Echocardiographic features in the study and control groups are given in the Table 2. LA size was significantly higher in diabetics than control (3.17 vz 2.89, P=.0001).Similarly mean aortic size was also significantly more in diabetics

Table 1 Clinical characteristics of study population	Parameter	Males	Females	Total	Z value	P value

	Age, Mean	51.38	50.3	50.84		
	Family H/O DM *	32	21	53	0.96	NS
	Family H/O CAD*	11	6	17	0.79	NS
	Control of diabetes					
	By diet		1	1		
	Oral hypoglycemic drugs	47	28	75	2.09	0.04
	Insulin	9	15	24	−2.09	0.04
	Duration of diabetes					
*Family H/O DM family history	<5 years	28	19	47	0.68	NS
of diabetes mellitus, *Family	5–10 Years	14	13	27	−0.51	NS
H/O CAD family history of	>10 years	14	12	26	−0.26	NS
coronary artery disease						

Table 2 Echocardiographic features in the study and control groups

Parameter	Patients		Controls		Z value	P value
	Mean	SD	Mean	SD		
LA	3.17	0.29	2.89	0.28	6.96	0.00
AORTA	2.52	0.28	2.29	0.25	6.14	0.00
LVIDD	4.39	0.43	4.33	0.36	1.07	NS
LVIDS	2.79	0.39	2.81	0.38	0.36	NS
EF	73.17	6.43	72.47	6.95	0.741	NS
IVSD	0.93	0.15	0.84	0.15	4.25	0.00
LVPWD	0.77	0.18	0.70	0.14	3.08	0.00
Mitral E	0.75	0.17	0.85	0.15	−4.42	0.00
Mitral A	0.72	0.21	0.54	0.14	7.15	0.00
EDT	245.55	49.51	174.5	29.5	12.36	0.00
IVRT	110.22	22.17	84.84	16.63	9.1	0.00
LV Mass	124.06	31.81	108.18	27.26	3.83	0.00

LA left atrium, LVIDD left ventricular internal diastolic diameter, LVIDS left ventricular internal systolic diameter, EF ejection fraction, IVSD interventricular septal diameter, LVPWD left ventricular posterior wall diameter, LV Mass left ventricular mass, E early diastolic flow velocity, A late diastolic flow velocity, EDT early deceleration time

than control (2.52 vz 2.29, P=.0001). There were no significant difference in mean LVIDD (4.39 vz 4.33, P = NS), mean LVIDS (2.79 vz 2.81, P = NS) and ejection fraction 73.17 vz 72.47, P = NS) between diabetics and controls. The mean IVSD(.93 vz .84, P=.0001) and mean LV posterior wall diameter (0.77 vz 0.70, P=.0001) was significantly higher in diabetics than controls .Regarding mitral inflow E/A ratio was significantly lower in diabetics than control (1.1 vz 1.38 p=0.0001).

The mean EDT (245.55 vz 174.5, p=0.0001), mean IVRT (110.22 vz 84.84, P=0.0001) and mean LV Mass (124.06 vz 108.18, P=0.0001) were significantly higher in diabetics than control.

Mean LV mass was significantly higher in males than females both in diabetic patients (133.28 vz 112.21, P=0.001) and controls (124.42 vz 95.04, p=0.0001).

Echocardiographic parameters and duration of diabetes (Table 3)

Patients were divided into three groups depending on the duration of diabetes ie, less than 5 year, 5 to 10 year and more than 10 year. The parameters like E/A ratio, EDT, IVRT, LV Mass and ejection fraction were compared between the groups.

There was significant difference in mean EDT between less than 5 year and more than 10 years (230.38 vz 267.85, P= .001). There was a progressive increase in EDT as the duration of diabetes increased. Regarding mean IVRT there was significant difference between less than 5 year and 5 to 10 year duration of diabetes (104.49 vz 117.56, P=001).There was no significant difference in E/A ratio between the groups. LV mass and ejection fraction had no significant differ-ence between the groups.

Echocardiographic parameters and age (Table 3)

Patients were divided into three groups depending on age, less than 40 years, 40 to 50 years and more than 50 years. The parameters like EDT, IVRT, and LV MASS, E/A ratio and ejection fraction were compared between the groups. There was no significant difference in E/A ratio between the groups even though non-significant trend was seen between age less than 40 and more than 50.There was a significant increase in EDT with age less than 40 and more than 50 years (239.56 vz 269.53, P=.001).There was significant increase in IVRT between less than 40 year and more than 50 year (102.31 vz 111.51, P=001).No significant difference was noted in LV Mass between the groups. Ejection fraction also had no significant change between the groups.

Echocardiographic parameters and modes of control of diabetes (Table 3)

The parameters like EDT, IVRT, and LV Mass, E /A ratio and ejection fraction were compared in patients who were controlled by drugs or insulin. There was no significant difference in these parameters on the mode of control of diabetes.

Discussion

Diabetic patients are susceptible to heart failure and have a higher prevalence of coronary heart disease, hypertension, and cardiomyopathy [1, 3]. Epidemiological data indicate a greater risk of cardiovascular morbidity and mortality, in diabetic subjects as compared with those without diabetes [4]. The present study provides an integrated approach to

Table 3

Echocardiographic features in relation to age, duration of diabetes, and modes of control

Echocardiographic parameters	Age					
	40–49 years (A)		50–59 years(B)		>60 years (C)	
	Mean	SD	Mean	SD	Mean	SD
E/A	1.15	0.31	1.15	0.36	0.83	0.2
EDT	239.56	55.07	241.29	44.28	269.53	41.57
IVRT	102.31	23.30	111.51	21.28	111.63	19.57
LV Mass	124.17	29.18	125.66	29.98	126.94	44.88
EF	74.49	5.86	72.67	6.07	71.54	6.80
	Duration of DM					
	<5 Years (A)		5–10 years (B)		>10 years(C)	
E/A	1.16	0.36	1.14	0.33	2.97	0.32
EDT	230.38	44.30	250.88	49.85	267.85	49.72
IVRT	104.49	20.78	117.56	23.14	113.42	21.67
LV Mass	113.19	33.11	110.85	22.81	121.30	33.41
EF	74.01	6.10	73.21	6.39	71.59	6.97
	Modes of control					
E/A	OHA		Insulin			
EDT	1.12	0.36	1.04	0.33	Z=0.93	p = ns
IVRT	248.27	44.19	235.82	65.66	Z=1.03	p = ns
LV Mass	111.19	21.67	108.86	22.60	Z=0.43	p = ns
EF	124.61	30.79	122.23	36.28	Z=0.30	p = ns
	72.80	6.45	73.95	6.28	Z=−0.74	p = ns

the assessment of diabetic cardiac function using non-invasive echocardiography evaluation. In this study total of 100 Type 2 asymptomatic diabetic patients were evaluated by echocardiography. There were 56 males and 44 females. The mean age was around 51 in both sexes. Family history of diabetes was present in 53% and family history of CAD in17%. Diabetes is the only condition that causes women to have heart disease rates similar to those of men. The reason for this effect is uncertain. It is unlikely that it reflects differential loss of men with coronary heart disease through excess mortality. The increased heart disease risk of diabetic women could be mediated in part by the loss of women's usually favorable lipoprotein profile in the presence of diabetes.75% of the patient were controlled by drugs and 24% by insulin in this study group. In the study group insulin use was significantly more in females and OHA use more in males.

In this study mean left atrial (3.17 vz 2.89, p=.0001), aortic size (2.52 vz 2.29, p=.0001), mean IVSD (.93 vz .84, p=.0001) and mean LV posterior wall thickness (.77 vz .7, p= .0001) were significantly higher in diabetics than control. There was no significant difference in LVIDD, LVIDS between diabetics and control. The IV septal thickness and left atrial diameter were increased in the diabetics as compared with the control group ($p<0.01$, $p<0.01$ respectively) in the study. The mean E/A ratio (1.1 vz 1.38, p=.0001) was significantly lower in diabetics than control. Mean EDT (245.55 vz 174.5, p=.0001), mean IVRT

(110.22 vz 84.34, p=.0001) were significantly higher in diabetics than control. In this study there is prolonged EDT, IVRT and reduced E/A ratio indicating abnormal-ities of diastolic function. It indicates that abnormalities of diastolic function occur earlier in totally asymptomatic patients. Thus the impairment of the LV diastolic function was observed in patients free of diabetic complications, hypertension and symptomatic coronary artery disease. This study led to the conclusion that LV diastolic dysfunction could be much more common than previously reported in this population. Numerous studies have shown that impairment of the LV diastolic function may be detected in diabetic population by using several non-invasive studies methods.

In the large majority of studies, abnormalities of LV diastolic function have been demonstrated in diabetic patients with intact systolic function. This has been illustrated by the study of Raev et al which showed a high prevalence of diastolic dysfunction with preserved systolic function in asymptomatic, young, diabetic patients [5].

In the study of Paillole et al 16 type1 diabetics free of microangiopathy, hypertension or coronary artery disease and with diabetes duration of at least 10 years, were compared to 16 healthy control subjects [6]. A significant reduction in mitral E wave, E/A ratio and an increase of isovolumic relaxation time was observed in the diabetic group. Early diagnosis of left ventricular diastolic impair-

ment has been demonstrated to have important therapeutic implications. Several invasive or non-invasive methods to investigate diastolic properties of the left ventricle have been described; a large number of studies compared different parameters of diastolic function in order to find the most accurate: this is of particular prognostic relevance since diastolic dysfunction may remain asymptomatic for a long period before resulting in overt heart failure.

In this study mean LV mass (124.06 vz 108.18, p=.0001) significantly higher in diabetics than control. There was significant gender difference in mean LV mass both in diabetics (133.28 vz 112.21, p=.0001) and controls (124.42 vz 95.04, p=.0001). The LV mass index was increased in the diabetics as compared with the control group ($p<0.02$) in the study. LV mass and relative wall thicknesses were higher in diabetic than nondiabetic subjects independent of covariates. Insulin resistance positively but weakly related to LV mass and relative wall thickness. Thus type 2 diabetes was associated with higher LV mass, more concentric LV geometry, and lower myocardial function, independent of age, sex, body size, and arterial BP. In the Framingham cohort, diabetes was associated with higher LV mass in women but not men [1]. Improved glycemic control induces regression of left ventricular mass in patients with diabetes mellitus. Improved glycemic control in patients with 1 diabetes mellitus is associated with regression of septal thickness and left ventricular mass without significant effect on systolic or diastolic function.

Parameters on duration of diabetes

In this study,there was significant difference in mean EDT between less than 5 years and more than 10 years (230.38 vz 267.85, P=.001). There was a progressive increase in EDT as the duration of diabetes increases. Regarding mean IVRT , there was significant difference between less than 5 year and 5 to 10 year duration of diabetes (104.49 vz 117.56, P=001). Evidence of an alteration in LV diastolic function at an early stage of diabetes without correlation with specific complication is suggested in a few studies. Attali et al observed LV diastolic dysfunction in asympto-matic type1 and type2 patients (n=49) compared with controls. All patients were free of cardiovascular diseases and diabetes mellitus for less than 5 years [7]. Di Bonito et al observed diastolic dysfunction in 16 normotensive type 2 diabetic patients, free of

microvascular complications with a disease duration of less than 4 years and even less than 1 year [8].

Parameters on modes of control

The parameters like EDT, IVRT, and LV Mass, E /A ratio and ejection fraction were compared in patients who were

controlled by drugs or insulin. There was no significant difference in these parameters on the mode of control of diabetes in this study.

In a study by Grandi AM et al, on effect of glycemic control on left ventricular diastolic function in diabetes mellitus [9]. A close relation was found between glycemic control and LV diastolic function, which improves when glycemic control improves. Therefore, diastolic dysfunction can be prevented or reversed, at least partly, by tight glycemic control. Several studies have suggested that hyperglycemia alters the metabolism of cardiac myocytes and could be the primary insult in the pathogenesis of diabetic cardiomyopathy [10]. Even in type 2 diabetic patients without cardiac involvement, uncontrolled hyper-glycemia is known to provoke diastolic LV dysfunction [9, 11]. Nichols et al demonstrated that a reduction in HbA1C coupled with a lower baseline HbA1C was predictive of a decreased incidence of heart failure in a multi-variate model, emphasizing the importance of glycemic control for prevention of heart failure [12].

Parameters and age

In this study, there was no significant difference in LV mass, Ejection fraction and E/A ratio between the groups even though non-significant trend was seen between age less than 40 and more than 50.A significant increase in EDT, IVRT with increasing age less than 40 and more than 50 years was noted. Masugata H et al demonstrated that cardiac diastolic dysfunction without LV systolic dysfunc-tion in patients with well-controlled type 2 diabetes is related neither to hypertension nor LV hypertrophy, but rather to aging and the duration of type 2 diabetes [13]. Diabetes mellitus is the strongest independent correlate of left ventricular diastolic dysfunction. Mishra TK et al also showed that asymptomatic diabetic patients have reduced left ventricular systolic and diastolic function as compared with healthy subjects [14]. Left ventricular systolic and diastolic abnormalities are correlated with the duration of diabetes and with diabetic microangiopathies, like retinop-athy and neuropathy.

Myocardial involvement in diabetics may occur rela-tively early in the course of disease, initially impairing early diastolic relaxation and when more extensive, it causes decreased myocardial contraction [15]. Early subclinical diastolic dysfunction previously been regarded as benign has only recently been recognised as it could lead to diastolic heart failure and diabetic cardiomyopathy.

Factors involved in the development of diabetic myo-cardial dysfunction are altered insulin signalling, glyco- and lipotoxicity, increased cytokine activity and intramyocyte and/or interstitial deposition of triacylglycerol and endo-thelial dysfunction may all affect myocardial function

directly or indirectly. Three patterns of abnormal diastolic function have been recognised, i.e.,impaired relaxation, pseu-donormalisation, and restrictive filling [16]. Diastolic dysfunc-tion is seen even when the DM is present at younger age, is of shorter duration, and has no effects on other body systems and it is suggestive of pre-clinical diabetic cardiomyopathy.

In conclusion, diastolic dysfunction is much more common than previously reported in subjects with well controlled asymptomatic type 2 diabetes.

Diastolic dysfunction can be used as an early indicator, as it is a precursor to increased LV hypertrophy and clinical left ventricular dysfunction.

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Conflict of interest None declared

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Int J Diab Dev Ctries

LETTER TO EDITOR

Once-weekly exenatide in type 2 diabetes

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Sir,

Glucagon-like peptide-1 (GLP-1) analogues (incretin mimetics) represent new class of anti-diabetic agents for the treatment of type 2 diabetes. Exenatide, the first in this new class of drugs, has been approved by the Food and Drug Administration (FDA) in April 2005. A very large number of clinical studies have demonstrated the efficacy of incretin mimetic exenatide in terms of reduction in glycosylated haemoglobin (HbA_{1c}), fasting and postprandial glucose and reduction in body weight as well as its safety in terms of a low rate of hypoglycemic events [1]. Exenatide is rapidly absorbed reaching peak concentrations in approximately 2 h with a half-life of approximately 2 h after subcutaneous administration. Following subcutaneous injection of the maximally tolerated dose, significant elevation of exenatide in plasma may be observed for 5 to 6 h and exposure is negligible after 12 h post dose, explaining why twice-daily dosing is needed in order to obtain full effect on glycemic control [2]. GLP-1 receptor agonists with extended half-lives entailing fewer injections and presumably an improved throughout-the-day glycemic control are in clinical development [3]. Currently available formulation of exenatide requires once- or twice-daily injections; a once-weekly subcutaneous formulation of exenatide is under FDA review.

The efficacy and safety of exenatide administered once weekly have been investigated in the clinical trials known as Diabetes Therapy Utilization: Researching Changes in

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A_{1c}, Weight and Other Factors Through Intervention with Exenatide Once Weekly (DURATION). The DURATION-1 trial was designed as a two stage protocol. In the first phase, Drucker et al., [4] compared a long-acting release formulation of exenatide 2 mg administered once weekly to 10 μg exenatide administered twice a day, in 295 patients with type 2 diabetes (inadequately controlled on lifestyle intervention and/or monotherapy or double therapy with metformin, sulphonylurea or thiazolidinedione) over 30 weeks period in a randomized, non-inferiority study. At 30 weeks, the patients given exenatide once a week had significantly greater changes in HbA_{1c} than those given exenatide twice a day (−1.9% versus −1.5%, $p=0.0023$). The HbA_{1c} was

reduced to 7% or less in 77% patients on exenatide once weekly and 61% on exenatide twice a day ($p=0.0039$). Overall, exenatide once weekly had shown significantly greater improvements in glycaemic control than exenatide given twice a day, with no increased risk of hypoglycaemia and similar reductions in bodyweight during this study.

Buse et al., [5] conducted a randomized, multicenter, comparator-controlled, open label trial (second phase of DURATION-1) enrolling 258 patients and reported the safety and efficacy of exenatide once weekly in patients who continued treatment for an additional 22 weeks (to complete 52 weeks) and who switched from exenatide twice daily to exenatide once weekly after 30 weeks. Patients continuing exenatide once weekly maintained HbA_{1c} improve-ments through 52 weeks (baseline HbA_{1c}: −2.1% at week 30 and −2.0% at week 52), patients switching from exenatide twice daily to exenatide once weekly achieved further HbA_{1c} improvement (HbA_{1c} reduction: −1.8% at week 30 and −2.0% at week 52) while both groups achieved a mean HbA_{1c} of 6.6% at 52 weeks of treatment. Body weight was reduced by more than 4 kg after 52 weeks in both treatment groups. Side effects like mild nausea occurred

less frequently while no major hypoglycemia was ob-served in this assessment period of 22 weeks.

The DURATION-2 was a 26-week randomized, multi-centre, double-blind, double-dummy, superiority trial conducted by Bergenstal et al., [6] to compare once-weekly exenatide with sitagliptin or pioglitazone as an adjunct to metformin in 491 patients with type 2 diabetes. Patients received 2 mg injected exenatide once weekly plus oral placebo once daily; 100 mg oral sitagliptin once daily plus injected placebo once weekly; or 45 mg oral pioglitazone once daily plus injected placebo once weekly. After 26 weeks, exenatide lowered the mean HbA_{1c} level significantly more than sitagliptin (−1.5% versus −0.9%, $p < 0.0001$) or pioglitazone (−1.5% versus −1.2%, $p=0.0165$). Weight loss with exenatide was significantly

greater than	with sitagliptin (−2.3	kg versus −0.8 kg,
$p=0.0002$)	or pioglitazone (−2.3	kg versus +2.8 kg,

$p<0.0001$). No major hypoglycemic episodes were reported. Nausea and diarrhea were the most common adverse events associated with exenatide and sitagliptin; upper respiratory infection and peripheral edema were the most common adverse events with pioglitazone.

The DURATION-3 was a 26-week open-label, randomized, parallel study, conducted by Diamant et al., [7] for comparison of once-weekly exenatide with once-daily insulin glargine in 456 patients with type 2 diabetes who had less-than-ideal glycemic control despite maximum tolerated doses of blood-glucose-lowering drugs for at least 3 months. Patients received exenatide (2 mg, once-a-week injection) or insulin glargine (once-daily injection, starting dose 10 IU, target glucose range 4.0-5.5 mmol/L) to their blood-glucose-lowering regimens. Patients receiving exenatide had a −1.5% reduction in HbA_{1c} compared with −1.3% for patients receiving once-daily insulin glargine. During the study, weight loss in patients receiving exenatide was significantly more than those receiving insulin glargine (−2.6 kg versus +1.4 kg, $p<0.001$). Significantly more patients had discontinued exenatide than insulin glargine treatment by the end of this study period, at 5% and 1%, respectively. Although, Misra et al., [8] agreed with the conclusion of DURATION-3 trial, they also noted that the some important ‘unknowns’ remain about treatment with long-acting exenatide

including that the long-term effects of sustained use of a GLP-1 receptor agonist on pancreatic beta cells in human beings are not known as well as the need of continuing pharmacovigilance on recently observed renal dysfunction during exenatide therapy.

The DURATION-4, [9] a 26-week, double-blind, ran-domized, four-arm parallel study compared exenatide once weekly monotherapy to sitagliptin, pioglitazone or metformin in 820 patients with type 2 diabetes who were not achieving adequate HbA_{1c} control on diet and exercise and were not on any diabetes therapy when they entered the study. After 26 weeks of treatment, patients randomized to exenatide

once weekly had a −1.5% reduction in HbA_{1c} from baseline, which was significantly greater than the reduction of −1.2% for sitagliptin. Patients randomized to metformin and pioglitazone had a reduction in HbA_{1c} of −1.5% and −1.6%, respectively. An average reduction in HbA_{1c} of less than 7% was achieved in patients receiving exenatide once weekly, pioglitazone and metformin treatment by study end. Treatment with exenatide once weekly produced statistically significant greater weight loss than patients with sitagliptin and pioglitazone (−2.0 kg versus −0.8 kg with sitagliptin and +1.5 kg with pioglitazone) Patients with metformin treatment experienced weight loss of −2.0 kg. There were no major hypoglycemic events in any treatment group. Nausea and diarrhea with exenatide; diarrhea and headache with metfor-min; upper respiratory tract infection, headache, hypertension and peripheral edema with pioglitazone and upper respiratory tract infection and headache with sitagliptin were the most frequently reported adverse events during the study.

To sum up, currently available clinical data suggests that long-acting exenatide likely will become an important new option for managing patients with type 2 diabetes.

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LETTER TO EDITOR

Comparison of Framingham Risk Score and Indian diabetes risk score by obesity status and lipids abnormality in women of Asian Indian origin: Santiniketan women study

Minakshi Bhagat, Arnab Ghosh

Int J Diab Dev Ctries. 2011 ; 31 : 123-124

Dear Sir,

Type 2 diabetes mellitus (T2DM) is a significant risk factor for coronary heart disease (CHD) and stroke [1]. T2DM removes the normal sex related differences in the preva-lence of CHD. Asian Indian women are comparatively worse off than men with regard to many of the risk factors for CHD [2]. Hence the present cross-sectional study was aimed to compare Indian Diabetes Risk Score (IDRS) and Framingham Risk Score (FRS) by obesity and lipid abnormality status in women of Asian Indian origin. The study was conducted from April 2008 to May 2009 in the Bolpur-Santiniketan area, West Bengal, India on 214 healthy women aged 25 to 65 years. The study was approved by the institutional ethics committee of the 'Human Genetic Engineering Research Center' (HGERC), Calcutta, India. Anthropometric and body composition measures, blood pressure measures, metabolic profiles were all collected using standard techniques [2, 3]. Participants were considered as underweight when they had body mass index (BMI) <18.5 kg/m^2, normal with BMI≥18.5 to <23.0 kg/m^2 and overweight when they had BMI≥23 kg/m^2. Cut off values of central obesity measure and IDRS are given elsewhere [4]. Similarly, FRS of <9 and ≥9 was also used to dichotomize the study population. Atherogenic index (AI) was calculated using the following equation: (Total Cholesterol-High density lipoprotein cholesterol)/ High density lipoprotein cholesterol. The atherogenic index of <3.42 and ≥3.42 (3.42 was equivalent

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to 85th percentile of AI in the study participants) was used to identify the subjects who were at risk of lipids abnormality.

