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Table 1 Demographic and clinical characteristics of study groups

	Reference	Normo albuminuria		Micro albuminuria		Macro albuminuria	
		Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR
N		175	108	112	162	54	72
Age (years)	46(31–65)	49(30–73)	51(51–77)	50.50(33–68)	51.45(33–68)	50(32–70)	48(32–72)
Men (%)	59	54	52	52	57	50	59
Duration (years)	–	11(1–30)	12(1–28)	13(4–32)	13(5–28)	16(5–29)	17(5–29)
BMI (kg/m^2)	23.33±2.03	26.63±3.42	26.89±4.10	26.13±3.07	26.13±3.31	25.62±5.3	25.63±5.29
Smoker (%)	13.00	9.70	12.03	9.82	9.87	7.40	13.88
Fasting (mg/dl)	83.62±5.45	116.00±18.25	121.83±33.56	169.99±25.46	167.78±25.05	174.96±27	177.59±27.81
2hr PPBG (mg/dl)	116.32±7.02	198.27±32.09	199.43±32.08	249.54±30.63	248.64±33.08	264.03±37.71	272.52±41.07
Glycated Hb %	5.31±0.44	7.63±0.80	8.32±0.99*	9.06±1.88	9.06±1.9	9.69±2.58	9.93±2.55
SBP (mmHg)	115±9	119±7	126±10*	134±16	134±17	148±19	147±21
DBP (mmHg)	75±7	77±4	79±7*	82±13	82±12	88±10	91±10
Cholesterol (mg/dl)	141.32±36.54	175.43±45.160	189.74±40.75	207.05±46.99	211.53±42.55	233.59±61.19	235.08±54.36
Triglycerides (mg/dl)	116.72 (35.07–197.85)	133.01 (71.20–198.61)	136.42 (72.96–207.53) *	152.60 (80.21–359.07)	170.05 (72.150–384.56)	237.11 (91.94–382.95)	217.34 (91.03–395.67)
HDL(mg/dl)	46.66±13.27	44.64±13.23	47.03±13.74	52.05±12.51	52.72±12.60	42.44±13.05	44.83±12.03
LDL (mg/dl)	115.27±29.95	126.99±38.75	132.98±36.10	157.42±47.26	152.04±46.92	163.10±46.94	166.52±47.42
eGFR ($ml/min/1.73\ m^2$)	95.390 (78.55–118.11)	60.07 (39.39–113.44)	51.43 (35.38–89.27) *	49.93 (35.00–99.72)	43.12 (29.62–87.75) *	42.35 (31.27–72.11)	42.18 (26.89–89.43)

Data are means±SD or median (Range). Paired t test for significance: *P<0.05

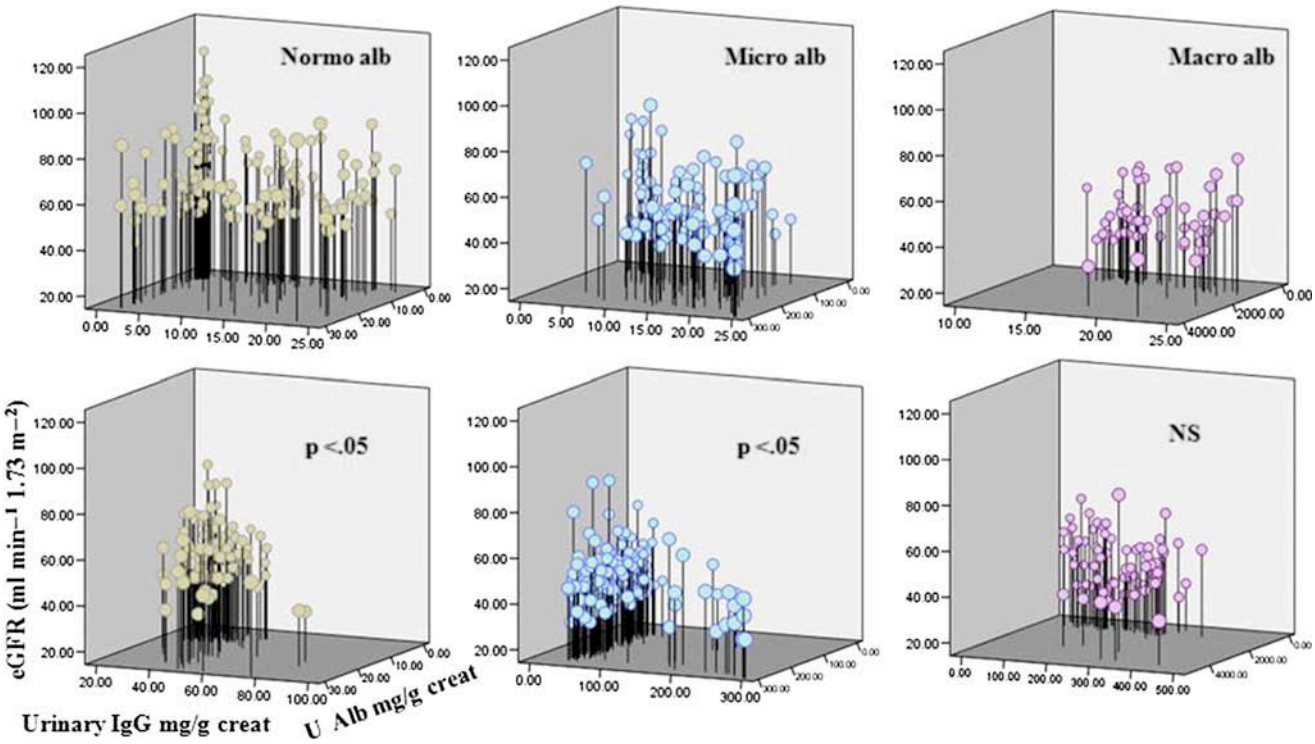

Fig. 2 Showing eGFR in studied groups: normoalbuminuric, micro and macroalbuminuria with low and high UIgGCR respectively. Difference in eGFR was estimated by applying paired t test for each albuminuric group within low and high UIgGCR, P<0.05, which was considered significant

tions through a number of mechanisms, including interaction with their receptors, (RAGEs). A cascade of dramatic events follows this interaction, which include oxidative stress and activation of inflammatory pathways that all cause endothe- lial dysfunction [10]. The levels of AGEs significantly increased with proteinuria which clearly indicated a strong association of proteinuria with higher AGEs. It has been suggested that AGEs leads to proteinuria by multiple mechanisms. Accumulation of AGEs in the ECM occurs on proteins with a slow turnover rate, in addition to this AGE formation have been suggested to increase cross-linking that can trap macromolecules in the vicinity. In this way, AGEs altere the properties of the large matrix proteins like collagen, vitronectin, and laminin. AGE cross-linking on type I collagen and elastin lead to the increased area of ECM, resulting in increased stiffness of the vasculature. Formation of AGEs on structural and functional proteins alters their properties, and it has been shown that AGE modification of laminin has lead to the reduced binding of type IV collagen, reduced polymer elongation, and lower binding of heparan sulfate proteoglycan [4, 26]. Therefore, a slight change in AGE levels leads to a great impact on protenuria. Moreover, type I and type IV collagens, which play a pivotal role in the integrity of the basement membrane, lead to the inhibition of adhesion of the endothelial cells and matrix glycoproteins. These mechanisms have been suggested for the formation of glomerular crescents and shunt like pores in GBM [27] which easily allows macromolecules to escape in the urine. It has been observed that the recovery of these lesions in diabetic nephropathy exhibits poor reversibility [28]. AGE bound to RAGE on the endothelium has been suggested for altering the surface antithrombotic properties which affect blood flow. The interaction of AGEs with RAGEs in monocytes induces their activation to macrophages [29], which leads to the induction of various proinflammatory cytokines and chemokines. In addition to this, AGE-RAGE interaction promotes polymorphonuclear neutrophils and monocytes, activated polymorphonuclear cells generate the cascade of highly reactive oxygen species (ROS, H_2O_2 and $HOCl^-$) which ultimately leads to production of lipid peroxides, lipid hydroperoxides and AOPP.

Lipid peroxidation products deleteriously create endothelial and glomerular basement membrane injury as numerous glomerular basement proteins like nephrin and connectin are prone for oxidation. Loss of functionality of these molecules suddenly increases the protein level in urine. It has been suggested that plasma ALEs and lipid hydroperoxides actively damage GBM. Moreover, pathological significance of lipid hydroperoxides in type 2 diabetic patients have been reported previously and increased levels of these molecules have independent relation with proteinuria [30]. The increased plasma lipid hydroperoxide may get accumulated due to their

Table 2 Plasma antioxidant statuses of studied groups

	Reference	Normo albuminuria		Micro albuminuria		Macro albuminuria	
		Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR
Catalase (U/mg protein)	1.14±0.10	0.87±0.11	0.84±0.13	0.78±0.13	0.71±0.14	0.68±0.14	0.66±0.16
Glutathione (U/mg protein)	0.47±0.11	0.39±0.12	0.37±0.11	0.32±0.16	0.31±0.13	0.28±0.17	0.28±0.15
SOD (U/mg protein)	25.98±3.04	19.60±3.29	19.21±3.18	13.98±4.49	13.72±3.69	10.56±5.24	10.43±5.35
PON activity (nmol/min/ml)	324.36 (289.20–357.75)	274.27 (234.06–275.43)	208.98 (191.09–237.06)*	198.10 (173.72–224.61)	128.03 (122.94–176.33)*	167.30 (125.44–168.83)	145.12 (117.85–157.28)*
FRAP (μ mol/L)	1053.6±85.42	847.34±92.54	819.73±92.93	769.71±85.86	741.67±101.31	642.23±83.43	602.22±79.05
Thiol (μ mol/ L)	252.78±32.56	220.35±33.43	245.14±36.57	198.28±22.54	193.09±29.83	177.58±37.42	173.35±22.16

Data are means±SD or median (Range). Paired t test for significance: *P<0.05

Table 3 Plasma and urinary proteins, and associated risk factors among study groups

	Reference	Normo albuminuria		Micro albuminuria		Macro albuminuria	
		Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR	Low UIgGCR	High UIgGCR
Total Plasma Protein (g/dl)	6.89±0.49	6.66±0.82	6.50±0.77	6.40±0.78	6.22±0.69*	5.61±0.88	5.55±0.92
Plasma Albumin (g/dl)	4.06±3.31	3.85±7.63	3.80±7.14	3.74±9.38	3.70±8.77	3.47±11.98	3.78±10.18
Plasma IgG (mg/dl)	841.26 (763.24–912.23)	1129.0 (1096.2–1119.8)	1068.0 (1040.0–1069.0)	997.61 (987.39–1024.9)	996.81 (982.91–1012.9)	981.17 (918.14–970.96)	913.26 (897.96–942.67)
Uinary Albumin (mg/g Creatinine)	3.01 (0.24–5.19)	4.6 (0.54–28.68)	7.80 (0.40–29.95)	147.29 (31.69–295.91)	161.19 (32.88–298.75)	709.42 (323.94–3705.7)	760.10 (312.67–4436.7)
Uinary IgG (mg/g Creatinine)	1.17 (0.16–3.51)	2.37 (0.12–24.24)	35.92 (25.09–82.95)**	13.79 (2.02–24.67)	41.27 (25.44–296.80)**	17.20 (12.59–24.48)	165.29 (25.84–427.02)**
AGEs (nmole/mg protein)	3.02±0.58	3.82±0.61	4.06±0.60	4.07±1.06	4.11±1.06	4.85±0.81	4.92±0.78
AOPP (μ mol/L)	75.88±19.01	110.07±37.31	117.76±40.55	127.66±38.31	128.07±40.68	154.65±54.21	166.16±49.10
Lipid Hydroperoxides (μ mol/L)	3.50±1.27	3.73±1.18	4.27±1.07	5.02±1.55	5.09±1.59	4.73±1.05	5.67±2.09
ALEs (μ mol/L)	3.35±0.25	4.02±0.59	4.26±0.64	4.54±0.62	4.65±0.63	5.03±0.69	5.21±0.74

Data are means±SD or median (Range). Paired t test for significance: *P<0.05, **P<0.001

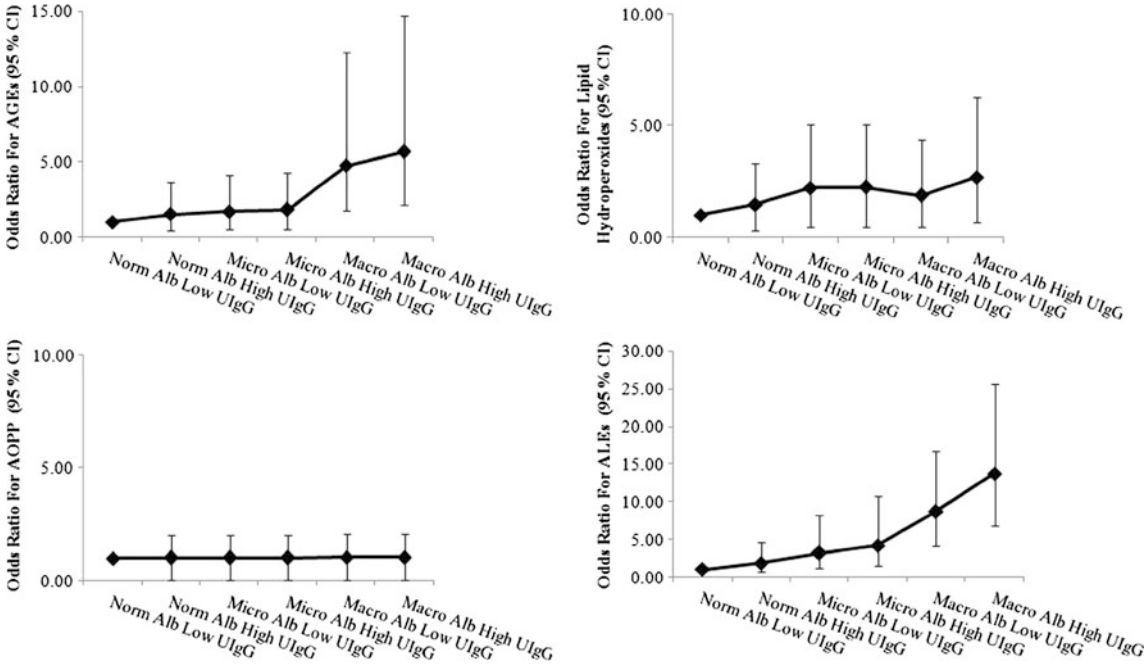

Fig. 3 Association of proteinuria level with the odds ratio for hazardous molecules. The centers of squares are placed at the point estimates, and vertical lines represent the corresponding 95% CIs. The estimates are adjusted for baseline covariates, including age, gender, and duration of diabetes

diminished catabolism. This view was further supported by decreased PON activity in high UIgGCR sub group in the present study. Recently, it has been reported that PON is a glycation prone anti-oxidative enzyme, which actively hydrol- yses lipid hydro-peroxides. A study by other researchers has shown that the PON activity was associated with decreased renal function and increased proteinuria in type 2 diabetic patients [31]. The decreased PON activity imparts increased lipid peroxidation products and membrane lipid hydroper- oxides [32]. This alters membrane fragility and membrano- sclerosis, ultimately leading to proteinuria observed in the present study. AOPP accumulation has been reported in renal cells, and these oxidized proteins causes' loss of functionality which acts as potential threat for activation of intrinsic pathways. Lipooxidative and glucoxidative products have been co-localized in renal tissue of diabetic patients which indicates co-existence of glucotoxicity and lipotoxicity. In addition to this AOPP has been reported to deliver stress to the lipids and other normal carbohydrate moieties. Numerous authors have supported the view that the stress produced at the time of onset of nephropathy produces deleterious effects leading to rapid progression of protenuria [33]. In the present study role of AOPP in progressive proteinuria seems statistically insignificant, similar finding has been observed in type 1 diabetic children [34]. However, a study on the type
2 diabetes has reported increased AOPP with vascular complication and higher BMI patients when compared to control subjects [35]. Therefore, it is concluded that the plasma AOPP has a less adverse effect on proteinuria than macrovascular complications. That's why odds for AOPP remain static over the proteinuria in type 2 diabetic patients.

The present study has suggested that hyperglycemia induced ROS, is a central player in deterioration of glomerular membrane which leads to the escape of large molecular size protein in urine. The odd for these potential mediators has increased with every step of proteinuria in diabetic patients. However antioxidant enzymes were unable to attain statistical significance with a subtle difference in proteinuria. However, the increased pool of AGEs, ALEs and lipid hydroperoxide potentially increased the odds for every of proteinuria. In addition, these conditions frequently occur in those patients who stop/ interrupts medications and face frequent hyperglycemia.

In conclusion, the incidence of diabetic nephropathy after therapeutic intervention are matter of speculation and biomarkers are unable to predict the relative risk for progression of diabetic nephropathy. Present cross-sectional study represents a striking impact of AGEs, ALEs, lipid hydroperoxide metabolism on increased excretion of UIgGCR in type 2 diabetic patients. These intermediates are potentially able to deliver stress into other molecules, and participates in metabolic memory, thus producing long term pathogenesis. On the other hand, short term therapeutic interventions maintain the homeostasis but do not retard pathogenesis of diabetic nephropathy. Therefore, these end-products have high impact on proteinuria, and higher odds were reported for high UIgGCR within all three groups of

UACR. These results potentially indicate that high UIgGCR increases relative risk for diabetic nephropathy.

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ORIGINAL ARTICLE

Diabetic nephropathy and associated risk factors for renal deterioration

Sandesh Mohan, Kiran Kalia, Jyoti Mannari

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Abstract Predominantly diabetic nephropathy starts with glomerulosclerosis, when plasma molecules cross the dismantled glomerular basement membrane (GBM) and subsequently appear in urine. Therefore proteinuria is a sensitive criterion to diagnose progressive renal impairment and the presence of immunoglobulin like large molecules is potentially able to predict severity of nephropathy. Directly, or indirectly hyperglycemia induces proteinuria and high urinary excretion of IgG appears with progression of glomerular injury. This is an observational study of 683 patients with type 2 diabetes mellitus. Patients with varying degree of proteinuria were enrolled and classified into three groups according to the urinary albumin creatinine ratio (UACR, <29, 30–299, >300 mg/g creatinine.) and each group was further sub-classified into the low and high urinary IgG creatinine ratio (UIgGCR) based on the median value. Biochemical parameters were analyzed by standard laboratory methods. The association of proteinuria and odds ratio for risk factors of diabetic nephropathy was estimated using multino- mial logistic regression models. The normoalbuminuric and microalbuminuric patients with high UIgGCR had shown lower eGFR ($p < 0.05$). There was no interaction observed between higher UIgGCR and lower eGFR in regression model analysis. Multinomial logistic regression model estimated the odds ratio for the AGEs, AOPP, lipid hydroperoxides and lipid peroxidation products were increased; 5.64 (95% CI 3.52–9.04), 1.03 (95% CI 1.02–1.04), 2.71 (95% CI 2.05–3.57) and 13.72 (95% CI 6.98–26.95) respectively with high UIgGCR in diabetic patients. High UIgGCR has shown a significant association with decreased eGFR and increased odds for potential hazardous factors. The current study has shown UIgGCR as an increased relative risk, and threat for rapid progression of diabetic nephropathy, possibly because GBM is the primary vulnerable site for deterioration by several hazardous metabolites. In conclusion, association between fractional clearance of IgG and relative risk of GBM threatening factors should be useful for prediction of progressive nature of diabetic nephropathy.

Keyword Advanced Oxidation Protein Products (AOPP) · Advanced Glycated End products (AGE) · Advanced Oxidized Lipid Products (ALEs) · Diabetic Nephropathy · Glomerular Basement Membrane (GBM)

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Introduction

The pathogenesis of diabetic nephropathy initiated at the onset of diabetes when hyperglycemia initiates increased production of ROS [1]. Metabolic memory and stress at initial stage decide the progression of diabetic nephropathy with an association of disturbed homeostasis of metabolites. Several factors, including oxidants and anti-oxidants, and their end products deleteriously affect glomerular membrane [2]. The oxidized and glycated end products of carbohydrates, lipids,

and proteins cumulatively disturb the homeostasis which leads to activation of several destructive pathways. The studies made on markers of these intermediate/end products (AGEs, ALEs, AOPP) have widely reported their deleterious effect, on microvessels. [3, 4]. In normal healthy kidney crossing of proteins at GBM is strictly monitored by several mechanisms, including the barriers of glycocalyx, size/charge selectivity, and urinary excretion is prevented by selective clearance of proteins through FcRn, megalin, and cubulin opsonisation and internal- ization of proteins and peptides [2, 5, 6]. On the other hand, several factors allow plasma proteins to cross the GBM, like shunts, scanty podocytes and foot processes and increased blood pressure [7]. Hyperglycemia in diabetes leads to increased pool of AGEs, ALEs, AOPP, and hydro-peroxides which are responsible for renal deterioration [8–10]. In the present study, we determined the impact of these factors on renal function.

Material and methods

The present study was conducted on patients attending the OPD and diabetic clinic of the Department of Medicine, S. K. Hospital and P. S. Medical Collage, Karamsad, Gujarat, India. Only cases of type 2 diabetes were enrolled in our study referred by diabetologists. A written and informed consent was taken from all patients. This protocol was approved by an ethical committee. All patients included in the study underwent a detailed questionnaire. The patients were taking metformin, glibenclamide or glipizide as anti-hyperglycemic drugs. Patients with hypertension were receiving calcium-channel blocker, angiotensin-converting enzyme inhibitors or angiotensin receptor blocker. None of them was taking immunosuppressive drugs. Pregnant and lactating females were excluded from the study. All the patients were free from any chronic illness and lesions like diabetic foot. Patients in this study were from almost similar economic status, food habits, and physical activity. The blood pressure was recorded by trained nurses, in sitting position after 10 min of rest, in right arm. Blood and urine samples were collected in the morning period with medical history and anthropological data. Samples were collected in pre-sterile vial procured from BD Biosciences and were brought to the laboratory in cool condition, aliquot were stored in −25°C till further use.

Biochemical tests were performed by standard laboratory methods and protocols, plasma protein was measured according to method of Lowry et. al. [11], Creatinine was measure by Zaffs reaction [12], Glycated hemoglobin [13], plasma albumin and lipid profile was estimated by commercially available kits from Eve's diagnostics Ltd., Urinary Albumin was measured by IMMULITE auto analyzer. The activity of plasma anti-oxidative enzymes, Catalase [14], Super Oxide Dismutase [15], Glutathione Peroxidase [16], Paraoxonase [17] was measured along with total plasma protein thiol content [18] and total plasma antioxidant capacity by FRAP [19]. AGEs [20], AOPP [21], Lipid hydroperoxides [22], and ALEs by TBARS [23] in plasma were measured by colorimetric methods. Plasma and urinary IgG was quantified using sandwich ELISA as described elsewhere [24]. All chemicals were of analytical grade and procured from Sigma chemicals.