The mean and standard deviation (SD) of age, BMI, waist circumference (WC), total cholesterol (TC), triglyc-eride (TG) and systolic blood pressure (SBP) was 38.52 (10.33), 22.63 (4.34), 76.33 (11.01), 184.95 (25.03), 137.69 (32.19) and 115.68 (19.11) respectively. Comparison of IDRS and FRS by obesity (both generalized and central obesity) status revealed that there was significant difference ½#^{2} ð2Þ ¼ 23:61& between medium and high risk of IDRS for both generalized (as measured by BMI categories) and central obesity (as measured by WC categories) status. No individual was eventually found in the low IDRS category. Moreover, interestingly, no significant group difference was observed for FRS by obesity (both generalized and central obesity) status. Unlike FRS, a significant group difference ½#^{2} ð1Þ ¼ 4:37& for IDRS was also evident by atherogenic index.

Unlike FRS, significant group differences (medium vs. high risk category) for IDRS by obesity status and atherogenic index hinted that IDRS can predict cardiovas-cular and diabetic risk more effectively than FRS in the people of Asian Indian origin. It is noteworthy to mention that in a case-control retrospective study [5], it was also argued that in the Indian population, the Framingham risk prediction protocol fails to identify a large proportion of high risk non-diabetic patients. The simple and cost effective IDRS could thus serve as a tool for a primary care physician or a health worker to identify at risk individuals for diabetes and cardiovascular diseases. How-ever, a more comprehensive risk prediction protocol for Indian population is urgently required to identify at risk individuals in the coming years.

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Early remission of type 2 diabetes mellitus by laparoscopic ileal transposition with sleeve gastrectomy surgery in 23–35 BMI patients

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Abstract To assess the efficacy of ileal transposition with sleeve gastrectomy (SGIT) surgery in remission of Type 2 Diabetes Mellitus (T2DM) in patients with 23–35 BMI. Diabetes is considered a life style disease. Despite medications and lifestyle changes, (HbA1c) - remains > 7 in 56 % of diabetics, predisposing them to high risk of diabetes related complications. Bariatric surgery results in remission of diabetes in over 84% patients with BMI>35 m2s/m^2. Based on hindgut hypothesis suggesting role of incretins like GLP-1, early trials of ileal interposition surgery have displayed consistent HbA1c levels below 7 in over 80% patients with BMI>30 m2/m^2. In developing countries majority of T2DM patients are not morbidly obese and surgical procedures are to be evaluated for their efficacy in this group. In this study we have assessed the efficacy of ileal transposition with sleeve gastrectomy (SGIT) in 23–35 BMI T2DM patients. Selected T2DM patients [HbA1c>7, C Peptide>-1 ng/ml] underwent Lap SGIT by a single surgeon. Data of first five patients with minimum 6 months follow up was analyzed for glycemic control and altered need for medications. Data were analyzed using SPSS (SPSS release 16; SPSS Inc. Chicago). The study target (HbA1c<7) was achieved in 60% of patients within 1 month and in 100% of patients within 6 months. Requirement of medications reduced significantly within 6 months and their HbA1c levels reduced from 9.65% to 6.22%. Laparoscopic SGIT may represent a new paradigm for the treatment of T2DM even in non morbidly obese patients.

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Keywords SGIT • T2DM • HbA1c • Incretin

Introduction

Diabetes mellitus is a group of metabolic diseases character-ized by hyperglycemia resulting from defects in either insulin secretion, insulin action, both or factors not yet well understood. Chronic hyperglycemia of diabetes is associated with dysfunction & failure of various organs.

Several pathogenic processes are involved in the devel-opment of diabetes ranging from, autoimmune destruction of beta cells, secretion of excess insulin to compensate for insulin resistance in peripheral tissues, and increased endogenous glucose production, [1– 3]. Abnormalities of carbohydrate, fat, and protein metabolism are due to deficient action of insulin on target tissues. Insulin resistance, or hyperinsulinemia, is key in the pathogenesis of metabolic syndrome, with the term “insulin resistance syndrome” also commonly used. The deterioration of secretion and insulin action is present for many years before the diagnosis of diabetes [4]. However, some patients with T2DM are not obese [5], and for some non obese T2DM individuals, insulin resistance is not an etiology essential [6]. Moreover, diabetes still can persist even when complete restoration of normal insulin sensitivity after weight reduction has been achieved, implying an essential role for impaired insulin secretion [7].

Role of incretins

Incretins role in glucose homeostasis is being increas-ingly investigated. In patients with T2DM, the incretin effect is either greatly impaired or absent, which significantly reduces the patient's ability to adjust their insulin secretion to their needs. It is recognized that early-phase plasma insulin response to glucose (0– 20 min) is impaired but significantly enhanced by both glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), as compared with glucose alone. A defective amplification of the so called late-phase plasma insulin response to glucose (20–120 min) by GIP also is observed, whereas GLP-1 enhances the late-phase plasma insulin response markedly [8]. Loss of early-phase insulin secretion has severe consequences for glucose homeostasis and includes inability to suppress glucagon secretion, free fatty acid secretion, and hepatic glucose output adequately, determining a continued delivery of glucose in the circulation [9].

It has been observed that in diabetics, secretion of GIP generally is normal, whereas the secretion of GLP-1 is reduced. It probably is an even more important observation that the effect of GLP-1 is preserved and that the effect of GIP is severely impaired [10]. After continuous infusion of GLP-1, findings demonstrated a lowered effect on fasting and average plasma glucose concentrations, a decrease in glycosylated hemoglobin (HbA1c) levels, and an improve-ment in insulin sensitivity [11].

Role of ileal transposition in remission of T2DM

Bariatric surgery, especially gastric bypass [12] and malab-sorptive surgeries [13], are effective in achieving long-term control of obesity and in controlling T2DM. Various hypotheses to explain glycemic control in 84%–97% diabetic patients undergoing these surgeries include exclusion of duodenum (foregut hypothesis) or early delivery of partially digested chyle to distal ileum stimulating release of GLP-1 (hindgut hypothesis) [14]. Animal studies were performed to assess impact of duodeno-jejunal bypass based on foregut theory & ileal interposition surgery based on hindgut hypothesis.

Subsequently, human trials have started on these novel gastro-intestinal surgical procedures. Auero de Paula et al. [15] performed a technique termed "neuroendocrine break" characterized by transposition of an ileal segment in proximal jejunum. The mechanism involves providing early exposure of ingested nutrients to the interposed ileum aimed at determining an early rise in glucagon like peptide 1 (GLP-1) and consequently influencing the defective early-phase insulin secretion. Subsequently, he added sleeve gastrectomy to improve results by restricting calorie intake & ghrelin effect. They reported complete remission of T2DM for morbidly obese patients [16].

Since most of the studies have shown efficacy of this surgery in morbidly obese, a large segment of T2DM patients with BMI<35 are not benefited by this surgery. This study aimed to evaluate the results of laparoscopic transposition of an ileum segment to the proximal jejunum along-with sleeve gastrectomy on glycemic control in T2DM patients with a body mass index (BMI) lower than 35.

Materials & methods After due approval by the ethical committee & registration with Clinical Trial Registery of India: CTRI/2008/091/00206), patients were selected for SGIT.

The inclusion criterions are as follows Inclusion criteria

1. Diagnosed Type 2 Diabetes over 1 year
2. Inadequately controlled blood glucose (HbA1c>7)
3. BMI between 23.0 and 35.0 kg/m^2
4. Stable weight as determined by no more than a 3% change in body weight in the last 3 months
5. Age between 25 and 65 (both men and women to be included)
6. Able to provide Informed Consent
7. Able to comply with follow-up procedures

The patients were investigated to assess their suitability for surgery. These specific investigations included

- ➢ Fasting Insulin
- ➢ Fasting C peptide
- ➢ Anti islet anti bodies

The patient comprised 2 male and 3 female with a mean age of 47.33 (44–53) years for female patients and 47.5 (43–52) years for male patients. The mean age of all the patients is 47.4 (43–53) years. Mean

preoperative BMI was 29.4 (25–35) m2/m^2 and mean preoperative HbA1c was 9%. All five patients were taking diabetic medication as prescribed by their endocrinologist/physician. 2 patients were only on oral hypoglycemic agents (OHA) and 3 patients were on both insulin as well as oral agents. The mean amount of insulin used was 91 (60–138) units/day. The preoperative demographic data of each patient as well as their mean values is summarized in Table 1.

Technique/procedure of surgery The patient was placed on the OR table in split leg position. A pneumatic compression stocking was placed, and the patient received antibiotic prophylaxis. Pneumoperitoneum was established in stan-dard fashion using closed approach (Verres) and optical trocar (Excel). The pneumoperitoneum pressure did not exceed 14 mm Hg. Four other trocars were placed under direct vision, two 5 mm and two 12 mm all in the upper abdomen. The reduction of the gastric volume (sleeve gastrectomy) was made by excising the greater curvature of the stomach. This was done by devascularizing the greater curvature using ultrasonic scalpel. A 36 French bougie was introduced by the anesthesiologist and positioned next to

Table 1 Preoperative demographic data: Individual as well as mean profile of the patients

Pat. ID No.	IT1	IT2	IT3	IT4	IT5	Mean/ Ratio
Age (Years)	52	43	44	45	53	47.33
BMI (kg/m^2)	25	32	26	35	29	29.4
Sex	M	M	F	F	F	2 : 3
Duration of Diabetes (yrs)	2	16	11	4	9	8.4
OHA/Insulin	OHA	INSULIN (91U)	INSULIN (75U)	OHA	INSULIN (60U)	2 : 3 (138U)
Co-morbidities	Impaired renal function (IRF)	Retinopathy (Blind), CAD, IRF	NIL	NIL	NIL	_
Pre op HbA1c	8.5	9.0	11.7	7.6	8.1	9.0

the lesser curvature toward the pylorus. The stomach was transected 5 cm from the pylorus to the angle of His by using a linear stapling device. The resected portion of the stomach was removed at the end of the procedure. The iso-peristaltic ileal loop transposition was made with the patient in mild Trendelenburg position. The jejunum was divided 20 cms from the ligament of Trietz, by linear stapler. Point A (proximal) and point B (distal) on the jejunum were identified and marked. Next, 20 cms of distal ileum was measured from I-C junction. The ileum was divided at this point with a linear stapler. Two points, C and D, proximal and distal respectively on the cut ileum were marked. Division of a new ileal point 170 cm proximal to C with the linear cutter was performed. Determination of the two points on the ileum E and F, proximal and distal respectively was made and marked. An intracorporeal anastomosis between the points of the ileum identified as E and D was performed with re-establishment of the continuity of the ileum. The anastomosis was constructed with a GIA stapling device. The common opening at the end of the stapling procedure was hand sewn/stapled intracorporeally. The mesentery was re-approximated with sutures. The 170 cm isoperistaltic segment ileal segment F and C was anastomosed—jejunal-ileal (A to F) and ileo-jejunal (C to B). Each mesenteric segment was closed to prevent herniation.

All patients were managed in intensive care for atleast one night with two hourly blood glucose and other parameter monitoring and management. Oral intake was started 48–72 h after surgery. Early mobilization was encouraged and LMWH was continued for the hospital-ization period. The patients were evaluated at regular

intervals for the following parameters: Fasting and post prandial blood glucose, fasting insulin, creatinine, HbA1c.

Statistical analysis Data (expressed as Mean±Standard Error Mean) were compared using the two-tailed student's

t-test for paired data. $p<0.05$ was considered statistically significant. Data were analyzed using SPSS (SPSS release 16; SPSS Inc. Chicago)

Results

The mean operative time was 270 (240–310) min. The average hospital stay after surgery was 8.33 (5–12) days. Initial two patients were converted to open surgery (in first patient to reinforce the second small bowel anasto-mosis while second patient has had dense adhesions following an earlier surgery for ruptured appendicular abscess).

Mean fasting plasma glucose before surgery was 256.8± 40.991 mg/dl which reduced to 123±16.084 mg/dl at 1 month, 112.4±5.354 mg/dl at 3 months & 109± 5.550 mg/dl at 6 months after surgery. Simultaneously, mean postprandial glucose levels reduced from 313.8± 43.379 mg/dl preoperatively to 162.2±24.136 mg/dl at 1 month, 149±22.309 mg/dl at 3 months & 158± 25.507 mg/dl at sixth months after surgery. These values indicate 57.55% reduction in fasting & 49.64% reduction in post prandial blood sugar levels within 6 months of surgery, Table 2.

Mean glycosylated hemoglobin (HbA1c) before surgery was 9±0.7144% which reduced to 6.6±0.4% at first month, 6.9±0.4% at 3 months & 6.2±0.2% at sixth month after the surgery. These values indicate 30.8% reduction in HbA1c within 6 months of surgery, (Table 2).

Mean fasting plasma insulin before surgery was 85.6± 66.3 mU/ml which was 78.9±69.4 mU/ml (p=0.412) at 1 month, 7.0±1.4 mU/ml (p =0.299) at 3 months & 6.4±1.5 mU/ml (p =0.302) during sixth month. These values indicate 91.46% reduction in fasting plasma insulin levels within 6 months of surgery. The high pre-op mean insulin level was due to exceptionally raised insulin (>300 mU/ml) in one patient.

Table 2 Glycemic status

	FBG	p	PPBG	P	HbA1c	P
Pre-Op	256.8±40.9	–	313.8±43.3	–	9±0.7	–
Post-Op						
1st Month	123±16.0	0.01	162.2±24.1	0.017	6.696±0.4	0.031
3rd Month	112.4±5.3	0.019	149±22.3	0.011	6.9±0.4	0.009
6th Month	109±5.5	0.02	158±25.5	0.014	6.2±0.2	0.009
% Reduction	57.55%	–	49.64%	–	30.8%	–

The insulin levels were calculated by HOMA index values=glucose (mM) X insulin (pM)/22.5. Insulin resis-tance level reduced by 95.01% in 6 months period. (p values—0.214) (Table 3).

Mean creatinine level before surgery was 1.68± 0.5286 mg/dl which was 1.29±0.20 mg/dl (p=0.345) at 1 month, 1.17±0.1895 mg/dl (p=0.220) at three months & 1.156±0.194 mg/dl (p=0.262) after sixth months. These values indicate 31.19% reduction in creatinine levels within 6 months of surgery.

Mean systolic blood pressure before surgery was 141± 11.662 mg/dl which reduced to 140±11.402 mg/dl (p=0.956) at 1 month, 118±7.348 mg/dl at (p =0.082) 3 months & 118±3.742 mg/dl (p =0.077) at 6 months after surgery. Simultaneously, mean diastolic blood pressure reduced from 83±4.359 mg/dl preoperatively to 80±4.4 mg/dl (p =0.468) at 1 month, 76±2.4 mg/dl (p =0.325) at 3 months & 82±2.000 mg/dl (p=0.854) at sixth months after surgery.

Out of 5 operated patients, 3 were on insulin therapy and 2 were only on OHA. All 3 patients on insulin, with a mean requirement of 90.33 units/day, went off insulin within 5 months after surgery. Both OHA patients and one insulin patient continue to require reduced OHA (metformin only) for glycemic control.

Mean weight reduction was 23.2 (45–69) m2s and mean BMI reduction was 8.4 (17.57–27.54) m2/m^2 within 6 month after surgery, (Table 4).

No patient had any major postoperative complication requiring reoperation for gastric leak, internal hernia, massive bleeding or intestinal obstruction.

Discussion

T2DM in morbidly obese is usually attributed to increased body weight besides other factors. American Diabetes Association in their 2009 guidelines [17] has recommended bariatric surgery in T2DM patients with BMI>35 m2/m^2. However, in a country with maximum diabetic patients in world, [18] a recent consensus statement [19] has estab-lished high adiposity at lower BMI amongst Indians. This results in higher co-morbidity association at lower BMI levels. This study has been designed to evaluate impact of surgery on T2DM control in <35 BMI diabetics.

Our study results show that SGIT is associated with high rate of T2DM remission/control within 6 months of surgery even in non-morbidly obese subjects with T2DM. The T2DM control (HbA1c<7) is comparable to the previously reported series following similar surgical techniques [16]. Our study has two distinct features: first, the patients were overweight or obese (BMI 23–35 m2/m^2) and not morbidly obese. Second, the selection of patients was based on C-peptide and fasting insulin levels and not on the duration of illness/ insulin intake. Even patients with over 10 years of diabetes were included.

Our study has shown some interesting results –

1. Even though our study has included T2DM patients with duration of illness of more than 10 yrs, the diabetes remission rate remained similar. This is in contrast to previous retrospective study results with lower remission rate amongst long-standing diabetics [20, 21]. The poor remission of diabetes reported in these studies may be due to non-assessment of β cell

Table 3 Mean HOMA values

	Mean HOMA Values
Pre–Op	33.4±21.4
1st Month	15.7±12.5
3rd Month	2.0±0.4
6th Month	1.6±0.3
% Reduction	95.01%

Table 4 Mean reduction in weight and BMI after 6 months

	Mean Weight (kgs)	Mean BMI (Kg/m^2)
Pre – Operative	79.8	29.4
1st Month	71.6	26.2
3rd Month	64.2	23.8
6th Month	56.6	21.0
Mean reduction over 6 months	23.2	8.4

	mass or presence of anti islet antibodies in patients

	primarily chosen for bariatric surgery. In our study
	these patients were excluded.
2.	Primary end point (HbA1c<7) of the study was
	achieved by all the patients on OHA within 1 month.
	However both patients on OHA continued to require
	small/reduced anti diabetic medication. All the patients
	on insulin therapy achieved similar level within
	6 months after surgery. 66% patients on pre op insulin
	discontinued all anti-diabetic medications within
	1 month. Even patients on long term insulin therapy
	responded after surgery. Vidal et al. [22, 23] in their
	study have concluded that patients on pre operative diet
	or OHA had better response than those on insulin
	therapy. Even though T2DM patients on dietary control
	alone were excluded from our study, better glycemic
	control was achieved after SGIT, in patients on insulin
	therapy, compared to the results of the particular study.
	This variation in result could be attributed to absence of
	incretin effect in a restrictive surgery or truncated
	GLP-1 response after GBP as compared to SGIT.
3.	Fasting insulin: The fasting insulin levels of our
	patients reduced from mean pre-operative level of
	85.626±66.3597 to 6.452±1.5365. This decline
	indirectly indicates increased peripheral insulin sen-
	sitization. 95.01% reduction of HOMA IR can explain
	reduced insulin requirement. Reduced basal insulin
	secretion is expected to delay β cell burn out. GLP - 1
	is believed to increase insulin secretion [11], however, its
	role in reducing insulin resistance is not yet established.
	Ghrelin may also be a factor affecting insulin resistance,
	through mechanism not well understood.
4.	All patients with hypercreatinemia had improved
	creatinine values after surgery. Though not significant
	(p=0.262), this observation reflects improvement in
	renal function or at least cessation of deterioration.

5. Blood Pressure – The blood pressure values became normal in both hypertensive patients and medicines were discontinued.
6. Complications – No significant surgical complication including leak, bowel ischemia, infection or major bleeding was seen. One of the patients required blood transfusion in immediate post operative period. 3 patients complained of persistent nausea for 1–3 months.

All patients lost significant weight in 6 months & thus issue of doing this surgery in non-obese T2DM patients remains questionable. Though a matter of major concern for our patients, the weight loss, always stabilized in 3–5 months.

The procedure designed by Dr. A. L. DePaula was specifically for the treatment of patients with diabetes. Classical T2DM is characterized by impaired insulin secretion or insulin resistance along with impaired incretin

effect. Also there is insensitivity of beta cell to glucose which may be due to reduction in early GLP-1 response [23]. The basic mechanism of the surgery is the early exposure of the ingested nutrients to the interposed ileum thus stimulating the early secretion of GLP-1 resulting in resumption of the early phase insulin secretion.

Sleeve Gastrectomy was also instrumental in restricting calorie intake, resulting in weight loss. In patients with diabetes, irrespective of adipose tissue mass, caloric restriction and weight management are vital in delaying or preventing further diabetes-related co morbidity [24, 25]. All 5 patients undergoing this surgery achieved 29.07% of weight loss from their original weight. However, weight loss alone cannot be a valid predictor for the remission of glucose control [23]. Even before significant weight loss, 2 of the 5 patients achieved normalization of blood glucose levels within a month of surgery, with one patient being hypoglycemic within 9 days of surgery.

IT involves few technical complexities as the need of enteroanastomosis has a potential risk to leaks, intestinal obstruction and internal hernia. Use of suture techniques, closure of all possible internal hernia sites, and over sewing of stapler lines probably represented additional safety lines, thereby averting complications in the initial series [26].

IT-SG can potentially cause nutritional problems. All patients are advised regular intake of iron, calcium, B12, and multivitamins. Hypertrophy of the pancreas with nesidioblastosis [27] has been reported in post gastric bypass patients, though no causal relationship has been established. Various hypotheses have been proposed to explain this condition. Proliferative effect of GLP-1 on β cells is also considered. Only a long term follow up of these patients will be able to establish this possibility.

To conclude, in our study, laparoscopic ileal transposition with sleeve gastrectomy proved to be an adequate treatment for the components of the metabolic syndrome and some other important independent factors. Though partial/ complete remission of diabetes has been defined as glycemic control with discontinuation of pharmacologic therapy or ongoing procedures for 6–12 months [28], all of our patients achieved study target of HbA1c <7% within 6 months.

The limitation of this study were the small number of patients (n=5) and the lack of relevant control group. The findings are adequate control of glucose, both fasting as well as post prandial, significant discontinuation of the insulin requirement post operatively by patients achieving HbA1c within the limits requirement of <7%, and, significant weight loss. Thus, it can be inferred that this surgery may be considered an option for the treatment of the T2DM.

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Conflict of Interest The authors do not have any disclosable conflict of interest.