The eGFR was calculated by CG/BSA (Cockcroft-Gault's equation corrected for body surface area, a most prevalence equation for Indian population) [25]. The diabetic subjects were categorized into the three groups; normoalbuminuric, microalbuminuric and macroalbuminuric which were defined on the bases of urinary albumin to creatinine ratio (UACR) <29, 30–299 and >300 mg/g creatinine respectively. These groups were further sub grouped into low and high urinary IgG excretion (UIgGCR) on the bases of median value of IgG to creatinine ratio (25 mg/g creatinine) for pooled samples. These three groups were analyzed by independent sample t-test for low and high urinary IgG excretion subgroups. The difference in decreased eGFR was analyzed by paired t-test. A multinomial logistic regression model was used to calculate odds ratio and calculated odds was adjusted for age, gender and duration of diabetes. All statistical analysis was done using SPSS 17 software.

Results

Table 1 shows the baseline characteristics of the 683 type 2 diabetic patients. Diabetic subjects had a higher BMI, fasting and 2-hr post prandial blood glucose value, glycated Hb, cholesterol, triglycerides and LDL as compared to normal healthy individuals. High UIgGCR in normoalbuminuric group was significantly associated ($P<0.05$) with increased glycated hemoglobin, blood pressure and triglyceride levels when compared to low UIgGCR subgroup. The eGFR in normoalbuminuric and microalbuminuric group was significantly decreased in low to high UIgGCR subgroups ($P<0.05$) as compared to healthy individuals as shown in Table 1. Moreover, estimated GFR was compared in three groups with different grade of UACR, and a significant decrease ($P<0.05$) was observed with higher UIgGCR in normoalbuminuric and microalbuminuric group. The decrease in eGFR was statistically insignificant in macroalbuminuric group as shown in Fig. 2.

Table 2 shows the plasma antioxidant status of the studied groups and all the diabetic subjects showed decreased antioxidant enzyme activity and decreased total

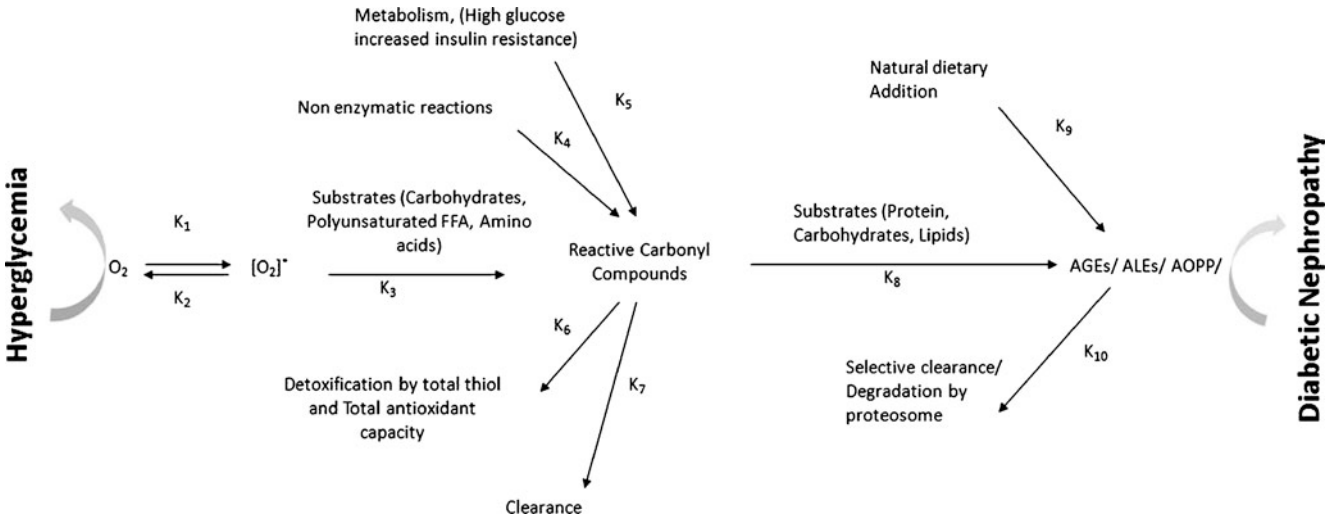

Fig. 1 Showing chemical kinetics model for the accumulation of hazardous molecules (advanced glycated /oxidized products). K_1 is enhanced by increased glucose flux in four pathways (Polyol), K_2 is enhanced by good glycemic control and diet rich in antioxidants, K_3 is increased by uncontrolled blood glucose and nonenzymatic pathways, K_4 is resultant of Millard reaction, K_5 is increased by anaerobic metabolism, increasing insulin resistance, and hypoglycemia, K_6 is increased by high total antioxidant capacity (enzymatic + nonenzymat- ic antioxidants), K_7 is receptor mediated clearance by macrophages and other scavenger cells, K_9 is increased food stuffs, K_{10} decided by clearance of AGEs, ALEs and AOPP selectively by proteosome. K_8 is subsequent resultant of $[(K_1 + K_3 + K_4 + K_5 + K_9) - (K_2 + K_6 + K_7 + K_{10})]$

antioxidant capacity when compared to healthy individuals. Diabetic patients of normo, micro and macroalbuminuric group were lacking association with antioxidant status when compared within the group of low and high UIgGCR. Only the decreased paraoxonase enzyme activity was significantly associated with high UIgGCR subgroup within each respected albuminuric group. We further analyzed the independent relationship between grades of proteinuria and odds for hazards factors (AGEs, lipid hydroperoxides, AOPP and AELs). The values of these factors along with plasma and urinary proteins (albumin and IgG) are shown in Table 3. The odds ratio calculated for the hazards factor was adjusted for the conventional risk factors including age, gender, duration of diabetes and smoking status, as shown in Fig. 3. The group exhibiting normoalbuminuria and low UIgGCR was set as a reference. The adjusted odds for AGEs in normoalbuminuric group with high UIgGCR was 1.51 (95% CI 1.10–2.09, $p<0.05$). It increased in microalbuminuric group from 1.71 (95% CI 1.21–2.39, $p<0.001$) to 1.80 (95% CI 1.3–2.49, $p<0.001$) with higher UIgGCR. In macroalbuminuric group odds ratio with increased UIgGCR ranged from 4.72 (95% CI 2.97–7.52) to 5.64 (95% CI 3.52–9.04, $p<0.001$). This clearly indicated a relationship between higher odds for increased UIgGCR. Similar findings were observed for the adjusted odds for lipid hydroperoxides in normoalbuminnuric group with high UIgGCR (1.48; 95% CI 1.18–1.85, $p<0.001$), which continuously increased through microalbuminuric group with high UIgGCR (2.25; 95% CI 1.79–2.83, $p<0.001$), to macroalbuminuric group (1.88; 95% CI 1.4–2.52 to 2.71, 95% CI 2.05–3.57, $p< 0.001$), from low to high UIgGCR respectively. The adjusted odds for AOPP was increased but unable to achieve a statistical significance and only slight difference was observed in macroalbuminuric group with high UIgGCR (1.03, 95% CI 1.02–1.04). Moreover, ALEs has shown the highest relation with proteinuria, adjusted odds in normoalbuminuric group with high UIgGCR, which was 1.80 (95% CI 1.17–2.79, $p<0.001$). Odds were further increased in microalbuminuric group from low to high UIgGCR (3.17; 95% CI 1.97–5.08 to 4.17; 95% CI 2.65–6.55, $p<0.001$), respectively. Macroalbuminuric group has shown the highest odds for ALEs and was 8.72 (95% CI 4.6–16.55, $p<0.001$), which markedly increased with high UIgGCR to 13.72 (95% CI 6.98–26.92, $p<0.001$).

Discussion

Strict blood glucose control is desirable in type 2 diabetic patients to prevent secondary complications, but it is not always achievable. Hyperglycemia directly or indirectly alters normal functioning of antioxidative enzymes. Accelerated chemical modification of proteins and lipids during hyperglycemia leads to the formation of AGEs, ALE, hydroproxides and AOPP. Proteinuria in diabetic patients leads to the loss of antioxidants and leads to increased pool of a hazardous molecules as shown in Fig. 1. AGEs contribute to the development and progression of diabetic vascular complica-

ORIGINAL ARTICLE

Macrosomic infants of nondiabetic and diabetic mothers: The challenges for obstetric practices in low resource community

Mahjabeen Khan

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Abstract To estimate and compare the obstetric outcome of fetal macrosomia in both diabetic and non-diabetic mothers as challenges in obstetrics practice Karachi, Pakistan. Study Design: comparative cross sectional, Study duration: From June 2008-May 2009, Study population: All singleton pregnant women, Sample size: 229. Neonates with birth weight of 3,500 gms or greater born to diabetic and non-diabetic mother. Babies with 3,500 gms birth weight and more were considered as macrosomic. The major outcome measures were obstetrics outcome: live births, perinatal mortality, mode of delivery and APGAR scores of both groups. We compared demographic, obstetric and neonatal outcomes on diabetic & non-diabetic mothers delivering macrosomic babies. Data were entered and analyzed using SPSS windows version 15. Significance of difference was calculated using t test, Chi square test as applicable. There were 72 diabetic and 157 non-diabetic pregnant women. Uncomplicated diabetic and non-diabetic women of single index pregnancy had age range of 19–35 years. Overall incidence of macrosomia (≥3,500 gms) in this study was 72(31.4%). In this study there were significantly more macrosomic newborns in diabetic women; (52.8%) compared to (47.2%). Fetal macrosomia in our study was 31.4% in both diabetic and non-diabetic mothers. The obstetric challenges of diagnosis and management of fetal macrosomia in low resource country like Pakistan require screening for macrosomia as an integral part of antenatal care.

Keywords Macrosomia · Diabetes · Non-diabetes · Body mass index · Obstetric practice · Low resource

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Introduction

Fetal macrosomia represents a continuing challenge in developing countries for community obstetrics practices. Fetal Macrosomia has been defined as birth weight above a value for the defined ethnic, social and demographic population based on resources available in obstetrics practices. The overall incidence of macrosomia has been rising [1–3]. Heiskanen from Finland reported the incidence as 3.4% [4]. The prevalence of macrosomia is 4.9%–15.3%.with a perinatal mortality 58/1000 [5, 6]. Akhtar et al determined fetal complications of macrosomia in 13.1% [7]. Perinatal mortality remains high among macrocosmic infants of both diabetic and non-diabetic mothers. These are higher in Pakistani women compared to western countries possibly due to poor glycemic control. Most morbidity occurs in newborns weighing less than 4,000 gms in the developed world. Akinbi found macrosomia in non-diabetic mothers associated with chronic fetal hyperinsulinemia [8]. Macrosomia in non-diabetic mothers had less neonatal complications with vaginal delivery [9].

One of the obstetric challenges in developing countries is that the detection of macrosomia has poor predictive value. The pre-pregnancy maternal weight, ante-partum risk factors and scan reports for fetal weight in our obstetric

practices due to low resource and poor expertise at secondary and primary maternity hospitals have not been standardized and validated. Another challenge is that there has been no consensus regarding ante-partum prediction and management in diabetic and non-diabetic macrosomia as accurate diagnosis have been made retrospectively. The prenatal diagnosis of macrosomia remains imprecise.

Induction of labour and prophylactic caesarean delivery has not altered the incidence of shoulder dystocia among non-diabetic patients. Caesarean section and induction of labour have been associated with increased risk of operative morbidity and mortality with added cost implications in obstetrics care in low resource community and refusal for operative delivery results in fetal mortality.

This case control study estimated the frequency of macrosomia and compared the obstetric outcome of macrocosmic babies in both diabetic and non-diabetic mothers.

Subjects and methods

In this case control study, 229 singleton pregnancies were recruited. Mothers were stratified by the diagnosis of diabetes mellitus with birth weight of 3,500 gms or greater as cases and birth weight <3,500 gms as control. This study was conducted in a private, secondary care maternity home, at Karachi. The hospital caters for mothers from medium to low socio-economic community. The study was conducted from June 2009 to May 2010. All booked cases of any parity delivered at the same facility were recruited in the study. Exclusion criteria were grand multigravidae, late booking, previous 2 or more caesarean sections, maternal complications such as pregnancy-induced hypertension, thromboembolism, infections, and other operative delivery (Episiotomy, Vacuum extraction and low forceps). The major outcome measures were obstetrics outcome, live births, perinatal mortality, mode of delivery and APGAR scores of both groups. We compared demographic, obstetric and neonatal outcomes between diabetics & non-diabetics mothers delivering macrosomic babies. Odds among diabetics and non-diabetics with chi-square test for comparing means in both groups were implied after control for confounders.

Data were entered and analyzed using SPSS version 15. Means with standard deviation were calculated for numerical variables and proportions for categorical variables. Significance of difference was calculated using t test and chi square test as applicable

Results

A total of 229 macrosmic neonates were observed for fetal obstetric outcome. There were 72 diabetics and 157 non-diabetic pregnant women. Uncomplicated diabetics and non-diabetic women of single index pregnancy with age range of 19–35 years were observed. Overall frequency of macrosomia (≥3,500 gms) was 31.4% compared to 68.6% in those with birth weight ≤3,500 gms. The proportion of macrosomic infants in diabetics were 52.8% and non-diabetic patients 47.2%. The odds ratio (OR) of macrosomia in diabetic and non-diabetics was 1.54. Fetal outcome of macrosomia in both groups have been compared in Table 1. The fetal outcome showed OR 4.8 among other associated risk factors which showed the stronger relationship and dependency on obstetrics practices at hospital levels. Other associated risk factors have also been shown in Table 2.

Discussion

Worldwide studies of hyperglycemia in pregnancy are gradually establishing acceptable diagnostic criteria, appropriate screening procedures and an evidence-based study for obstetrics care. Several studies have been conducted to determine association between macrosomia and adverse pregnancy outcome in different obstetric resources. These studies have been conducted in different times and in variety of clinics and hospitals in public and private sectors. In this study the major outcome measures of fetal outcome (alive / dead), mode of delivery (vaginal, operative and cesarean sections), gender outcome, APGAR Score among diabetic and non-diabetic mothers have been estimated and compared.

The ability to predict macrosomia is poor in low resource settings and requires tool to develop policy management for macrosomia [10]. Saleh showed significantly more macrocosmic newborns in nondiabetic women (73% versus 27% in diabetic women) [11]. However in this study there were significantly more newborns in diabetic women (N=38; 52.8%) compared to non diabetics (N=34; 47.2%).

Maternal anthropometric factors as predictor of fetal weight outcome have been shown in some studies. High pre-pregnant weight and increased weight gain during pregnancy have been associated with macrosomia in developed world. Serial ultrasonography contributes to detect macrosomia during antenatal care. In low resource setting serial scanning is difficult but in women with previous large babies there is a high risk of macrosomia and this subgroup require serial sonography for early detection of macrosomia.

Several non-invasive and nonoperative factors have been evaluated for prenatal diagnosis of macrosomia. They require future studies to find the best parameters to predict macrosomia in low resource setting. Colman from a developed and well established center reported

Table 1 Risk Factors associated with macrosomia in a low resource community N0 229

Factors	Macrocosmic infant N0 72	Non-macrosomic infant N0 157	P-value	OR(95%CI)
RH Factors				
• Positive	70	150		1.63(0.33 to 8.06)
• Negative	2	7	0.54	1.00
Previous Abortion				
• Yes	17	28		1.42(0.72 to 2.81)
• No	55	129	0.37	1.00
Booking status				
• Booked	31	68		0.99(0.56 to 1.74)
• Non Booked	41	89	0.97	1.00
Fetal presentation				
• Cephalic	70	149		1.87(0.38 to 9.08)
• Non Cephalic	2	8	0.42	1.00
Mode of admission				
• Emergency	56	104		1.78(0.93 to 3.41)
• OPD	16	53	0.07	1.00
Mode of delivery				
• SVD	53	117		0.95(0.51 to 1.8)
• Cesarean Section	19	40	0.88	1.00
Fetal outcome				
• Alive	70	138		4.81(1.09 to 21.27)
• Death	2	19	0.03	1.00
Fetal congenital abnormality				
• Yes	0	1		–
• No	72	156	0.99	
Baby Gender				
• Male	47	95		1.23(0.68 to 2.19)
• Female	25	62	0.49	1.00
Diabetic status				
• Diabetic	38	66		1.51(0.88 to 2.70)
• Non Diabetic	34	91	0.13	1.00

that the reliability of ultrasound estimation of fetal weight to detect macrosomia was poor in term singleton pregnancy measured within 7 days after delivery [12]. Ultrasound estimation has been controversial in obstetrics practice and accepted as related to subjective skills of the ultrasound operator.

Gender dimorphism occurs as the risk of macrosomia attributed to maternal glucose tolerance status [13, 14]. This

Table 2 Comparison of the obstetric outcome of macrosomic infants in diabetic and non-diabetic mothers (N0 72)

Obstetric Outcome of Macrosomia	Diabetic N0 38	Non Diabetic N0 34	Total	P-Value
Fetal outcome				
Alive	36	34	70	0.371
Death	2	0	2	
Gender				
Male	23	24	47	0.46
Female	15	10	25	
APGAR Score				
At 1 minute	8.05±0.52	8.09±0.29	–	0.72
At 5 min	9.37±0.75	9.53±0.51	–	0.29

study showed 47 macrocosmic male compared to 25 females (P value00.46)

A direct correlation occurs between macrosomia and a higher incidence of secondary cesarean section and assisted vaginal delivery (P<0.002) . This study suggested that all alive neonates (N=142) had cesarean section compared to 13 spontaneous vaginal deliveries and assisted vaginal deliveries. Cesarean section among macrocosmic births has been controversial. Currently cesarean section as predictive measure to avoid vaginal delivery complications has not been accepted in low resource community practice. This study has compared the mode of delivery in macrocosmic babies among diabetic and non-diabetic mothers. Astrid study showed that altogether 13.5% of the fetuses were delivered by cesarean section. [15] This study has shown that the cesarean sections were 19(26.38%) compared to 53(73.61%) vaginal deliveries in low resource setting. The explicit reason for these cesarean sections were to avoid complications during vaginal delivery, non-prediction of macrosomia and no validated accepted tool available for detection of macrosomia in low resource setting.

Every woman with suspicion of macrosomia should receive individual guidance regarding special intrapartum and perinatal conditions. In this study perinatal outcome of macrosomia was 18/229 (78.6/1000 live births per year). Yang reported perinatal mortality of 17.4/1000 compared to this study [16]. He also reported that the overall adverse perinatal outcome was 3–9 times greater in infants of diabetes mellitus compared to non-diabetics on time scale. The current study showed that it was about five times higher in diabetic mothers. Therefore early detection through early trimester glucose estimation to avoid adverse consequences of macrosomia can reduce frequency and complications of macrosomia. [17–19] There is an association of increased weight gain during pregnancy and macrosomia [20]. Therefore mothers showing raised maternal weight gain during pregnancy compared to normal weight require antenatal assessment of risk for macrosomia.

Fetal macrosomia has underpinning of childhood obesity, which needs to be prevented. A normogram based on actual birth weight tool had accurately predicted the risk of cesarean delivery in cases of macrosomia. In Pakistan normogram has not been developed for general population yet therefore the development of this tool will reduce the perinatal complica- tions among macrosomic babies in our obstetrics practice.

Mimouni found that the rate of birth trauma was 3.4% in diabetic mothers compared to 2.5 % in controls [21]. In this study most macrocosmic were born alive, had vaginal delivery (142 compared to cesarean sections 59) with complications of perineal laceration and shoulder dystocia in four each. There were no significant differences in perinatal or maternal morbidity among macrosomic babies of diabetic and non-diabetic mother with regard to mode of delivery.

In diabetic mothers vaginal deliveries are complicated by shoulder dystocia without any risk of perinatal morbidity compared to non-diabetic mothers. The risks for shoulder dystocia in diabetic mothers are not significantly different from nondiabetic mothers. The frequency of shoulder dystocia has been reported as 0.2-3 % of all births in obstetrics emergency. [22] The current study found that the diabetic mothers had shoulder dystocia in 14(5.5%) compared to non-diabetic mothers 4(25.4%) with an outcome of all live births.

In conclusion, fetal macrosomia in our study was 31.4% in both diabetic and non diabetic mothers. The obstetrics challenges of diagnosis and management of fetal macrosomia in low resource country like Pakistan require screening for macrosomia as an integral part of antenatal care.

Our study suffers from the limitation that the perinatal consequences of macrosomia have not been studied because of resource constraints.

Conflict of Interests The author declares no conflict of interest for this study.

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ORIGINAL ARTICLE

Neutrophil Gelatinase-Associated Lipocalin (NGAL): an early marker for diabetic nephropathy

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Abstract Neutrophil gelatinase-associated lipoprotein (NGAL) represents a novel biomarker for early identification of acute kidney injury. This study evaluates the usefulness of urine NGAL as a marker for the early detection of diabetic nephropathy. This is a cross-sectional study which involved ninety patients with diabetes mellitus and thirty healthy controls. The diabetic patients were categorized into three groups based on their urine albumin/creatinine ratio (UACR); normoalbuminuria (<3.5 mg/mmol), microalbuminuria (3.5–35 mg/mmol), macroalbuminuria (>35 mg/mmol). In addition to urine NGAL, HbA1C, serum creatinine, urine albumin/creatinine ratio, serum cystatin C, and urine protein were assessed to determine their correlation with urine NGAL. Data analysis was done by using SPSS and MiniTab. Urine NGAL was elevated in all groups of diabetic patients with respect to controls. It was increased proportionately to the severity of kidney function. It was also elevated in some normoalbuminuria diabetic patients. Analysis of correlation revealed that urine NGAL was not correlated with glycemic indices (HbA1C and fasting blood glucose). However, urine NGAL correlated significantly with cystatin C, serum creatinine, urine albumin/creatinine ratio, and inversely with eGFR. Besides, it is also shown to have a significant correlation with eGFR in advanced kidney disease (eGFR<30 ml/min per 1.73 min^2). Urine NGAL can be used as a non-invasive tool for the early detection and assessment of the severity of diabetic nephropathy.

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Keywords NGAL · Diabetic nephropathy · Chronic kidney disease

Introduction

Diabetes has been recognized as a worldwide health problem, affecting people at variable ages with increasing incidence and prevalence, leading to various complications [1]. Longer duration of diabetes, earlier age at diagnosis with poor glycemic control are part of the risk factors that lead to the development of diabetic kidney disease. Other risk factors include positive family history, smoking, and hypertension. Diabetic nephropathy is a major concern for the development of microvascular and macrovascular complications of diabetes and for diabetes-related and overall mortality. Besides that, with an inadequate treatment and late diagnosis, diabetic nephropathy has become the most common diagnosis at initiation of renal replacement therapy [2].

The key pathophysiologic event in diabetic nephropathy is likely to involve an interaction of metabolic and haemodynamic pathways which lead to the development of basement membrane damage [3]. In addition, active inflammation caused by the passage of macromolecules through the basement membrane will result in secondary damage to the membrane. In relation to the glomerular lesion, there will be persistent diabetic proteinuria. The sustained passage of this molecule within the tubular lumen may activate intratubular complement cascade and eventually contribute to tubular injury [4]. This last condition can lead first to tubular inflammation and then to tubulointerstitial fibrosis, which ultimately signals the appearance of an irreversible renal impairment [5].

Historically, diabetic kidney disease has been diagnosed by the presence of proteinuria in the diabetes patients with the reduction of glomerular filtration rate and increased serum creatinine.