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Efficacy of ethanolic extract of ginger on kidney lipid metabolic profiles in diabetic rats

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Abstract The present study was carried out to investigate the antihyperglycemic and hypolipidemic effect of ginger in streptozotocin (STZ)-induced diabetic rats. Forty two male wistar rats were divided into seven groups and treatment was given as stated in experimental protocol. The two doses (100 mg/kg and 200 mg/kg bw) of ginger on blood glucose levels in diabetic rats were studied and the levels of malondialdehyde (MDA) and tissue lipids like total cholesterol (TC), triglyceride (TG) and phospholipids (PL) were estimated in the kidney tissue of diabetic rats. The effects were compared with glibenclamide, a reference standard. Diabetic rats showed a significant increase in kidney MDA, TG, TC and PL levels. Treatment with ginger and glibenclamide resulted in a significant diminution of blood glucose levels. Oral administration of ginger for 30 days to diabetic rats decreased the levels of MDA, TC, TG and PL. The decreased lipid peroxides and tissue lipids clearly showed hypolipidemic effect of ginger apart from its antidiabetic property. Our study suggests that phytochem-icals and other bioactive compounds present in ginger may

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play an important role in suppressing the elevated tissue lipids in diabetic rats. Hence, ginger may be useful in the treatment of diabetes.

Keywords Diabetes • Ginger • Lipid metabolic profiles • Kidney • Rats

Introduction

Diabetes is a major endocrine disorder and growing health problem in most countries [1]. Diabetes produces disturbances in lipid profiles and especially, an increased susceptibility to lipid peroxidation [2]. Accumulation of lipids in diabetes is mediated through a variety of derange-ments in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic more prone to hypercholesterolemia and hypertriglyceridemia [3, 4]. The metabolism of all fuels including carbohydrates, fats and proteins are altered in diabetic subjects, leading to lipid disorders and an increased risk of coronary heart disease, peripheral vascular disease and cerebrovascular disease [5].

Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its complica-tions continue to be a major medical problem in the world population. Currently, a few medicinal plants are used to treat diabetes, among them are: momordica, stevia, and ginger. In the present study, we used ginger to assess its antidiabetic and antihyperlipidamic properties in diabetic rats. The pharmological effects of ginger rhizomes include antimicrobial, analgesic, antiulcer, anti-diabetic, cardioton-ic, anti-inflammatory, immuno-stimulant and antioxidant [6– 8]. Zingiber officinale rhizome has been reported to contains hundreds of chemical compounds, including (6)-gingerol, α-zingiberene, phenolic compounds, essential oils

and oleoresin resins [9]. These compounds are known to posses the antioxidant, hypolipidemic, hypocholesteremic properties [10, 11]. There are very limited reports on the effect of ginger in diabetic rats with special reference to lipid metabolic profiles in the kidney tissue. Hence, in this study we investigate the effect of ginger on lipid metabolic

profiles in the kidney tissue of diabetic rats.

Materials and methods

Wistar strain male albino rats aged 6 months weighing 180 ±200 g were obtained from Indian Institute of Science Bangalore (IISc). The rats were housed in clean polypro-pylene cages having 6 rats per cage and maintained under temperature controlled room (27±2°C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were fed with a standard rat pellet diet and water ad libitum.

Streptozotocin was obtained from Sigma chemicals (USA). All the other chemicals used were of analytical grade.

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were considered as diabetic if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The blood glucose levels were measured from the rat tail vein by using Accucheck glucometer (Roche, Germany).

The fresh rhizomes of ginger were purchased locally and washed with water to remove the waste. The outer layer of ginger was peeled off and was air dried. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted in cold percolation with 95% ethanol for 24 h. The extract was recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanolic extract was air-dried, finally yielding 80 g of dark brown, gelatinous extract of ginger dried rhizomes. Without any further purification, the crude ethanolic extract was used for the experiments. Dose equivalent to 100 mg/kg and 200 mg/kg bw of the ginger, was calculated and suspended in 2%, v/v Tween 80 solution for the experiment [12].

The rats were divided into 7 groups, six rats in each group and treated as follows:

I). Normal Control (NC): This group of rats received vehicle solution (2% of tween 80).

II). Ginger treatment (Gt 1): This group of rats received ginger ethanolic extract via oragastric tube for a period of 30 days at the dose of 100 mg/kg body weight.

III). Ginger treatment (Gt 2): This group of rats received ginger ethanolic extract via oragastric tube for a period of 30 days at the dose of 200 mg/kg body weight.

IV). Diabetic control (STZ 50 mg/kg body weight) (DC) : Streptozotocin is given intraperitonially for the induction of diabetes to this group.

V). Diabetic on Ginger treatment, (D+Gt 1) : Diabetic rats received ginger ethanolic extract as described in group II for a period of 30 days.

VI). Diabetic on Ginger treatment, (D+Gt 2): Diabetic rats received ginger ethanolic extract as described in group III for a period of 30 days.

VII). Diabetic on Glibenclamide treatment (D+Gli): Diabetic rats treated with glibenclamide 600 µg/kg body weight in aqueous solution orally for a period of 30 days.

After completion of 30 days treatment the animals were sacrificed by cervical dislocation and the kidney tissue was excised at 4°C. The tissues are washed with ice-cold saline, and immediately stored in deep freezer at −80°C for further biochemical analysis. The selected Lipid metabolic profiles such as lipid peroxidation (MDA), total cholesterol (TC), triglycerides (TG) and phospholipids (PL) levels were monitored by the meth-ods of Ohkawa et al., (1979) Liebermann Burchard reaction as described by Natelson (1971), Natelson (1971), Zilversmidth and Davis (1950) respectively. The experiments were carried out in accordance with guide-lines and protocol approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/ dt.17.07.2001) in its resolution number 9/ IAEC/SVU/2001/ dt. 4.03.2002).

The data has been analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at $p<0.05$.

Results

Effect of ginger on blood glucose levels and body weight changes

The blood glucose levels in STZ injected diabetic rats were drastically increased than that of normal control rats. This increase of blood glucose was almost three-fold higher even after 30 day compared to control rats. However, we found that the elevated blood glucose levels in diabetic rats were significantly ($P<0.001$) decreased after 30-day ginger

administration. Glibenclamide, which has been used as standard antidiabetic reference drug to compare the bene-ficial effects of ginger extract, also showed significant ($P< 0.001$) decrease in blood glucose levels and this is almost equal to the normal control rats. (Table 1).

Body weight significantly ($P<0.001$) decreased from 0 day to 30th day in diabetic rats than that of normal control rats, whereas with ginger treatment in diabetic rats, the body weights significantly increased. (Table 1).

Effect of ginger on kidney lipid metabolic profiles

We investigated the effects of ginger on renal lipid metabolic profiles like MDA, TC, TG, and PL in all experimental groups.

MDA is one of the oxidative stress markers. Renal MDA levels in diabetic rats are significantly ($P<0.001$) increased than that of normal control rats. However, diabetic rats treated with ginger for a 30-days period resulted marked depleted in MDA level (Fig. 1).

The renal TC was significantly ($P<0.001$) increased in diabetic rats. In this study we demonstrated that the increased TC in diabetic rats was decreased by ginger treatment. This decreased TC in ginger treated diabetic rats was similar with that of glibenclamide treated diabetic rats (Fig. 2).

Figure 3 depicts the renal PL in diabetic rats. PL level was significantly ($P<0.001$) increased in diabetic rats, whereas PL level was significantly decreased in ginger and glibenclamide supplemented diabetic groups than that of diabetic control group. When we compared, the ginger extract and glibenclamide treatments showed same result regarding the restoration of PL against diabetes-induced rise (Fig. 3).

The estimated renal TG level in all experimental groups was represented in Fig. 4. STZ injection resulted a significant ($P<0.001$) increase in the level of TG in

diabetic group as compared to the normal control rats. Interestingly, we found decreased TG level by 30 day ginger treatment in diabetic rats than that of diabetic control rats (Fig. 4).

Impact of ginger on histopathological changes in kidney tissue of diabetic rats

Figure 5 illustrate the pathological changes in the kidney of diabetic rat, include severe tubular degeneration, degener-ation of glomeruli, focal necrosis of tubules, cystic dilatation of tubules and fatty infiltration. The above mentioned pathological changes were reduced with ginger treatment in diabetic rats. The histological picture of ginger treated diabetic rats showed regeneration of glomeruli, tubules and renal cells.

Discussion

Diabetes is a pathologic condition, resulting in severe metabolic imbalances and non-physiologic changes in many tisues, where oxidative stress plays an important role in the etiology [13]. STZ causes diabetes by rapid depletion of beta cells which leads to reduction in the insulin release [14]. Hyperglycemia causes oxidative damage by genera-tion of ROS [15] leading to the development of diabetic complications [16]. The administration of ginger to STZ diabetic rats reduced blood glucose levels. This is in accordance with earlier reports [7]. In diabetic rats, decreased body weight was observed. The loss of weight may be due to excessive break down of tissue protein and protein wasting due to unavailability of carbohydrate as an energy source and catabolism of fats. Oral administration of ginger for 30 days to diabetic rats improved the body weight. Ginger contains many bioactive and pharmacolog-ical compounds. They may help in suppressing the free

Table 1 Effect of ginger and glibenclamide on blood glucose level and body weight change in diabetic rats

	Blood Glucose (mg/dl)			Body Weight (grams)		
Groups	0th Day	15th day	30th day	0th Day	15th day	30 day
Group I (NC)	81±1.41	88±1.032	94±2.8	195±9.66	197.5±2.73	215±14.28
Group II (Gt 1)	81±0.98	80±0.83	80.5±1.64	205±4.42	201±3.76	196±3.76*
Group III(Gt 2)	83±1.47	79±1.70	78±1.87*	200±7.07	194±8.94	190±8.01
Group IV (DC)	253±3.53	277±7.23*	269±15.6*	187±2.73*	170±4.47	150±6.83
Group V (D+Gt 1)	286±5.819	217±10.42*	191±3.50	180 ±3.76	187±7.52	195±3.763
Group VI (D+Gt 2)	259±4.09	177±6.43*	138±5.84*	185±6.32	176±3.7*	190±4.08
Group VII (D+Gli)	260±1.79	143±8.16*	94±3.71*	190±3.12*	186±7.35	205±0.07

All the values are mean, ± SD of six individual observations, *significant at p<0.001

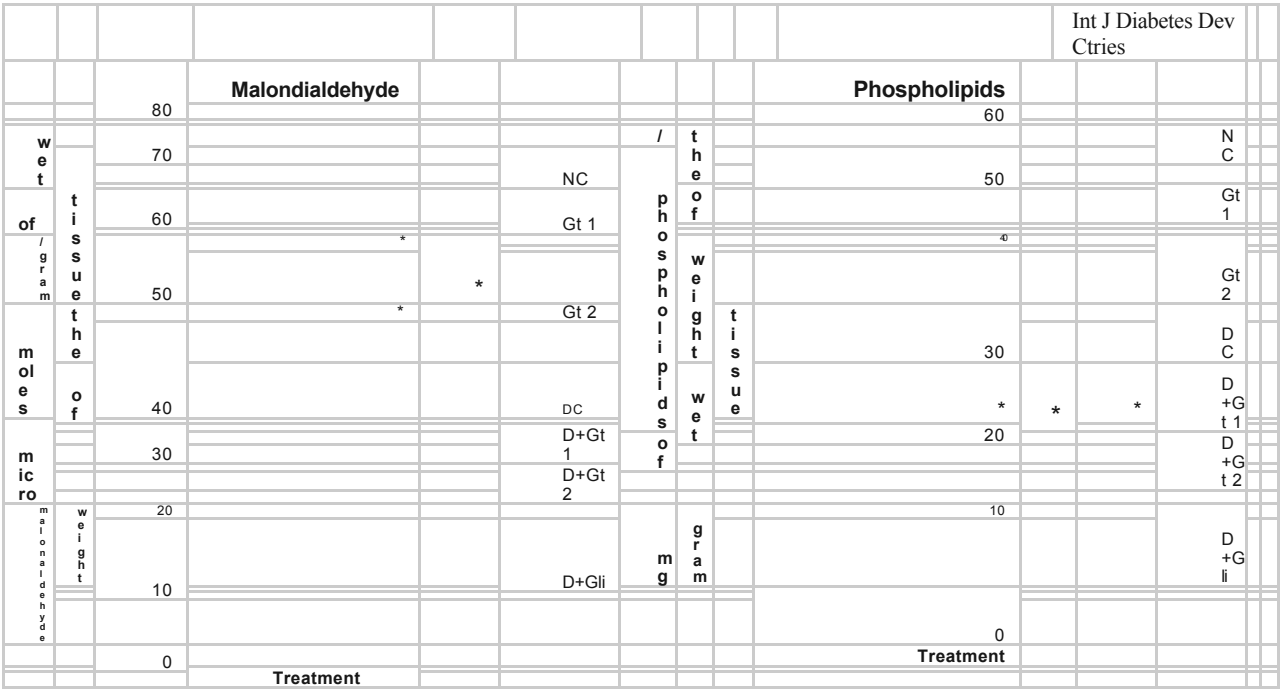

Fig. 1 Effect of ginger and glibenclamide on MDA level in kidney tissues of of normal and diabetic rats. * The values are significant compared to the following: control (*P<0.001), (Dunnett's multiple comparison test). NC: normal control, Gt 1: ginger treatment 1, Gt 2: ginger treatment 2, DC: diabetic control, D+Gt 1: Diabetic on ginger treatment 1, D+Gt 2: Diabetic on ginger treatment 2, D+Gli: Diabetic on glibenclamide treatment

Fig. 3 Effect of ginger and glibenclamide on Phospholipids (PL) in kidney tissues of normal and experimental rats. The values are significant compared to the following: control (*P<0.001), (Dunnett's multiple comparison test). NC: normal control, Gt 1: ginger treatment 1, Gt 2: ginger treatment 2, DC: diabetic control, D+Gt 1: Diabetic on ginger treatment 1, D+Gt 2: Diabetic on ginger treatment 2, D+Gli: Diabetic on glibenclamide treatment

radicals in uncontrolled diabetes. This will ultimately lead to decreased levels of blood glucose and increased level of body weight.

In the current study, the content of renal MDA was significantly increased in diabetic rats. Hyperglycemia generates reactive oxygen species which in turn cause lipid peroxidation and membrane damage in this study [17]. Earlier studies have reported that lipid peroxidation in liver, kidney, and brain of diabetic rats was increased [18]. Lipid peroxide mediated

tissue damages have been observed in type I and type II diabetes. Diabetic rats had significantly higher levels of lipid peroxides in plasma urine and renal proximal tubules [19] suggesting increased oxidative stress in diabetic kidneys. In the present investigation, ginger treatment to diabetic rats lowered the MDA content. Afshari et al [20]. reported that administration of ginger powder caused significant decrease in TBARS levels. This decrease in TBARS levels may increase the activity of GPx in treated rats and hence cause inactivation of lipid

peroxidation reaction [21]. The significant reduction of MDA levels in liver, kidney tissues of ginger fed rats at 0.5%, 1%, 5% suggests that decreased content of MDA. Dehydrogingerone a significant anologue of zingerone showed mild inhibition of lipid peroxidation by acting as free radical scavenger [22]. Shobana and Akhilender Naidu [23] reported that ginger contain antioxidants-gingerol and hexahydrocurcumin which are responsible for significant inhibition of lipid peroxidation.

In the present study, we reported increased level of total cholesterol in diabetic group. Saravanan and Pari [24] reported that higher levels of cholesterol and triglycerides in diabetic rat tissues (Liver and Kidney). Similar results have been reported by other workers in diabetic rats [25]. The significant increase in total cholesterol levels in diabetic rats indicate inefficient scavenging of reactive oxygen species which might be implicated in the oxidative inactivation of enzymes and especially the deleterious effects due to accumulation of superoxide radicals [26].

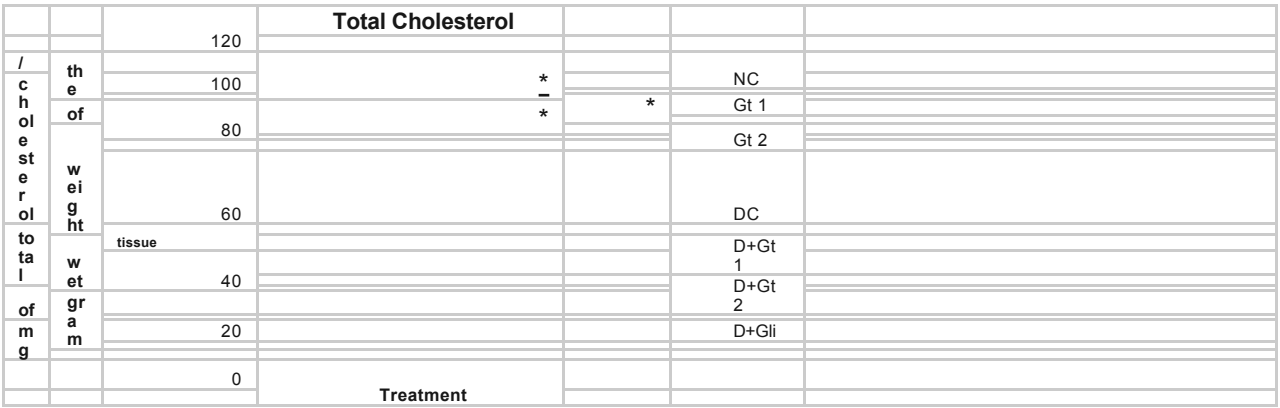

Fig. 2 Effect of ginger and glibenclamide on Total cholesterol (TC) in kidney tissues of normal and diabetic rats. * The values are significant compared to the following: control (*P<0.001), (Dunnett's multiple comparison test). NC: normal control, Gt 1: ginger treatment 1, Gt 2: ginger treatment 2, DC: diabetic control, D+Gt 1: Diabetic on ginger treatment 1, D+Gt 2: Diabetic on ginger treatment 2, D+Gli: Diabetic on glibenclamide treatment

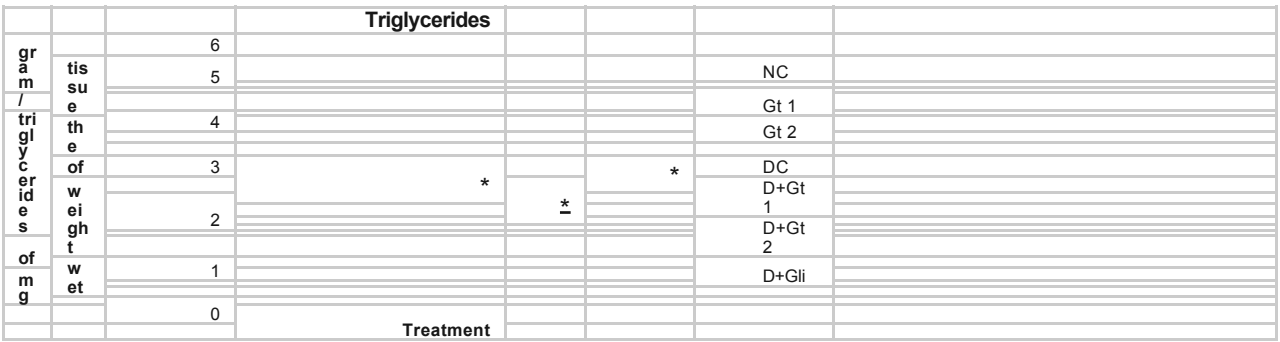

Fig. 4 Effect of ginger and glibenclamide on Triglyceride (TG) in kidney tissues of normal and experimental rats. * The values are significant compared to the following: control (*P<0.001), (Dunnett's multiple comparison test). NC: normal control, Gt 1: ginger treatment 1, Gt 2: ginger treatment 2, DC: diabetic control, D+Gt 1: Diabetic on ginger treatment 1, D+Gt 2: Diabetic on ginger treatment 2, D+Gli: Diabetic on glibenclamide treatment

Fig. 5 Effect of ginger on histopathological changes in STZ induced diabetic rat kidney. Photomicrograph of Normal control (NC) kidney showing

normal architecture 1. Normal glomeruli, 2. Normal tubules. Photomicrograph of Ginger treated (Gt 1) kidney showing,

1. Normal glomeruli, 2. Con-gestion of blood, 3. Normal tubules. Photomicrograph of Ginger treated (Gt 2) kidney showing, 1. Normal glomeruli,
2. Normal tubules. Photomicro-graph of Diabetic Control (DC) kidney showing 1. Degeneration of glomeruli, 2. Severe tubular degeneration, 3. Focal necrosis of tubules. Photomicrograph of Diabetic + Ginger treated (D+Gt 1) rat kidney showing 1. and 2 showing the regeneration of damaged of glomeruli, 3. Renal cells appears to restored, 3. Photomicrograph of Diabetic + Ginger treated (D+Gt 2) rat kidney showing 1. Regeneration of glomeruli, 2. Renal cells appears to restored, 3. Renal tubule appears to be restored

These effects may be due to a higher activity of cholesterol biosynthesis enzymes and or high levels of lipolysis. The increased level of cholesterol in kidney is due to the decreased level of HDL-cholesterol [27]. The most com-mon lipid abnormalities in diabetes are hypertriglyceride-mia and hypercholesterolemia [28]. These results confirm that there is a strong correlation between oxidative stress and diabetes occurrence. A variety of derangements in metabolic and regulatory mechanisms due to insulin deficiency are responsible for the observed accumulation of lipids [29]. Administration of ginger to diabetic rats normalises the levels of cholesterol in the kidney tissue. The hypocholestemic effects of ginger may be due to the inhibition of cellular cholesterol synthesis. Srinivasan and Sambaiah [11] suggested that ginger stimulates the conver-sion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. The ginger ethanolic extract produced a significant fall in serum total cholesterol levels indicating profound lipid lowering activ-

ity of the test drug [12]. Ginger may improve hypercholes-terolemia by modifying lipoprotein metabolism enhanced uptake of LDL by increasing LDL receptors. Hence, in ginger-treated diabetic rats decreased levels of total choles-terol levels were observed.

Phospholipids are vital part of biomembrane rich in Poly Unsaturated Fatty Acids (PUFA), which are susceptible substrate for free radicals, such as $O_2^{\bullet-}$ and OH^- radicals. In our study phospholipid levels are increased in diabetic group. Earlier reports also confirmed the presence of increased levels of phospholipids in tissues and serum in diabetic rats [30]. The levels of glycemic control and elevated levels of HDL cholesterol and decreased triglycer-ides in blood are correlated with phospholipid levels [31]. Elevated levels of phospholipids in kidney tissue have been observed in diabetic rats which may results in a number of deleterious effects due to accumulation of H_2O_2 [32]. The phospholipid level was decreased in ginger treated diabetic rats, this was due to the presence of antioxidant compounds

in ginger. Ginger directly decreases oxidative stress and also scavenges the free radicals involved in the process of oxidative stress, thus lowers the concentration of phospho-lipid [33]. Aeschbach et al [34]. reported that the gingerol, an antioxidant compound of ginger is a powerful inhibitor of phospholipid peroxidation. Hence, in ginger treated rats phospholipids content was decreased.