In view of the role of the tubular injury in the progression of kidney damage in diabetes, several tubular factors have been recognized as a marker for chronic kidney disease. These include Monocyte Chemoattractant Protein-1 (MCP-1), N-acetyl-B-D glucosaminidase, cathepsin and few others [6].

Neutrophil gelatinase-associated lipocalin (NGAL) is a small 25 kDa protein which was recently discovered by nephrologists. It is a small 25-kDa protein, consists of a polypeptide chain of 178 amino acids and belongs to the 'lipocalin' superfamily [6]. It is expressed by neutrophils and various epithelial cells. This protein is released in blood and urine following ischemic and nephrotoxic injury from injured tubular cells after various conditions [3]. Variable degrees of NGAL gene expression is demonstrated in human tissues like uterus, prostate, salivary glands, lung, trachea, stomach, colon, and kidney [7].

NGAL is currently considered as one of the most promising biomarkers in clinical nephrology and has been extensively studied in acute kidney disease. Acute kidney disease is closely related to high morbidity and mortality. Therefore researchers have aggressively searched for a new biomarker and NGAL has been found to improve the accuracy of the current biomarkers used to detect kidney damage.

A study done by Mishra et al. has revealed that NGAL was easily detected in the urine within 2 h following ischemia [8]. This has preceded the appearance of other urinary markers such as B2 microglubulin and N-acetyl-B-D-glucosaminidase. In this study, they have confirmed that NGAL is originated from the tubule cells. Other findings from this study include the increased NGAL following the administration of nephrotoxic agent, where they have administered cisplastin to the rats. These result in the presence of early, non-invasive urinary biomarkers for ischemic and nephrotoxic renal injury [8].

Materials and methods

This is a cross-sectional study, approved by the Institutional Review Board of University Malaya Medical Centre. Sample size calculation was done by using Epi Info Version 6.0 with a power of 80% and 95% confidence interval giving a sample size of 32. With a recruitment of ninety subjects, the power of study was inferred to be more than 80%. Subjects were patients with type 2 diabetes with various degrees of renal impairment from the medical clinic in University Malaya Medical Centre. They were divided into three categories based on urine albumin/creatinine ratio (UACR); normoalbuminuria (<3.5 mg/mmol), microalbuminuria (3.5–35 mg/mmol) and macroalbuminuria (>35 mg/mmol). The macroalbuminuria group was further divided into four stages of chronic kidney disease (CKD) based on National Kidney Foundation Criteria [9]. Those with infections, neoplasias, and inflammation were excluded in order to avoid any interference in the NGAL measurement.

To minimize the confounding factors, few exclusion criteria had been considered. These include subjects with infections, neoplasia, and any inflammation that could interfere with the urine NGAL measurement [10].

Inclusion criteria included those who agreed to participate and consented to this study. They were patients in University Malaya Medical Centre with Type 2 diabetes mellitus, stable renal function and diabetic nephropathy.

The control group consisted of thirty normal healthy subjects with no history of hypertension, diabetes mellitus, inflammation, neoplastic disorder, and cardiovascular disease. None of the subjects were on medical treatment.

The following data were collected: demographic characteristics, disease duration, and renal function. Besides that, other blood markers for renal function (serum creatinine, cystatin C, eGFR, urine albumin/creatinine ratio (UACR), urine protein, and urine NGAL) were also taken to determine their correlation with NGAL.

Estimated glomerular filtration rate (eGFR) of each subject was calculated from cystatin C by using the Hook et.al formula. This eGFR was then compared with the eGFR obtained from the MDRD formula to observe the correlation between these two formulas. Patients with eGFR of less than 30 ml/min per 1.73 min^2 were identified to study their
correlation with urine NGAL and cystatin C.

Urine NGAL was measured by ARCHITECT (Abbott, Longford, Ireland). It was carried out in a double batch. This assay is an automated method using a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of NGAL in the human urine. It has a coefficient of variation of less than 5%.

Specimens used for urine NGAL were centrifuged at ≥ 400 RCF (Relative Centrifugal Force) for a minimum of 5 min within collection. After that, the clarified specimens were transferred to a sample cup or secondary tube for storage and were stored at −80°C if testing was delayed for more than 7 days.

Cystatin C was measured by particle-enhanced immunonephelometry using SIEMENS kit (Marburg, Germany). The Intra-Assay CV determined at 0.6 mg/L was <4.15%. All tests were carried out in a single batch. Serum creatinine was measured by using Dimension Vista (SIEMENS, Newark, USA). Urine albumin/creatinine ratio (UACR) was assayed by the ARCHITECT (Abbott, Longford, Ireland).

Table 1 Demographics and disease-related characteristics

Variables	Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
Age	23±5.2	63.3±12.04	68.17±9.39	62.33±12.83
Gender				
Male	12 (40%)	15 (50%)	17 (57%)	10 (33%)
Female	18 (60%)	15 (50%)	13 (43%)	20 (67%)
Race				
Malay	22 (73%)	16 (53%)	11 (37%)	13 (43%)
Chinese	4 (13%)	3 (10%)	10 (33%)	11 (37%)
Indian	4 (13%)	11 (37%)	9 (30%)	6 (20%)
eGFR (ml/min per 1.73 m^2)	122.94±12.7	94.92±19.92	61.67±51.92	49.87±28.47
Serum creatinine (mmol/L)	72 (40–103)	70 (46–138)	129.5 (31–442)	139.5 (37–485.0)
Serum Cystatin C (mg/l)	0.631±0.07	0.78±0.28	1.44±0.87	1.65±1.12
Urine NGAL (ng/mL)	4.75 (0.1–27.5)	19.05 (1.1–60)	26.9 (3.7–603.5)	28.55 (0.7–1500)
HbA1C	5.27±0.315	6.23±1.56	7.62±2.73	6.4±3.29
Urine protein	0.12±0.18	0.13±0.09	0.31±0.36	1.08±2.33
Urine albumin/creatinine ratio	0.6 (0.1–1.7)	0.95 (0.1–2.8)	13.15 (4.1–30)	97 (32.4–568.4)

Statistical analysis was performed by using SPSS and MiniTab. For normally distributed values, data were presented as mean ± SD, whereas for non-normally distributed values, data were presented as median ± SD. Normality test was done by using Anderson Darling normality test. Correlation between serum NGAL and other tests were performed by using Pearson coefficient. All results were considered significant when $p<0.01$. Log transformed was done to normalize the non-normally distributed data and Pearson coefficient study was then used for the correlation analysis. Analysis of the non-normally distributed data was performed with Spearman coefficient study. All results were considered significant when $p<0.01$. In order to compare the three groups; (normoalbumiuria, microalbuminuria, and macroalbuminuria), log transformed of urine NGAL was analyzed by using ANOVA. Results were considered significant when $p<0.05$. The ANOVA was then followed by Bonferroni test as the post hoc test. Bonferroni showed statistically significant difference between the normoalbuminuric group and macroalbuminuric group with p value of 0.01.

Results

The demographic and disease-related characteristics of the patients are presented in Table 1. This study involved ninety patients with Type 2 diabetes mellitus and thirty healthy controls. The study population consisted of 66 female

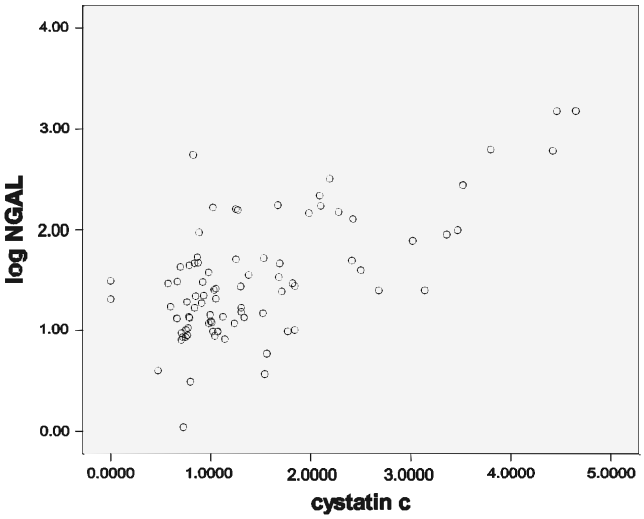

Fig. 1 Urine NGAL and cystatin C

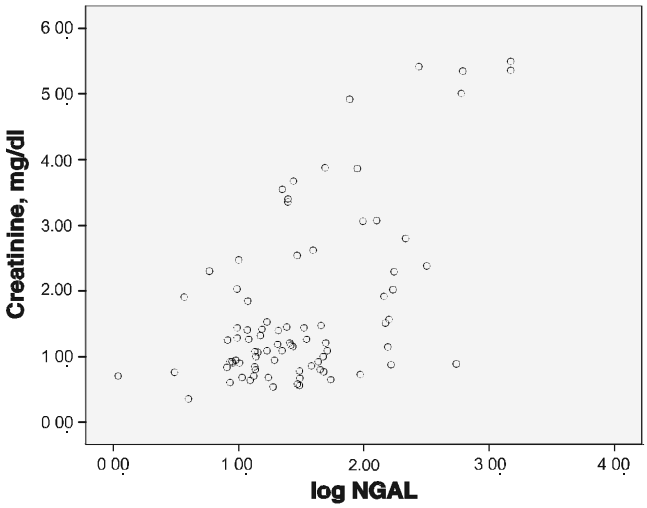

Fig. 2 Urine NGAL and serum creatinine

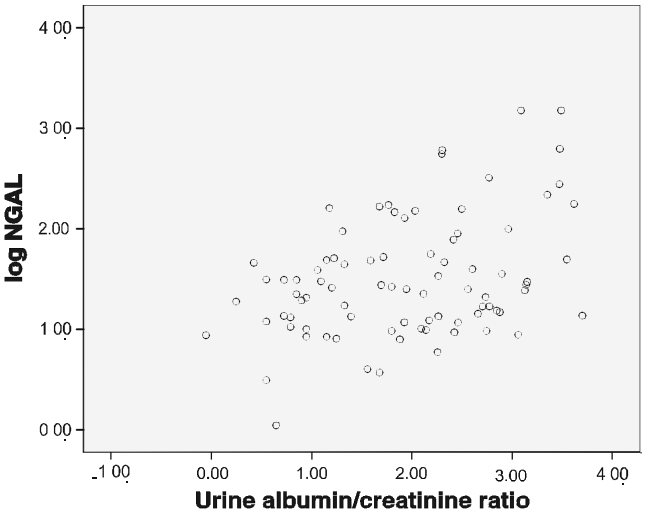

Fig. 3 Urine NGAL and urine albumin/creatinine ratio

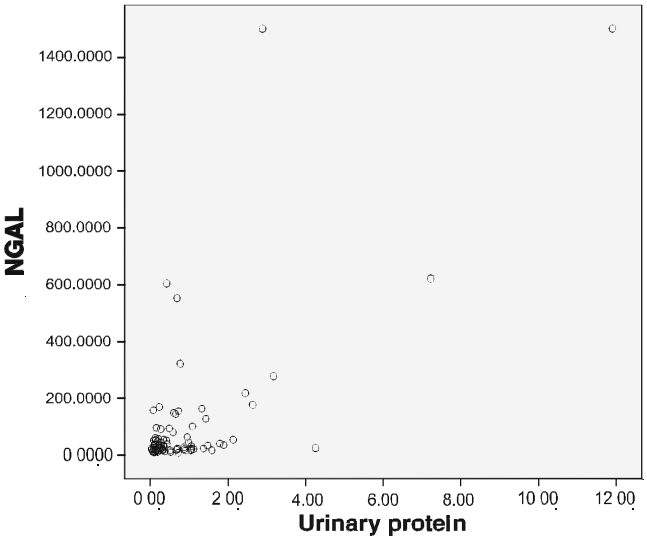

Fig. 5 Urine NGAL and urine protein

patients (55%) and 54 male patients (45%) with ages ranging from 22 to 89 years old.

The ethnic distribution (Table 1) was 52% Malay, 23% Chinese, and 25% Indian respectively. They were categorized into normoalbuminuria (N=30), microalbuminuria (N=30), and macroalbuminuria (N=30), depending on their urine albumin/creatinine ratio.

Control group showed a perfectly conserved renal function with a mean eGFR of (122.94±12.7 ml/min per 1.73 m^2 and mean creatinine of 71.9±16.7 mmol). The group with macroalbuminuria showed a reduced eGFR (49.87±28.47, mean) thus signalling the presence of diabetic nephropathy. Mild reduction in renal function was seen in patients with micro and normoalbuminuria.

The levels of urine NGAL were found to be significantly elevated in patient with macroalbuminuria as compared to the control, normoalbuminuria, and microalbuminuria (mean 28.55; min 0.7, max 1500) (Table 1). However, urine NGAL was also found to be elevated in some patients with normoalbuminuria. This is an interesting finding and supports the hypothesis that urine NGAL can be used as a marker for the early detection of diabetic nephropathy [11]. Besides, the mean of urine NGAL was also observed to be increased concomittently with the degree of renal impairment. This finding indicates that urine NGAL can also be used in determining the severity of renal disease.

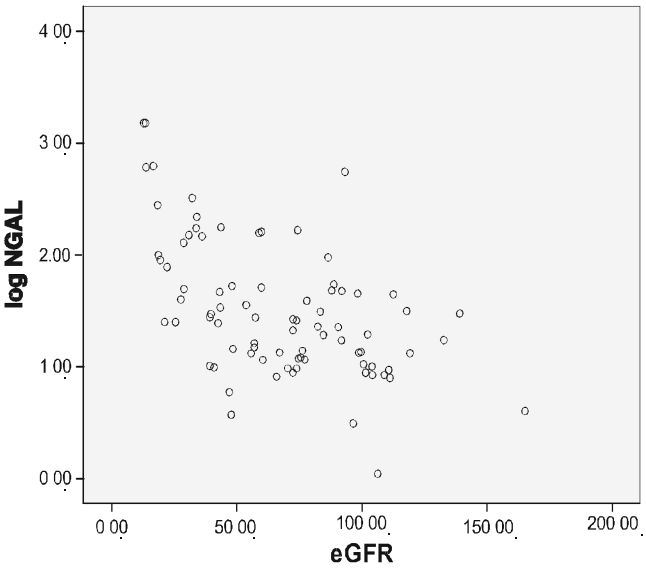

Fig. 4 Urine NGAL and eGFR

Urine NGAL and other renal indices

Urine NGAL did not correlate with any demographic parameters (age, gender, and race). All p values were more than 0.01(Table 1)

Figures 1, 2, 3, 4 and 5 display the statistical correlations (r) between urine NGAL and other biomarkers for renal impairment. Urine NGAL correlated significantly with cystatin C, serum creatinine, UACR, eGFR and urine protein with the r values ranging from 0.39 to 0.7 and $p<0.01$.

Analysis of patients with advanced stages of kidney disease (eGFR<30 ml/min per 1.73 min^2), showed significant correlation of both the cystatin C and urine NGAL with eGFR. This suggests that urine NGAL may be a superior marker for advanced cases of renal impairment as compared to creatinine. However this study shows that cystatin C has higher correlation as compared to urine NGAL in patients with eGFR >30 ml/min per 1.73 min^2. eGFR calculated from the cystatin C using the Hook formula shows a significant correlation with the eGFR obtained from the MDRD formula (r: 0.88) (Fig. 6).

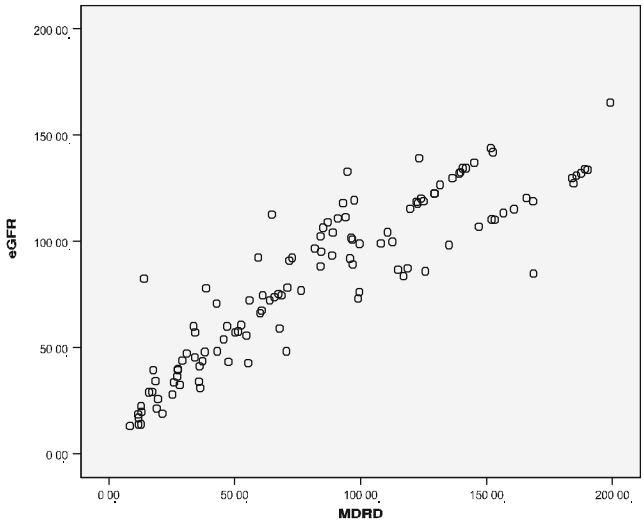

Fig. 6 MDRD and eGFR

The three groups (normoalbuminuria, microalbuminuria, and macroalbuminuria) were compared by using ANOVA. The sample means for the three groups of NGAL were: 19.05 ng/ml for normoalbuminuria, 26.9 ng/ml for microalbuminuria and 28.55 for macroalbuminuria. The difference in urine NGAL between the groups was statistically significant (p=0.012). Bonferroni test revealed statistically significant differences between the normoalbuminuric and macroalbuminuric group (p=0.01).

Discussion

Neutrophil gelatinase-associated lipocalin (NGAL) is a small 25 kDa protein recently discovered by nephrologists [12]. It is a small 25-kDa protein, consisting of a polypeptide chain of 178 amino acids and belongs to the 'lipocalin' superfamily. It is expressed by neutrophils and various epithelial cells [7]. This protein is released in blood and urine following ischemic and nephrotoxic injury from the tubular cells. Variable degrees of NGAL gene expression is demonstrated in human tissues like uterus, prostate, salivary glands, lung, trachea, stomach, colon, and kidney [12].

Present studies have reported that NGAL is a marker that has outperformed other recent renal markers. They demonstrated that NGAL may represent a novel early urinary biomarker particularly for the detection of acute renal injury [13–16]. This is because NGAL is able to manifest in the urine after 3 h of tubular injury. In comparison, serum creatinine will only be elevated after 24 h of reperfusion [8]. In this study, we are trying to demonstrate that urine NGAL can be used as an early marker for the detection of nephropathy in these patients, particularly in those with incipient nephropathy.

However, a few studies have demonstrated that NGAL might also be elevated in some other conditions, not necessarily pertaining only to renal injury [14–16]. Therefore, we have excluded those likely to interfere with our results. These factors include patients with infection, neoplasia and inflammation.

This study which involved 90 patients with diabetes and 30 controls. It has demonstrated that urine NGAL does not correlate with other diabetic indices; such as HbA1c and dermographic factors such as age, race, and gender. These findings are in accordance with reports from Bolignano et al. [11]. However, urine NGAL was noted to be correlating significantly with urine protein, urine albumin/creatinine ratio, cystatin C, and eGFR. Few studies have reported the same findings in patients with chronic kidney disease [17–20], hence supporting idea that urine NGAL may be used as a marker for nephropathy.

Interestingly, in this study urine NGAL and cystatin C were elevated in some normoalbuminuria diabetic patients. The results obtained showed a few patients with normal UACR demonstrating an elevated urine NGAL and cystatin C. From this point of view, we can conclude that urine NGAL may be used as an early marker for diabetic nephropathy, particularly in incipient nephropathy. This is very important because early identification of CKD and timely detection are global priorities as it can prevent further complications and reduce the cost of treatment.

Generally, in the advanced stages of CKD, creatinine does not correlate well with eGFR [21]. However, in this study, we have managed to demonstrate that both urine NGAL and cystatin C were elevated in patients with advanced kidney disease thus further supporting the utility of urine NGAL in advance kidney disease.

However, in contrast to previous studies [22], this study showed that cystatin C has a superior correlation with eGFR as compared to urine NGA in the early detection of diabetic nephropathy. These contradictory findings may result from the different sample size, different methods and assays that has been adopted, and different populations. However, a few studies have evaluated NGAL in diabetic nephropathy as compared to acute kidney injury. This is because cystatin C may be more useful in the early detection of chronic nephropathy for some unknown reasons. Therefore, more research on urine NGAL should be done in the setting of chronic kidney disease.

In conclusion, we have demonstrated that urine NGAL has outperformed other renal markers that are currently used to diagnose diabetic nephropathy. It is able to predict renal impairment at an early stage in diabetic nephropathy. However, there are a few limitations of the present study. First of all, this is a single-centre study. The small sample size and limited number of tests may minimize the validity of the results. Secondly, as mentioned above, the eGFR was

established by using cystatin C, which may have produced a biased correlation.

Further research on urine NGAL in the setting of chronic kidney disease seems warranted.

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ORIGINAL ARTICLE

The prevalence of Type 2 Diabetes Mellitus in the United Arab Emirates: justification for the establishment of the Emirates Family Registry

Habiba Alsafar, Khadra A. Jama-Alol, Ahmed A. K. Hassoun, Guan K. Tay

Abstract To study the prevalence of Type 2 Diabetes Mellitus (T2DM) in a population of United Arab Emirates (UAE) residents through the creation of the "Emirates Family Registry" (EFR). Major hospitals and diabetes centres in the UAE were contacted to establish a bio-banking facility referred to as the EFR. Through assistance made available by the Ministry of Health and collaborators of this network, demographic data of T2DM patients were collected and collated in a database for analysis and longitudinal studies. Clinical specimens were collected for biochemical profiling (such as; glucose, lipids, HbA_{1c} levels). In the first 24 months of the operation the EFR recruited 23,064 adult volunteers from three major hospitals and nine primary care centres throughout the UAE. Within this cohort, 88% were patients classified as T2DM patients from the medical records. The cohort was divided into age categories with 59% of T2DM patients aged between 40 and
59 years old. UAE nationals comprised 30% of the database of which 21% were diagnosed with T2DM. However the percentage of adults with T2DM was higher in other ethnic groups effecting almost 33% of the Indians who live in the UAE. A total of 741 UAE Nationals consented to donate blood; in phase I of the study; for biochemical testing after which 23% were diagnosed with T2DM, 30% with pre-T2DM and 47% were healthy. This study is consistent with the previously reported high prevalence of T2DM in the UAE. Furthermore, analyses of the factors that predispose to the disease have revealed that obesity, a large waist circumference, consanguineous marriage, family history, lack of physical activity, unhealthy dietary practices, high total cholesterol, and high triglycerides levels were more prevalent in T2DM patients. The classification of these features will contribute to defining more effective and specific plans to screen for and manage diabetes and its complications in UAE and other developing countries.

Keywords Type 2 Diabetes · UAE · Arab · Emirates Family Registry

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Background

The United Arab Emirates (UAE) has a cosmopolitan population of about 4.7 million and exhibits a unique demographic structure. The UAE sits at a crossroad of the trade routes between Asia and Europe. It has flourished as a contemporary centre of trade and commerce over the last four decades. People from every part of the world arrive in search of jobs, trade and business. UAE national makes up only 19% of the total population with balance comprising expatriates of different ethnic backgrounds. The largest ethnic group are people of South Asian origin (approximately 50%). Those from other parts of Asia include Philippines, China, Hong Kong, Indonesia, Singapore and Thailand.

These East Asians are grouped with Caucasian and compromise up to 8% of the population. Iranians comprises 8% and the rest of the population are from other Arab states (15%). These estimates are based on the results of the 2005 census that included a significantly higher estimate of net immigration of non-citizens than estimates in July 2009 [1, 2].

T2DM, has become a major public health problem in the UAE. A survey completed by UAE's Ministry of Health reported that the overall percentage of people with diabetes was between 13% and 19% among expatriates who live in UAE. Furthermore, Malik and his colleagues [3] have estimated that 25% of UAE nationals suffer from diabetes; mainly T2DM; and the prevalence of the disease is increasing.

In addition, another study conducted by Reed and colleagues in the year 2005 [4] on a random sample of UAE citizens over the age of 30 living around the city of Al-Ain reported that 20% of subjects studies suffered from T2DM (14% rural to 25% urban). However, the methodology used may have resulted in underestimation of prevalence by as much as 20% as a recent study reported by Centre for Arab Genomic Studies (CAGS) indicated that the prevalence of T2DM in UAE rises with increasing age reaching 40% in people over 30 years. These observations emphasise the necessity of considering prevention for diabetes in the UAE.

The Emirate Family Registry (EFR) project was conceived to provide a means to more accurately estimate prevalence through a longitudinal approach. Secondly it represents an important tool and resource as the genomic era gains momentum towards assisting in deciphering the complexity of diseases in humans [5]. Similar approaches to assess risk factors of diabetes in other populations have been conducted [6–9] . When the EFR project commenced, the requirement was to establish a registry with well defined description of the disease (ie. the phenotype) as well as the genetic background of populations of interest (ie. the genotype). This resource is currently not available for the ethnic groups of the Arab world. Therefore the EFR was developed to address this deficiency. The EFR can be used by local research groups to systematically study common diseases throughout the Middle East region. It will also be used to develop regional and international collaborations in biomedical science. The EFR is a register containing information on the local ethnic population of the region designed specifically to study the genetic factors that are unique to this region which will lead to better patient care, disease management and improved quality of life.

Methods

Emirates Family Registry (EFR)

Three major hospitals and nine primary care centres in the United Arab Emirates (UAE) were contacted to establish EFR. Through this collaboration, data from all patients attending these clinics and hospitals was collected and tabulated in a database. This study was approved by the UAE Ministry of Health and Dubai Police Head Quarter. A verbal consent was obtained from those patients who agreed to allow their name to be added to the registry and an informed consent was obtained from all individuals who donated blood before commencement of the study procedures.

The database of the registry was constructed using Visual Studio 2006. The EFR comprises two components: [1] a computer database documenting the details of participants of the registry and [2] a DNA and bio-specimen repository. Data from patients include demographic data, biochemical results such as haemoglobin A_{1c} (HbA_{1c}), fasting blood glucose, oral glucose tolerance test (OGTT), lifestyle variables (healthy diet, daily physical activity, smoking, quality of life), disease complications (neuropathy, nephropathy, retinopathy) and family history. There are provisions to expand the registry to include different diseases and their associated clinical and genetic features.

Subjects

A total of 23,064 adult who reside in the UAE volunteered to participate in this study on their routine visit to the three major hospitals and nine primary care centres. Of the total group 20,374 were diagnosed with T2DM. Overall 741 UAE Nationals donated blood for biochemical tests to confirm their diagnosis (Diabetic, Pre Diabetic and healthy) and to study the risk factors which contribute to developing T2DM.

Biochemistry profile

Up to 5 ml of peripheral blood was drawn from 741 UAE national and collected in EDTA, Heparin and Fluoride vacutainers for biochemical tests. Fluoride and Heparin tubes were centrifuged at 3,000 rpm for 5 min and serum was collected. Serum from the Fluoride tubes was used to measure fasting glucose, total cholesterol and oral glucose tolerance, and serum from Heparin tubes was used to measure triglycerides, urea and creatinine level. 25 μl of blood from EDTA tube were used to measure haemoglobin A_{1c} (HbA_{1c}).

An individual was classified as diabetic if the subject [1] was diagnosed with diabetes by a qualified physician [2], was on a prescribed drug treatment regime for diabetes and [3] had biochemical test results that was consistent with the criteria laid by the World Health Organization (WHO) consultation group report that specifies a fasting plasma glucose level of at least 126 mg/dl. Glucose tolerance was preformed only on subject that did not suffer from diabetes when enrolled in this study. Individuals were classified in the prediabetic group if the 2-hour post glucose level in the subject was more than 140 mg/dl and more and normal

glucose tolerance group if the 2 h post glucose level was less than 140 mg/dl.

All the biochemical tests were performed at Al-Baraha Hospital using the Cobas Integra 800 clinical chemistry system (Roche Diagnostics, Indianapolis, USA).

Statistical analysis

The p-values (probability value) for each phenotype studied were calculated using Dunnett's Multiple Comparison Test in GraphPad Prism version 5.0. The standard deviation, mean and percentages were calculated from data input into a Microsoft Excel spreadsheet. A p value <0.05 was regarded as statistically significant for a two-sided test.

Results

The establishment of a registry which contains essential clinical information linked to genomic data is vital towards great understanding of disease mechanisms in the local ethnic groups of the UAE. All patients who volunteered to participate in the EFR went through a well defined process. The patient was interviewed and consent was obtained in their routine visit to the primary care centre or hospital. The data collected from the patient was entered into the database and become part of the overall data of the registry. The patient's disease status was assessed and specimen types were recommended and collected. Subsequently, as data from the analysis became available it was entered into the database.

Table 1 Characteristic of the 23,064 individuals in the Emirates Family R~~eg~~istry

Characteristic	Value (n)	Percent (%)
Age (years)		
18–20	1,014	4.40
21–39	3,281	14.22
40–59	13,126	59.91
+60	5,642	24.46
Gender		
Male	10,059	43.61
Female	13,005	56.39
Ethnicity		
UAE National	6,904	29.93
Others[a]	16,160	70.07
Disease Affection		
Type 2 Diabetes Mellitus	20,374	88.34
Healthy	2,690	11.66

[a] consist of: (124;0.54%) African, (14,587;63.25%) Asian, (348;1.51%) Caucasian, (1,097;4.76%) Middle Eastern (except UAE) and (4;0.017%) Southern American who are residence of the United Arab Emirates during the study period

Table 1 provides a summary of the data that has been entered into the registry at the time this manuscript was compiled. As the patients' data were entered, it accumulated and increased the amount of information available for analysis. Over the lifetime of the registry this information became an important resource. To date the EFR contains information on 23,064 individuals, of which 60% were between the ages 40 to 59 years old. Female volunteers comprise 56% of the entries in the database. Almost 30% are UAE nationals and 88.3% were diagnosed with T2DM.

The registry was set up to estimate the percentage of those who are burdened with diabetes by age group. In time, the overall prevalence of disease throughout the population was determined. Figure 1 shows a breakdown of the information collected for the separate age categories studied. Approximately 3% of T2DM patients were under the age of 20, about 13% of adult were aged between 20 and 39 years, 59% of adult were aged 40 and 59 years and more than 24% of adults were aged 60 years or older. This was included in the study design because of the fact that most studies are showing that younger populations throughout the world are succumbing to T2DM.

The EFR reflects the ethnic diversity of the UAE population. In Table 2; the volunteers are separated into East Asia, Central Asia, and Middle East. Unfortunately, the local Ministry of Economy has chosen to combine the minority groups into one category, which combines the distinct genetic groups in the orient (East Asia) with Caucasians (Western group). Apart from this discrepancy, information is readily available country-wise giving data that is more specific to each population. Overall, the population of UAE nationals with T2DM is 21%. However the EFR revealed a higher percentage of T2DM in other ethnic groups such as Indian (33%) as one of the major hospitals had most of their patients of Indian origin.

In 2009, UAE's population was estimated at 4.7 million, of which 19% were UAE nationals, while the majority of the population were expatriates. The largest group were of South Asian origin (50%). Those from other parts of Asia (includes Philippines, China, Hong Kong, Indonesia, Singapore and Thailand) and those of Caucasian origin compromised up to 8% of the population, while Iranian comprised 8% of the population. The rest of the population were from other Arab states (15%). It was estimated that close to 20% of UAE national have Type 2 Diabetes Mellitus. However the percentage of adults with Type 2 diabetes mellitus is higher in other group effecting almost 52.67% of Southern Asians who live in the United Arab Emirates.

The issue of screening for T2DM is important both in terms of an individual's health and day-to-day clinical

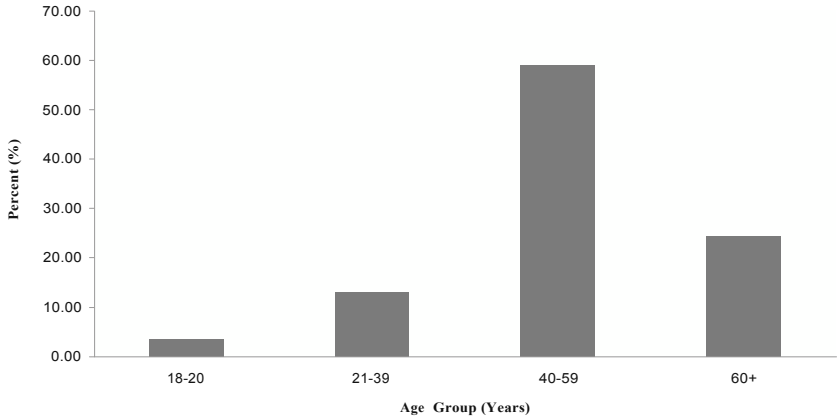

Fig. 1 Proportion of Type 2 diabetes mellitus patients by age group in emirates family registry

practice as well to a country's overall public health system. One of the advantages of the screening process set out in the EFR program is to identify individuals at risk of having undiagnosed T2DM or at risk of developing T2DM. Tables 3 and 4 show the risk factors that could affect the 741 UAE nationals who are diagnosed with T2DM, pre-T2DM through their physical appearance, life style, inheritance and biochemical test. In regard of the physical appearance 26.18% of the UAE participants are overweight and 7% are obese. Of the 741 UAE national 39% have large waist circumference (male and female). Additionally, lifestyles features can be seen in Table 3 which shows that 58% of the patients were having unhealthy food in their diet such as fast food, 45% not performing any kind of exercises (minimum of 30 min walking a day). Genetics heritability is another risk factor in developing the disease; Table 3 shows that 65% have a history of T2DM in their family (at least one parent diagnosed with T2DM) with 35% of consanguine marriages. As far as the biochemical tests performed the percentage of the population with results association with the disease are summarised in Table 3.

Table 5 summarises the predicted p value between healthy group and pre-T2DM and between healthy group and T2DM using Dunnett's Multiple Comparison Test. Age represents the most significant risk factor in developing T2DM among the physical appearance features (p00.0065). Lipids profile such as cholesterol and triglyceride shows a significant p value (0.0018, 0.0023 respectively) when healthy individuals are compared to diabetic patients.

Discussion

The importance of a thorough and well maintained database for significant disease entities cannot be overstated. Diabetes is an overwhelming healthcare problem throughout the world and in the UAE studies have shown that around 20% of the population have T2DM.

The EFR was conceived as a tool to manage T2DM in the UAE. It provides data that is available through clinical testing and DNA screening. The data is stored in a systematic way within a database and is coupled to a DNA and bio-bank repository to facilitate future longitudinal studies. To the best of our knowledge such an effort has not been undertaken for the Arab population. By breaking down the data in different ways, it is easy to establish what particular strategy might be employed to improve disease management.

The process works quite the same for each patient and is carried out the same way allowing simplifying the decision making process for healthcare workers, allowing for consistency in the material collected. At the initial consultation; the status of each individual is assessed and specific protocols are followed. A specific questionnaire and assessment of clinical information within the UAE healthcare database, allows for a first pass screen to determine disease status. The volunteers are categorised according to disease status, and the decision is made as to the nature of bio-specimens that need to be collected. Using T2DM as a case in point, biochemical test relating to glucose, triglycerides and others

Table 2 Ethnicity and prevalence of T2DM

Ethnic Group	Percent of Ethnic Group in UAE	Number of T2DM in EFR	Percent of T2DM in EFR	Prevalence of T2DM per100,000
South Asian	50%	10,732	52.67%	447.31
UAE national	19%	4,214	20.68%	462.21
Other Arab	15%	3,961	19.44%	550.31
Caucasian + East Asian[a]	8%	504	2.47%	131.29
Iranian (Persians)	8%	487	2.39%	126.86

[a]According to the UAE census bureau, Caucasian and East Asians were consolidated in minority group

Table 3 Characteristics of clinical data for 741 (UAE National) Type 2 Diabetes, pre Type 2 Diabetes mellitus and healthy adult individuals

Category		Subcategory	Value (n)
Physical Appearance	Gender	Male	470 (63.43%)
		Female	271 (36.57%)
	Age	18–20	5 (0.67%)
		21–39	246 (33.20%)
		40–59	396 (53.44%)
		60+	76 (10.26%)
	Body Mass Index (BMI)		
	Underweight	<18.50	7 (0.94%)
	Normal range	18.50–24.99	319 (43.05%)
	Overweight	25.00–29.99	194 (26.18%)
	Obese	≥30.00	53 (7.15%)
	Waist Circumference		
	Male	≤40 in	315 (42.51%)
		>40 in	155 (20.92%)
	Female	≤35 in	135 (18.22%)
		>35 in	136 (18.35%)
Lifestyle	Smoking	Yes	185 (24.97%)
		No	556 (75.03%)
	Physical Activity	Yes	405 (54.66%)
		No	336 (45.34%)
	Diet	Yes	314 (42.38%)
		No	427 (57.62%)
Inheritance	Family History	Yes	479 (64.64%)
		No	262 (35.36%)
	Consanguinity Marriage	Yes	259 (34.95%)
		No	482 (65.05%)
Biochemistry Test	Fasting Plasma Glucose	<100 mg/dl	340 (45.88%)
		100–125 mg/dl	247 (33.33%)
		≥126 mg/dl	154 (20.78%)
	Oral Glucose Tolerance	<140 mg/dl	349 (47.10%)
		≥140 mg/dl	224 (30.23%)
	HBA1c	<6.5%	542 (73.14%)
		≥6.5%	199 (26.86%)
	Cholesterol	<200 mg/dl	495 (66.80%)
		≥200 mg/dl	246 (33.20%)
	Serum Triglycerides	<150 mg/dl	430 (58.03%)
		≥150 mg/dl	311 (41.97%)
	Urea	<43 mg/dl	707 (95.41%)
		≥43 mg/dl	34 (4.59%)
	Creatinine	<1.3 mg/dl	675 (91.09%)
		≥1.3 mg/dl	66 (8.91%)

are requested along with a sample for research purposes. Since there is a lack of information on genetic factors that predispose Arabs to T2DM, DNA samples are stored for present and future studies. Its value as a DNA data bank will increase over time as more volunteers are recruited and genetic studies are completed. It has, thus far, been quite successful. By increasing the numbers of patients that are in the DNA pool, we also increase our ability to identify gene polymorphisms that may be related to T2DM in Arabs.

When T2DM is identified in its early stages and treated, the impact of the disease and severity of the complications is reduced. However, there are even greater possibilities if the disease could be detected through DNA testing. By having a large data base of patients, we are more likely to determine

Table 4 Clinical and biochemical features of 391 patients diagnosed with T2DM, pre T2DM and 350 healthy individuals

Characteristic		Type 2 Diabetes Mellitus		Pre-Type 2 Diabetes Mellitus		Healthy	
		Male n0 85	Female n0 83	Male n0 167	Female n0 56	Male n0 218	Female n0 132
Physical appearance	Age	51.75±9.17	50.96±11.01	37.94±9.83	30.66±9.17	48.39±12.95	44.93±9.93
	BMI	33.47±7.21	31.94±7.97	28.37±6.75	29.11±8.40	23.92±4.01	23.84±4.06
	Waist Circumference	41.42±10.16	41.62±6.78	44.52±13.36	42.43±11.41	33.71±6.94	33.50±8.29
Life Style	Smoking	49.41	10.84	40.72	1.79	28.90	1.52
	Physical Activity	32.94	28.92	28.74	12.50	87.16	81.82
	Diet	23.53	16.87	3.59	5.36	75.69	80.30
Inheritance	Family History	63.53	71.08	55.69	78.57	3.67	3.03
	Consanguinity Marriage	50.59	51.81	38.92	30.36	29.82	19.70
Biochemical Test	Fasting Plasma Glucose	179.91±48.88	160.52±38.99	105.37±9.41	109.25±7.78	89.77±6.36	88.98±7.54
	Impaired Glucose Tolerance	–	–	159.61±13.16	158.18±14.03	99.73±9.61	98.91±8.37
	HbA_{1c}	8.24±1.73	7.86±1.90	6.42±1.48	5.98±0.46	4.97±0.63	4.92±0.60
	Cholesterol	239.78±35.95	227.98±54.31	195.37±15.42	203.13±15.40	116.11±19.48	122.43±16.40
	Serum Triglycerides	188.85±35.32	178.11±51.18	153.22±17.98	155.98±19.29	91.60±19.16	100.16±11.38
	Urea	43.47±6.77	32.27±7.35	25.17±5.98	23.93±5.92	21.95±5.13	21.55±5.31
	Creatinine	1.24±0.16	1.04±0.23	0.81±0.19	0.80±0.22	0.88±0.13	0.85±0.12

the nature of the genetic polymorphisms that predisposes to disease and the possibly the underlying mechanisms, giving rise to the potential of therapy. DNA research in general has just come into its own over the last two decades but research in diabetes and particularly an understanding of genetic makeup that is associated with T2DM in Arabs is desperately needed at this time.

This database will allow clinicians and researchers to have access to information that can make a tremendous difference to the nature of treatment that might be put in place to manage the disease. For example, as illustrated in Table 2, there is a higher number of females who are affected by T2DM in the UAE. Further, the prevalence of the disease increases with age (Fig. 1), in addition specific physical attributes and lifestyle habits (Table 4) are associated with the disease. A complication related to T2DM is metabolic syndrome. Hypercholesterolemia is one of many significant problems with levels above 200 mg/dl indicative of the abnormality (Tables 3 and 4). With this information, and in combination with other factors, physician can monitor patient with hypercholesterolemia and be aware of signs that could indicate a progression to T2DM.

Currently over 170 million people around the globe suffer from T2DM. Most of these patients are middle aged, however, variations in this regard are not rare, and are affected by factors such as lifestyle, heredity, as well as behavioural factors [10]. In this study, Table 3 shows young patients of 30 year age with a large waist circumference have fasting blood glucose at 108 mg/dl or greater and HbA_{1c} levels at 6.42% (data not

Table 5 Dunnett's multiple comparison test between healthy individuals and pre-diabetic and diabetic patients

Risk Factors		p value	
		Healthy vs. Pre-Diabetic	Healthy vs Diabetic
Physical Appearance	Age (years)	0.0079	0.0065
	BMI (kg/m2)	0.0121	0.0113
	Waist Circumference (inch)	0.0083	0.0085
Biochemical Test	Fasting Plasma Glucose (mg/dl)	0.0032	0.0025
	HbA1c (%)	0.0565	0.0484
	Total Serum Cholesterol (mg/dl)	0.0020	0.0018
	Serum triglycerides (mg/dl)	0.0025	0.0023
	Urea (mg/dl)	0.0138	0.0107
	Creatinine (mg/dl)	0.2792	0.2498

shown). It is also noted in Fig. 1 that the 40 to 59 year old group is the largest group but the group of 20 to 39 year olds are not far behind with 10% diagnosed with T2DM.

T2DM risks increase as an individual grows older, especially after the age of 45 years. It has been estimated that one out of five people aged 20 to 79 lives with this disease. Part of the reason is that as people grow older they tend to become less physically active and they gradually lose muscle mass and gain weight [11]. However over recent years, a dramatic rise in T2DM among individuals in their 30s and 40s has been observed and more children and teenagers are being diagnosed with the disease.

Public awareness can be increased using campaigns to reverse the alarming trend of increasing prevalence of diabetes. Moreover, over the past decade it has been obvious that the prevalence of T2DM is increasing rapidly. Unless appropriate action is taken, it is predicted that there will be at least 350 million people in the world with T2DM by the year 2030 [12].

Risk factors for T2DM are well defined. These include obesity, physical inactivity, family history of diabetes and those with a lower tolerance for glucose. Table 4 illustrates the importance of maintaining a healthy physique, especially BMI and waist circumference, life style, and biochemicals testing to monitor physiological changes. Abnormal fasting glucose above 126 mg/dl, triglycerides above 150 mg/dl, cholesterol above 200 mg/dl, and an elevated BMI and waist circumference can mean that the patient already has metabolic syndrome.

Previous studies have shown that a family history of T2DM is a very important indicator for developing T2DM [13, 14]. Among the 741 UAE national who donated blood for this study, 63% of males and 71% of females who are diabetic have first degree relatives with T2DM, where only 3.6% of males and 6.06% females without the disease have history of T2DM in their family. EFR has focussed on collecting DNA from families to study T2DM on the premise that having one or more first-degree relatives with T2DM increases the odds of having the disease. Further, the use of families provides a degree of redundancy with a registry. Over time patients are lost to the system due to migration. These individuals can be readily tracked by contacting family members to detect their whereabouts.

The prevalence of T2DM was more common among individuals in consanguineous marriages with first degree relatives compared with the healthy group, an observation that is in consonance with the study by Bener et, al., which showed that consanguineous marriages were more prevalent in T2DM patients [15]. This study also confirms previous studies [14] that showed T2DM closely associated with overweight and obesity (BMI >25). Okosun and his group showed in their study that a large waist circumference is the strongest indicator of T2DM risk [16]. Data in Table 4 show that males patients have larger waist circumferences than their females counterparts.

Additionally, the data in Table 4 shows that the smoking among T2DM and pre-T2DM is higher than the healthy individual. It has been suggested that smoking increases the risk of diabetes but the evidence has been inconclusive. It is not surprising that smoking plays an important role as there is evidence that smoking is bad for the pancreas, causes internal inflammation and increases the hormones that increase abdominal fat even in thin smokers, which would increase insulin resistance [17]. Table 4 summarises information to be used especially by those in primary care clinics. With access to a public health database such as the EFR, physicians can establish deficiencies and where diagnostic processes are resulting in diabetics being missed. This in turn allows them to determine processes that might allow for earlier diagnosis, follow up and prevent complications.

As with the association between disease and family relationships, ethnicity is another risk factor. African Americans, Hispanic or Latino Americans, American Indians, and some Asian Americans and Pacific Islanders are at particularly high risk for T2DM [18]. Some component of this factor is most likely related to genes carried from earlier times, passed down through generations. The data collected in the EFR and tabulated in Table 2 supports this showing varying degrees of prevalence among populations from different races.

The nature of DNA profile or genetic makeup is generally population specific and can provide leads toward best practice for care. To date, the nature of the genetic lesions that leads to T2DM in Arabs is not known. One of the primary objectives of the EFR was to provide a resource material to study genes of indigenous Arab populations. The DNA repository, when coupled with longitudinal data, will provide opportunities for researchers to dissect different variations of the disease and for physicians to determine what the long term management procedures might be used for monitoring patients.

In summary, the EFR data collected from volunteers has revealed that obesity, increased waist circumference, consanguineous marriages, positive family history, lack of physical activity, unhealthy diet with high total cholesterol and triglycerides levels were more prevalent in T2DM (Table 5).