In the present study, we reported that triglyceride levels were increased in kidney tissue of diabetic rats. The

concentration of tissue lipids such as cholesterol and triglycerides, were significantly higher in diabetic rats than in control groups. Bruan and Severson [35] have reported that deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. Hypertriglyceridemia is also associated with the metabolic consequences of hypercoagulability, hyperinsuli-nemia, insulin resistance, and glucose intolerance [36]. The increase of TG-rich lipoproteins that have been shown in diabetes could be a consequence of the reduction of lipoprotein lipase (LPL) activity due to its glycation [37]. Hence, in diabetic rats TG levels were high. However, with ginger treatment of diabetic rats, TG levels decreased. Ginger extract produced a significant fall in serum triglyceride levels. Al-Amin et al [38]. have clearly shown that an aqueous extract of raw ginger effectively lowers serum glucose and triglycerol in diabetic rats. Ginger treatment significantly decreased serum triglyceride in STZ induced diabetic rats [39].

The histopathological studies of kidney showed severe tubular degeneration, degeneration of glomeruli, focal necro-sis of tubules, cystic dilatation of tubules and fatty infiltration in diabetic control rats, which might be due to increased diuresis and renal hypertrophy in diabetic rats. The collecting tubules showed dilation, probably under the pressure of increased urine flow. These observed pathological changes were decreased in diabetic rats which were treated with ginger. The glomeruli appear to be restored, tubules also appears to be regenerated and less fatty infiltration was observed in the ginger-treated diabetic rats, which may be due to a protective effect of the ginger. Thus, in addition to blood glucose lowering effect, histopathological observations also supports the concept that ginger at 100 mg/kg and 200 mg/kg doses produced significant reduction in blood glucose levels and lipid metabolic profiles and protected the renal tissue from diabetic oxidative stress.

From this study, it is evident that ginger effectively decreases the blood glucose levels in diabetic rats and ameliorates the lipid abnormalities in STZ-induced diabetic rats by virtue of its antioxidant bioactive compounds, antidiabetic compounds and phytochemicals. The synergetic role played by ginger compounds is attributed to the protection of diabetic rats against lipid abnormalities. It is hoped that the activity-guided isolation of ginger may yield

valuable therapeutic compounds which will be useful for developing powerful hypoglycemic and hypolipidemic drugs. Further pharmacological and biochemical studies are underway to know the hypoglycemic and hypolipidemic properties of ginger.

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Insulin-like growth factor-1 in correlation with bone mineral density among Egyptian adolescents with type 1 diabetes mellitus

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Abstract Diabetes is associated with alterations in bone health in children and adolescents. The natural history and etiopathogenesis of osteoporosis in type 1 diabetes is not clear. This study was designed primarily to estimate the prevalence of osteoporosis in a sampleoftype1diabeticadolescentsandtoassesthelevelofIGF-I and its association with bone mineral density. A total of 60 type 1 diabetic patients and 40 healthy controls aged 13 to 18 years participated in the study. DEXA scan was performed in patients, serum IGF-I, urinary albumin excretion, serum calcium, phospho-rus and serum alkaline phosphatase were assessed in both patients and controls. Our results revealed that 50% of our patients have impaired BMD Z score (−2.10 to −1.20), diabetic cases showed significantly lower mean IGF-I when compared to control group ($P<0.0001$) and there was positive correlation between BMD and serum level of IGF-I ($r=0.66$, $P<0.0001$) and not with disease duration, insulin dose, HbA1c and serum metabolic bone markers. Diabetic osteopenic patients had significantly lower mean weight, height and BMI than diabetic non-osteopenic patients ($P<0.0001$, $P<0.0001$, $P<0.001$ respective-

ly). Moreover, age at menarche in diabetic osteopenic females is significantly delayed than non-osteopenic ($P=0.02$), a higher frequency of smoking in diabetic osteopenic was observed ($p< 0.0001$). Impairment of bone mineral density was identified in Egyptian adolescences with T1DM. Lower serum IGF-I levels correlates with decreased mineralization, which suggests its prominent role in the pathophysiology of osteoporosis.

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Keywords Insulin-like growth factor-1 • Bone mineral density • Adolescents • Type 1 diabetes

Introduction

Type 1 diabetes (T1DM) is an autoimmune disorder resulting in the loss of pancreatic insulin-producing β-cells, occurring in childhood or early adulthood. Along with increased risk of complications including retinopathy, nephropathy, neuropathy, and cardiovascular events, adults with T1DM have decreased bone mineral density (BMD) compared with control subjects [1]. In fact, osteoporosis is the most significant metabolic bone disease in individuals with diabetes and may have risk of complications, including hip fracture [2].

Recent studies demonstrate that diabetes is associated with alterations in bone health in children and adolescents [3]. Prepubertal and pubertal patients with T1DM (aged<15 years) have decreased bone mass measured both by dual-energy X-ray absorptiometry (DEXA) scan and quantitative ultrasound [4, 5]. These observations suggest that adverse effects on bone health may occur early after the diabetes diagnosis. Understanding the natural history of BMD changes in young adults with T1DM may elucidate how the disease progresses and provide opportunities for prevention of significant bone loss and presumably, fractures [6, 7].

Boneremodelingisregulatedbysystemichormonesandlocally produced factors acting in concert to maintain bone anabolic regulatorofbonecellfunctione.g.decreasingcollagendegradation,

increasing bone matrix deposition and increasing osteoblastic cell recruitment[8]. The effects of insulin –like growth factor I (IGF-I) are modulated by at least six different IGF-binding proteins (IGFBPs) and (IGBP) proteases [9].

This study was designed primarily to estimate the prevalence of osteoporosis in a sample of T1 diabetic adolescents and to asses the level of IGF-I and its association with BMD.

Subjects & methods

Subjects

The present study included 60 (18 males and 42 females) type 1 diabetic adolescents who were recruited from the Diabetes Specialized Clinic Children's Hospital, Ain Shams University over a 10 months period. The patients are regular attendees at the Clinic which cares for more than 1,500 children, adolescents, and youngadultswithT1DM. Tobeeligibleforthestudypatientshad to satisfy the following criteria: 13–18 years age and suffering from T1DM for at least 3 years with normal vitamin D levels.

Exclusion criteria included: secondary or genetic types of diabetes, T2DM, systemic illness affecting BMD, any endocrine disorders (other than diabetes), long term corticosteroid treatment, juvenile osteoporosis, Paget's disease, metastatic bone lesions and immobilization.

Patients' mean age was 14.67±1.53 years, their disease duration ranged between 3 and 14 years with a mean of 6.86±3.12 years, the patients included used human insulin in a dose ranging from 0.5 to 2 IU/kg/day with a mean of 1.09± 0.39 IU/kg/day, all participants were on intensive insulin therapy. Forty six patients were on preprandial injections of short- acting insulin plus intermediate acting isophane insulin & 14 patients were on long acting insulin analogue (insulin glargine) at bedtime plus preprandial short acting insulin analogue.

Forty healthy adolescents with no obvious medical disorder (any disease or drug therapy) with a mean age of 14.98±1.83 years served as a control group.

Subjects participated in the study after written information and informed consent from their parents. The study was approved by the Ethical Committee of Ain Shams University.

Methods

All subjects underwent the following:

Detailed Questionnaire

Complete history taking including their age, diabetes duration, complications, insulin regimen, menstrual his-tory, calcium intake assessed by a semi quantitative food frequency questionnaire [10] and medication intake including calcium supplements, life style habits (weekly

physical activity was determined according to three categories: no sport at all "A"; physical education classes only, with an average 3 h per week "B" and physical education classes and organized extracurricular sports C), smoking history(passive and active), and reported prior fracture history.

Clinical assessment

Physical examination included: anthropometric meas-ures; weight in kg, height in cm and body mass index (BMI). Blood pressure was measured and recorded. Additionally, the stage of pubescence was determined according to Tanner's classification [11].

Biochemical investigations

Venous blood samples were obtained in the morning time from all patients after an overnight fast from venous blood .After centrifugation, part of serum was taken for the direct assay of calcium, phosphorus, alkaline phosphatase and other portion was frozen at −20°C for further analysis of IGF-I.

Additionally 2 ml blood in EDTA tube was taken for direct assay of HbA_1c.

Serum calcium, phosphorus, alkaline phosphatase were performed by spectrophotometer methods on Roche/Hitachi 917.Glycated hemoglobin concentration, was measured by high performance liquid chromatography (HbA_1c) supplied by Bio-Rad Diagnostic Group. Horcules C.A. Milano-Muchon- Paris.

Levels of 1, 25 Dihydroxyvitamin D(3) was

performed by 1,25-Dihydroxyvitamin D

supplied by DiaSorin inc.USA based upon competitive radioimmunoassay.

Serum IGF-I levels were assessed in both patients and controls by enzyme linked immunosorbant assay using a commercial kit [Bio Source IGF-I ELISA, KAPB2010, Biosource Europe SA—Beligium].

Receiver Operating Characteristic (ROC) curve was used to define the best cutoff level of IGF-I to detect osteopo-rosis which was 316 ng/ml with a sensitivity of 100%, specificity 77%, PPV 87% and NPV 100% with a diagnostic accuracy of 91%., likelihood ratio(LR)+=4.44 and AUC of 97%. Also IGF-I was found to be reliable to differentiate between DO & DNO patients. The best cut off value of IGF-I was 223 ng/ml with a sensitivity of 100%, specificity 67%, PPV 75% and NPV 100% with a diagnostic accuracy of 83%. Cases with IGF-I >223 ng/ml were not likely to have osteoporosis, (LR+=3 and AUC of 97%).

Urinary albumin excretion was measured by immuno-turbidimetric method. The kit used was from SERA-PAK (Bayer Corporation, NY, USA).

The result was expressed as albumin to creatinine ratio (ACR) in urine to avoid diurnal variation in albumin excretion. Urinary creatinine was estimated on Synchron Cx7 (Beckman, California USA).

Microalbuminria was defined as urinary albumin/creat-inine ratio (ACR) 30–299 mg/g in at least two urine samples, 2 month apart [12].

BMD measurements

DEXA were used to measure bone mineral density in the whole body (DEXA; LUNAR-DPX).

All scans were performed on the same device, a single technician performed 50% of cases, three additional technicians performed the remainder of cases.

BMD was interrupted as z – score, a reference to standard values for a given age and sex. Osteopenia was defined as a BMD Z score of $< -1$and ≥ -2.5, and osteoporosis as a BMD Z score of< -2.5 [13].

Statistical Analysis

The data were coded, entered and processed on computer using SPSS (version 15). The level $P<0.05$ was considered significant.

Chi-Square test X^2 was used to test the association variables for categorical data.Fisher exact test was per-formed in table containing value less than five. The Mann– Whitney U test: non-parametric test to assess the statistical significance of the difference between two population means in a study involving independent samples.

Correlation analysis: assessing the strength of associa-tion between two variables. The correlation coefficient denoted symbolically r, defines the strength and direction of the linear relationship between two variables.

Results

Clinical and biochemical characteristics

Characteristics of the diabetic patients and controls enrolled in the study is demonstrated in Table 1. There was no statistical difference between patients and control group as regards mean age, weight, height, BMI ($p=0.36$, $P=0.32$, $P=0.78$, $P=0.22$ respectively), also no statistical difference in the same studied parameters between T1DM females and males. The only significant difference observed while comparing anthropometric measurements were lower mean weight, mean height and mean BMI in diabetic osteopenic and osteoporotic

(DO) patients than diabetic non-osteopenic (DNO) patients (Table 2). Mean blood pressure, pubertal staging, calcium intake and exercise performance did not differ between diabetic patients and controls. The same was observed comparing diabetic males and females. Median pubertal staging for control and diabetic cases was 4 (Table 1). Twenty two patients had pubertal stage 3, 24 stage 4 and 14 had stage 5. Median pubertal staging for male diabetic cases was 3 (IQR 3–4) and for female cases 4

(IQR 3–5). The median pubertal staging in diabetic osteo-penic and osteoporotic was 3 (IQR 3–4) and diabetic non osteopenic patients 4 (IQR 4–4.5). Moreover age at menarche in DO patients is significantly delayed than DNO (P=0.02) Table 2.

Overall, mean IGF-I levels were significantly lower in cases when compared to control group (P<0.0001, Fig. 1), as well as in DO compared to DNO (P<0.0001, Fig. 2). Moreover female cases showed lower IGF-I (196.33±67.94 ng/ml) when compared to males (212.0±59.43 ng/ml) but the difference did not reach a significance level (P=0.4).

Interestingly, higher rate of smoking was observed in DO patients when compared to DNO patients (P<0.0001) (Fig. 3, Table 3), but there was no statistical differences between males and females patients.

Bone mineral density

The present study revealed that median BMD Z score in adolescents with T1DM was −1.00 (range −1.20 to −0.20), 50% of cases (n=30) had median BMD Z score −1.20 (range −2.10 to −1.20) and proved to be osteoporotic (n=4) and osteopenic (n=26), the reminder had normal median BMD Z score −0.2 (range −0.50 to 1.00). Female cases showed lower median BMD (−1.10) when compared with male cases (−0.50) but the difference did not reach a significant level (p=0.54). Dietary intake of calcium as well as physical activity did not alter the BMD results.

Relationship between serum biochemical markers, BMD

No correlation could be detected either between BMD nor IGF-I and diabetes duration,insulin dosage, metabolic control(measured by HbA1c), serum biochemical markers parameters including Ca, Ph,AlP, 1, 25 Dihydroxyvitamin D(3), tanner stage and ACR. However, there was positive correlation between BMD and serum level of IGF-I (r= 0.66, P<0.0001, Table 4).

We explored the relation between microvascular com-plications and BMD and found that DO had higher rate of neuropathy, nephropathy and retinopathy when compared to DNO but the difference did not reach a significance level (P=0.12, P=0.59, P=0.49 respectively).

Discussion

The effect of insulin therapy on BMD in patients with T1DM have been studied [14].So far studies on the dependency between IGF-I and BMD in diabetic children and adolescents have been limited .Here we present the BMD status in a sample of adolescents with T1DM and its correlation with IGF-I levels.

Table 1 Characteristics of the diabetic patients and controls enrolled in the study

	Groups								
	Control					Cases			
	Mean	±SD	Range			Mean	±SD	Range	
Age (yrs)	14.98	±1.83	13.00	18.00		14.67	±1.53	13.00	18.00

Weight (kg)	51.73	±12.52	31	71	54.27	±12.37	30	78
Height (cm)	153.50	±9.99	136	167	154.00	±8.07	136	167
Diabetes duration (yrs)					6.86	±3.12	2.90	14.000
Insulin dose (U/kg/day)					1.09	±0.39	.50	2.000
BMI (kg/m^2)	21.65	3.32	16	26	22.54	±3.70	16	300
Tanner stage	4.00		3.5	5.0	4.00		3.00	4.000 a
HbA1C (%)					8.06	±2.01	6.60	10.300
Serum Ca (mg/dl)					9.15	±0.75	8	11
Serum Ph (mg/dl)					4.38	±0.72	3	6
Alkaline Phosphates(U/l)					472.17	±178.45	134	910
1, 25 Dihydroxyvitamin D(3) (pg/ml)	38.6	±4.1	30.3	45.2	37.2	±2.1	28.1	38.4
IGFI (ng/ml)	362.48	56.92	273	440	201.03	±65.41	104	315

a Values presented as median and Interquartile range(IQR)

The present study revealed impaired BMD in 50% of adolescents with T1DM. Most studies reported that bone formation rate are low [15, 16] or did not differ from those in healthy subjects in patients with T1DM [17, 18] others suggested higher osteoblast function [19]. It was previously suggested that diabetes control does not play a major role in the pathogenesis of bone loss in T1DM. Moreover, if the relationship between diabetes and bone loss was only related to hyperglycemia, one would expect a similar incidence of osteoporosis in patients with T1DM and those with T2DM, but osteoporosis is rather an uncommon feature of T2DM [20].

Table 2 Comparison between diabetic osteopenic and osteoporotic and diabetic non osteopenic according to clinical and laboratory characteristics

	Diabetic osteopenic and osteoporotic (30)		Diabetic non osteopenic (30)		t	P
	Mean	±SD	Mean	±SD		
Age (yrs)	14.80	±1.54	14.53	±1.53	0.67	0.50
Weight (kg)	46.87	±10.23	61.67	±10.03	4.00	<0.001
Height (cm)	149.00	±7.36	159.00	±5.42	4.24	<0.001

BMI (kg/m^2)	20.88	±2.89	24.21	±3.72	3.87	<0.0001
Disease duration (yrs)	7.19	±3.40	6.53	±2.83	0.82	0.42
Insulin dose (U/kg/day)	1.01	±0.32	1.17	±0.44	1.69	0.10
Age at Menarche (yrs)	13.50	±1.35	12.30	±1.13	2.57	0.02
HbA1C%	8.23	±2.65	7.91	±1.37	0.04	0.97
Serum Ca (mg/dl)	9.17	±0.69	9.13	±0.82	0.20	0.84
Serum Ph (mg/dl)	4.49	±0.74	4.27	±0.70	1.18	0.24
Alkaline Phosphate (U/l)	494.27	±184.31	450.07	±1172.66	0.96	0.34
1, 25 Dihydroxyvitamin D(3) (pg/ml)	37.0	±2.3	37.3	±1.7	0.52	0.60
IGFI (ng/ml)	148.13	±34.90	253.93	±41.18	10.74	<0.0001
Tanner stage	3(median)	3–4(IQR)	4(median)	4–4.5(IQR)	1.54[a]	0.12

Student's t test(t)

[a] Mann-Whitney test(z), IQR Interquartile range

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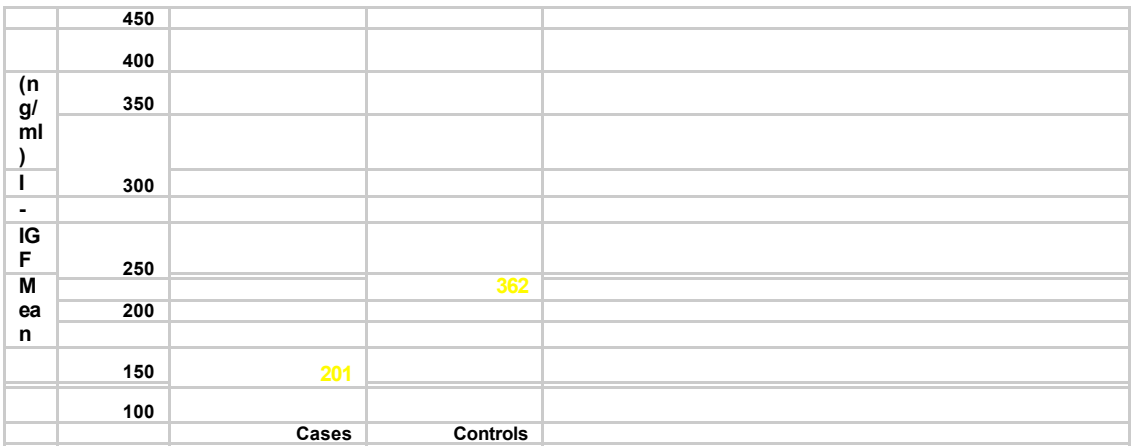

Fig. 1 Comparison between cases and controls as regards to IGF-I levels (P<0.0001)

There may be differences between two types of diabetes other than glycemic control that affect BMD decrease. Several possible factors have been investigated but the actual mechanisms involved in lower BMD in T1DM are not known. Increasing evidence suggests that insulin deficiency in T1DM may interfere with new bone forma-tion, possibly due to defective function of osteoblasts. The impaired recruitment and function of osteoblasts might be an effect of increased osmolarity in poorly controlled diabetes [21], lower levels of IGF-I [22] or accumulation of advanced glycation end-products in the type I collagen matrix [23]. Other possible contributors to reduced BMD in T1DM patients include a trend to insufficient intake of regular calcium intake as well as exercise performance.

Short term measures of control such as glucose level or HbA1c results would not be expected to reflect cumulative bone damage measured by BMD as majority of studies found no correlation between BMD with HbA_1c [17, 18, 24]; perhaps cumulative life-time glycemic control is a better indictor of osteoporotic risk [24]. This hypothesis is supported by studies demonstrating that decreased lumbar spine and femoral neck BMD in adults with T1DM is

associated with progressive microvascular complications that appears later in life [4, 14, 15].

In addition, our data did not find correlation between BMD and insulin dosage. However other study

[1] demonstrated, for the first time, that insulin requirements is negatively correlated with bone mineral content .It remains unclear how the effects of exogenous and endogenous insulin levels differ in terms of their impact on the skeleton.

In our study 73.3% of the patients enrolled who proved to be osteopenic were females. Our result is consistent with the hypothesis that androgens protect the bone mass by promoting periosteal bone formation, whereas estrogen either inhibit or have no effect on periosteal bone formation [25]. Conflicting data has been observed on gender effect on BMD; some studies have reported no sex difference [18, 24] whereas others have reported that males more affected than females in lowering of BMD [26]. On the other hand, another study [27] reported that BMD was higher in girls with T1DM than in boys. This finding may be attributed to the markedly higher BMI of many of the girls, because obesity is known to induce an increase whole body mineral content during childhood and adolescence [28].