There are both monogenic as well as polygenic forms of T2DM. While the simple classification method of Type 1 and Type 2 diabetes are helpful in unlocking the secrets of the disease, these have not resulted identifying key clear cut factors between both forms of the disease in the Arab population, therefore, a more extensive period of continuous research is required to understand the true nature of this disease. We believe that the longitudinal nature of the EFR will allow the researchers to assess whether or not there are confounding environmental factors or if a different set of genes account for earlier onset T2DM.

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Conflict of Interest All the authors declare no conflict of interest.

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ORIGINAL ARTICLE

Spironolactone treatment in patients with diabetic microalbuminuria and resistant hypertension

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Abstract Resistant hypertension is common in diabetes. Spironolactone by inhibiting aldosterone not only exerts antihypertensive effect but also antiproteinuric effect. In this study, the mean decrease in systolic and diastolic blood pressure after 4 weeks of spironolactone was 25.5±7.8 and 11.4±3.5 mmHg respectively and it increased further at 8 weeks and at 12 weeks, while there was no significant change in blood pressure in the control group. At 12 weeks, significantly greater reductions in both the systolic and diastolic blood pressure were observed in patients treated with spironolactone having serum potassium less than 4 meq/l vs those having serum potassium more than 4 meq/l. Reduction in urine microalbumin, though higher in patients with serum potassium less than 4 meq/l was not significant at any intervals.

Keywords Resistant hypertension · Spironolactone · Microalbuminuria · Rennin-angiotensin-aldosterone-axis

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Introduction

Resistant hypertension is defined as failure to control blood pressure (BP) to <140/ 90 mmHg with the use of three or more different classes of drugs, one of which includes a diuretic, in a reasonable combination and at full doses [1]. It accounts for 30% cases of hypertension [2]. A sustained reduction in blood pressure appears to be the most important single intervention to prevent progressive nephropathy in diabetes, with optimal BP control defined as below 125/ 75 mmHg [3]. In the United Kingdom Prospective Diabetes Study (UKPDS), a reduction in BP from 154 to 144 mmHg was associated with a 30% reduction in microalbuminuria [4].

Angiotensin Converting Enzyme (ACE) inhibitors are the mainstay of therapy [5]. By reducing BP and decreasing intraglomerular pressure, they reduce proteinuria and retard progression of chronic kidney disease [6]. ACE inhibitors not only retard the development of overt proteinuria, but also decrease urinary albumin excretion by more than 30%, leading to normoalbuminuria in some [7]. Two land mark trials, Reductions of Endpoints in patients with Non-insulin-dependent diabetes mellitus with Losartan (RENAL) and Irbesartan in Diabetic Nephropathy Trial (IDNT), examined the effects of Angiotensin Receptor Blockers (ARB) losartan and irbesartn respectively [8, 9]. Aldosterone, the end product of Rennin-Angiotensin-Aldosterone System (RAAS) is an important mediator of renal and cardiovascular disease [10]. Consequently aldosterone blockade improves survival in patients of chronic heart failure [11]. The recent reappraisal of European Society of Hypertension Guidelines [12] confirms that initiation of therapy in the high-normal range is reserved for diabetic patients with some degree of Target Organ Damage (TOD), in particular microalbuminuria.

Our study was carried out to observe the antihypertensive benefit of spironolactone as an add-on therapy in patients

with resistant hypertension currently being treated with a multidrug regimen including a diuretic, ACE inhibitors or ARBs and Calcium Channel Blockers (CCB).

Methods

We selected a total of 35 type 2 diabetes patients with resistant hypertension and microalbuminuria diagnosed on the basis of any of the following criteria like 1) 24 h urine albumin 30–300 mg/ 24 h, 2) spot urine albumin 20–200 μg/min, 3) spot urine albumin / creatinine 30–300 μg/mg or 4) spot urine albumin 30–300 mg/L.

All patients were subjected to history taking, physical examination including measurement of BP and routine investigations including glycemic profile, serum electrolytes, renal parameters, urine examination. The patients were included in the study when their BP was not controlled in 2 or more clinic visits with pharmacologically optimum doses of diuretic, ARB/ ACE inhibitors and CCBs and the patients were required to be on stable doses of antihypertensive agents for at least previous 6 weeks. After taking due signed written informed consents from the patients, they were segregated into 2 groups. Secondary causes of hypertension like renal parenchymal, renal vascular, primary hyperalosteronism, Cushing's syndrome or pheochromocytoma were excluded by biochemical analysis or radiological imaging when indicated. Group1 having 19 patients were designed to receive spironolactone 25 mg daily and group 2 having 16 patients received placebo. The patients in both the groups were matched for age, sex and antihypertensive drug intake.

Patients were asked for follow up visits at week 4, 8 and 12. During the study period, all patients were counseled to take less than 6 g salt a day. At follow up visits, patients were subjected for BP measurement according to American Heart Association Guidelines. Basing on the control of blood pressure, spironolactone dose was adjusted. Similarly the laboratory estimation of renal parameters, serum electrolytes and glycemic profile was also carried out.

Values were expressed as mean ± standard deviation. Values between groups and time periods were compared by student's "t" test and one-way analysis of variance (ANOVA). P values of <0.05 were considered statistically significant.

Results

The baseline clinical parameters of the study subjects are depicted in Table 1. It highlights the gross similarity in characteristics of the patients in both groups.

The comparison of the response to therapy is shown in Table 2. The mean decrease in systolic and diastolic blood pressure after 4 weeks of spironolactone was 25.5±7.8 and 11.4±3.5 mmHg respectively and it was 35.2±8.5 and 13.4± 2.1 mmHg at 8 weeks. It was maintained after 12 weeks of spironolactone wherein the values were 46. 7±10.4 and 17.5± 2.3 mmHg respectively. There was no significant change in blood pressure in the control group. At 12 weeks, significantly greater reductions in both the systolic and diastolic blood pressure were observed in patients treated with spironolactone having serum potassium less than 4 meq/l vs more than 4 meq/l (Table 3). Reduction in urine microalbumin, though higher in patients with serum potassium less than 4 meq/l was not significant at any intervals. Hyperkalemia (serum potassium >5.5 meq/l) was noted in one patient in the spironolactone group at the end of 12 weeks.

Table 1 Baseline clinical parameters of the patients. Data expressed as mean ± SD

	Group 1 (Spironolactone)	Group 2 (Placebo)
Number of patients	19	16
Age (in years)	45.6±13.1	48.1±12.5
M: F	12: 7	10: 6
BMI (Kg/ m^2)	32.5±4.6	31.2±3.8
Systolic BP (mm Hg)	175.6±13.4	171.4±10.6
Diastolic BP (mm Hg)	96.8±8.4	95.4±6.3
Fasting blood sugar	110.6±13.4	105.7±12.9
Post lunch blood sugar	150.8±25.6	145.2±24.5
Serum sodium (meq/l)	135.7±3.5	138.4±2.8
Serum potassium (meq/l)	4.1±0.6	4.4±0.8
Serum creatinine (mg/dl)	1.1±0.2	1.0±0.2
Urine for microalbumin (mg/day)	235.6±34.9	212. 9±40.5

Discussion

The results of our study demonstrate that spironolactone can induce substantial BP reduction when added to multidrug regimens in patients with diabetic microalbuminuria and resistant hypertension. Nishizaka MK et al., have shown that spironolactone could reduce BP in patients with resistant hypertension without hyperaldosteronism [13]. Another study by Abolghasmi et al. has demonstrated the efficacy of low dose hypertension in chronic kidney disease with resistant hypertension [14]. A surveillance study has also substantiated the efficacy of low dose spironolactone in resistant hypertension [15].

Extra-adrenal tissue may synthesize aldosterone. The vasculature, kidney and heart have been reported as sites of synthesis [16]. Besides the well documented effect of aldosterone to expand extra cellular volume with the net result of

Table 2 Follow up data of patients in both groups. Data expressed as mean ± SD and these data were compared with respective baseline values mentioned in Table 1 for statistical significance

	4 weeks		8 weeks		12 weeks	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Systolic BP (mm Hg)	140.1±8.3 *	168.8±8.3	132.4±8.9 *	165.7±9.6	127.1±7.4 *	169.4±10.1
Diastolic BP (mm Hg)	85.4±6.9 *	90.1±6.3	82.3±6.3 *	91. 4±7.5	78.3±4.4 *	90.6±6.9
Serum sodium (meq/l)	139.7±3.2	139.5±5.1	140.5±4.2	139.7±5.8	140.6±3.7	140.2±4.9
Serum potassium (meq/l)	4.2±0.3	4.6±0.3	4.3±0.4	4.8±0.2	4.2±0.9	4.7±0.5
Serum creatinine (mg/dl)	1.2±0.1	1.1±0.5	1.2±0.5	1.1±0.6	1.1±0.9	1.2±0.1
Urine for microalbumin (mg/day)	185. 6±23.9 *	210.4±36.7	175. 4±21.4 *	215. 0±32.4	169. 3±27.4 *	208. 6±31.7

*P<0.0001

hypertension, direct vascular actions of aldosterone have been proposed. Systemic vascular resistance changes modestly in response to acute aldosterone infusion in normal humans [17].

Chapman N et al. have provided an evidence base for an increased use of spironolactone in patients with resistant hypertension [18]. It appears that less than half the subjects defined as being resistant had been exposed to diuretics and their response to spironolactone was not different. It may no longer be necessary to have prior use of diuretics to define resistant hypertension. The preferential reduction in systolic BP in that study was attributed to the effect of spironolactone on arterial stiffness [19]. In another study, a significant relationship between the aldosterone to rennin ratio and aortic systolic BP and arterial stiffness was found in untreated hypertensive sub- jects [20]. The reduction in both brachial and aortic BP an arterial stiffness, measured by wave reflection, were all posi- tively related to baseline aldosterone to rennin ratio in patients receiving aldosterone only [20]. We did not find any similar finding of preferential systolic BP reduction in our subjects.

A previous study by Mahmud A et al. has shown a significantly greater BP reduction in response to spironolactone 50 mg daily in resistant hypertension individuals with serum potassium of under as opposed to above 4 meq/ l [21]. In our series though the reduction in BP was higher in patients with serum potassium <4 meq/l at all intervals, it was staastifically significant at 12 weeks only.

One study showed a strong correlation between aldosterone levels and degree of proteinuria [22], and others supported this association [23]. Patients in our series receiving spironolactone have shown persistent and significant reduction in microalbuminuria at all intervals. Nitta K et al. have concluded that adding spironolactone 25 mg/ day to an angiotensin receptor blocker in patients with chronic glomerulonephritis resulted in further BP reduction and a 13% decrease in proteinuria [24].

In our study, assymptomatic hyperkalemia was observed in one patient. None developed any other adverse events. Another study showed a significant increase in serum potassium with eplerenone use regardless of renal function [25].

In conclusion, addition of spironolactone would provide a significant additive BP reduction in patients with diabetic microalbuminuria and resistant hypertension. However, the limitations of our study were the brevity of the study period and inclusion of small number of patients. Larger clinical studies with long term follow up data would strengthen our preliminary findings on safety and efficacy of aldosterone antagonists in this category of patients.

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Table 3 Comparison of BP in patients treated with spironolactone based on serum potassium levels. Data expressed as mean ± SD

	4 weeks		8 weeks		12 weeks	
	Serum K^+ <4 (N0 6)	Serum K^+ >4 (N0 13)	Serum K^+ <4 (N0 4)	Serum K^+ >4 (N0 15)	Serum K^+ <4 (N0 5)	Serum K^+ >4 (N0 14)
Systolic blood pressure	139. 5±12.4	144.6±8.7	131.4±8.2	137.8±9.9	121.5±6.7 *	135.5±8.7
Diastolic blood pressure	82.5±7.8	86.8±6.7	80.3±7.9	84.8±5.8	73.1±4.2 *	84.7±5.7
Urine for microalbumin	182. 6±30.9	190.6±24.7	168. 4±22.9	177.4±27.9	165.4±32.5	176.7±27.8

*P<0.05 was significant

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ORIGINAL ARTICLE

Prevalence and impact on prognosis of glucometabolic states in acute coronary syndrome in a middle eastern country: The GLucometabolic abnOrmalities in patients with acute coronaRY syndrome in Jordan (GLORY) study

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Abstract Western studies have shown that diabetes mellitus and other glucometabolic states are highly prevalent among patients with Acute Coronary Syndrome (ACS), and are associated with worse adverse cardiovascular outcome. Whether this also applies to Middle Eastern patients is largely unknown. We studied the prevalence of glucometabolic states (known diabetes, newly diagnosed diabetes, impaired fasting glucose (IFG), and no diabetes) in 656 ACS patients, who were followed up prospectively for total mortality, and composite events of death, readmission for myocardial infarction, or urgent coronary revascularization for 1 year after admission. Results: Of the whole group, 291 (44.6%) were known diabetics, 69 (10.6%) had newly diagnosed diabetes, 86 (13.2%) had impaired fasting glucose, and 206 (31.6%) were nondiabetics. The overall in-hospital mortality rate was 2.6%, and was not significantly different between the four groups. At 1 year; overall mortality was 7.2%, and was significantly higher (p=0.002) among diabetics (newly diagnosed; 17.1%, and known diabetics; 7.8%) compared with patients who had IFG (3.4%) and nondiabetics (4.4%). Composite events at 6 months was significantly higher (p=0.016) in known diabetics (14.7%), compared with newly diagnosed diabetics (7.1%), IFG (9.2%) and nondiabetics (6.3%). At 1 year, composite events occurred in 15.9% of the whole group, and was significantly higher (p=0.049) in known diabetics (20.1%), compared with newly diagnosed diabetics (10%), IFG (11.5%) and nondiabetics (13.6%). Conclusions: In Middle Eastern ACS patients, 70% have abnormal glucometabolic states. Newly diagnosed diabetics and known diabetics have higher risk of cardiovascular events than patients with impaired fasting glucose and nondiabetics after 1 year of admission.

Keywords Glucometabolic states · Acute coronary syndrome · Adverse cardiovascular events

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Introduction

Cardiovascular disease (CVD) is the most common cause of death in the Middle East [1–4]. High prevalence of diabetes mellitus (DM), smoking, obesity and dyslipidemia contribute to the rising incidence of CVD in this region even among young individuals [5–8]. DM is an independent predictor for adverse outcomes in patients with acute coronary syndrome (ACS) [9–11], but data on the prevalence and impact of glucometabolic states, i.e., pre-existing or known DM, newly diagnosed DM, and impaired fasting glucose (IFG), on patients with ACS in the Middle East are largely unknown. The GLORY (GLucometabolic AbnOrmalities in patients with Acute CoronaRY Syndrome in Jordan) study is a prospective study of the prevalence of glucometabolic states and their impact on prognosis in patients with ACS who were followed up for 1 year after the index admission.

Methods

Between December 2007 and December 2008, 656 consecutive ACS patients admitted to the coronary care unit were enrolled in the study. Clinical features, electrocardiographic and laboratory findings, medical treatments, coronary diagnostic and revascularization procedures, and adverse events were documented during the index admission. Mortality and composite endpoints (mortality, readmission for myocardial infarction (MI), or urgent coronary revascularization) were evaluated at 1, 6, and 12 months. These endpoints were defined as follows: mortality was defined as death from any cause, including cardiovascular causes (fatal MI or stroke); MI as a reason for readmission, was defined as the presence of chest pain suggestive of myocardial ischemia associated with typical electrocardiographic changes and cardiac enzyme elevation changes (see below) and treated during a subsequent hospital admission. Urgent coronary revascularization was defined as, either primary coronary intervention for acute ST-segment elevation MI, or coronary intervention for non-ST-segment elevation ACS within 48 h of admission due to persistent chest pain despite medical therapy. Data on endpoints were obtained from patients during subsequent admissions during the first year after admission, and through periodic outpatient evaluation visits, or by phone calls at 1, 6, and 12 months.

All patients had chest pain suggestive of myocardial ischemia. ACS was classified as (1) acute ST-segment elevation MI (STEMI), defined by ST-segment elevation of ≥2 mm in at least 2 contiguous leads on the 12-lead electrocardiogram, and elevated cardiac troponin or CPK-MB >2 upper limit of normal, (2) non-ST-segment elevation MI (NSTEMI) defined by ST-segment depression and/or inverted T wave and elevated cardiac troponin or CPK-MB >2 upper limit of normal, or (3) unstable angina (UA) defined by ST-segment depression and/or inverted T wave and normal cardiac troponin on admission and 8–12 h later. Patients were treated by either invasive or conservative strategies according to the discretion of the treating cardiologists. Glucometabolic states were defined according to current guidelines as: (1) known DM, defined as past medical history of DM made by a physician, or patient being on antidiabetic treatment; (2) newly diagnosed DM, defined as either the presence of unequivocal hyperglycemia with classical symptoms of DM (polyuria, polydipsia, and unexplained weight loss) and casual plasma glucose ≥200 mg/dl, or fasting plasma glucose 126 mg/dl; (3) impaired fasting glucose (IFG), defined as a fasting plasma glucose 100–125 mg/dl; or (4) nondiabetic state, defined as a fasting plasma level of <100 mg/dl and no past history of DM or intake of antidiabetic treatment [12, 13]. Fasting plasma levels were obtained after at least 8 h of admission. We excluded patients whose chest pain was of non ischemic nature or did not fulfil the electrocardiographic criteria of ACS. The study protocol was approved by the participating hospitals' ethics committees, and written consent was obtained from all the patients.

Statistical analysis

Data were entered into computer using the Statistical Package for Social Sciences software, SPSS (SPSS Inc., Chicago, IL, USA) version 15. Percentages were compared using χ^2 test for categorical variables. Outcomes after 1 year of follow-up for patients admitted with ACS were compared between patients according to their glucometabolic states in stratified analysis by discharge diagnosis. A p-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the 656 patients are shown in Table 1. About 50% of the patients were younger than 60-year old. There was a high prevalence of hypertension, dyslipidemia, obesity, and smoking among the patients. STEMI was diagnosed in 28.5%, NSTEMI in 26.5%, and UA in 44.9% of the whole group. Coronary angiography was performed in 79% of all patients (87% of STEMI, 79% of NSTEMI, and 72% of UA patients). Percutaneous coronary revascularization was performed in 47% (63% in STEMI, 48% in NSTEMI, and 37% in UA patients), and 7% of the whole group underwent coronary artery bypass surgery. Aspirin was used in 95%, beta-blockers 62%, statins 90%, heparin (unfractionated or low molecular weight) 89%, clopidogrel 55%, angiotensin converting

Table 1 Baseline characteristics of 656 patients with acute coronary s
yndrome

Variable	N (%)
Age (year), mean (±SD)0 60.0 (±12.6)	
≤50	151 (23.0)
51–60	173 (26.4)
>60	332 (50.6)
Gender	
Women	191 (29.1)
Men	465 (70.9)
Hypertension	402 (61.3)
Dyslipidemia	403 (61.4)
Smoking	
No	279 (43.3)
Current	277 (43.0)
Ex-smoker	88 (13.7)
Family history of CVD	275 (41.9)
Weight category	
Normal (BMI<25)	103 (20.3)
Overweight (BMI 25–29.9)	204 (40.1)
Obesity (BMI 30–34.9)	140 (27.6)
Severe obesity (BMI ≥35)	61 (12.0)
Past history of CVD	
None	423 (64.5)
CAD, no revascularization	81 (12.3)
Percutaneous coronary intervention	121 (18.4)
Coronary bypass surgery	21 (3.2)
Past stroke	19 (2.9)

ACS acute coronary syndrome; BMI body mass index (weight (kg)/ height (m) ^{2}); CAD coronary artery disease; CVD cardiovascular
disease; SD standard deviation

enzyme inhibitors or angiotensin I receptor blockers 45%, and tirofiban in 32%.

The glucometabolic states among the patients are shown in Table 2. Overall, only 31.6% were free of abnormal glucometabolic states (i.e., non diabetics). On the other hand, 44.6% were known DM, 10.6% had newly diagnosed DM, and 13.2% had IFG. The prevalence of known DM was higher in patients with NSTEMI than patients with STEMI (52.0% vs. 37.1%). Newly diagnosed DM was more common among the STEMI patients (14.0%) compared with NSTEMI (11.0%) and UA (8.2%) patients.

Distribution of glucometabolic states among patients according to gender, age, body mass index (BMI), and other clinical features is shown in Table 3.

Of patients with new onset diabetes; 34% were diagnosed by fasting glucose measurements, 28% by random glucose measurements, and 38% by both measurements.

The majority of women (80%) and 2/3 of men had abnormal glucometabolic states. The prevalence of newly diagnosed DM was higher in men than women, but the prevalence of known DM was higher in women than men. DM (known and newly diagnosed) was higher among older age groups, higher BMI groups, smokers, and patients with hypertension. Patients with a diagnosis of known or new-onset diabetes were discharged taking oral hypoglycemic agents (38%), insulin (50%). Of patients with IFG; 4% developed diabetes by 1 year.

In-hospital outcomes are shown in Table 4. Overall, in-hospital mortality was 2.6%, and was not significantly higher (p=0.304) among patients with abnormal glucometabolic states [known DM (2.1%), newly diagnosed DM (5.7%) or IFG (3.4%)] compared with nondiabetic patients (1.9%). Patients with newly diagnosed DM were more likely to have cardiogenic shock, stroke and ventricular arrhythmia during admission, compared with patients with known DM, IFG and nondiabetic patients. The incidence of all adverse events was significantly higher (p=0.001) among patients with newly diagnosed DM (17.0%) and known DM (7.9%) compared with nondiabetic patients (3.4%).

Patients' outcomes after 1, 6, and 12 months are shown in Table 5. At 1 month, mortality was 4.0% in the whole group, and was significantly higher (p=0.0001) among newly diagnosed DM (14.3%), compared with mortality among known DM (3.1%), IFG (3.4%), and nondiabetic patients (1.9%). At 6 months, mortality was significantly higher (p=0.003) among diabetic patients (newly diagnosed 15.7%, and known DM 6.8%), compared with patients who had IFG (3.4%), and nondiabetic patients (3.9%). After 1 year of follow up, mortality was 7.2% in the whole group, and was the highest (p=0.002) among newly diagnosed DM (17.1%)

compared with known DM diabetic patients (7.8%), patients

with IFG (3.4%), and nondiabetic patients (4.4%). Composite events at 1 year occurred in 104 (15.9%) of the whole group. Known DM was associated with higher rate of composite events (20.1%, p=0.049) compared with nondiabetic patients (13.6%), patients with IFG (11.5%), and newly diagnosed DM (10%).

Discussion

To our knowledge, this is the first Middle Eastern prospective study that shows a high prevalence of abnormal glucometabolic states (up to 70%) among patient admitted with ACS, and an increased rate of adverse cardiovascular events among patients with abnormal glucometabolic states compared with nondiabetic patients.

Previous studies showed that the prevalence of known DM (about 50%) of Middle Eastern patients with ACS is higher than that reported among ACS patients in the West [14, 15], which is as low as 17% of patient with NSTEMI and as high as 21–32% [16],

Table 2 Glucometabolic states in patients with acute coronary syndrome

Glucometabolic states	STEMI (186 patients) N (%)	NSTEMI (173 patients) N (%)	UA (293 patients) N (%)	All patients (652) N (%)
Nondiabetics	64 (34.4)	48 (27.7)	93 (31.7)	206 (31.6)
Known DM	69 (37.1)	90 (52.0)	133 (45.5)	291 (44.6)
Newly diagnosed DM	26 (14.0)	19 (11.0)	24 (8.2)	69 (10.6)
IFG	27 (14.5)	16 (9.2)	43 (14.7)	86 (13.2)

DM diabetes mellitus; IFG impaired fasting glucose; NSTEMI non-ST-segment elevation myocardial infarction; STEMI ST-segment elevation myocardial infarction; UA unstable angina

depending on the electrocardiographic changes on admission [2, 10, 16–19].

The criteria for diagnosis of known DM in most of these studies followed the EUROSPIRE study criteria, which defined known DM as "a prior history of DM diagnosed by another physician, or the use of insulin or oral antidiabetic agent" [12, 13]. It is evident that this widely used definition may potentially underestimate the true prevalence of glucometabolic states because it overlooks the frequently-encountered ACS patients with no prior diagnosis of DM whose plasma glucose levels upon admission are higher than normal levels and fall in the ranges of IFG or DM. We found that an additional one-fourth (23.8%) of patients had newly diagnosed DM or IFG, who could have been excluded from being diagnosed as ACS patients with abnormal glucometabolic states had the EUROSPIRE

Table 3 Glucometabolic states in patients according to relevant characteristics

Variable	Glucometabolic states				P-value
	Non-diabetics N (%)	Known DM N (%)	Newly diagnosed DM N (%)	IFG N (%)	
Gender					
Women	40 (20.9)	108 (56.5)	15 (7.9)	28 (14.7)	0.001
Men	167 (35.9)	184 (39.6)	55 (11.8)	59 (12.7)	
Age (year)					
≤50	76 (50.3)	41 (27.2)	16 (10.6)	18 (11.9)	
51–60	61 (35.3)	80 (46.2)	13 (7.5)	19 (11.0)	0.0001
>60	69 (20.8)	172 (51.8)	41 (12.3)	50 (15.1)	
Body mass index					
<25	45 (43.7)	37 (35.9)	10 (9.7)	11 (10.7)	
25–29.9	66 (32.4)	83 (40.7)	25 (12.3)	30 (14.7)	0.037
30–34.9	34 (24.3)	70 (50.0)	17 (12.1)	19 (13.6)	
≥35	12 (19.7)	36 (59.0)	6 (9.8)	7 (11.5)	
Hypertension					
No	97 (50.3)	72 (26.6)	30 (47.6)	37 (45.7)	0.001
Yes	96 (49.7)	199 (73.4)	33 (52.4)	44 (54.3)	
Current smokers					
No	83 (22.4)	194 (52.4)	36 (9.7)	57 (15.4)	0.0001
Yes	129 (43.2)	95 (34.2)	34 (12.2)	29 (10.4)	
Past history of stroke					
No	199 (31.5)	280 (44.4)	68 (10.8)	84 (13.3)	0.788
Yes	5 (26.3)	10 (52.6)	1 (5.3)	3 (15.8)	

DM diabetes mellitus, IFG impaired fasting glucose

Table 4 In-hospital adverse events in acute coronary syndrome patients according to glucometabolic states

	Glucometabolic states					
	Non-diabetics (N0206) N (%)	Known DM (N0293) N (%)	Newly diagnosed DM (N070) N (%)	IFG (N087) N (%)	All (N0656) N (%)	P*
In-hospital death	4 (1.9)	6 (2.1)	4 (5.7)	3 (3.4)	17 (2.6)	0.304
Cardiogenic shock	6 (2.9)	22 (7.5) *	11 (15.9) *	1 (1.1)	40 (6.1)	0.0001
Bleeding	8 (3.9)	12 (4.1)	5 (7.2)	5 (5.7)	30 (4.6)	0.619
Stroke	0 (0)	3 (1.0) *	3 (4.3) *	0 (0)	6 (0.9)	0.008
VT	6 (2.9)	3 (1.0)	7 (10.1) *	3 (3.4)	19 (2.8)	0.001
Recurrent Ischemia	5 (2.4)	10 (3.4)	2 (2.9)	1 (1.1)	18 (2.7)	0.705
AV block	2 (1.0)	3 (1.0)	1 (1.4)	0 (0)	6 (0.9)	0.786
Renal failure	3 (1.5)	12 (4.1)	3 (4.3)	1 (1.1)	19 (2.9)	0.215

Definitions of events:

Cardiogenic shock was defined as systemic arterial hypotension (systolic blood pressure ≤90 mmHg) due to left ventricular pump failure determined by left ventricular ejection fraction ≤35% on transthoracic echocardiography or contrast left ventriculography; bleeding was defined as the presence of one or more of the following: gastrointestinal bleeding, drop in serum haemoglobin concentration of ≥2 g/dl, a major hematoma at the vascular access site (>4 × 4 cm), or one requiring blood transfusion; stroke was defined as the development of a new neurological deficit due to thrombotic occlusive disease in the cerebral circulation involving the internal carotid or vertebral arteries territories, or intra-cerebral haemorrhage, associated with a computed tomography scan finding of cerebral infarct or haemorrhage; VT ventricular tachycardia, defined as the presence of ≥3 consecutive premature ventricular beats necessitating the administration of antiarrhythmic medication or electric cardioversion; recurrent ischemia was defined as recurrence of the angina pain the patient complained of initially upon admission, with or without new electrocardiographic changes; AV block Atrio-ventricular block, defined as third degree atrioventricular block with a ventricular rate <50 beats per minute, associated with hypotension and necessitating the insertion of percutaneous transvenous temporary pacemaker; renal failure was said to be present when it had been previously diagnosed, when serum creatinine on admission was >2 mg/dl, or when there was a rise in serum creatinine of ≥50% above baseline value

DM diabetes mellitus, IFG impaired fasting glucose

P* value comparing non-diabetics with the other groups

definition been used. Several prospective studies have reported a prevalence of newly diagnosed DM in patients with coronary artery disease and no previous diagnosis of DM ranging from 10% to 22% [12, 13, 20–22].

Patients with impaired fasting glucose accounted for only 3% of ACS patients in one study, much less that in our study (13.2%) [12, 13]. Use of oral glucose tolerance test (OGTT) in ACS patients is recommended for accurate diagnosis of

Table 5 Patients' outcomes after 1, 6, and 12 months of follow up according to glucometabolic states

	Glucometabolic states						
	Non-diabetics (N0206)	Known DM (N0293)	Newly diagnosed DM (N070)	IFG (N087)	All (N0656)	P*	P-value
Mortality, N (%)							0.304
1 month	4 (1.9)	9 (3.1)	10 (14.3) *	3 (3.4)	26 (4.0)	0.0001	0.0001
6 months	8 (3.9)	20 (6.8) *	11 (15.7) *	3 (3.4)	42 (6.4)	0.003	0.619
12 months	9 (4.4)	23 (7.8) *	12 (17.1) *	3 (3.4)	47 (7.2)	0.002	0.008
Composite events[a], N (%)							0.001
1 month	3 (1.5)	15 (5.1) *	2 (2.9)	0 (0)	20 (3.0)	0.033	0.705
6 months	13 (6.3)	43 (14.7) *	5 (7.1)	8 (9.2)	69 (10.5)	0.016	
12 months	28 (13.6)	59 (20.1) *	7 (10.0)	10 (11.5)	104 (15.9)	0.049	

DM diabetes mellitus; IFG impaired fasting glucose

[a] Composite events: mortality, readmission for MI, or urgent coronary revascularization

P* value comparing non-diabetics with the other groups

impaired glucose tolerance and can detect more patients (up to 22%) with glucometabolic states other than newly diagnosed DM or IFG [13]. However, performing OGTT in ACS patients during their critical care unit stay was not recommended by the study's ethics committee, similar to recommendations of other studies' ethics committees, a fact that explains the low percentage (53%) of the OGTT data in such patient population [13, 23].

Glucometabolic states and adverse cardiovascular events

Several clinical studies [9–13], including Middle Eastern ones [4, 7, 15], have shown higher rates of mortality and adverse events during hospital admission for ACS and after variable periods of follow up (≥1 year) in association with DM (known and newly diagnosed) compared with nondiabetic patients. Our study confirmed that diabetic patients had higher incidence on in-hospital complications, including cardiogenic shock, ventricular arrhythmias and stroke. Higher mortality rate compared with nondiabetic patients were evident at 1 month of follow up to 1 year after index admission.

We also observed that the 1-year mortality in the newly diagnosed DM was higher than that of known DM (17.7% vs. 7.8%, respectively, p0 0.002), a finding that is not in agreement with other studies which observed that patients with newly diagnosed DM presented as an intermediate risk group with 1-year mortality in-between that of known DM and patients with normal glucose regulation [13].

Higher mortality among newly diagnosed DM could be explained by the possibility that those patients were actually suffering from unrecognized diabetes probably for a considerable time before sustaining ACS, but did not seek medical attention to have their DM diagnosed earlier. Treatment bias was an unlikely explanation of higher mortality among newly diagnosed DM due to the fact that there was no difference in the rate of utilization of the ACS standard medications (antiplatelets, anticoagulants, beta blockers, renin-angiotensin system blockers, and statins), or coronary revascularization procedures among patients with different glucometabolic states.

IFG could not be identified, in our study, as an independent predictor for adverse outcomes, contrary to other studies that observed an increase of future cardiovascular events in IFG after acute MI as compared with non-diabetics [23]. It should be noted, however, that all participants of prior studies were followed for almost 3 years, whereas the present study included a heterogeneous group of patients with ACS who were followed for only 1 year. This period may have been not long enough to observe an increased risk for adverse events in patients with ACS and IFG.

This study has several important implications for cardiac care offered to ACS patients in our region. Glucometabolic states among these patients should be looked for, and hyperglycemia among patients not known previously to have diabetes should not be dismissed as a stress-related phenomenon [24–26]. Due to the fact that newly diagnosed and known diabetes are strongly associated with adverse outcomes, endocrinologists should be actively participating in the acute care of such patients, and cardiologist should utilize the recommended pharmacological agents, and should also follow an aggressive coronary diagnostic and revascularization strategies in these high-risk patients.

A limitation of our study is that we did not perform multivariate analysis to control for possible confounders which are many in this study. However, the number of patients sustaining individual clinical events among each glucometabolic states category and among each of the ACS subgroup (STEMI or NSTEACS) is small. For example; the number of patients sustaining in-hospital death, cardiogenic shock, stroke, significant arrhythmias, recurrent ischemia, or renal failure in the IFG category ranges from 0 to 5. Such small number of events doesn't allow sufficient power to conduct multivariate analysis using logistic regression or Cox proportional hazard regression analysis.

In conclusion, in this large population of patients with ACS, we found that about 70% patients have abnormal glucometabolic states. We observed that patients with known diabetes are at high risk for mortality and cardiovascular events, and demonstrated that patients with newly diagnosed diabetes are at even higher risk for adverse outcomes.

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ORIGINAL ARTICLE

Performance of four risk scores for predicting insulin resistance in Thai adults

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Abstract This study aims to evaluate the performances of four scoring systems, previously designed for screening type 2 diabetes, in predicting insulin resistance in healthy Thai adults. A cross-sectional study was conducted on 1,195 participants aged ≥ 35 years old who were undergoing annual health check-ups in 2008. The four sets of scores used were The Royal College of Physicians of Thailand Score, Thailand Ministry of Public Health Score, Aekplakorn's and Keesukphan's risk scores. Predictive performances of the four methods were assessed by using a homeostasis model assessment insulin resistance index (HOMA-IR), with cut-points of ≥ 1.56 and ≥ 1.64 respectively for men and women, as a gold standard. All four scoring systems are associated with HOMA-IR, with the correlation coefficients ranging from 0.335 to 0.442 and $p<0.001$. Sensitivities and specificities of all methods were 68.8- 81.2% and 38.1- 68.8%
respectively. Aekplakorn's method provides the best overall performance as indicated by the AUC (0.755), with the sensitivity of 71.3% and the specificity of 68.8% in predicting insulin resistance in the study population. Diabetes risk scores available for Thai adults, particularly Aekplakorn's, could also be used as practical methods to identify those with insulin resistance.

Keywords Insulin resistance · Sensitivity and specificity · Screening Instruments · Risk score

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Introduction

Diabetes Mellitus (DM) is a metabolic disorder which has a large impact on humanity around the world. Many people suffer from late complications of the disease, e.g. irreversible blindness, chronic kidney disease, cardiovascular disease, foot ulcers, etc. prevalence of diabetes continues to rise globally [1].

Data suggests that by the time fasting plasma glucose gets elevated, some degree of complications will have already developed [2, 3]. Therefore, the ability to detect the pathology before the rise in blood glucose occurs will lead to a better prognosis. We have conducted this study to find an alternative way to detect insulin resistance, which is the condition that develops earliest in the disease course [4]. From the literature, there are several proposed ways to detect insulin resistance. Euglycemic clamps are the gold standard for identifying the pathology; however, this method is costly and only useful for intensive physiological studies on small numbers of subjects [5]. Another method, the homeostasis model assessment (HOMA), is widely used and more appropriate for large epidemiological studies [6]. This method, proposed by Matthews et al., is calculated by using fasting plasma glucose and fasting plasma insulin.

There is also a study which evaluates the ability of a scoring system, initially developed to estimate the probability of asymptomatic type 2 DM, to detect insulin resistance

in subjects with high risk for diabetes [7]. The results indicate a good applicability.

In this study, we aim to assess the ability of four scoring systems, initially developed in Thailand for either detecting undiagnosed type 2 diabetes or predicting future DM, to detect insulin resistance among employees of a university hospital in Thailand, by using HOMA-IR as a gold standard. These methods are proposed by: (1) The Royal College of Physicians of Thailand (RC) [8], (2) Thailand's Ministry of Public Health (PH) [9], (3) Aekplakorn's (ADR) [10], (4) Keesukphan's (KDR) [11]. These scoring systems are non-invasive and inexpensive; features that are appropriate and important for developing countries like Thailand.

Materials and Methods

Subjects

A cross-sectional study was conducted on hospital personnel who participated in annual health check-ups at King Chulalongkorn Memorial Hospital in Bangkok during August through October 2008. The total participant population comprised 1,265 subjects (246 men and 1,019 women) who were ≥ 35 years old at the time of their annual health examination.

Participants who were currently taking anti-diabetic medications at the time of the annual health examination, who had previous medical diagnoses of diabetes, or whose data needed for calculations was missing, were excluded from this study. 1,195 participants (223 men and 972 women) with no known history of diabetes comprised the sample population for this study.

The research was carried out with the approval of the Ethical Committee of the Faculty of Medicine, Chulalongkorn University, and informed consents were obtained before data was collected from the participants.

Data collection

Self-answered questionnaires were used for collecting participants' information, including age, marital status, occupation, educational attainment, medical history, the use of anti-hypertensive, anti-diabetic, or lipid-lowering medications, smoking status, and alcohol consumption habits.

All participants underwent routine physical examinations that included measurements of height, weight, waist circumference and resting blood pressure. Standing height was measured to the nearest 0.5 cm, when the subject was barefeet. Weight was measured with an automatic electronic scale (Seca, Inc., Hamburg, Germany) to the nearest 100 g when subjects were lightly clothed and barefeet. Waist circumference was measured midway between the inferior margin of the last rib and the iliac crest at the end of expiration with a heavy-duty inelastic plastic fiber measuring tape to the nearest 0.5 cm while the subject stood balanced on both feet. Systolic and diastolic blood pressures were measured using an automatic sphygmomanometer (UDEX-IIα, UEDA, Corp., Tokyo, Japan), after the subjects had been in a rested seating position for at least 5 min.

Laboratory procedures

Venous blood samples after an overnight fast were collected from all participants. Fasting plasma glucose (FPG) concentration was measured by the hexokinase method, and fasting serum insulin concentration was measured using a solid-phase, two-site chemiluminescent immunometric assay (Immulite 1000, Insulin). Glucose and insulin concentrations were reported as mg/dl and μIU/ml, respectively.

HOMA-IR index

The Homeostasis Model Assessment— insulin resistance (HOMA-IR) index was used as a surrogate measure of insulin resistance because it has been shown to correlate
well with values obtained by the "gold standard" clamp technique [6]. HOMA-IR is calculated through the following mathematic formula:

HOMA IR ¼ FI FPG=405

FI 0 fasting serum insulin (μIU/ml); FPG 0 fasting plasma glucose (mg/dl); 405 is a constant.

According to a previous epidemiological study on the Thai population, the mean HOMA-IR score of subjects with a normal insulin resistance status is below 1.56 in men and below 1.64 in women [12]. Subjects with a greater than normal value are considered insulin resistant.

Variables definition

Central obesity was measured by waist circumference. The cut-points for South Asians were ≥ 90cms in men, and ≥ 80cms in women [13].

Hypertension is defined as sustained systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg [14]. Family history of DM is defined as the positive history of diabetes in first-degree relatives such as parent or sibling.

Impaired fasting plasma glucose (IFG) is defined as FPG 100 mg/dl to 125 mg/dl. Fasting is defined as no caloric intake for at least 8 h [15]. Diabetes Mellitus is defined by FPG ≥ 126 mg/dl, 2-h plasma glucose ≥ 200 mg/dl during an OGTT, or by classic symptoms of hyperglycemia or hyperglycemic crisis with a random plasma glucose ≥ 200 mg/dl [15].

Screening instruments

All subjects completed a set of screening questionnaires which included items from all four screening methods. Characteristics of the screening methods are described in detail in Table 1.

Statistical analysis

Population characteristics were summarized by mean and standard deviation for continuous variables, or frequency and percentage for categorical variables. Screening risk scores, as well as the HOMA-IR, were summarized by median and interquartile range (IQR), as the data were not normally distributed.

The associations between each scoring system and HOMA-IR were assessed by the Spearman correlation test. Performances of screening methods in identifying insulin resistance were then analyzed. Sensitivities and specificities of the screening methods were determined at the different cut-off points for each method. Receiver operator characteristic (ROC) curves were then constructed by plotting sensitivity against 1-specificity for each cut-off value, and the areas under the curve (AUC) were calculated in order to compare the impacts of screening methods [16]. The positive and negative predictive values were derived using Bayes' Theorem. The prevalences used in the formula were obtained from the previous epidemiological study in Thailand [12].

All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc. Chicago, IL, USA.).

Results

Subjects characteristics

The characteristics of the study population are shown in Table 2. Among the population included in the study, there are similar percentages for each age group. The mean values of Body Mass Index (BMI) and waist circumference (WC) for men were 25.4 kg/m^2 and 86 cm respectively. For women, the mean BMI was 24.2 kg/m^2 and the mean WC was 76 cm. The prevalence of hypertension was 38.1% and 18.7% for men and women respectively. The percentage of normal FBG in

Table 1 Summary of four screening methods. Cut-off-points presented below are from the original studies. Note, the cut-off-points for detecting insulin resistance differ in 1 and 4 methods

Methods	Variables included	Remark
1) The Royal College of Physicians of Thailand(8)	Age >40 years old, Body mass index (BMI) >27 kg/m^2 Family history of diabetes Previous delivery of large infant (≥ 4000 gm) or diagnosed with GDM Hypertension(BP≥ 140/90 mmHg) History of HDL-C ≤ 35 mg/dl or triglyceride ≥ 250 mg/dl and IFG or IGT	Total score 0–6, cut-off point ≥ 2
2) Thailand Ministry of Public Health(9)	Age ≥ 35 years old Body mass index (BMI) ≥ 25 kg/m^2 Family history of diabetes Previous delivery of large infant (≥ 4000 gm) or diagnosed with GDM Hypertension (BP ≥ 140/90 mmHg or history of hypertension) History of HDL-C ≤ 35 mg/dl or triglyceride ≥ 250 mg/dl and IFG or IGT	Total score 0–6, cut-off point ≥ 2
3) Aekplakorn's(10).	Age Gender BMI Waist circumference ≥ 90 men, ≥ 80 women Hypertension (BP ≥ 140/90 mmHg or history of hypertension) History of diabetes in parent or sibling	Total score 0–17, different weighing applied , cut-off point ≥ 6
4) Keesukphan's(11).	Age BMI History of hypertension	Using formula "3Age +5BMI +50HHT[a]", cut-off point ≥ 240

[a] HHT: History of Hypertension; presence 0–1, absence 0–0

Table 2 Baseline Characteris- tics of Study Population (N01,195). Data are means±SD HOMA-IR

Number of subjects	Men		Women	
	223	(18.7)	972	(81.3)
Age (years)	46	±7	46	±7
Age group				
35- 39 years	47	(21.1)	244	(25.1)
40- 44 years	60	(26.9)	214	(22.0)
45- 49 years	50	(22.4)	240	(24.7)
≥ 50 years	66	(29.6)	274	(28.2)
BMI (kg/m^2)	25.4	±4.2	24.2	±4.0
BMI group				
<23 kg/m^2	62	(27.8)	440	(45.3)
23- 27.4 kg/m^2	109	(48.9)	350	(36.0)
≥ 27.5 kg/m^2	52	(23.3)	182	(18.7)
Waist circumference (cm)	86	±9	76	±10
Central obesity[a]	68	(30.5)	333	(34.3)
Hypertension	85	(38.1)	182	(18.7)
Family history of DM	64	(28.7)	345	(35.5)
Previous delivery of large infant (≥ 4000 gm)	N/A	N/A	29	(3.0)
Previous diagnosed with GDM	N/A	N/A	17	(1.7)
History of IFG 100- 125 mg/dl	13	(5.8)	24	(2.5)
History of IGT 140- 199 mg/dl	3	(0.3)	6	(0.6)
FPG (mg/dl)	90	±18	87	±12
FPG status				
Normal FPG	194	(87.0)	900	(92.6)
IFG range	27	(12.1)	62	(6.4)
DM range	2	(0.9)	10	(1.0)
History of HDL ≤ 35 mg/dl	44	(19.7)	120	(12.3)
History of TG ≥ 250 mg/dl	49	(22.0)	140	(14.4)
HOMA-IR [median(Inter-quartile range)]	0.871	(1.16)	0.899	(1.03)
Insulin resistance[b]	59	(26.5)	207	(21.3)

[a]Waist circumference ≥ 90 men, ≥ 80 women

[b]HOMA-IR ≥ 1.56 for men and ≥ 1.64 for women

men was 87.0% and in women was 92.6%, whereas the rest had abnormal fasting blood glucose levels, either in the IFG range or the diabetic range. By using the HOMA-IR values ≥ 1.56 for men and ≥ 1.64 for women as the cut-off points to indicate insulin resistance, we found that 59 (26.5%) men and 207 (21.3%) women were considered positive. Other histories indicating the risks of DM are also exhibited in Table 2.

Calculated risk scores

The summaries of calculated scores for the four scoring systems are shown in Table 3. Overall, calculated scores for all scoring system were skewed to the lower values. For example, while calculated Royal College of Physicians of Thailand Scores (RC scores) for all participants ranged from zero to five, the majority of the study population scored 1 or 2 (38.1% and 28.8%, respectively). Calculated Thailand Ministry of Public Health Scores (PH scores) also ranged from zero to 5, with the majority of participants scoring only 1 or 2 (34.7% and 36.7% respectively).