The data presented here demonstrate that cases had a lower IGF-I levels .The results of our study are consistent with those with other authors [1, 7, 29], in contrast to others [17] who showed that IGF-I values were not lower in diabetic patients compared with control subjects. This may be due to the fact that serum concentrations do not necessarily reflect autocrine production. Hyperglycemia suppress IGF-I, failure of current insulin therapy to restore IGF-I levels to normal is the result of non physiological insulin concentration in the liver and if insulin is directly infused into the portal circulation, a prompt return of IGF-I production occurs. Hepatic insulin concentration influence

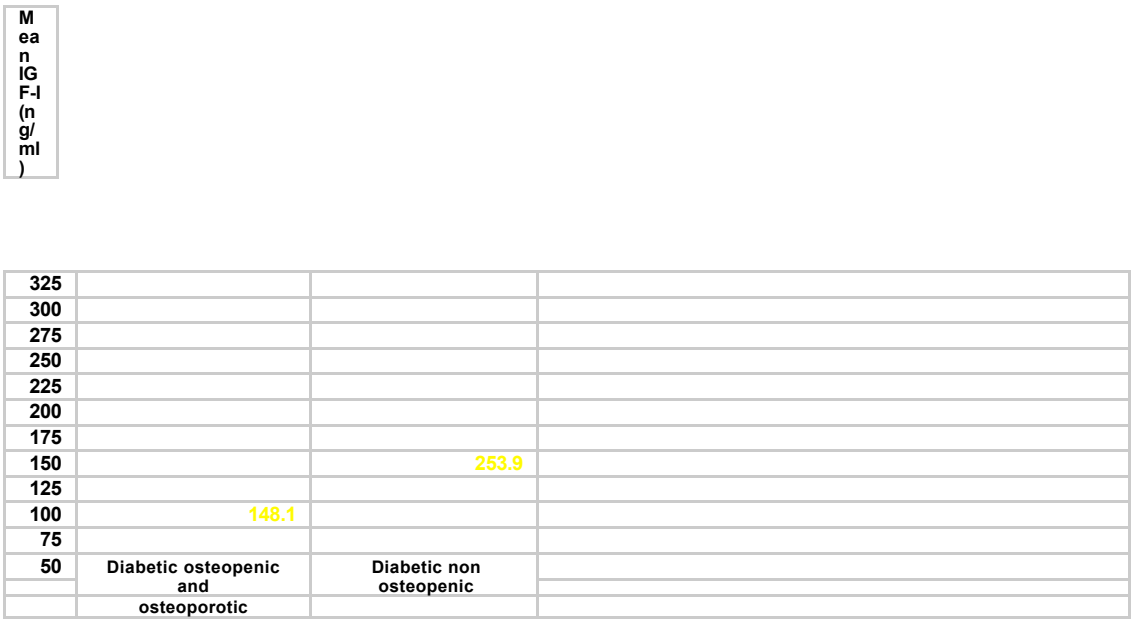

Fig. 2 Comparison between diabetic osteopenic and osteoporotic and diabetic non osteopenic as regards their mean IGF-I ($P<0.0001$)

Fig. 3 Diabetic osteopenic and osteoporotic showed significantly higher rate of passive smoking when compared to diabetic non osteopenic ($P<0.0001$)

Table 3 Comparison between diabetic osteopenic and osteoporotic and diabetic non osteopenic according to life style habits and

microvascular complications

		Diabetic osteopenic and osteoporotic (30)		Diabetic non osteopenic (30)		χ^2	P
		N	%	N	%		
Sex	Male	8	26.7%	10	33.3%	0.32	0.57
	Female	22	73.3%	20	66.7%		
Exercise	No	20	66.7%	15	50.0%	1.71	0.19
	Yes	10	33.3%	15	50.0%		
Smoking	No[1]	6	20.0%	22	73.3%		
	Passive	22	73.3%	8	26.7%	15.63	<0.0001
	Active	2	6.7%	0	.0%		1.00
Medication as Ca	No	22	73.3%	18	60.0%	1.20	0.27
	Yes	8	26.7%	12	40.0%		
Fracture history	No	28	93.3%	24	80.0%		0.25
	Yes	2	6.7%	6	20.0%		
Detailed history of Feeding	regular Ca intake	8	26.7%	14	46.7%	2.58	0.11
	Irregular Ca intake	22	73.3%	16	53.3%		
Fundus Exam	Normal	28	48.3%	30	51.7%		0.49
	Affected	2	100.0%	0	.0%		
Neuropathy	Normal	14	46.7%	20	66.7%	2.44	0.12
	Affected	16	53.3%	10	33.3%		
Nephropathy	Normal	18	47.4%	20	52.6%	0.29	0.59
	Affected	12	54.5%	10	45.5%		

IGF-I bioactivity by regulation of hepatic IGFBP-1 pro-duction [30].

In our study, as in others [7, 31, 32] lower IGF-I concentration correlates with decreased mineralization and not with the serum biochemical markers of bone. It has been postulated that in addition to insulinopenia, relative IGF-I deficiency, whether systemic or local, contributes to low BMD in diabetes. IGF-I levels are lower in individuals with T1DM, and poor glycemic control negatively impacts IGF-I production by the liver. This suggests a prominent

role of IGF-I in the pathogenesis of osteoporosis in the developmental age [7].

In addition, our data demonstrated that DO had lower weight and height indicating that growth retardation is a common complication of T1DM and may play role in the development of diabetic osteoporosis and osteopenia. Moreover, they had a lower BMI which is a risk factor for osteoporosis and being overweight protects against osteoporosis, either by increasing the calcium accretion due to body weight or through the hormone leptin [33].

Table 4 Correlation between IGF-I (ng/ml), BMD (Z score) and other studied parameters

	IGFI		DEXA	
	r	P	r	P
BMD (Z score)**	0.66	<0.0001		
Diabetes duration (years)	−0.14	0.28	−0.22	0.10

		Insulin dose (U/kg/day)	−0.02	0.87	0.21	0.11
		HbA_1c%	−0.07	0.60	0.02	0.90
		Serum Ca (mg/dl)	−0.14	0.27	0.02	0.90
		Serum Ph (mg/dl)	−0.24	0.06	−0.17	0.19
		Alkaline phosphate (U/l)	0.06	0.67	−0.12	0.38
		1, 25 Dihydroxyvitamin D(3) (pg/ml)	−0.21	0.26	−0.08	0.68
		Tanner stage	0.15	0.25	0.30	0.10
		Urinary ACR (mg/g)	−0.15	0.26	0.15	0.28

Of particular significance in our study is the delayed age at menarche in DO. A later age at menarche was found to be associated with lower BMD in the spine and proximal femur [34] and higher risk of vertebral [35] and hip fracture [36] in adulthood. Indirect evidence from a retrospective epidemiological survey suggests that this association is likely to be related to the influence of pubertal timing on bone mineral attainment. This association is usually considered as the expression of later and thereby short exposure to estrogen [37]. In contrast to our results others [7, 17] have reported that age at menarche was not significantly different in patients with diabetes compared with control subjects.

The small size of the study population, limited its ability to detect true differences regarding microvascular complications in DO that may still exist. Peripheral neuropathy in T1DM may be an independent risk factor for reduced BMD in the affected limbs as well as in the skeleton in general, suggesting a systemic effect of peripheral neuropathy or factors associated with periph-eral neuropathy such as microangiography [38]. Diabetic patients who had sustained fracture had more peripheral neuropathy than those without fracture [39], also patients with retinopathy were at higher risk of osteopenia or osteoporosis than patients without retinopathy [14, 40]. Both diabetic retinopathy and peripheral neuropathy may lower BMD through impaired physical activity and neuromuscular/skeletal interactions, and enhance the pro-pensity of falls [41].

In this cross sectional study a higher rate of smoking was observed among DO. Moreover the population-based Gothenburg Osteoporosis and Obesity Determinant (GOOD) cohort study [42] on 1,068 young men aged 18.9±0.6 years who demonstrated that smoking was associated with lower BMD and reduced cortical thickness in young men. In agreement with those finding meta-analysis including subjects with various ages showed that smoking is more strongly associated with low BMD and more fracture in men than in women [43, 44]. Smoking is implicated as a risk factor for osteoporosis by affecting calcium and vitamin D metabolism and therefore in-creased susceptibility for fracture [45].

The mechanism by which smoking affects bone metabolism and bone mass remains inadequately eluci-dated. Nicotine, the principal pharmacologically active component of cigarette smoke, has been investigated in relation to bone cell function. It has been shown to have direct effects on osteoblast cell proliferation, mediated by specific receptors, and to be able to induce expression of the bone matrix

protein osteopontin [46], suggesting a direct toxic effects of nicotine on the bone cells.

Because T1DM typically occurs in children and adolescents, one might argue that calcium and vitamin

D3 deficiency could be a contributing factor towards osteopenia. In the current study, patients had no prior history of metabolic bone disease, vitamin D deficien-cy, parathyroid disease and malabsorption. Our findings suggest that vitamin D deficiency is not the cause of diabetic osteopathy in our patients group, although alterations of calcium and Vitamin D3 balance have been implicated as contributing factors of diabetic osteopenia by some but not all authors [47– 50] and even shortly after diagnosis with T1D children presented with decreased lumbar spine BMD and decreased bone formation markers concentration of the carboxy-terminal propeptide of type I collagen [51]. In most studies biochemical parameters of calcium and bone metabolism showed no clear relationship to the bone mineral density measurements [52, 53]. From few bone histology studies in humans and experimental studies there is evidence that a decreased bone formation is one major mechanism leading to reduced bone mass in diabetics. Microangi-opathy at the bone tissue was also discussed as a possible reason for diabetic osteopenia [53]. The study conducted by Di Cesar and coworkers reported that mean vitamin D levels in type 2 diabetic patients were in the deficient range whereas mean levels in the type 1 diabetic patients reached the expected level, unrelated to age, sex, or insulin treatment. Although higher BMI was associated with lower 25-OH-D levels, the difference in vitamin D levels between type 2 and type 1 diabetic individuals persisted after adjusting for BMI [54]. Moreover, further adjustment for dietary calcium intake and Vitamin D as well as physical activity did not alter the BMD results [7]. Also, the inclusion of patients without a significant elevation of serum creatinine level that may alter their renal function could partially exclude abnormal vitamin D metabolism that will lead to increase bone resorption.

It has been suggested that, in diabetic patients with chronic kidney disease, bone loss is detectable and progressive during follow-up, and apparently more severe at the femoral neck [55]. In fact, hydroxyproline excretion is increased in those with diabetes and micro-albuminuria [56]. Furthermore, in patients with chronic kidney disease stage 5, those who were also diabetic were at greater risk of 25-hydroxyvitamin-D deficiency, and there was a positive association between 25-hydroxyvitamin D levels and BMD Z scores [57]. For some time, hypercalciuria has been considered a potential risk factor for osteoporosis in patients with poorly controlled type 1 [58] or type 2 [59] diabetes, but glycemic control can reduce hypercalciuria [58].

Finally, the causes of bone loss in diabetes are still unclarified though the predominant defect in DM1 is a decrease in bone formation, rather than increased

resorption, supporting a decreased osteoblast number and/or maturation. Diabetes can impact bone through multiple pathways, some with contradictory effects, including obesity, changes in insulin levels which serves as a bone anabolic factor, higher concentrations of advanced glycation end products in collagen, hyper-calciuria associated with glycosuria, increased urinary excretion coupled with lower intestinal absorption of calcium [60, 61]. Other suggested mechanisms were inappropriate homeostatic response of parathyroid hor-mone secretion, complex alterations of vitamin D regula-tion, reduced renal function, lower insulin-like growth factor-I, microangiopathy, and increased production of inflammatory cytokines [62, 63]. A better understanding of how diabetes metabolism and treatments affect bone would improve bone status and help fracture prevention efforts in diabetic adolescents.

In conclusion, BMD is affected during period of adolescence in patients with T1DM where females are more prone. Screening of bone density testing in children and adolescent in T1DM should be a routine follow up and counseling regarding lifestyle interventions that may improve bone health including adequate intake of calcium, vitamin D and exercise should be emphasized. As low serum IGF-I levels have independent deleterious effects on bone in diabetic patients of all ages, newer approaches for manipulating the IGF regulatory system may become effective as therapeutic adjuncts for the treatment of osteoporosis.

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

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ORIGINAL ARTICLE

Effect of human umbilical cord blood CD34+ progenitor cells transplantation in diabetic mice

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Abstract Shortage of donor organs spurs research into alternative means of generating β cells. Stem cells might represent a potential source of tissues for cell therapy protocols, and diabetes is a candidate disease that may benefit from cell replacement protocols. We examined the effect of transplanted human umbilical cord blood CD34+ cells on some detailed parameters in streptozotocin- (STZ) induced diabetic mice. An experimental study was con-ducted in the departments of clinical pathology, physiology and pathology of Faculty of Medicine, Suez Canal University. Thirty male albino mice 8–12 weeks were included and subdivided into 3 groups, first group served as normal control group, second group as diabetic control after

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induction of diabetes with STZ and third group treated diabetic mice by injection of positively selected CD34 progenitor cells from human umbilical cord blood (HUCB) with a dose of one million cells/mouse. Blood glucose and serum insulin were measured at specific time interval and immunohistochemical (IHC) analysis and histopathology on pancreas were conduced. Data were analyzed using chi square between groups. Intravenous injection of CD34+ cells caused significant improvement in blood glucose level (277.9±102.5 mg/dl in treated group vs 530.3±99 mg/dl in untreated group, $p<0.01$). Blood level of mouse insulin was higher in the treated group as compared with untreated diabetic mice (0.77±0.2 ng/ml in treated group versus 0.26± 0.09 in untreated group, $p<0.001$). IHC analysis for detection of human insulin producing cells in pancreas of treated mice revealed that 33.3% positive cellular staining and 55.6% positive sinusoidal staining were detected. In conclusion, Transplantation of HUCB-CD34+ cells appear to be a modality of stem cell therapy in diabetes mellitus.

Keywords CD34 cells • Human umbilical cord blood • Streptozotocin induced diabetic mice

Introduction

Diabetes mellitus is a chronic metabolic syndrome charac-terized by increased levels of blood glucose, referred to as hyperglycaemia. Type 1 diabetes generally results from autoimmune destruction of pancreatic islet ß cells, with consequent absolute insulin deficiency and complete dependence on exogenous insulin treatment. Type 2 diabetes is associated with insulin resistance and pancreatic insufficiency and generally progresses to a state of insulin dependence [1].

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The observation that bone marrow-derived cells may become insulin-producing cells is of enormous importance. Since the human umbilical cord blood and bone marrow contain similar cell populations, it is expected that human umbilical cord blood may contain cells with a potential to develop into insulin-producing cells [2].

It was found that HUCB mononuclear cells can be used as another modality of stem cell therapy to prevent a variety of disease states, including diabetes. They also noted that the transplanted mice did not demonstrate any clinical or histological evidence of either acute or chronic graft-vs-host disease. Also, no other evidence of adverse effects was noted in the transplanted mice [3].

Until recently, blood that remained in the umbilical cord and placenta after delivery was routinely discarded. Now that this blood is known to contain both hematopoietic stem cells and pluripotent mesenchymal cells, there has been a substantial increase in the clinical use and research investigation of umbilical cord blood in hematopoietic transplantation and regenerative medicine [4]. The aim of the study was to investigate the effect of human umbilical cord blood (HUCB) CD34 progenitor cells transplantation on blood glucose and insulin level in diabetic mice.

Materials and methods

Animals

Thirty male CD1 albino mice, aged 8–12 weeks were acclimatized for 1 week and kept with free access to standard pellet animal diet and tap water. The study was approved by our university ethical committee for research. Mice were equally divided into the following three groups, each containing ten mice: group 1 to serve as a normal control group, Untreated group 2 (diabetic control): this group was subjected to induction of diabetes mellitus with streptozotocin (180 mg/kg IP) without any type of treatment and group 3 was subjected to induction of diabetes mellitus with streptozotocin (180 mg/kg IP) and received HUCB CD34+ cells with a dose of 1×10^6 cells\mouse IV in the tail vein. Portions from pancreas were taken from all animals and prepared for light microscopy examination and immunohistochemistry (IHC). All slides were reviewed by two pathologists who were blinded to the source of the material; difference in readings was reconciled by simultaneous review using a double-headed microscope.

under non fasting condition. Serum insulin was measured by ELISA (DRG Insulin (rat\mouse) EIA-4127 ELISA insulin kit) blood was obtained from retro-orbital venous plexus at the time of sacrifice.

Transplantation of HUCB CD34+ cells Sterile collection tubes (50 ml) containing citrate phosphate dextrose adenine-1 (CPDA-1) as anticoagulant (5 ml) was used for collection of the human umbilical cord blood. Separation of CD34 positive progenitor cells was carried out with immunomagnetic separation technique by Dynal CD34+ progenitor cell selection system. 0.2 ml of PBS solution was added to the CD34+ cell pellet for final dilution and injection IV in the tail vein in a dose of 10^6 cells/mouse. After transplantation all groups were followed up for 4 weeks with regular measurement of blood glucose every 2 days and body weight weekly. On death or sacrifice, blood was collected from the retro-orbital venous plexus and was kept in room temperature to be clotted. Thereafter, it was centrifuged for 15 min. Serum was collected and stored at −20°C for insulin measurement.

Immunohistochemistry (IHC) of pancreas Two paraffin Sections 5 μm from each paraffin block in the treated group were stained with mouse monoclonal antibody for insulin (1:50), (ThermoScientific Lab vision corporation).

Results

Table 1 demonstrates minimum, maximum and mean values ± SD of serum insulin level in the studied groups and show a highly significant difference among all groups as regards to mean serum insulin level ($P<0.01$).

Table 2 shows that positively stained human insulin cells are detected in 33.3% of the treated group based on the presence of granular cytoplasmic specific staining of anti-insulin. Endothelial cells within islets capillaries are

Table 1 Descriptive statistics of the serum insulin (ng/ml) in the studied groups

Study groups	Control	Untreated	Treated	P value
Serum insulin level (ng/ml)	group	group	group	
Minimum	0.8	0.2	0.4	0.000*
Maximum	2	0.5	1.1	0.000**
Mean ± SD	1.41±0.4	0.26±0.09	0.77±0.2	0.002***

Methods	*Control group versus untreated group
Blood glucose was measured every week by Medismart	**Untreated group versus treated group
glucometer, blood was obtained by snipping the mouse tail	***Control group versus treated group

Table 2 Immunohistochemical stain findings in treated mice group

*P<0.05

	Islets cell stain N%	Endothelial cells stain N%	P value
Positive	3(33.3%)	5(55.6%)	0.0047*
Negative	6(66.7%)	4(44.4%)	

positively stained in 55.6% of the treated group. This finding was highly significant (p<0.01).

Discussion

In the current study hyperglycemia significantly improved in the treated group as the blood glucose level was significantly lowered relative to untreated group at the end of the study (277.9 mg/dl±102.5 vs 530.3 mg/dl±99, p< 0.0 1). This result comes in conformity with that reported by others [5] as they demonstrated that after 4 days of HUCB mononuclear cells transplantation into STZ diabetic mice, serum glucose level was significantly decreased in comparison with pre-transplantation. In another study [6] diabetic mice were treated with human bone marrow stromal cells and they demonstrated that human bone marrow stromal cells lowered blood glucose levels in the treated diabetic mice relative to untreated controls (330.12 mg/dl± 20.16 SE vs 500 mg/dl±44.1 SE, P=0.0019). Others [3] transplanted HUCB mononuclear cells into NOD mice and demonstrated that, among the treated mice, the glucose level was significantly lower in the mice receiving high dosage of HUCB cells (untreated: 440 mg/dl±68; treated ($100–150\times 10^6$ cells): 310 mg/dl±82; and treated (200×10^6 cells): 170 mg/dl±42). One study [7] reported that blood glucose levels increased with duration of diabetes in control mice, reaching up to 344±1.88 mg/dL. In contrast, the blood glucose levels were significantly lower (P<0.001) in trans-planted mice. At the time of sacrifice, the blood glucose levels were 262±1.76 mg/dL. One study implanted one million mouse ES-derived insulin-secreting cells into the spleen of diabetic mice and reported normalization of blood glucose level within a week [8].

We found that the improvement of hyperglycemia was reversible and relapse occurred after 3 weeks, this finding was reported by many investigators [5, 8] as they demonstrated that after 14 days of improvement, serum glucose level was returned to the pre-transplantation level. Others reported that the relapse occurred in 40% of the transplanted animals after 12 weeks. The difference in duration may be explained by the different types of stem cells used in the transplantation, and different method of transplantation. It is suggested that this finding is probably depending on the half-life of the implanted cell cluster.

The possible explanation of the relapse of hyperglycemia in the transplanted mice might be that hematopoietic stem

cells self-renewal is not a perfect process and daughter cells have progressively reduced proliferative capacity,

due in part to progressive telomere erosion at each cell division. This, in turn, leads to proliferative senescence that can be observed both in vivo and in vitro. The best-characterized compensatory mechanism for maintenance of telomere length is mediated by telomerase enzyme that synthesizes terminal telomere repeats. CD34+ cells exhibit low telo-merase activity which can be transiently upregulated upon cytokine stimulation but activity increases significantly in both HSC and progenitor cells during the progression in the cell cycle. In studies using cytokine combinations that were effective in activating stem cells into the cell cycle, but were less effective in maintaining long-term HSC prolifer-ation, telomerase was up-regulated at early stages of culture but declined rapidly after 3 to 4 weeks [9]. Another possible interpretation of the relapse of hyperglycemia is rejection of the transplanted cells as reported by [10] who transplanted diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells and found that islet grafts could reduce the hyperglycemia of diabetic rats and after day 20, glucose levels increased again which was ascribed to allograft rejection.

In the present study serum insulin was significantly higher in the treated group than untreated group. This observation was reported by many researchers [5, 6, 8, 11].

In the current study we found that the treated group had significantly more pancreatic islets per section than untreated group, the pancreatic islets in treated group were larger and contained more cells, while the untreated group had smaller islets with fibrosis and lymphocyte infiltration. This finding was in agreement with [6] who stated that pancreases from the STZ-diabetic mice contained smaller islets and a decreased number of islets per section. In pancreases from treated diabetic mice, the islets appeared larger compared with islets from untreated diabetic mice. Also, there was an increase in number of islets per section.

Human insulin was detected in the pancreatic islets in 33% of treated mice, this finding was based on immuno-histochemical study using primary antibody against human insulin that gave positive result with human β cells in positive control and gave negative result with normal mouse β cells in negative control. This finding indicates that human umbilical cord blood CD34+ cells might change to insulin secreting cells in vivo and contribute in improvement of hyperglycemia in diabetic mice.

This finding comes in conformity with the result of a study that was demonstrated that bone marrow harbors cells have the capacity to differentiate into functionally compe-tent pancreatic endocrine β cells and that represent a source for cell-based treatment of diabetes mellitus [12] others found that the transplantation of bone marrow cells after diabetes induction with STZ supports the recovery of pancreatic cell mass and partial reversal of hyperglycemia in mice and noted that after STZ administration, up to 2.5% of insulin-positive cells were donor marker positive. Also found that up to 9% of donor cells in the pancreases of STZ-administered animals stained positive for the vascular marker platelet endothelial cell adhesion molecules and suggested that bone marrow cells might differentiate into endothelial cells within islets capillaries and secrete factors that might contribute to the improvement of β cells function and the reduction of diabetic hyperglycemia [11]. Another researchers isolated and characterized single cell-derived stem cell lines obtained from mouse BM and demonstrated that in vitro differentiation of these cells resulted in populations meeting a number of criteria set forth to define functional insulin-producing cells. These cells were transplanted into streptozotocin-induced diabetic mice and caused reversal of hyperglycemia [13]. In contrast to previous studies, other researchers demonstrated that there is no evidence for significant transdifferentiation of bone marrow into pancreatic cells in vivo neither under steady-state conditions nor after tissue injury through partial pancreatectomy or STZ administration. It has been suggested that experimental conditions, such as the trans-plantation protocol, the method used for the identification of cell phenotype and the model of pancreatic damage might explain these differences [14– 16].