Correlation of the four scoring systems and HOMA-IR

All of the four scoring systems are positively correlated with HOMA-IR, with the correlation coefficients ranging from 0.335 to 0.442 and $p<0.001$ (Table 4). The ADR scoring system vs. HOMA-IR shows the highest correlation (correlation coefficient00.442, $p<0.001$).

The predictive performance of the four scoring systems to detect insulin resistance

The receiver operating characteristics (ROC) curves were plotted in order to find the optimal cut-off point for predicting insulin resistance, by using the HOMA-IR value (≥ 1.56 in men vs. ≥ 1.64 in women) as a state variable (Fig. 1). The

Table 3 Calculated scores for the four scoring systems

Screening Method	Median	(IQR[a])	(Min, Max[b])
The Royal College of Physicians of Thailand Score (RC score)	1	(1)	(0- 5)
Thailand Ministry of Public Health Score (PH scores)	2	(2)	(0- 5)
Aekplakorn's Diabetes Risk Score (ADR scores)	5	(6)	(0- 15)
Keesukphan's Diabetes Risk Score (KDR scores)	258.15	(48.85)	(189.52-
HOMA	0.89	(1.06)	(0.02- 39.79)

[a]Inter-quartile Range,
[b]Minimum, Maximum

optimal cut-off points for the RC and PH scoring systems were 1 and 2, while those for the ADR and KDR were 6 and 260 respectively.

Overall, sensitivities and specificities of the screening methods ranged between 68.8- 81.2% and 38.1- 68.8% respectively. Their positive and negative predictive values (PPV and NPV) in male subjects ranged from 30.5% to 43.4% and 84.8% to 87.7%, respectively. Their PPVs and NPVs in women ranged from 26.4% to 38.5% and 88.0% to 89.7%, respectively (Table 5). While the RC scoring system had the highest sensitivity (81.2%), its specificity was the lowest (38.1%). When using the AUCs as the indicators for overall performance of the screening methods, their values ranged between 0.695- 0.755, with the ADR scoring system having the highest AUC (0.755). The ADR scoring system also had the highest specificity (68.8%), PPV (69.6%), and NPV (70.6%), while its sensitivity was the second highest (71.3%).

When excluding participants with abnormal fasting plasma glucose (≥ 100 mg/dl) and those in the diabetic range (≥ 126 mg/dl) from the analysis, the sensitivities and specificities, PPVs and NPVs, and AUCs are slightly decreased (Table 5).

Discussion

Several tools have been developed in order to identify individuals at high risk of developing diabetes. Each of those tools varies in terms of variables needed to calculate the risk and were invented by different organizations using different study populations. Therefore, their applicabilities may be altered if applied to a different group of subjects. These tools, along with their sensitivities, specificities and other relevant information are listed and reviewed in a recent study [17].

The attempt to examine the ability of a questionnaire, originally designed for assessing the diabetes risks and detecting undiagnosed type 2 DM, to predict insulin resistance has already been made. The Finnish Diabetes risk score (FINDRISC) has been evaluated by Schwarz et al. for this purpose [7]. The study confirmed that the FINDRISC was significantly correlated with HOMA-IR index. Analysis of our data indicates correlation between all four scoring systems and HOMA-IR. Aekplakorn's Diabetes Risk score shows the strongest correlation as compared with the other three tools. The analyzed overall performance, indicated by the AUC, was also highest in Aekplakorn's method, with the sensitivity and the specificity of 71.3% and 68.8%, respectively. Because insulin resistance occurs early in the development of DM, using the ADR score to detect this condition can lead to a better prediction. The earlier people recognize insulin resistance in themselves, the more conscientious they will be about their health. This may slow the development of DM. The simplicity and inexpensiveness of the tools used in Aekplakorn's method make them suitable for large-scale usage, especially in developing countries such as Thailand.

After we excluded participants with abnormal fasting blood glucose and those in the diabetic range, 1,094 of subjects with normal FBG remained for analysis. The subgroup analysis was done using the same cut-off points as previously indicated. It subsequently revealed a slight decrease of both sensitivities and specificities in all four scoring systems.

As stated above, there was a previous attempt to examine the ability of a questionnaire, originally designed for assessing the diabetes risks and detecting undiagnosed type 2 DM, to predict insulin resistance. In the study conducted by Schwarz et al., the Finnish Diabetes risk score (FINDRISC) was found to have a significant correlation with HOMA-IR [7] and it has also been encouraged to be used as a diabetes

Table 4 The correlation coeffi- cients for each scoring system and HOMA-IR

Screening Method	Correlation Coefficient (p-value)
The Royal College of Physicians of Thailand Score (RC score)	0. 335 (<0.001)
Thailand Ministry of Public Health Score (PH score)	0.368 (<0.001)
Aekplakorn's Diabetes Risk Score (ADR score)	0.442 (<0.001)
Keesukphan's Diabetes Risk Score (KDR score)	0.386 (<0.001)

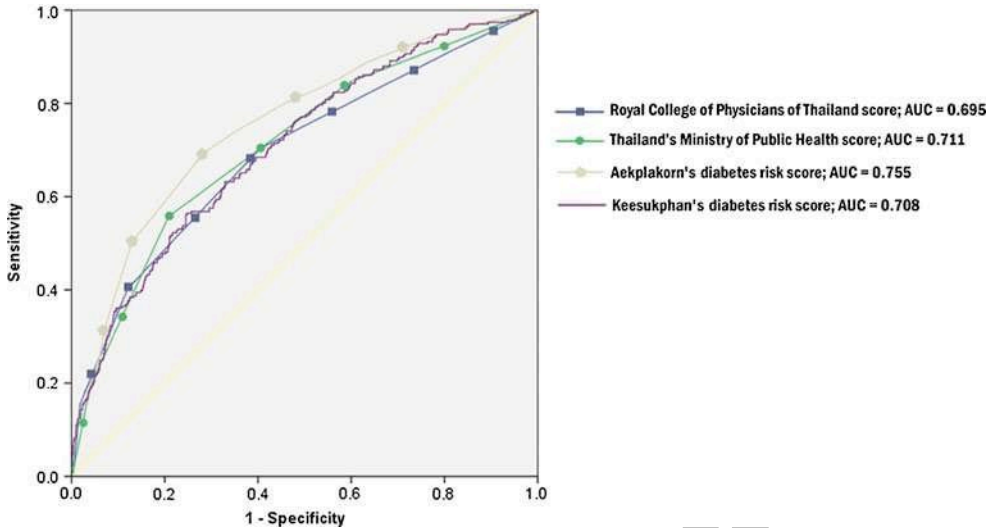

Fig. 1 ROC curves of all four scoring systems, providing the best cut-off HOMA-IR values for predicting insulin resistance

risk screening tool in a recent study [18]. As compared to Aekplakorn's scoring system, FINDRISC (in a 1997 baseline study) at the optimal cut-off point has a higher sensitivity (72.7% vs. 71.3%) but a lower specificity (68.2% vs. 68.8%) to predict insulin resistance in populations with no known diabetes. The overall performance as demonstrated by AUCs is higher in Aekplakorn's method than in the FINDRISC. However, the difference in study populations should be taken into consideration when comparing the sensitivities and specificities between FINDRISC and the four scoring systems in our study. The questionnaire we used did not include some of parameters needed to assess the FINDRISC scores. As a result, the authors cannot evaluate the sensitivity and the specificity of the FINDRISC from the study population. FINDRISC may also be one of the appropriate tools to detect insulin resistance in Asian population. A further study to compare the sensitivities and specificities between FINDRISC and the four methods, especially Aekplakorn's method, is suggested, and can be accomplished by applying these methods to the same Asian study group.

Using the same study populations as ours, the performance of the four scoring systems to identify abnormal FPG was conducted by Srichang, et al. [19]. The sensitivities and specificities of all four methods range from 71% to 92% and 31% to 57%, respectively. The corresponding AUCs were 0.68‑0.73. When comparing this data to the performance (as demonstrated by AUCs) of these four scoring systems in identifying adults with abnormal FPG, the performance in identifying those with insulin resistance was higher in three scoring systems, excluding Keesukphan's method.

The use of history of low HDL or high triglycerides and IGT or IFG in The Royal College of Physicians of Thailand score and The Thailand Ministry of Public Health score may not be practical in general population use. Not everybody has taken their blood test, so negative lab history does not necessarily indicate normal levels of lipids and glucose. As

Table 5 Sensitivities, specificities, Positive Predictive Values (PPV), Negative Predictive Values (NPV) of the scoring systems to detect insulin resistance

Scoring Systems	Sensitivity (%)	Specificity (%)	PPV (%) male	NPV (%) male	PPV (%) female	NPV (%) female	AUC
All participants (N01,195)							
The Royal College of Physicians of Thailand Score (RC score)	81.2	38.1	30.5	85.8	26.4	88.1	0.695
Thailand Ministry of Public Health Score (PH score)	70.1	59.8	36.9	85.6	32.3	88.0	0.711
Aekplakorn's Diabetes Risk Score (ADR score)	71.3	68.8	43.4	87.7	38.5	89.7	0.755
Keesukphan's Diabetes Risk Score (KDR score)	68.8	58.2	35.5	84.8	31.1	87.2	0.708
Excluding participants with abnormal FPG (N01,094)							
The Royal College of Physicians of Thailand Score (RC score)	77.6	39.1	29.9	83.9	25.9	86.4	0.665
Thailand Ministry of Public Health Score (PH score)	66.1	60.6	36.0	84.2	31.5	86.7	0.686
Aekplakorn's Diabetes Risk Score (ADR score)	65.6	69.8	42.1	85.8	37.3	88.1	0.729
Keesukphan's Diabetes Risk Score (KDR score)	62.9	59.2	34.1	82.6	29.7	85.4	0.676

a result, those participants will have lower scores. Those lower scores will decrease the sensitivities of these two methods. In contrast, Aekplakorn's and Keesukphan's tools are much simpler and do not require the lab data. This may account for the better performance of these methods.

According to the parameters used in the four risk scores, it should be noted that only anthropometry data, family history and history of abnormal biochemical blood test (low HDL or high triglycerides and IGT or IFG) were used in those questionnaires. As diabetes is a multi-factorial disease, there are more risk factors involved in the development of the disease; modifiable, non-modifiable and environmental risk factors. Modifiable risk factors include BMI, physical activities, diet, depression, hyperglycemia, hypertension, and lipid disorder. Non-modifiable risks are age, sex, family history, ethnicity, and low birth weight. Environmental factors include health promoting system, socio-economic status, culture, religious constraints, and stress. Adding more parameters such as physical activities or dietary habit to the questionnaire may help to create more accurate tools for predicting or screening diabetes in Thailand.

From a previous study in an Indian population, the Indian Diabetes Risk Score (IDRS) can be applied as a screening tool for arterial stiffness [20]. The parameters used in IDRS are age, abdominal obesity, family history of type 2 DM and physical activities. Three of the factors, excluding physical activities, are also used as parameters in Aekplakorn's diabetes risk score. We hypothesize that a correlation between the Aekplakorn's score and arterial stiffness might exist and a further study will be needed to confirm this hypothesis.

Some limitations exist in our study. Firstly, all subjects included were hospital employees of King Chulalongkorn Memorial Hospital in Bangkok, so they do not represent the general Thai population. Difference in backgrounds, lifestyles, eating habits, basic knowledge and health concerns between the study population and the whole Thai population may contribute to some degree of inaccuracy in the results, as these factors are important for developing DM. Moreover, all subjects participated in the annual health check-up. This indicates that this study population is more conscientious about personal health than the general population, and it might also influence data when the tool is used on a large scale. Additionally, the significantly larger number of female subjects as opposed to male subjects in our study population might warrant consideration when applying the test to detect insulin resistance in men. The applicabilities of the methods to the overall population can be further improved by conducting a similar study on a different study population with different race, baseline characteristics, lifestyles, socio-economic status, level of education and health awareness, and more even male-to-female ratio.

In conclusion, the four scoring systems which are used in identifying or predicting type 2 diabetes in Thai adults are also useful for detecting insulin resistance among populations with no known type 2 diabetes. Aekplakorn's Diabetes Risk score shows the strongest correlation with HOMA-IR and the highest overall performance in identifying individuals with insulin resistance.

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CASE REPORT

Tonic drink and poor glycemic control: a forgotten issue

Hai Err, Viroj Wiwanitkit

Int J Diab Dev Ctries. 2012 ; 32 : 60.

Sir, the control of blood glucose is the aim in management of the patients with diabetes mellitus. There are many foods that the diabetic patients should avoid in order to achieve good glycemic control. Those include desserts, soft drinks and fruits. However, a diabetic may ingest many foods inadvertently, thus leading to poor metabolic control. Here, the authors present a case study on poor glycemic control due to a tonic drink intake. The index case was a 45 years old male diabetic patient with diabetes mellitus for the past 5 years. This patient regularly visited (trimonthly) the physician and the blood glucose control was good (blood glucose levels had been between 97–110 mg/dL and HbA1C levels had been between 5.0–5.5%). However, in the present visit, there was an unexplained poor glycemic control (blood glucose level was equal to 217 mg/dL and HbA1C was equal to 7.4%). The repeated analysis confirmed the results. In this case, the patient was examined in-depth for any behavioral change but gave no history of intake of dessert, soft drinks or fruit juices. However, there was an interesting history of drinking tonic drink (Red Bull Energy Drink) for 3 months. One bottle (100 cc) of tonic drink was drunk daily in order to derive energy and strength for driving. The patient was suggested to stop the tonic drink and the glycemic control improved dramatically (blood glucose=108 mg/dL and HbA1C06.0%)

The tonic drink is a commonly drunk beverage in many developing countries. The labor and factory workers are the main groups who drink this beverage. There are some publications indicating the usefulness of the tonic drink. Many papers published show that drinking tonic drink can help promote body performance [1–3]. In the index case, the main active ingredients in tonic drink included taurine, caffeine and glucuronolactone. However, there are limited reports on its adverse effects on diabetic patients. Focusing on the ingredients of the tonic drink, the glucose level is about 17.5 g in 100 cc. Indeed, this level can be considered problematic for the diabetic patient. Of interest, an important ingredient in tonic drink, taurine has effect on blood glucose as modulator of glucose metabolism. The taurine might help control the high glucose level [4]. However, in the tonic drink, the amount of taurine is low (800 mg/100 cc; basic body requirement about 1.5 g/day) and this is not comparable to the very high glucose content. In addition, the tonic drink might also induce some other unwanted side effects such as hypokalemia [5]. Hence, the use of tonic drink should be carefully recorded. Since the use of tonic drink is widely spreading in large sections of population at present, the effect of this beverage on diabetes mellitus and other metabolic disorders should not be forgotten.

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EDITORIAL

Risk factors in diabetic nephropathy

R. V. Jayakumar

Int J Diab Dev Ctries. 2012 ; 32 : 1-3

Diabetes morbidity and mortality is mainly determined by the late chronic complications of diabetes. Though the macrovascular complications affecting the heart is the leading cause of mortality in diabetes, nephropathy is slowly becoming an important factor in determining the final outcome. This is because of better prevention and treatment of cardiovascular problems with the availability of newer drugs and devices, thereby making diabetic patients live longer. Also it is a well accepted fact, that patients of diabetic nephropathy are more predisposed to cardiovascular and peripheral vascular diseases. Even though the hyperglycemia is closely related to the development of microvascular complications, the evidence of direct relationship between hyperglycemia and nephropathy is less convincing in humans, as only 30% of diabetic patients develop clinical nephropathy [1]. Majority of patients escape renal failure even though some histological evidence of renal damage is present in many. It is unclear why some diabetic patients are more susceptible than others for the development of renal disease. Inherited factors may be providing protection from susceptibility to diabetic nephropathy, but evidence is lacking as to which factors are important. Familial predisposition to raised arterial pressure increases the susceptibility to renal disease in patients with diabetes. Studies have demonstrated that mean blood pressure levels are significantly higher in those who progress to microalbuminuria, than in those who do not, indicating that hypertension is an important risk factor for diabetic nephropathy [2]. There is evidence of familial clustering of diabetic nephropathy in type 2 diabetes and the affected sib-pair linkage analysis have identified loci associated with diabetic nephropathy in type 2 diabetes [3, 4]. Interstingly, in Pima Indians blood pressure levels before the onset of diabetes, predicts the future risk of developing nephropathy [5]

Factors determining the occurence and progression of diabetic nephropathy include hyperglycemia, hypertension, hyperlipidemia and genetic factors. From a clinical patient management point of view, reversible or treatable factors like hypertension, hyperlipidemia and hyperglycemia are important. At present genetic factors may be important only for theoretical discussion, but with the rapidly advancing field of gene therapy, it may be possible to modify the risk factors for diabetic nephropathy favourably by gene therapy.

Genes involved in the genetic predisposition to diabetic nephropathy, are likely to be those involved in renin-angiotensin system, nitric oxide pathway, aldose reductase pathway, GLUT-1, and lipoproteins metabolism. These have been investigated, but studies have, by and large, been inconclusive or shown only weak associations [6]. However, a strong association between polymorphism in the 5′ end of aldose reductase gene and the development of diabetic nephropathy in type 1 diabetes has been confirmed by many investigators [7].

Renoprotective effects of good glycemic control has been demonstrated in many longitudinal and intervention studies, the most famous being DCCT and UKPDS studies. Also reversal of already established structural changes in the kidney has been achieved by maintaining near-normal glycemia by pancreatic transplantation in a small number of type-1 diabetic patients [8]. Hyperglycemia increases the risk of progression of diabetic nephropathy predominantly by altering the functioning of anti-oxidant system. It accelerates the chemical modification of the proteins and

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lipids leading to the increased formation of Advanced Glycation End products (AGE), Advanced Oxidized Lipid End Products(ALE) and Advanced Oxidation Protein Products (AOPP).

The AGEs have been shown to produce renal damage in both humans and experimental diabetes. AGEs directly alter the structural and functional properties of extra-cellular matrix proteins, increase their rigidity, and favour the trapping of LDL and immunoglobulin-G. The interaction of AGEs with its receptors in the kidney induces the synthesis and release of many cytokines like TGF-b1, and IGF and results in enhanced production of collagen, laminin and fibronectin. Also there is over expression of receptors of AGE (RAGE) in glomerulus and tubular epithelial cells and the AGE-RAGE complexes can produce tubulointerstitial fibrosis[9].In this issue of the journal, Sandeesh Mohan et al. has shown that increased pool of AGEs, ALEs and lipid peroxidases in diabetics, increase proteinuria in all stages of diabetic nephropathy [10].

AGE-RAGE interaction promotes polymorphonuclear leukocyte generated cascade of highly reactive oxygen species, which ultimately lead to production of lipid peroxidases and AOPP. Lipid peroxidation products like ALEs and lipid hydroxyperoxidase produce endothelial and glomerular basement membrane injury by altering proteins like nephrin and connectin and thereby resulting in proteinuria. Lipo-oxidation and gluco-oxidation products have been co-localized in renal tissues of diabetic patients indicating co-existance of glucotoxicity and lipotoxicity.

Hyperglycemia induced activation of polyol pathway leading to kidney damage has been postulated in the pathogenesis of diabetic nephropathy. In animal studies there has been evidence that aldose reductase inhibitors have reduced the albumin excretion rates, but no convincing effect of aldose reductase inhibitors has been shown in controlled studies in humans. So far it appears that activation of polyol pathway may be more likely to be epiphenomena in the setting of diabetic nephropathy, rather than the main factor in the pathogenesis.

High glucose concentration in experimental studies, involving isolated glomeruli, have shown alteration of extracellular matrix formation. High concentration of glucose in mesangial cells cause hypertrophy, increase gene expression and protein secretions like collagen, laminin and fibronectin. [11]. Hyperglycemia also reduces the activity of metalloproteases, enzymes responsible for extracellular matrix degradation.

One of the important predictors of decline in renal function in diabetic nephropathy is the amount of proteinuria. Increasing quantity of proteinuria is a risk factor, indicating progressive renal damage in diabetic nephropathy. Similarily urinary excretion of immunoglobulins, which are large molecules, will help to predict the severity of nephropathy. In this issue of the journal, Sandesh Mohan et al. has shown that urinary immunoglobulin-G/creatinine ratio has a significant association with eGFR and increased odds for potential hazardous factors. Excessive protein overload, leads to excessive protein reabsorption and consequent accumulation of protein in the tubular epithelial cells and these induce the release of vasoactive and inflammatory cytokines. These cytokines lead to local injury, infiltration of mononuclear cells and ultimately renal scarring and insufficiency. The tubular toxicity of protein raises the possibility that the beneficial effect of ACE inhibitors is also through its anti-proteinuric effect other than its hemodynamic effects.

Numerous studies looking at the cellular and molecular mechanism of renal damage in diabetic nephropathy, lead to the unifying concept that the insults of hyperglycemia, hypertension and proteinuria converge at the cellular level by using similar molecular signaling pathways and influencing the expression of common cytokines. The important cytokines that are implicated in the diabetic nephropathy are Transforming Growth Factor b1(TGF-b1),Connective tissue growth factor (CTGF), Insulin like growth factor (IGF), Vascular Endothelial Growth Factor (VEGF) and Angiotensin-2. All these cytokines induce tissue injury, thicken the basement membrane and alter the permeability producing proteinuria and scarring.

During the last couple of decades, a number of advances have been made in understanding the pathogenetic mechanisms and risk factors for the development of diabetic retinopathy. In a recent study from India, the risk factors determining the diabetic nephropathy in urban Asians are the duration of diabetes, the diabetic control and systolic blood pressure [12]. The consensus is that hyperglycemia by its action through glucotoxicity and lipotoxicity, hypertension and proteinuria contributes to nephrotoxicity in diabetics. All these factors induce oxidative stress which trigger the release of various tissue damaging cytokines which produce the renal damage. The amount of proteinuria and the renal loss of immunoglobulin-G may help to assess the risk in diabetic retinopathy. From the treatment point, tight control of blood glucose, blood pressure, and using drugs affecting renin-angiotensin system, cytokine production and anti-oxidants, may all help to salvage the renal damage. Finally, the prevention or slowing the progression of diabetic nephropathy will significantly improve both the patient's quality of life and reduce the public health expenditure.