Endothelial cells within the islets capillaries were positively stained with anti-human insulin in more than 50% of the treated group, we suggested that HUCB CD34+ cells might differentiate into endothelial cells and secrete insulin. Insulin secretion from non-β cells was reported by in a study [17] where they found widespread insulin mRNA and protein expression in different diabetic mouse and rat models, including streptozotocin-treated mice and rats. They detected proinsulin- and insulin-positive cells in the liver, adipose tissue, spleen, bone marrow, and thymus of diabetic mice and showed that 3-day exposure of mice to elevated blood glucose was sufficient to activate extrap-ancreatic insulin-1 and insulin-2 gene expression. Another alternative explanation of this finding is that the presence of insulin in endothelial cells was a consequence of cellular uptake, not expression [18]. This finding may be also explained by non specific staining occurred with paraffin embedded specimens.

The observations made here raise the possibility that HUCB CD34+ cells transplantation supports the recovery

of pancreatic islets and the endogenous regeneration of pancreatic islets causes the observed improvement in blood glucose level and CD34+ cells give origin to a few human insulin-positive cells in vivo.

In conclusion, transplantation of human umbilical cord blood CD34+ cells appears to be a modality of stem cell therapy in diabetes mellitus.

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Int J Diab Dev Ctries

ORIGINAL ARTICLE

Difference in prevalence of diabetes, obesity, metabolic syndrome and associated cardiovascular risk factors in a rural area of Tamil Nadu and an urban area of Delhi

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Abstract This study compared difference in diabetes, obesity, metabolic syndrome (MetS), C-reactive protein (hs-CRP), homocysteine, and other cardiovascular risk factors between rural and urban Asian Indians using similar/standardized field measurements. The design used a cross-sectional and population-based study among rural (Tamil Nadu) and urban (Delhi) Asian Indians aged 18 years and older. 574 rural Indians and 508 urban Indians completed face-to-face interviews, and anthropometric measurements. Fasting venous blood samples were obtained for fasting plasma glucose and serum lipid tests. The mean age was 42.6±11.8 y (urban) and 39.5±13.9 y (rural). Although the prevalence of type 2 diabetes (T2DM) was lower in rural (8.4%) than urban (13.6%) areas, rural Asian Indians had a higher percent of undiagnosed cases (25%), poorer glycemic control, and unawareness of diabetes than their urban peers. Urban Indians had elevated rates of the MetS (as defined by NCEP and IDF criteria), hs-CRP, total cholesterol, LDL, and hypertension than their rural peers. Females in general had significantly higher central obesity and lower HDL-C than males. Homocysteine levels (measured only among urban respondents) was higher among males than females ($p=.04$). Prevalence of hypertension increased with age ($r=.37$, $p<.001$) and correlated with respondents' blood glucose levels ($r=.11$, $p<.001$). There was a step-wise worsening of risk factors as individuals progressed from normal to IFG to T2DM. High burden of diabetes and other cardiovascular risk factors in urban and rural Asian Indians provide basis for tailored and cost-effective prevention and intervention programs, in such resource-constrained settings.

Keywords Diabetes . Prevalence . C-reactive protein . Metabolic syndrome . Urban . Rural . India

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Background

The burden of diabetes is more pronounced in India than any other country in the world [1]. An estimated 46.5 million Indians have diabetes (2007) with a projected increase to 80 million by 2030 [1].Type 2 diabetes mellitus (T2DM) occurs a decade earlier than in other ethnic groups and may lead to more complications [2, 3]. Increased urbanization, nutrition and lifestyle transitions are associated with the rapid rise of T2DM in urban as well as rural areas [4-6]. In particular, affluence and automation has resulted in pronounced physical inactivity and consumption of diets rich in fat, sugar and calories [7].

With more than a billion people, India is home to significantly diverse groups of people in terms of ethnicity, caste and religion, socioeconomic status, educational level, and lifestyle and food habits [6]. Approximately 70% of India's population lives in rural areas and in resource-poor settings where the increasing prevalence and chronic nature of T2DM poses considerable health and economic burden [3]. Studies consistently show high rates of T2DM and the Metabolic Syndrome (MetS) and cardiovascular disease (CVD) in the Asian Indian population as compared to Caucasians and other ethnic groups [8-11]. Insulin resistance, a key pathophysiologic factor, is highly prevalent in Asian Indians, despite low rates of obesity [9, 12-14]. Impaired glucose tolerance is more common in Asian Indians at a young age. Most of the studies have been carried out on the prevalence of diabetes in urban areas [15-20] with a few in rural areas [21-23]. The study by Mahadik et al. [9] and Zargar et al. [15] examined both rural and urban populations but the former study assessed only MetS and the later was limited to Kashmir state in North India. The prevalence of diabetes in India study (PODIS) [6] is the only country-wide prevalence study on diabetes that took into account the marked heterogeneity of the Indian population.

The purpose of this population-based study was to determine the prevalence of pre-diabetes, T2DM, MetS and association with hs-CRP, homocysteine, and lipid profile in rural and urban sites in India using similar design and field measures.

Methods

Subjects

A cross-sectional and population-based study design was used among rural and urban Indians ≥18 years. The period of study was 2005 to 2007. Urban sample was selected based on multi-stage cluster sampling technique, according to the modified World Health Organization Expanded Program of Immunization Sampling Plan [24]. First, a list of residential colonies within 10 km of the All India Institute of Medical Sciences was prepared. Colonies were randomly selected from this list for inclusion. The number of houses in each colony was determined and the Resident Welfare Associations of these colonies were approached to conduct the study in their locality. The households were selected randomly and one member from each was invited to participate in the study. 900 urban respondents from a wide range of socioeconomic strata were contacted for participation and 610 completed the face-to-face interviews (response rate 67.7%) and 508 (56.4%) completed anthropometric measurements, and provided venous blood sample after an overnight fast of at least 8 h for blood tests. The questionnaire (in both Hindi and English) used for the study was initially pretested in a smaller population (n=10 in each site) and validated. Most of the subjects who refused to participate in Delhi did not want to spare time for the survey and blood work since they had to report to work early in morning.

In the rural site, sample was randomly selected from eight hamlets (out of 30) classified as 'rural' by the Indian government land records. 850 individuals were contacted through door-to-door visitations, 599 rural Indians participated in the face-to-face interviews by trained interviewers and 574 completed the anthropometric measurements and fasting blood work (response rate 67.5%) at the Gandhigram Rural Institute. Most of the respondents who refused to participate in the rural areas were migrant workers. The All India Institute of Medical Sciences (AIIMS), Delhi was used for urban Indians and also served as the core laboratory for biochemical analysis. The study was approved by the Institutional Review Board of Texas A & M University and AIIMS.

Data collection

Information was collected on demographic profile, anthropometrics, T2DM and CVD risk factors e.g., blood pressure, smoking, generalized and abdominal obesity, fasting blood glucose values, serum lipids, fasting plasma insulin, and high-sensitivity C-reactive protein (hs-CRP) levels. The primary endpoint of the study was the prevalence of diabetes, MetS, and CVD risk. Prior to beginning the study, a workshop was held to train the field staff about the anthropometric measurements. The measurements were taken by one observer each for males and females and the intra- and inter-observer variation was less than 10%. Study protocol and data collection procedures were standardized, calibrated equipments used at both sites, and all research assistants were trained at AIIMs and monitored during the study period.

Demographic profile Comprised of age, gender, marital status, education, employment status, income, and personal and family history of diabetes and chronic diseases.

Anthropometrics profile Height, weight, waist and hip circumference.

Fasting blood glucose and serum insulin test Venous blood sample was obtained after an overnight fast of at least 8 h for fasting plasma glucose (FPG). Subjects were diagnosed to have diabetes if FPG was >=126 mg%. Plasma insulin was measured using the Radio Immunoassay procedure (RIA; Linco Research, Inc., St. Louis, MO), and glycosy-

lated hemoglobin (HbA1c) was measured using the Thiobarbituric acid method.

Serum lipids Serum levels of total cholesterol, triglycerides and HDL-c were estimated using commercially available reagent kits (Randox Laboratory, San Francisco, CA, USA) on a semi-automated analyzer (das srl, palombara, Sabina, Italy). Value of LDL-c was calculated using the Friedewald's equation.

Definitions Overweight and obesity was defined as BMI ≥23 kg/m^2 and ≥25 kg/m^2 respectively [25]. Abdominal obesity was defined using the cut-off points of waist circumference (WC) as defined by NCEP, ATP III and also according to the Asian Indians and WHO Asian Pacific Guidelines specific cut-off points [26]. High waist-to-hip circumference ratio (W-HR) was defined as >0.90 in males and >0.80 in females [27]. Diabetes was labelled if FPG was >=126 mg% and/or a self-reported admission to the question "Have you ever been told by a doctor or health professional that you have diabetes or are on treatment for diabetes". Hypertension was defined as blood pressure >140/90 mmHg and/or self-reported admission for the question "Have you ever been told by a doctor or health professional that you have hypertension or high blood pressure?" Subjects were classified as the MetS according to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III criteria): (1) WC, men >102 cm, women >88 cm; (2) triglyceride level ≥150 mg/dL; (3) HDL cholesterol (HDL-C), men, <40 mg/dL, women, 50 mg/dL; (4) blood pressure ≥130/85 mmHg or known treatment for hypertension; and (5) fasting glucose level of ≥100 mg/dL or known treatment for diabetes. For each criterion, subjects received a score of 1 if present or 0 if absent, therefore allowing a range of 0-5. A score of ≥3 indicated MetS. International Diabetes Federation (IDF) criteria: central obesity for South Asians (WC, men >90 cm, women >80 cm), plus any two of the following four criteria: (1) triglyceride level ≥150 mg/dL; (2) HDL-C, men <40 mg/dL, women <50 mg/dL ; (3) blood pressure ≥130/85 mmHg or known treatment for hypertension; and (4) fasting glucose level of ≥100 mg/dL or known treatment for diabetes. For each criterion, subjects received a score of 1 if present or 0 if absent, therefore allowing a range of 0-5.

High-sensitivity C-reactive protein Elevated level of hs-CRP was defined as >1.0 mg/dL [28].

Statistical analysis

The first stage of analysis was to assess normal distribution of variables, descriptive statistics and differences by rural and urban Indians and data expressed as mean (SD) and proportions of the sample. The prevalence and mean (SD) of obesity, abdominal obesity, T2DM and the MetS was determined for rural and urban subjects. Analysis of Covariance (ANCOVA) (adjusted for age since there was difference in age distribution between the two groups) was used to compute all indicators. All analyses were done with the SPSS system (version 17.0). The sample size was based on power calculations for an unadjusted pair wise comparison of MetS and T2DM between rural and urban Indians. The power was set to 80% with a goal to detect differences in mean for our primary outcomes (type 1 error rate of 0.05, two-tailed significance tests). Calculations indicated that 956 participants would provide over 80% power to detect important rural urban differences in MetS, T2DM and CVD risk factors.

Results

Demographic & socioeconomic characteristics

The mean age was 39.5±13.9 y, 18-88 y (rural) and 42.6± 11.8 y 18-77 y (urban). The majority of respondents (>75%) were married. Level of education varied between sites with 84.7% of rural Indians and 53.5% of urban Indians reporting a high school diploma or below. The modal income was ≥$200/ month for urban Indians and <$25/month for rural Indians. However, 86% of rural Indians and 9% of urban Indians had income below Rs 3,500/month (approximately $70.00). The majority of urban and rural Indians were employed or self-employed (56% and 53% respectively); 37% and 33% of urban and rural respondents were homemakers. Twenty four percent of urban respondents and 19% of rural respondents reported a family history of diabetes (includes siblings and parents). Thirty percent of rural Indians and 28% of urban Indians used some form of tobacco (sometimes or always; cigarettes, beedi, gudka, snuff, and tobacco with Betel leaves). Significantly more rural women (20%) used tobacco than urban women (5.2%).

Prevalence of pre-diabetes and diabetes

Urban Indians had significantly higher rate of diabetes and pre-diabetes (p=.002 and .008 respectively). Among the urban Indians, 22% of the respondents had pre-diabetes (Table 1) and 13.6% had T2DM (11.2% with known diabetes and 2.4% with undiagnosed diabetes). Approximately one-fifth (17.4%) of the T2DM patients did not know they had the disease; 50% and 16.6% of the undiagnosed cases had HbA1c greater than 7.0 and 8.0%, respectively. The prevalence of pre-diabetes and diabetes in the rural sample was 12.5% and 8.4% (6.3% with known

Table 1 Prevalence of diabetes and pre-diabetes in rural and urban Indians

Group	Urban Indians in New Delhi		Rural Indians in Tamil Nadu		Urban rural difference
	(n=508) Prevalence (% frequency)	(n=508) Blood glucose Mean (SD)	(n=574) Prevalence (% frequency)	(n=574) Blood glucose Mean (SD)	χ^2value (p-value)
Normoglycemia	64.6 (328)	84.6 (9.3)	79.1 (450)	82.7 (10.1)	2.56 (.011)
Prevalence. of pre-diabetes	21.9 (111)	108.2 (8.2)	12.5 (71)	105.3 (5.0)	2.67 (.008)
Gender					
Males	25.9 (66)	107.6 (6.9)	13.6 (24)	105.3 (5.76)	11.40 (.001)
Females	17.8 (45)	108.9 (9.7)	12.0(47)	105.2 (4.6)	
Age groups					
18-29 years	13.5 (15)	107.6 (3.3)	17.1 (12)	103.2 (2.3)	0.73 (.692)
30-39 years	27.9 (31)	109.5 (7.2)	24.3 (17)	105.1 (3.9)	
40-49 years	31.5 (35)	107.5 (7.5)	31.4 (22)	105.9 (6.4)	
50-59 years	18.0 (20)	108.5 (4.5)	14.3 (10)	105.6 (5.1)	
≥60 years	9.0 (10)	109.6 (7.6)	12.9 (9)	107.1 (5.5)	
Prevalence. of diabetes	13.6 (69)	144.2 (46.3)	8.4 (48)	180.7 (76.7)	3.19 (.002)
Known diabetes	11.3 (57)	142.6 (47.1)	6.3 (36)	182 (78.9)	
Undiagnosed diabetes	2.4 (12)	152.2 (43.5)	2.1 (12)	174.4 (72.3)	
Gender					
Males	13.3 (34)	141.0 (46.8)	10.4 (18)	199.4 (94.2)	1.59 (.207)
Females	13.8 (35)	147.3 (46.3)	7.6 (30)	169.4 (63.1)	
Age groups					
18-29 years	1.4 (1)	157.0 (0)	4.2 (2)	103.5 (31.8)	1.17 (.882)
30-39 years	14.5 (10)	124.5 (55.2)	14.6 (7)	111.4 (46.2)	
40-49 years	30.4 (21)	132.6 (26.9)	25.0 (12)	159.5 (59.4)	
50-59 years	29.0 (20)	155.8 (62.2)	29.3 (14)	185.5 (52.1)	
≥60 years	24.6 (17)	144.3(46.3)	27.1 (13)	206.3 (84.3)	

diabetes and 2.1% with undiagnosed diabetes) respectively. One-fourth (25.0%) of the T2DM individuals were unaware they had the disease; 91.7% and 49.8% of the undiagnosed cases had HbA1c greater than 7.0 and 8.0% respectively.

Mean HbA1c was significantly higher among rural Indians with T2DM than urban Indians (p<.001). Although no gender differences were noted in rural areas, urban males (7.27±1.24) had poor glycemic control (p=0.90) as compared to their female peers (6.80±0.99). The prevalence of diabetes in different age groups showed an increase with age while the prevalence of IFG did not change (p<.001).

Obesity, hypertension, and the metabolic syndrome

The mean BMI (kg/m^2) was 24.7±4.8 and 21.3±4.0 for urban and rural respondents respectively. According to the Asian criteria, 16% of urban Indians and 14% of the rural Indians were overweight and 50% of urban Indians and 18% of rural Indians were obese. The mean WC was 89.6± 13.0 and 74.8±11.3 for urban and rural Indians respectively (Table 2). Using WC criterion, 66% of urban respondents and 24% of rural respondents had central obesity, significantly higher among females (76%, 27%) than males (57%, 17%) in both urban and rural areas. The mean WHR was 0.94±.09 and 0.84±.08 for urban and rural respondents respectively; WHR was elevated in 40% of urban & 16% of rural males and 43% of urban & 36% of rural females. Men and women living in urban areas had higher BMI, WC and W-HR, and were more likely to be overweight or obese than rural subjects (Table 3).

Prevalence of pre-hypertension (Urban, 38% systolic & 44% diastolic; Rural, 30% systolic & 20% diastolic) and hypertension (Urban, 20% systolic and 24% diastolic; Rural 9% systolic and 7% diastolic) was significantly higher (p=.001) among urban Indians than rural Indians, and increased with age (p=.001). There was a linear (significant) increase in systolic and diastolic blood pressure with the increase in fasting blood glucose levels. Approximately one-third (30.3%, males, 34.5%, females, 25.8%; p=0.03) of urban and 12.0% (males, 10.2%, females, 12.8%) of rural subjects were hypertensive. The

Table 2 Metabolic syndrome among rural and urban Indians by ATP/ NCEP III and IDF criteria

Metabolic syndrome	Indicator components	Total sample (n=508)		Male	Female	Gender difference[a]	Rural-urban difference[a]
		Mean (SD)	% at risk	(n=255)	(n=253)	T-value (p-value)	T-value (p-value)
Urban Indians				Male	Female	Urban Indians	
Blood pressure[b]	≥130/85 mmHg	123/82	42.9%	45.1%	40.6%	9.98 (0.327)	14.40 (<.001)
HDL[c]	≤40 mg/dl in M	46.93 (5.7)	40.0%	4.7%	68.0%	19.67 (<.001)	15.56 (<.001)
HDL[c]	≤50 mg/dl in W						
FBG[d]	≥100 mg/dl	97.82 (28.1)	35.6%	39.2%	31.9%	1.72 (0.085)	1.82 (.068)
Serum triglyceride[e]	≥150 mg/dl	146.50 (65.3)	40.0%	43.1%	36.8%	1.46 (0.143)	6.37 (<.001)
Waist circumference[f]	≥102 cm in M	89.61 (13.1)	38.6%	18.4%	58.9%	10.27 (<.001)	15.52 (<.001)
Waist circumference[f]	≥88 cm in W						
Waist circumference[g]	≥90 cm in M	74.75 (11.4)	66.3%	56.5%	76.3%	4.82 (<.001)	20.00 (<.001)
Waist circumference[g]	≥80 cm in W						
NCEP[b,c,d,e,f]	Met S%	30.8%		17.6%	44.2%	7.53 (<.001)	87.48 (<.001)
IDF[b,c,d,f,g]		39.2%		30.6%	47.8%	4.03 (<.001)	122.09 (<.001)
Rural Indians				Male	Female		
				(n=177)	(n=397)		
Blood pressure[b]	≥130/85 mmHg	115/73	18.6%	16.4%	19.6%	0.92 (0.355)	
HDL[c]	<40 mg/dl in M	52.04 (5.0)	27.9%	0%	40.5%	10.95 (<.001)	
HDL[c]	<50 mg/dl in W						
FBG[d]	≥100 mg/dl	95.42 (47.6)	21.2%	24.3%	19.8%	1.21 (0.225)	
Serum triglyceride[e]	≥150 mg/dl	121.17 (64.9)	22.8%	25.4%	21.6%	1.01 (0.311)	
Waist circumference[f]	≥102 cm in M	74.75 (11.4)	7.7%	3.4%	9.6%	2.58 (0.010)	
Waist circumference[f]	≥88 cm in W						
Waist circumference[g]	≥90 cm in M	74.75 (11.4)	23.9%	16.9%	27.0%	2.77 (0.006)	
Waist circumference[g]	≥80 cm in W						
NCEP[b,c,d,e,f]	Met S%	8.6%		4.6%	10.4%	4.48 (<0.001)	
IDF[b,c,d,f,g]		10.5%		6.2%	12.3%	2.49 (0.013)	

NCEP Criteria: metabolic syndrome is present if any three indicators [b,c,d,e,f] meet the criterion

IDF Criteria: Metabolic syndrome is present if central obesity and any two indicators[b,c,d,f,g] meet the criterion [a]

Significant difference between groups based on Student t-test

[b-g] Presents indicators of Metabolic Syndrome as defined by NCEP and IDF [b]

Blood Pressure ≥130/85 mmHg

[c] HDL <40 mg/dl in Men and <50 mg/dl in Women [d]

FBG ≥100 mg/dl

[e] Serum triglyceride ≥150 mg/dl

[f] Waist Circumference ≥88 cm in Women and ≥102 cm in Men

[g] Waist Circumference ≥80 cm in Women and ≥90 cm in Men

prevalence of the MetS was 30.8% and 8.6% among the urban and rural respondents respectively using the NCEP, ATP III definition, and 39.2% and 10.5% using the IDF definition (Table 2). The prevalence of the MetS is significantly higher among urban females (44.2% and 47.8% using NCEP, ATP III and IDF criteria, respectively) than males (39.2% and 47.8%, respectively); a similar pattern was noted among rural males and females (Table 2). In urban Asian Indians, the prevalence rates were higher for blood pressure (42.9%), serum triglyceride (40%), and low levels of HDL-C (40%; higher among females than males), and abdominal obesity (66.3%). The rates were lower in the rural Indians in all the individual MetS criteria, with the highest abnormalities noted in low levels of HDL-C (27.9%; higher among females than males), triglyceride (22.8%), and abdominal obesity (23.9%). These results show the prevalence of the MetS is significantly higher among urban Indians than rural Indians with significant gender differences in two of the five criteria (cholesterol and abdominal obesity). Both

Int J Diab Dev Ctries

REVIEW ARTICLE

Genetic etiology of type 2 diabetes mellitus: a review

Uma Jyothi Kommoju, Battini Mohan Reddy

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Abstract Type 2 diabetes mellitus (T2DM) is the most common form of diabetes characterized by elevated levels of plasma glucose caused by impairment in both insulin secretion and action. It is becoming an epidemic leading to various complications and its prevalence and incidence are increasing at an alarming rate in developing countries like India, raising a major public health concern. Both the genetic and environmental factors play a strong role in the manifestation of this complex genetic disorder. In the recent years, there has been a spate of molecular genetic investigations, including whole genome scans, to test the association of genetic variants with T2DM in different patho-physiological pathways. A large number of candidate genes have been identified to be associated with T2DM, albeit only a couple of them show consistency in association in different populations/ethnic groups. Given relatively high risk for T2DM in India and immense genetic heterogeneity and substructure of the constituent populations, the number of studies is too small to be able to characterize the genetic basis of the disease in India. The recent dramatic increase in number of affected people indicates that lifestyle factors related to urbanization and sedentary occupations may be particularly important in triggering the genetic elements that cause this type of diabetes. Therefore, it is imperative to precisely establish the underlying genetic and environmental factors behind this complex genetic disorder so that preventive measures can be initiated. We have attempted to review the molecular genetic studies conducted, till date, globally on T2DM along with the epidemiological, environmental and ethnic factors implicated in the manifestation of T2DM.

Keywords Insulin deficiency . Candidate genes . Genome-wide association scan (GWAS) . Ethnic variability . Indian scenario

Methods: Sources for the Articles including Review papers and Original studies were collected from NCBI database (PubMed) by search terms on 'Genetics of T2DM' including most recent papers till 2010. We have also gathered information from other sources including contacting author's personally.

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Introduction

Diabetes mellitus (DM) is a complex heterogeneous group of disorders characterized by persistent hyperglycemia and caused by an absolute or relative deficiency of insulin, which is an anabolic hormone, produced by the beta cells of the islets of Langerhans located in the pancreas. While the World Health Organization (W.H.O) [1, 2] describes DM as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, action or both, the American Diabetic Association (ADA) [3-5] defines DM as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The different types of Diabetes mellitus include Type1 & 2 and rare forms of MODY and Gestational diabetes.

Type 2 diabetes (T2DM) is the most common form of diabetes [1] constituting ~90% of the diabetic population [6]. This is a complex heterogenous disorder characterized by elevated levels of plasma glucose caused by impairment in both insulin secretion and action. It usually begins as insulin resistance disorder in which the cells do not respond to

insulin properly; as the need for insulin rises, the pancreas gradually loses its ability to produce insulin. It is a complex polygenic trait with a number of genes contributing to its susceptibility. It arises as a consequence of actions and interactions of many genetic and non-genetic factors [7] with onset usually in adulthood (>40 years) but recent studies revealed that T2DM is increasing even in children [8].

T2DM has become an epidemic which is the sixth largest cause of death in U.S. It is estimated that there will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in the developing countries. Thus, by the year 2025, 75% of people with diabetes will reside in developing countries, as compared with 62% in 1995. In developing countries, the majority of people with diabetes are in the age range of 45-64 years, as against the, majority age of ≥65 years in developed countries [9, 10]. Asia is the major site of a rapidly emerging epidemic of diabetes with India and China as the leading countries with large diabetic population. It is estimated that in the year 2025, China will contribute almost 38 million people while India with immense population size and high diabetes prevalence, will contribute 57 million to the global burden of diabetes [9, 11].

Studies on Type 2 diabetes are gaining importance, as it is becoming an epidemic leading to various complications, hence a major public health concern. There is a need for early diagnosis, screening, treatment as well as prevention. The recent dramatic increase in the number of affected people indicates that lifestyle factors related to urbanization and sedentary occupations may be particularly important in triggering the genetic elements that cause this type of diabetes. Therefore, it is important to know the underlying genetic aspects of this complex genetic disorder so that preventive measures can be initiated. In this review we focus on the genetic and environmental aspects of Type 2 diabetes mellitus (T2DM).

Genetic and environmental causes of T2DM

Genetic factors

Genetic susceptibility plays a crucial role in the etiology and manifestation of type 2 diabetes but the underlying pattern is complicated, since both impairment of beta cell function and an abnormal response to insulin are involved. The knowledge of the genetic aspects of T2DM is very important due to its scientific, prognostic, prophylactic and therapeutic significance [12]. There is strong evidence from twin, family, and epidemiological studies that genetic factors contribute to the etiology of T2DM [13-15]. The concordance of T2DM in monozygotic twins is about 70% compared with 20-30% in dizygotic twins. The lifetime risk of developing T2DM is 40% in the offspring of one parent with the disease, but the risk approaches 70% if both the parents are affected. Intriguingly, the risk in offspring seems greater if the mother rather than the father has T2DM. A first degree relative has a three-fold increased risk of developing the disease [16-18]. Women with gestational diabetes are at an increased risk of developing type 2 diabetes mellitus after pregnancy, while their offspring are prone to develop childhood obesity, with T2DM later in life. Foetal exposure to maternal diabetes is associated with a higher risk of abnormal glucose homeostasis in the offspring and among patients with gestational diabetes, a higher frequency of diabetes history has been reported in the mothers than in the fathers [19].

Molecular approaches for studying genetic predisposition of T2DM

With the advent of molecular genetic technology and rapid screening methods the focus has shifted to using molecular genetic markers for understanding genetic etiology of this complex disorder. Both nuclear and mitochondrial DNA (mtDNA) polymorphic markers have been implicated in the manifestation of T2DM. The most commonly used molecular approaches are linkage and association studies, using molecular techniques such as PCR - RFLP, Sequencing, Micro-array platforms. The association studies are carried out through the candidate gene approach and genome-wide scans. With advances in gene-mapping technology including Genome wide scans, a number of candidate genes have been identified, which have been further studied to understand their putative role in the etiology of T2DM.

Recently a novel approach has been developed to rank the candidates in the T2DM linked genomic regions as plausible disease genes, which is based on the domains of the interacting proteins of the disease genes and thereby constructing network motifs which represent specific biological functions and hence provide an insight regarding the physiological role of particular candidate. The etiology of T2D, though not very clear, involves multiple pathways wherein each probable disease gene confers only a modest risk. Thus, to understand the disease pathophysiology it is better to explore the global interaction network than single gene identities. These interactions if employed to prioritize genes might considerably increase the chance of detecting disease genes [20].

Linkage studies

The traditional way of mapping disease-causing genes has been to search for linkage between a chromosomal region and a disease by genotyping a large number of polymorphic markers (microsatellites) in affected family members [17].

Linkage-based studies in affected families have shown success in providing consistent results on linkage but they may lack the statistical power and mapping resolution to identify genes affecting common complex traits such as T2DM. Association studies are the alternative approach and are currently considered the method of choice for the identification of genes affecting common complex traits [21].

Association studies: The candidate gene approach

The genetic pre-disposition of T2DM can be known through the candidate gene approach in which variants of a particular candidate gene are analyzed based on it's patho-physiology. The research design may involve a case-control or a family study. Among them, case-control studies have gained importance and are widespread as they facilitate comparison of the risk allele frequency between cases and controls thereby determining whether it is a risk prone or confers protection against T2DM. Variants in genes encoding proteins that play a role in insulin control and glucose homeostasis pathways are excellent candidates for genetic pre-disposition of T2DM [22]. Recent insights into the molecular mechanisms of pancreatic development, insulin signaling, insulin secretion and adipogenesis and consequent physiologic changes in these and in other pathways, which were thought to contribute to diabetes, have lead to an explosion in the number of candidate genes for T2DM [23].

The genetic basis of T2DM is well illustrated in recent review articles [18, 22-26]. A list of candidate genes involved in the etiology of T2DM with respective chromosome locations and genomic regions along with their functions are presented in Table 1. Studies on T2DM candidate genes in different populations along with the variants and study-design are tabulated in Table 2. Figure 1 represents a bar diagram showing number of studies with and without association between T2DM and different candidate genes.

Among the large number of candidate genes, TCF7L2 emerges as one of the most promising genes in T2DM susceptibility. Though CAPN10 is considered as an important candidate gene, most of the studies did not show it as significantly associated with T2DM. PPARγ is another gene of interest in T2DM that shows ethnic variability. Some of the rare candidate genes for T2DM are PPP1R3, KCNJ10, IL-6, KCNQ1, RBP4, PTPN1, CETP, APOE, PGC-1A, LRP, IRS-2,IL-4 and IL-1RN. Except for a few, most of the candidate genes failed to show consistent association with T2DM. This may be due to inconsistent data with differences in study-design, method of study, small sample size and lack of statistical power of the study. Given that most of the studies with positive association are with case-control design, future studies should focus on case-control design with larger sample sizes to detect association of T2DM with various SNPs within each candidate gene.

TCF7L2 is shown to be consistently associated with T2DM in multiple ethnic groups. However, in Chinese population both positive & negative results were observed in the association of TCF7L2 with T2DM [35, 44]. Initially TCF7L2 variants were found associated in Icelandics [27]. Later, meta-analysis revealed strong evidence of association of TCF7L2 and T2DM in Caucasians, North Europeans, East Asians, Indians and Africans [99]. Replicative studies also confirmed that the variants of TCF7L2 were strongly associated with T2DM in the Indian population of Pune [40] and Chennai [41] as well as in migrant Indians [47]. The absence of heterogeneity among the studies suggests universality of the nature of contribution of this gene in the manifestation of T2DM. This situation is unique as other candidate genes for T2DM always show some degree of discrepancy between populations [100]. Though PPARγ shows high ethnic variability it is considered as promising gene as it is involved in the adipocyte differentiation; the frequency of the Ala allele appears to vary according to the ethnic background of the populations. It confers protection against T2DM in Czech republic, [63] Japanese [60, 61] and Caucasians but not in South Asians [64]. Given it's role in insulin secretion and action, CAPN10 is considered as one of the important candidate genes. However, more studies show negative than positive association with T2DM, besides showing ethnic variability in the haplotype combinations that show association with T2DM. While the heterozygous combination of 112/121 haplotype showed significant association in Mexican-Americans, [56] it failed to replicate in Polish, [12] Korean [54] and Scandinavian populations [52]. The other haplotype combinations found associated with T2DM were 121/121 [12] and 111/121 [51, 54] although others did not find these haplotypes to be associated with T2DM [48-50, 53]. The haplotype combination (1112/1121) of CAPN10 was found with increased risk of T2DM in the South Indians [57]. A recent study on Indian population revealed a new haplotype combination (111/112) to be associated with higher risk of T2DM [58]. KCNJ11 is another promising gene for T2DM though only a few studies were hitherto conducted. However, most of those studies show E23K polymorphism to be associated with T2DM [66-68]. The patterns of association of these 4 promising candidate genes are summarized in Table 3. Given the inconsistent nature of the findings, more studies are required before confirming the association of any particular gene with T2DM in a specific population and/or region. The result on the nature of association may differ due to difference in sample size, variation in the frequency of the risk allele(s) and statistical methods used, even among the populations of the same region or ethnic background.

The variants in mitochondrial DNA (mtDNA) polymorphisms also contribute to the pathophysiology of T2DM. An

Table 1 Names of the candidate genes studied for T2DM, along with chromosome location, genomic region and function

Gene ID	Gene Name	C'some Location	Genomic Region	Function
TCF7L2	Transcription factor 7-like2	10q25.3	Intron3,4,7	Blood-glucose homeostasis
CAPN10	Calcium-activated cysteine protease	2q37.3	Intron 3,6,13	Insulin secretion and action
PPARγ	Peroxisome proliferator-activated receptor gamma	3p25	Exon 2	Adipocyte differentiation
KCNJ11	Potassium inwardly rectifying channel	11p15.1	Exon,Promoter,3[1] UTR	Regulation of glucose induced insulin secretion
LPL	Lipoprotein lipase	8p22	Intron3,Promoter region	Insulin secretion and action
PPP1R3	Protein phosphatase1 regulatory subunit 3	7q31.1	Intronic region	Glycogen metabolism
KCNJ10	Potassium inwardly-rectifying channel, subfamily J, member 10	1q22-23	Intron,Promoter region	Insulin secretion
IL6	Interleukin −6	7p21	Promoter region	Insulin resistance
ENPP1 (PC-1)	Ecto nucleotide pyro phosphate	6q22-q23	Exon 4	Insulin receptor signaling
GLUT10/SLC2A10	Glucose Transporter 10	20q12-13.1	Exon,Intron	Glucose metabolism
IPF 1	Insulin promoter factor 1	13q12.1	Regulatory elements	Transcription Factor
HNF1B/TCF2	Hepatocyte nuclear factor 1-Beta	17q12	Exon 4,7	TranscriptionFactor expressed in pancreatic β cell
HNF4A	Hepatocyte nuclear factor 4-alpha	20q12-13.1	Intron2,Promoter region	Development of pancreas
PGC-1α	Peroxisome proliferator Activated receptor γ co-activator - 1 alpha	4p15.1	Exonic,3[1] UTR	Transcription co-activator
IRS-1	Insulin receptor substrate 1	2q35-36.1	Exonic region	Insulin signalling
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1	11p15.5	Intron 15	Insulin secretion
RBP4	Retinol binding protein	10q23-24	Promoter region	glucose uptake in adipocytes to systemic insulin resistance
PTPN1	Protein Tyrosine Phosphate	20q13.13	Intron 1	Insulin Signalling
CETP	Cholestrylester transferase	16q13	Intron1 Exon 15	Lipid metabolism
APOE	Apolipo protein	19q13	-	Lipid metabolism
GLUT2	Glucose transporter 2	3q26	Promoter region	Glucose uptake in pancreatic β cells & liver
FTO	Fat mass obesity associated	16q12.2	Intron	-
LRP	Lipoprotein related protein	1p31	Exon/Intron	Regulates adipose-tissue mass
IRS-2	Insulin receptor substrate 2	13q34	Exonic region	Insulin Signalling
IL-4	Interleukin-4	5q31.1	Intron3	Anti-Inflammatory Cytokine
ILRN-1	Interleukin 1 receptor antagonist	2q14.2	Exon2	IL-1 Inhibitor, beta cell function

A to G transversion mutation in the $tRNA^{Leu\ (UUR)}$ gene is found to be associated in about 1.5% of the diabetic population in different countries and races. The underlying pathomechanism is probably a delayed insulin secretion due to an impaired mitochondrial ATP production due to mtDNA defect [101]. Haplogroup J1 is also found to be associated with T2DM [102], whereas N9a confers resistance against T2DM in Asians [103]. mtDNA variants G10398A and T16189C were found susceptible to T2DM in north Indian population [104].

Genome wide scans/Association studies on T2DM

To overcome the shortcomings of candidate gene studies, investigators have applied a genome-wide linkage scan strategy in which regularly spaced markers are traced in families and sibling pairs for segregation with T2DM [22]. With advances in micro-array technology of high throughput screening of gene expression and information provided in databases such as Human Genome databases, SNP databases and haplotype maps combined with advances in large scale genotyping methodologies, help in identifying more novel genes for T2DM and better understanding of the genetic nature of the disease [18, 105]. The first major GWAS performed in French population identified new loci such as SLC30A8, HHEX and EXT2, containing variants that confer risk to T2DM, in addition to the known association of TCF7L2 [106]. Recent genome wide association studies (GWAS) that identified new susceptible loci are discussed in Table 4.

Table 2 The names of populations and study design in different candidate gene association studies of T2DM

Gene ID	Variants	Study design*	Population
TCF7L2	SNP	CC	Icelandic [27], Finnish [28], Italy [29], U.S [30, 31], Amish [32], Polish [33], Scandinavian [34], Han Chinese [35], Hongkong Chinese [36], Japenese [37, 38], Northern Sweden [39], Indians [40, 41], UK [42], Dutch [43], Chinese [44], Saudi [45], Brazil [46], UK resident South Asians [47]
CAPN10	SNP 43,19, 63 & 44	CC,F	Finnish [48], Samoan Polynesia [49], U.K [50], Polish [12], Caucasians [51], ScandinavianCaucasians [52], Chinese [53], Korean [54], Han Chinese [55], Mexican-American [56], South Asians [57], Eastern Indians [58].
PPARγ	Pro12ala	CC	Italian Caucasian [59], Japenese [60, 61], Finnish [62], Czech republic [63], South Asian in Dallas & Chennai [64]
KCNJ11	E23K	CC,F	U.K [65, 66], Korean [67], Saudi [68]
LPL	T93G, G53C	CC	Asian Indian (Chennai) [69], Koreans [70]
PPP1R3	ARE-1,ARE-2	CC	Japenese [71]
KCNJ10	SNP	CC	Pima Indians [72]
IL6	SNP	CC	U.S [73]
ENPP1 (PC-1)	K121Q	CC,F	Danish Caucasian [74], South Asians& Caucasians [75], Chinese [76], Korean [77]
GLUT10/SLC2A10	SNP	CC	Danish [78], Caucasians Americans [79]
IPF 1	PH1,PH2,PH3	CC	African Americans [80]
HNF1B/TCF2	S465R	CC	Japanese [81]
HNF4A	P2 Promoter	CC,F	Japenese [82], Finnish [83]
PGC-1α	Thr394Thr Gly482Ser	CC	Asian Indians [84], NorthIndians [85]
IRS1	G972R	CC	Finnish [86], Pima Indians [87], North American Polish & Scandinavian [88]
KCNQ1	SNP	CC	Chinese [89]
RBP4	G803A	CC	Rotterdam [90]
PTPN1	SNP	CC	Pima Indians [91]
CETP	Taq1B	CC	North Indians [92]
APOE	HhaI	CC	North Indians [92]
GLUT2	Nucleotide variants	CC	Danish Caucasian [93]
FTO	SNP	CC,F	India (Pune) [94]
LRP- 5, LRP-6	SNP	CC	Japenese [95]
IRS-2	G1057D	CC	India (Chennai) [96], Finnish & Chinese [97]
IL4, IL-1RN	VNTR	CC	NorthIndians [98]

*F family; CC case control

The new susceptible loci to T2DM identified through GWAS and showed consistent results in different populations are SLC30A8, HHEX, CDKN2B, IGF2BP2 and CDKAL1, in addition to the known genes such as TCF7L2 and PPARG. There may be still many susceptibility genes yet to be identified. Replication studies and Meta analysis provide support for involvement of a gene variant in disease risk and determines the susceptibility genes among the candidate genes. Recently a broad—based genetic re-analysis/meta-analysis of GWAS studies has been done which identified 12 new T2DM loci (BCL11A, ZBED3, KLF14, TP53INP1,CHCHD9, KCNQ1,CENTD2, HMGA2, HNF1A, ZFAND6,PRC1, DUSP9) [116]. The power to detect an association depends on the number of case and control subjects, the prevalence of disease and the effect of the associated alleles [91]. Most of the studies seem to fail due to lack of statistical power of the data. A complication of GWAS is the enormous number of tests of association required (at least one per SNP); thresholds of statistical significance are stringent, making it necessary to work with very large samples. One frequently used approach to managing size is the tiered design, in which a subset of SNPs found to be significant in the genomewide association study (sometimes called the discovery set) is genotyped in a second tier (a replication set), yielding a smaller subset of significantly associated SNPs that are then tested in a third tier (a second replication set), and so on. This process helps to identify false positive associations. Carrying forward a large number of SNPs identified through a genomewide association study into a test of

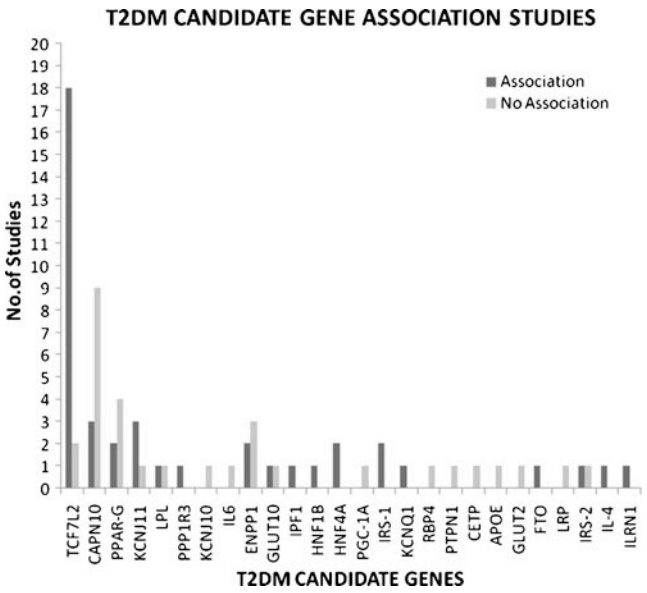

Fig. 1 Graphical representation of the status of association of different candidate genes with T2DM

replication also minimizes false negative results while raising the bar for the establishment of true positive results. The pooling of results obtained in genomewide association studies with the support of large consortia is often required for the detection of variants with small effects on the risk of disease. Such pooled studies, like all genetic association studies, must be examined and controlled for differences in allele frequency between groups that can lead to false positive associations. The most reliable evidence of a true genetic association, is replication of the association in multiple populations, especially in variants whose functional role is not clear [117].

GWAS have proved successful in identifying genetic associations with complex traits. Although this approach has proved powerful in identifying robust associations between many SNPs and traits, much additional work is needed to determine the functional basis for the observed associations so that appropriate interventions can be developed. Much more remains to be learned about how variations in intronic and intergenic regions (where the vast majority of SNP-trait associations reside) influence gene expression, protein coding and disease phenotypes [117].

Environmental factors

The important environmental risk factors for the development of T2DM emanate from life style changes due to urbanization including diet, physical inactivity, stress, smoking and alcohol consumption. The transition to modern diet lead to increased insulin resistance altering the glucose levels in the body along with decreased physical activity and sedentary life styles leading to increased prevalence of the T2DM. Recent studies showed that both active and passive smoking are associated with T2DM [118] as well as the combined effect of alcohol consumption and lifestyle risk behaviors [119].

Fu et al. [120] discussed different methods used in various studies to understand the associative pattern between diet and the risk for T2DM. Reduced fiber intake and increased consumption of animal fats and processed carbohydrates are the main changes in dietary habits of the westernized societies and adopted by migrant populations [121]. In the developing countries like India, energy intake has progressively increased during the last decades [122] and Indian lifestyle changes, especially in the dietary patterns, might be invoked to explain the increased susceptibility to glucose intolerance based on Neel's thrifty genotype hypothesis [123]. Traditional Asian diets are cereal-based, rich in fibre and low in saturated fat, cholesterol and meat. With gradual shift in socio-economic status, changes have taken place in both dietary structures and patterns [122], that triggered manifestation of T2DM resulting in high prevalence of T2DM in recent

Table 3 The most promising candidate genes for T2DM as revealed through their significant association in different ethnic groups

Candidate gene	Most commonly Studied Variants	Odds ratio (OR)	Remarks
TCF7L2	rs 12255372 rs 7903146 rs 7910695	1.04-1.98	Revealed significant and consistent association with T2DM in all populations except in Chinese [44] and Saudi [45].
CAPN10	SNP 43,19,63	0.24-2.58	Significant in Mexican-Americans [56], Indians [57, 58], Polish [49] but not in Han chinese [55], Samon Polynesia [49] and certain Caucasians [51].
PPARγ	pro12ala	0.002-0.66	Protective in Japenese [60, 61], Caucasians [64] but no association in South Asians [64], Italian Caucasians & Reduced risk of T2DM in Czech republic [63].
KCNJ11	E23K	0.93-.18	Significant in Saudis [68] and Koreans [67] but both negative & positive results were obtained in U.K [65, 66].

Table 4 Recent genome-wide association studies that identified new candidate genes

Populations	Study design**	Genes studied through GWAS	Genes strongly associated with T2DM*	Ref No:
EasternFinland, Ashkenzi Jews Germany, England	F	TCF7L2, LOC441171, STK32C	LOC441171	[107]
Japanese	CC	SLC30A8, HHEX, CDKN2B, IGF2BP2, CDKALI	CDKN2B,CDKALI	[108]
Japanese	CC	IGF2BP2,CDKN2A/B,HHEX,SLC30A8,EXT2, FTO,,PPARG,KCNJ11,LOC387761	CDKALI1, IGF2BP2,CDKN2A/B. HHEX,SLC30A8,KCNJ11	[109]
Japanese	CC	TCF7L2,CDKALI,HHEX,IGF2BP2,CDKN2A/B, SLC30A8,KCNJ11	TCF7L2,CDKALI,HHEX,IGF2BP2, CDKN2A/B, SLC30A8	[110]
(Asian ancestry) Hongkong & Korea	CC	TCF7L2,SLC30A8,HHEX,CDKALI,CDKN2A, IGF2BP2, FTO	HHEX,IGF2BP2	[111]
African Americans	CC	PKN2,IGF2BP2,CDKALI,SLC30A8, CDKN2A/B, HHEX,FTO,TCF7L2	CDKALI	[112]
Chinese	CC	PPARG,KCNJ11,CDKN2A-B,HHEX,IGF2BP2 SLC30A8,WFS-1,FTO,JAZF1, TSPANS-LGRS, THADA,ADAMTS9,NOTCH2 ADAM 30, TCF7L2	PPARG,KCNJ11,CDKN2A-2B, HHEX,IGF2BP2, SLC30A8	[113]
Han Chinese	CC	516212 SNP'S	PTPRD, SRR,KCNQ1	[114]
Han Chinese	CC	CDKN2A/B,CDKALI,TCF7L2,TCF2,MCR4, PPARG,JAZF1,HHEX, GCKR IGFEBP2, FTO, KCNJ11, TSPAN8/LGR5,CDC123/CAMKID, ADAM TS9 .	CDKN2A/B, CDKALI, TCF7L2	[115]

*CDKAL1 Cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1; CDKN2A/B Cyclin-dependent kinase inhibitor-2A/B; FTO fat mass- and obesity-associated gene; SLC30A8 solute carrier family 30 member 8; IGF2BP2 Insulin growth factor 2 binding protein 2; HHEX homeobox hematopoietically expressed; PTPR-D Protein Tyrosine Phosphatase; SRR- Serine Racemase

**F family; CC case control

years. Insulin resistance is considered as the characteristic feature of Indians and the influence of diet is described in the review articles [124, 125].

The most important implication of understanding gene-environment interactions is that it can help in suggesting approaches for modifying the effects of deleterious genes by avoiding environmental exposure, as both the genetic variant and environmental exposure must be present to produce disease. The interaction of environment with the genetic predisposition resulted in the manifestation of the disease thus increasing the incidence of chronic diseases particularly T2DM [126]. Most of the studies on T2DM focus on gene-diet (high fat and carbohydrates) interactions [127]. A study on interaction of TCF7L2 gene with dietary carbohydrate showed that quality and quantity of the diet modified risk of T2DM was associated with TCF7L2, which suggests that in situations of high glucose concentrations or insulin demand the changes in risk of T2DM attributable to TCF7L2-associated risk alleles is magnified [128]. A recent study on gene-diet interactions revealed that the IGF2BP2 (rs 4402960) influenced abdominal fat and TCF7L2 (rs12573128) influenced insulin sensitivity. This suggests that gene-dietary fat interactions may influence glucose homeostasis-related phenotypes and play an important role in determining the increased risk of diabetes associated with the T2DM susceptibility genes [129]. PPARG is another T2DM gene showing gene-environment interaction with dietary fats [130]. The interaction between PPARG and birth length of T2DM patients suggests that the manifestation of gene-environment interaction, whereby the genotype has different effects according to intrauterine growth during development, which influences gene expression and disease risk of T2DM [131].

Indian scenario

The knowledge of diabetes mellitus, which was known as Madhumeh among Hindu physicians, dates back to the sixth century AD [132]. India has a high prevalence of diabetes mellitus and the numbers are increasing at an alarming rate. The first major survey on diabetes was conducted by ICMR during 1972-1975 and subsequently two more surveys were done. The National Urban Diabetes Survey (NUDS) in 2001 and The Prevalence of Diabetes in India Study (PODIS) in 2004. NUDS (2001) revealed the highest prevalence of T2DM in the southern part of India (Kerala, Chennai, Bangalore, Hyderabad) when compared to other regions [133]. The CUPS (Chennai urban population study) and CURES (Chennai urban rural epidemiological study) showed age-standardized prevalence rates of 12% for urban India and a community based study was done in Pondicherry Institute of Medical Sciences (PIMS) in which the prevalence of known diabetes was estimated to be 5.6% [134]. These

studies suggest urban populations to show relatively greater frequency of T2DM than the rural populations [133, 135].

The risk factors for developing diabetes among Indians include high familial aggregation, central obesity, insulin resistance and life style changes due to urbanization [136]. The racial-pre-disposition to T2DM is explained by "Asian Indian Phenotype" which refers to certain unique clinical and biochemical abnormalities in Indians such as increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. This phenotype makes Asian Indians more prone to diabetes and premature coronary artery disease [133]. Within India, there are inter-regional difference in the prevalence of T2DM because of the varied lifestyles due to differences in the extent of urbanization and consequent differences between urban (modern lifestyle) and rural populations [137]. The intrusion of western culture into the lives of traditional indigenous communities has also had devastating results in terms of the rise in diabetes and related metabolic disorders [133]. Various studies on migrant Indians have consistently shown higher prevalence of diabetes among them when compared to the indigenous population of the host country. Excessive insulin resistance in migrant Asian Indians appears to be the likely mechanism for the excessive prevalence of diabetes; even a moderate degree of obesity can produce insulin resistance as fat tends to accumulate more in the abdominal region in people of this ethnic group [14, 121, 132]. Although there are no specific studies on gene-environment interactions in Indian populations, the environmental factors like diet and lifestyle changes due to urbanization coupled with Asian Indian Phenotype of high insulin resistance and greater abdominal fat deposition are tangible reasons to expect gene-environment interactions to have had played a significant role in the manifestation of T2DM as well its fast increasing prevalence, especially given evidence of gene-environment interactions in the non-Indian populations discussed earlier (genes with dietary interaction such as TCF7L2, PPARG and IGF2BP2 which influence the glucose levels, abdominal fat and insulin secretion). In this background it is also pertinent that the above genetic variants were found significantly associated with T2DM in the Indian populations [40, 41, 138, 139, 141, 142].

Genetic studies in India

A number of recent studies have examined molecular genetic association of T2DM using candidate gene approach. We have furnished results of these Indian studies in Table 5, which suggest strong role of common variants of T2DM susceptible genes such as TCF7L2, [40, 41, 138, 139, 142] PPARG [64, 139, 142] and CAPN10 [57, 58] and some rare variants of genes such as LPL [69], ENPP1 (PC-1) [75], FTO [94, 139], IRS-2 [96], PGC-1γ [84, 85] and IL-4 & ILRN1 [98].The replicative studies of GWAS also show strong association of KCNJ11, [142] IGF2BP2, [139, 142] CDKAL1, [142] SLC30A8 [142], CDKN2A [142, 144], HHEX [142, 143], CDKN2A/B, [143] and BAZ1 [143]. Of all the genes studied so far, TCF7L2 variants studied so far in the Indian populations (including the migrant Indians from UK [47]) show most significant and consistent association with T2DM, except for rs7901695 and rs12235572 [138]. Even rs 12235572 showed significant association in the Pune [40] and Chennai [41] populations and rs 7901695 in the population of Pune [40]. A recent study with large sample of 5146 of combined data (Delhi & Pune) [142] also showed rs7903146, consistent to the earlier findings in Pune [40]. The haplotype combination (1112/1121) of CAPN10 was found with increased risk of IGT as well as T2DM in the Chennai [57] population. A novel haplotype combination (111/121) of CAPN10 gene as well as co-existence of haplotype 112 with increased risk of T2DM and 121 haplotype with reduced risk of T2DM indicates the existence of risk conferring and protective haplotype in East Indians [58]. A study showed that pro12ala is not protective to T2DM in South Asians [64] but a recent study establish the strong protective effect of Ala allele against the development of type 2 diabetes in Indians [142].

Out of two polymorphisms studied in LPL, T93G was found to be associated with obesity and not with T2DM, G53C is protective against T2DM as well as obesity [69]. Inflammatory cytokines such as IL-4 and IL-1RN were found significantly associated in north Indian populations but failed to find gene-gene interactions with T2DM [98]. PCG-1A polymorphisms (Thr394Thr, Gly482Ser) were also found significantly associated in both north [84] and south Indians [85]. The ENPP1 gene (K121Q) polymorphism also confers susceptibility to T2DM in south Indians [75]. The CETP and APOE polymorphisms failed to show association with T2DM, while D442G polymorphism of CETP was not traced in either cases or controls of North Indian population [92]. The IRS2 G1024D polymorphism was only studied in Chennai population [96], was found to increase the susceptibility to type 2 diabetes in conjunction with obesity.

The polymorphisms in KCNJ11, SLC30A8, HHEX, CDKN2A,CDKALI were also studied among Indian populations. However, the association of these genes could not be validated in the population of Indian Sikhs [139], it was confirmed in the pooled data from Delhi and Pune populations [142]. On the other hand, the CDK5 which was studied only among the North Indian Sikhs [139] did not show any association with T2DM. Further, some rare variants such as FOXA2 [140] and TNFRSF1B [141] were

Table 5 The list of candidate genes studied in different Indian populations along with the polymorphic status and nature of association of different SNPS with T2DM

Serial no	Gene	SNPS studied	Polymorphism	Population	Confers risk/protective to T2DM
1	TCF7L2	rs 7903146	C/T	Pune [40], Chennai [41], Delhi & Pune [142], Khatri Sikhs- North India [138]	confers risk[b]
		rs 12255372	G/T	Pune [40], Chennai [41]	confers risk[b]
				Khatri Sikhs- North India [138]	[d]
		rs 4506565	A/T	Pune [40]	confers risk[b]
		rs 10885409	C/T	Khatri Sikhs- North India [139.138]	confers risk[b]
		rs 11196205	C/G	Khatri Sikhs- North India [138]	[d]
		rs 4918789	G/T	Khatri Sikhs- North India [138]	confers risk[c]
2	CAPN10	SNP 43- rs 3792267	G/A	South Asians - Chennai [57], Kolkata [58]	[d]
		SNP 44- rs 2975760	T/C	South Asians - Chennai [57]	[d]
		SNP 19- rs 3842570	32 bp indel	South Asians - Chennai [57], Kolkata [58]	[d]
		SNP 63- rs 5030952	C/T	South Asians - Chennai [57], Kolkata [58]	confers risk[b]
3	PPAR γ	rs 1801282	Pro12ala	Chennai [64]	[d]
				Delhi& Pune [142], Khatri Sikhs- North India [139]	protective[b]
4	LPL	[a]	T93G	Chennai [69]	confers risk[c]
			G53C		protective[b]
5	FTO	rs 9939609	A/T	Pune & Mysore [94], Khatri Sikhs- North India [139]	confers risk[b]
		rs7191344	A/G	Pune & Mysore [94]	confers risk[b]
6	IL-4	[a]	VNTR	North Indians [98]	confers risk[b]
7	ILRN1	[a]	VNTR	North Indians [98]	confers risk[b]
8	PGC-1α	[a]	Thr394Thr	Chennai [84], Kashmir, Punjab & Jammu [85]	confers risk[b]
			Gly482Ser	Chennai [84],	[d]
				Kashmir,Punjab &Jammu [85]	confers risk[b]
			A2962G	Chennai [84]	[d]
9	ENPP1/PC-1	rs 1044498	K121Q	Chennai [75]	confers risk[b]
10	IGF2BP2	rs 4402960	G/T	Khatri Sikhs- North India [139], Delhi & Pune [142]	confers risk[b]
11	CETP	[a]	Taq1B	North India [92]	confers risk[c]
			D442G		-
12	APOE	[a]	HhaI	North India [92]	[d]
13	IRS-2	rs1805097	G1057D	Chennai [96]	confers risk[b]
14	KCNJ11	rs 5219	E23K	Khatri Sikhs- North India [139]	confers risk[c]
				Delhi & Pune [142]	confers risk[b]
15	SLC30A8	rs13266634	C/T	Khatri Sikhs- North India [139]	[d]
				Delhi & Pune [142]	confers risk[b]
16	HHEX	rs1111875	A/G	Khatri Sikhs- North India [139]	[d]
				Delhi & Pune [142]	confers risk[b]
		rs7923837	A/G	Chennai [143]	confers risk[b]
17	CDKN2A	rs10811661	C/T	Khatri Sikhs- North India [139]	confers risk [c]
				Delhi & Pune [142]	confers risk[b]
18	CDKALI	rs10946398	A/C	Delhi & Pune [142]	confers risk[b]
		rs7756992	A/G	Chennai [143]	confers risk[b]
		rs7754840	C/G	Chennai [143]	confers risk[b]
		rs6931514	A/G	Chennai [143]	confers risk[b]
19	BAZ1B	rs12056034	A/G	Chennai [143]	confers risk[b]
20	CDKN2A/B	rs7020996	C/T	Chennai [143]	confers risk[b]
21	CDK5	rs7754840	C/G	Khatri Sikhs- North India [139]	confers risk[c]
22	FOXA2	rs1212275	C/T	North Indians [140]	[d]

Table 5 (continued)

Serial no	Gene	SNPS studied	Polymorphism	Population	Confers risk/protective to T2DM
		rs1055080	A/G		
		rs6048205	A/G		
		(TCC)n	[a]		confers risk[b]
23	TNFRSF1B	rs 1061622	G/T	North Indians [141]	[d]
		rs 3397	C/T		
		(CA)n	[a]		

[a] Information not available

[b] Significant association

[c] Non-significant Odds Ratio, although a trend of relatively greater frequency of the risk allele in the controls [d]

No association—The allele frequencies are almost identical in cases and controls

In CAPN10 gene, out of the 4 SNPS studied only SNP 63 showed significant association with T2DM. However, the haplotype combinations (1111/1221 and 1111/1121) [57] confer significant risk of T2DM in South Indian population [57]. On the other hand, while the haplotype combination 112 confers risk, 121 is found protective to T2DM in the Eastern Indians [58]

studied. TNFRSF1B polymorphisms did not reveal significant findings, although (TCC)n polymorphism of FOXA2 showed a strong association with T2DM. Out of the 45 SNPs of the AHI1, BAZ1B, CDKAL1, EXT2, HHEX, IGF2BP2, LOC387761, LOC441171, MC4R, MLXIPL, SLC30A8, STK32C, PPARG, and WFS1 genes tested, only BAZ1, CDKALI, CDKALI 2A/B and HHEX showed significant association with T2DM [143] in the population of Chennai, southern India.

Conclusions

Given that T2DM is a complex disorder with gene-environment interactions, the genetic factors may be triggered by the non-genetic factors resulting from rapid urbanization/westernization leading to change in the socio-economic status and lifestyles. The recent transition of these populations to more sedentary occupations and associated food consumption patterns that are atherogenic resulted in triggering the manifestation of many complex genetic disorders such as diabetes, thus increasing their prevalence at an alarming rate, especially in the developing countries like India. With increasing prevalence of T2DM in India, identification of new susceptible loci as well as validation of the genes already implicated in other populations has become imperative. It is necessary to study the effect of environment and ethnicity on the T2DM susceptible genes to better appreciate the "Asian Indian Phenotype" that is uniquely predisposed to develop the disease.

The unique population structure of India presents its population subdivided into strictly defined geographic and linguistic units. Within each geographic and/or linguistic zone the population is subdivided into endogamous and hierarchical castes, tribes and religious groups with rigid cultural and social laws that define the marital rules safeguarding the continued endogamy over generations. Therefore, the caste and tribal boundaries had been highly impermeable enabling caste/tribe specific gene pools to evolve independently over the number of generations. The much greater genetic heterogeneity observed within regional units in India as compared to the Europe is an ample testimony to the historical process of evolution [144]. The sociocultural and environmental variation across India induces variation in the food consumption patterns across populations in addition to the differential impact of urbanization/westernization across the regions and within the populations. This process has created many natural experimental situations in which a single genetically homogenous group presents its population into transitional groups such as urban, semi urban and rural with different degrees of urbanization, which may provide excellent study frame to gauge the role of specific environmental factors in the manifestation of complex disorders in a particular milieu.

The major challenge in identifying the underlying genetic cause of T2DM is the involvement of a large number of genes with small effects and the variable expression of some of the genes in different environments and ethnic groups. Despite rapid improvements in molecular genetic techniques enabling us access to vast array of genetic information, the potential role of many candidate genes involved in the pathogenesis of T2DM remains unclear, although some consistent findings are obtained through candidate gene approach. The need of the hour is to design appropriate GWA studies with large sample size in different ethnic and transitional groups/populations.

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Int J Diab Dev Ctries

EDITORIAL

Will the epidemic of diabetes in India subside?

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The epidemiologists have been prolific in disseminating the data on the cross-sectional and follow-up studies on the high prevalence of diabetes in India and predicting its alarming trends [1– 4]. This journal has carried several articles [5– 10] further bringing out the data on the differ-ences between urban and rural prevalence of diabetes. This issue of the journal contains an article showing the difference in the cardiovascular risk factors in the rural versus urban population [11]. This has served a useful purpose of bringing in focus the double-burden of transiting communicable diseases and newly entering non-communicable diseases. This has helped the government and Indian council of Medical Research to further strategize regarding the prevention of diabetes.

The rising trends of type 2 diabetes, both in the developed and developing world have been predicted mostly by using 2–3 points of observation 5–10 years apart in many countries [12] including in India [13, 14]. These observations are valid for the current period and probably for the near future. However, it will not be scientific to accept them as accurate predictions beyond the coming decade.

There are several important reasons why these predic-tions will be proven to be inaccurate. First of all, we are considering a biological phenomenon, with its inherent variability, which will influence the course of the predictive straight line. It is obviously a dynamic situation and we really do not know where the next point will come to lie. As a matter of fact if you continue to extrapolate the present

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straight line, you will reach a figure of 100% of Indians being diabetic in the distant future. This argument, although facetious, brings out the absurdities of such long term projections. As a matter of fact, CURES [14] has already shown some degree of leveling of the line, evidenced by the fact that the prevalence of diabetes in Chennai showed a rise of 39.8% between 1989–1995, 16.3% between 1995– 2000 and 6% between the year 2000–2004. Numerous dynamic factors that we know and understand and which we do not know can influence the prevalence of diabetes, although hard evidence and quantitation of their impact has not been assessed. (Table 1). So far, not many epidemiol-ogists have seriously looked at these possibilities, except for a brief reference to these factors and showing short term effect of modifying these factors individually and thus producing a favourable environment or behavioural changes. However, whether this will translate into reduced incidence of diabetes has not been studied. Many of the factors in Table 1 likely to influence the prevalence of type 2 diabetes are interdependent, like obesity is dependent on income, education and urbanization. Additionally, there may be many other factors like the quality of air, water, food, and exposure to chemicals influencing the prevalence of diabetes. The demographic structure of the Indian population as reported in the year 2001 [15] showed that 0–19 year age accounted for 45.6% while 50–80 year or above accounted

for 13.4% of the population. The current grim scenario exists inspite of the fact that the Indian population predominantly consists of young people. After 3–4 decades, when again older people account for a large proportion of population, a second wave of diabetes is possible. High prevalence of obesity in adolescents as reported from India [16] will aggravate the situation. These data will be further modified by the changing birth and death rates.

Table 1 Factors likely to influence prevalence of type 2 diabetes

	Factors	Factor likely to increase prevalence	Factor likely to decrease prevalence
1	Demography of the population	Increasing life span	Increase in younger age groups
2	Obesity	Increasing obesity	Decreasing obesity
3	Education	Continuing lower educational status	Rapidly increasing general and health education
4	Income	Increasing income (specially when accompanied by poor educational inputs)	Increasing income (specially when accompanied by enhanced educational inputs)
5	Genetic factors	Increased prevalence till adverse environment has acted on most genetically susceptible population	Stabilizing influence of genetically non-susceptible group
6	Physical activity	Decreased activity due to urbanization and poor built environment	Increased physical activity by introducing healthy lifestyle through education and better town planning
7	Diet	Increased calories, saturated fat, sugar and refined food intake	Decreased calories, saturated fat, sugar and refined food intake
8	Mental stress	High stress with poor quality of life	Peaceful and good quality of life

Influence of enhanced income on the prevalence of obesity and diabetes can be in either direction; when coupled with education the prevalence decreases but increasing income alone increases obesity and presumably diabetes. Women of higher socio-economic status tend to be overweight [17]. However, education has a dampening effect on the cardiovascular risk factors like hypercholes-terolemia, hypertension, obesity and smoking [18].

Urbanisation has a profound effect on the prevalence of diabetes. However, how urbanization leads to the escalation of obesity, is uncertain. It may be due to decreased physical activity and increased intake of saturated fat and calories [19].

Indian epidemiologists, diabetologists and healthcare personnel have not yet articulated the influence of poor built environment on the prevalence of diabetes, except for an occasional article [20]. Elaborating on this aspect may appear to be social activism, but without doing so, it will be difficult for the politicians to realize the importance of the same. Although screening for diabetes, diabetes education and promotion of healthy lifestyle has found a place in the public utterences of the Indian health planners, healthy town planning has not yet been appreciated as an important factor in the prevention of non-communicable diseases.

The genetic factors also require a close scrutiny. Although the diabetogenic genes of T2

DM are not clearly identified, it is accepted that the Asian Indians have a higher genetic susceptibility. Even so, it is possible that a section of the population has either non-susceptible genetic composition or a protective genetic composition against the development of T2 DM. An inkling of this fact is seen in the two important prevention studies [21, 22], where life style intervention led to almost same degree (58%) of prevention of type 2 DM. It can be conceptualized that with the assault of adverse environment factors, a certain percent of genetic population will succumb to diabetes until you

reach a residual population which can resist the disease. This fact will tend to blunt the slope of the diabetes curve. With increasing education, it is fervent hope of this author that the prevalence will get blunted. It is also hoped that if India adopts sound method of education, behavioural changes, and town planning, the second epidemic after 2–3 decades will also not visit its population.

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