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EDITORIAL

Predicting type 2 diabetes mellitus and insulin resistance

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Int J Diab Dev Ctries. 2012; 32: 4-6

Type 2 diabetes has now become a global health problem threatening the lives of millions of people. According to the latest Diabetes Atlas 5, released on 14th November 2011 by the International Diabetes Federation (IDF), there are currently 366 million people with diabetes globally and this is predicted to increase to 552 million by the year 2030 [1]. Unfortunately, type 2 diabetes is a silent disease. In the Chennai Urban and Rural Epidemiology Study (CURES), it was shown that the "Rule of Halves" is very much valid in the case of diabetes [2] just as in the case of hypertension [3]. Thus, half of those with type 2 diabetes in the community remain undiagnosed, of those diagnosed, less than half receive treatment and of those who take treatment, less than half have their diabetes under control [2]. One of the challenges for physicians and diabetologists therefore, is to detect undiagnosed type 2 diabetes in the community. Obviously, one way to do it is to screen everyone in the population for the disorder. In a country like India, however, this is not feasible due to sheer numbers of people with diabetes. According to the recent ICMR –INDIAB study, there are an estimated 62.4 million people with diabetes and 77 million people with pre-diabetes [4]. Hence the challenges of screening 1.2 billion Indians to identify all those with diabetes and pre-diabetes can well be imagined. There is therefore a need to develop simple tools to cost effectively identify type 2 diabetes in the population. This led to the establishment of several risk scores for diabetes such as the American Diabetes Association Risk Score [5] Finnish Diabetes Risk Score [6], German Diabetes Risk Score [7], Danish Diabetes Risk Score [8] Cambridge Risk Score [9] and the Spanish Risk Score [10]. Within India also different risk scores have been described based on population based studies [11, 12]. It has been shown that the Indian Diabetes Risk Score (IDRS) is useful not only to predict undiagnosed diabetes in the community [11] but also to predict incident diabetes [13], to classify the type of diabetes [14] and even to predict individuals who may have certain complications of diabetes like peripheral vascular disease and neuropathy [15]. The IDRS also serves as an effective indicator of metabolic syndrome and cardiovascular risk even among subjects with normal glucose tolerance [16]. Use of IDRS is more effective and less expensive than genotyping and makes it less costly than universal OGTT screening of the whole population to detect subjects with type 2 diabetes in India [17]. Thus it is clear that diabetes risk scores have come to stay, and if used judiciously, can lead to cost effective screening of diabetes.

While insulin secretory defects are common in all forms of diabetes, insulin resistance remains its hallmark of type 2 diabetes [18]. Several authors have tried to describe simple tools to predict insulin resistance in the community. In this issue of IJDDC, Srisung et al [19] describe the performance of four categories of risk scores in predicting insulin resistance in Thai adults. The four categories are (i) The Royal College of Physicians of Thailand (ii) Thailand Ministry of Public Health, (iii) the risk score of Aekplakorn et al and (iv) the risk score of Keesukpham et al. The Royal College of Physicians of Thailand Score includes almost all the criteria of metabolic syndrome (MS) such as history of hypertension, HDL cholesterol, triglycerides and IGT or IFG. The Thailand Ministry of Public Health criteria is also on similar lines. Using such sophisticated systems including laboratory investigations does not appear to be suitable for mass screening for diabetes or insulin resistance. However, the

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Aekplakorn and the Keesukpham criteria are much simpler and are based on simple anthropometry and historical details and hence would be much more cost effective. It is to be appreciated that the Aekplakorn criteria, in spite of not including biochemical details such as HDL cholesterol, triglycerides or IFG or IGT, performs better than the other scores. The study by Srisung et al is therefore a valuable contribution to the existing knowledge on the subject. However, one of the issues with this study is the female excess (almost 81% of the subjects studied were females) which is a serious limitation as the applicability to males would need to be established further.

One of the guiding principles behind using risk scores, is that it must be simple and inexpensive so that it can be applied at a population level for public health workers. It should also be easy to use by non-medical people, if it is to gain wide acceptance. For research purposes, sophisticated tests for diagnosing insulin resistance such as the euglycemic clamp technique or the Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) remain the gold standard [20]. However these tests are laborious, require large volumes of blood to be drawn, are observer dependent and need specialized training. Hence they are clearly unsuitable for large scale screening for epidemiological or public health purposes. Hence simpler tools are necessary.

The use of fasting insulin and the homeostatic model assessment (HOMA – IR) have widely been used for epidemiological studies [21]. However, the insulin assay is expensive and also needs careful standardization. Finally, they are not useful for people who already have diabetes particularly if treated with insulin injections and they are therefore best applied in a non-diabetic population. Hence, the necessity of simple risk scores to predict insulin resistance. It is here, that the paper by Srisung et al [19] where they describe the usefulness of the Aekplakorn criteria to assess insulin resistance, becomes important. It is obvious that risk scores are ethnic specific [22] as they are derived from the populations in which they have been tested. Hence using the risk score described in one country or region for another ethnic group or another region of the world, may not be appropriate and each region should ideally have its own risk score.

Use of risk scores are particularly important as they can help to cost effectively screen for diabetes. We have shown that IDRS can help in cost effective screening for diabetes in India as it uses simple, safe and inexpensive measures. Moreover it would help to do selective screening instead of universal screening. For example, if we were to screen a population of 1,00,000 adults in a city using a 2 h post load plasma glucose, assuming the cost of one glucose estimation including blood collection to be Rs.30/-, the cost would work out to Rs.30,00,000. For the same population, if a two step procedure is used for screening for diabetes, i.e. use IDRS first and then screen only those likely to have diabetes, only 43% of the population who have a score $\geq$60, will have to be screened. This would capture over 72% of the undiagnosed diabetic subjects. If the screening test is carried out on all these individuals then the cost would work out to Rs.12,90,000. Even if we add a cost of Rs.1,50,000 for collecting information on IDRS, the overall cost would only work out to Rs.14,40,000. Thus there would be a cost saving of almost 50%, which in this case, is Rs.15,60,000. Thus, using IDRS would help to drastically reduce the costs of screening for diabetes at a community level [23].

In summary, the use of simple clinical risk scores can help not only in cost effective screening for undetected type 2 diabetes, but also in its classification as well as to identify insulin resistance and metabolic syndrome in the community.

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LETTER TO EDITOR

Cancer cachexia as a model for treatment of obesity

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Int J Diab Dev Ctries. 2012 ; 32 : 61.

Dear Sir,

Cancer cachexia describes a syndrome of progressive weight loss, anorexia and persistent erosion of host body cell mass in response to malignant growth. Cachexia is a complex metabolic state with progressive weight loss and depletion of host reserves of adipose tissue and skeletal muscle mass. Cachexia seen in cancer patients is not only due to the persistence of anorexia, but is mainly due to both proteolysis and lipolysis constantly occuring in the body. This leads to both muscle as well as fat loss [1]. Factors implicated in cancer cachexia are eicosanoids, decreased nutrient intake, increased catabolism due to surgery and chemotherapy.

The cancer cells produce various factors like Lipid Mobilizing Factor (LMF) and Proteolysis Inducing Factor (PIF). Host cell and tumour cell interaction leads to pro-inflammatory cytokine production like TNF-α, Interleukin-1(IL-1), Interleukin-6 (IL-6) [2, 3] Tumor-host immune interaction also leads to neuroimmune activation, especially by IL-1 and TNF-α. These cytokines mediate "hypothalamic resistance" by hyper-activating anorexigenic neurones and suppressing prophagic neurons [4]. Neuro hormones and pro-inflammatory cytokines contribute to an imbalance in anabolic and catabolic pathways.

Leptin (released by adipocytes) helps in regulating food intake and energy expenditure by stimulating neuropeptide Y (orexigenic neuropeptide) in a negative feedback mechanism. In cancer, the hypothalamic actions of cytokines like IL-1, IL-6 and TNF-α stimulate and/or mimic the release of leptin alongwith other anorexigenic neuropeptides like Corticotropin releasing factor (CRF) thus inhibiting the negative relaease of NPY leading to its dysregulation, therefore producing anorexia and unopposed weight loss [5].

A small number of studies indicate the role of certain tumour-derived factors like Proteolysis-inducing factor (PIF) and Lipid mobilizing factor (LMF) which are closely related to weight loss in cachexia. PIF induces protein degradation in skeletal muscles while LMF induces lipolysis in murine adipocytes [6]. However, their definitive role in the mechanism of cachexia has yet not been established.

We know that both cachexia and anorexia coexist as an anorexia- cachexia syndrome in cancer patients. They act synergistically causing loss of muscle mass, reduction in total fat and reduction in bone mineral density. Although the cause of cancer cachexia is undoubtedly multi-factorial, cytokines play a pivotal role in the mechanism of weight loss in such patients. If we can identify those factors which only aggravate lipolysis without affecting muscle mass and bone density and use it in the management of obesity, one can hope to achieve positive results. Thus research could also be directed towards this cachexia model for further insights.

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REVIEW ARTICLE

A clinical score to predict survival from hyperglycemic crisis following general medical wards admission in a resource constrained setting

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Abstract This study aims to develop a risk score model, based on available clinical data to assess absolute risk of dying among admissions for hyperglycemic crisis in Eastern Cape, one of South Africa's most disadvantaged provinces. Data from 268 admissions for hyperglycemic crisis at Nelson Mandela Academic Hospital, Mthatha, for the 2 year periods of 2008 and 2009 were used to develop multivariate logistic regression and cox proportional hazards models for the time to onset of death and the area under curve (AUC) of the receiver-operating-characteristic curve (ROC). The risk score models included the following independent variables that were associated with mortality: systolic blood pressure (SBP) <90 mm/Hg, Odd's ratio (OR)=13.3 (95%CI 2.1- 83; p=0.006) and Hazard ratio (HR) =8.4 (95%CI 2- 36; p<0.0001), Leukocyte count $>10\times10^6$/L OR =16.7 (95%CI 2.1- 143; p=0.0008) and HR =5.7 (95%CI 1.8- 7; p=0.021) and Platelet count $<150\times10^6$/L OR =11.6 (95%CI 13- 43.5; p< 0.0001) and HR =5.1 (95%CI 2.2- 6.8; p< 0.001). The final models yielded good and significant area under the curve (AUC) for WBC $>10\times10^6$/L (AUC =0.642, 95% CI 0.557- 0.727; p< 0.001) and platelet count $<150\times10^6$/L (AUC=0.651, 95% CI 0.567- 0.741; p< 0.001) but lower and insignificantly discriminatory power of the models for SBP <90 mm/Hg (AUC =0.573, 95%CI 0.456- 0.691; p=0.163). The proposed scoring system is 5 points where all three independent predictors are absent with additional 3 points for each independent predictor that is present. Mortality rates were 4.8% (N=3/62), 17.1% (N=19/111), 52.2% (N=12/23) and 66.7% (N=2/3) respectively for admissions with none (total score=5points), any one (total score=8points), any two (total score=11points) and all three (total score=14points) independent predictors. The present risk scores developed in the Eastern Cape Province of South Africa using easily obtained clinical parameters can help identify high risk hyperglycemic crisis, total score ≥ 8 who would benefit most from management in the high risk care unit. These tools need to be validated in other limited resource settings.

Keywords Mortality · Vital signs · Laboratory parameters · Eastern Cape Province, South Africa

Introduction

Before the 1990s, diabetes mellitus (DM) in general and type 2 diabetes (T2DM) in particular was considered rare in sub-Saharan Africa [1]. However, contemporary literature [2, 3] shows that the prevalence and incidence of DM and T2DM is increasing in Sub-Saharan Africa. This is mostly due to epidemiologic and nutritional transition with increasing westernization and unfavorable lifestyle changes [4, 5]. Accompanying the increasing prevalence of diabetes particularly type 2 diabetes is an expected increase in patients presenting with hyperglycemic decompensation. The ideal setting for treating hyperglycemic crisis is in a high or intensive care unit. However, at the Nelson Mandela Academic Hospital Complex

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Mthatha in the Eastern Cape province of South Africa, which is the tertiary referral center for a population of about 1.7 million people, patients with hyperglycemic crisis were treated in the medical wards until the February 1, 2010 when a 4 bed medical high care unit became available. These 4 high care unit beds will however, be competed for by medical emergencies such as poisonings, unstable arrhythmias, myocardial infarction and pulmonary embolism. The optimal use of these limited high care unit beds will be enhanced by a triage system that can identify presentations likely to result in survival or demise following care in the general medical wards. In this study, we retrospectively assessed mortality in patients with hyperglycemic crisis and proposed a score based on characteristics at presentation and management time to guide in triaging patients to care in either the general medical wards or high care unit.

Methods

This was a retrospective review using the medical records of consecutive patients admitted with hyperglycemic crisis at the Nelson Mandela Academic Hospital Mthatha in 2008 and 2009. The Eastern Cape Province in which this hospital is located is the second poorest of South Africa s 9 provinces [6]. The study was performed in accordance with the declaration of Helsinki 11 and was approved by the Research and Ethics Committee of Walter Sisulu University, Mthatha, Eastern Cape. A total of 268 admissions were available for univariate analysis. Of these, 107 admissions had complete data for multivariate analysis. Data collected included gender, age, type of DM, systolic blood pressure (SBP), level of consciousness, precipitating cause for hyperglycemic crisis, laboratory parameters and status at discharge. The outcome variables at discharge were non-fatality or fatality. The choice of variables that were analyzed included those that have been associated with mortality in previous studies and typically assessed vital signs and laboratory parameters.

Biological data performed with quality control and according to routine standards of Central Laboratory, National Health Laboratories Services in our hospital, included plasma glucose, serum sodium, serum potassium, serum bicarbonate, serum creatinine, serum chloride, serum ketones, serum albumin, haemoglobin level, white blood cell and platelet counts.

Hyperglycemic crisis was defined and characterized as diabetic ketoacidosis (DKA), hyperglycemic hyperosmolar

Table 1 Mortality rates in relation to demographic and clinical
c haracteristics
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	Mortality rates % (N)	p value
Gender		
Female	24.3 (N0169/268)	0.03
Male	13.1 (N099/268)	
Age (years)		
≥ 40	23.9 (N0184/268)	0.02
<40	11.9 (N084/268)	
Type of diabetes		
Non-type 1	23.1(N0221/268)	0.01
Type 1	6.4 (N047/268)	
Type of hyperglycemic crisis		
Hyperosmolar	34 (N018/53)	0.005
Non-hyperosmolar	16.7 (N036/215)	
Precipitating factor		
Identified	30.5 (N0105/268)	0.0007
None identified	13.5 (N0163/268)	
Level of consciousness		
Unconscious	39.7 (N078/190)	<0.00001
Conscious	4.5 (N0112/190)	
Systolic blood pressure		
<90mmhg	42.1 (N019/203)	0.005
≥ 90mmhg	15.8 (N0184/203)	

Table 2 Mortality rates in relation to the presenting laboratory parameters

	Mortality rates % (N)	p value
White blood count (/L)		
$>10\times10^6$	25.6 (N0168/261)	0.002
$\leq 10\times10^6$	9.7 (N093/261)	
Platelet count (/L)		
$<150\times10^6$	56.3 (N032/261)	<0.00001
$\geq 150\times10^6$	14.8 (N0229/261)	
Haemoglobin (g/L)		
<10	8.6 (N049/261)	0.09
≥ 10	17.9 (N0212/261)	
Blood Glucose (mmol/L)		
>33.3	22.5 (N098/268)	0.5
≤ 33.3	20 (N0170/268)	
Serum Sodium (mmol/L)		
>150	45.5 (N022/268)	0.002
≤ 150	17.9 (N0246/268)	
Serum Potassium (mmol/L)		
<3.5	30.3 (N033/263)	0.3
3.5- 5.5	19.2 (N0188/263)	
>5.5	19.1 (N042/263)	
Serum Chloride (mmol/L)		
>104	30 (N060/266)	0.03
<104	17 (N0206/266)	
Serum Bicarbonate (mmol/L)		
<18	24.5 (N0139/268)	0.07
≥ 18	15.5 (N0129/268)	
Serum Creatinine (mmol/L)		
>200	35.7 (N056/260)	0.001
≤ 200	16.2 (N0204/260)	
Serum Albumin (g/L)		
<40	25.2 (N0111/171)	0.008
≥ 40	8.3 (N060/171)	

Table 3 Beta coefficients and odd's ratios (Exponential Beta) in the model to predict the probability of fatal admissions using logistic regression model

Independent variables	Beta Coefficient	Standard error	Wald Chi-square	p value	OR (95%CI)
SBP <90 mmHg	2.586	0.934	7.670	0.006	13.3 (2.1- 83)
WBC $>10\times10^6$/L	2.822	1.072	6.926	0.008	16.7 (2.1-
Platelets $<150\times10^6$/L	2.449	0.684	12.829	<0.0001	11.6 (3- 43.5)
Constant	4.831				<0.0001

CI Confidence interval, SBP Systolic blood pressure, WBC White blood count

state (HHS) and hyperglycemia (HG) using the American diabetes association (ADA) criteria [7]. The diagnosis of type 1 or 2 DM was as documented in the case records. New DM referred to the index hyperglycemic crisis as the first manifestation of DM. Anemia (haemoglobin levels <10 g/L), altered level of consciousness (Glasgow coma scale <15), age ≥ 40 years, thrombocytopenia (platelet count <150,000 × 10^6/L), leukocytosis (white blood cell count $>10\times10^6$), serum sodium >150 mmol/L, serum potassium <3.5 mmol/L, serum creatinine >200 mmol/L and serum albumin <40 g/L were explored as possible predictors of mortality.

Statistical analyses

Data were summarized as mean ± standard deviation (SD) with number (N) for continuous variables and proportions (%) with number (N) for categorical variables. All candidate

Table 4 Multivariate and independent association of individual clinical characteristics with in-hospital mortality using Cox's regression

Independent variables	Beta Coefficient	Standard error	Wald Chi-square	p value	OR (95%CI)
SBP <90 mmHg	2.124	0.574	13.676	<0.0001	8.4 (2- 36)
WBC >10×10^6/L	1.743	0.775	5.063	0.024	5.7 (1.8- 7)
Platelets <150×10^6/L	1.633	0.485	11.315	<0.001	5.1 (2.2-

CI Confidence interval, SBP Systolic blood pressure, WBC White blood count

predictors which were significantly associated with mortality in univariate analysis using Chi-square test were entered into multivariate (logistic and Cox) regression models. A logistic regression model (probability of dying during admission determined by the following formula: $1/1 + \exp^{-2}$ was estimated to

assess the independent contribution of patients demographic and bioclinical characteristics at presentation. Survival analysis was performed with Cox's proportional hazards regression, modeled with mortality and duration of hospitalization as the dependent variables. Multivariate Odd's ratio (OR) and relative risk (Hazard ratio 0 H) with their corresponding 95% confidence intervals (95%CI) were calculated in logistic and Cox regression models respectively. After Cox regression analysis, differences between exposed and non-exposed arms were assessed by log rank test (Chi-squared), while Kaplan-Meir survival curves were generated for each arm. A P-value<0.05 (two-tailed) was considered to be statistically significant.

The Receiver Operating Characteristic curves (ROC) were obtained for each independent predictor for not dying, identified by multivariate analysis. The optimal cutoff point was taken as the coordinate closest to the Y intercept (0.1) of the ROC curve, and at this point, the sum of the sensitivity and the specificity is maximal. Discriminatory power (diagnostic accuracy) was assessed by the area under the curve (AUC) [8] with its 95% CI. The risk-score for each study admission was calculated from the final model equation based on their individual values for the included candidate predictors. Using this score, sensitivity and false-positive fractions (1-specificity) were calculated for all possible threshold values. To estimate the in-hospital duration risk of an admission

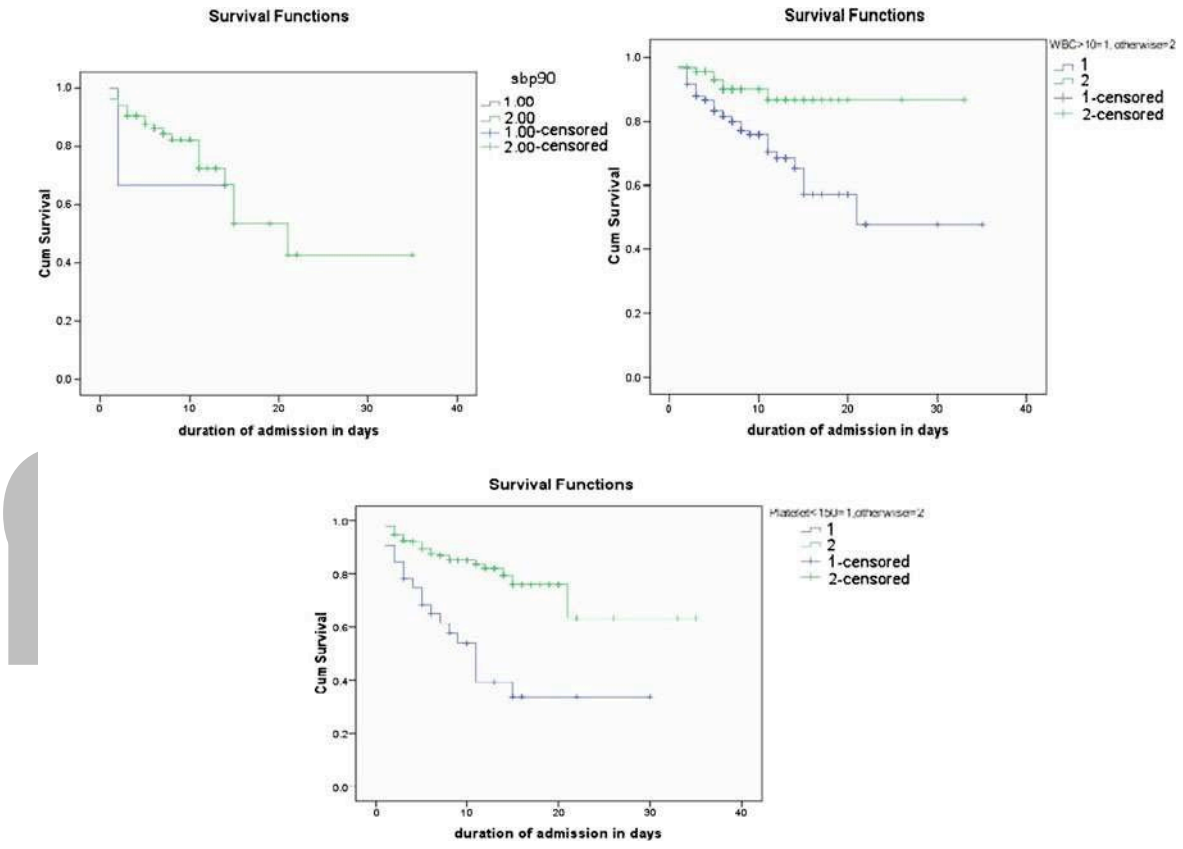

Fig. 2 Kaplan-Meir curves with the probability of dying in hyperglycemic crisis stratified for SBP, WBC and platelet count

with hyperglycemic crisis to die, the following formula was used: probability 01-mortality rate $^{exp\ (Z)}$, Z being the equation. All analyses were performed using the statistical packages for social sciences (SPSS) software version 15 for windows (SPSS Inc, Chicago, Il, USA).

Results

One admission was excluded as there was no data on mortality. Analysis was limited to the 268 admissions that had data on mortality. The majority of admissions (63.1%) were female related. The mean age of all admissions was 50.3±19.9 years.

The overall mortality rate was 20.1% as 58 of 268 admissions resulted in death. The mortality rates were 6.4%, 22.1% and 25%; p00.03 respectively for types 1, 2 and new diabetes related admissions. Syndromic specific mortality rates were: NHDKA (13.4%, N013/97), HDKA (37.5%, N09/24), HHS (31%, N09/29) and HG (19.5%, N023/118); p00.03. Table 1 shows female gender, age ≥40 years, non-type 1 diabetes, hyperosmolality, presence of precipitating factor, altered level of consciousness and hypotension as the demographic and clinical factors that were associated with deaths. Table 2 shows leukocytosis, thrombocytopenia, hypernatremia, hyperchloremia, hypoalbuminemia and elevated serum creatinine as the laboratory parameters that were associated with

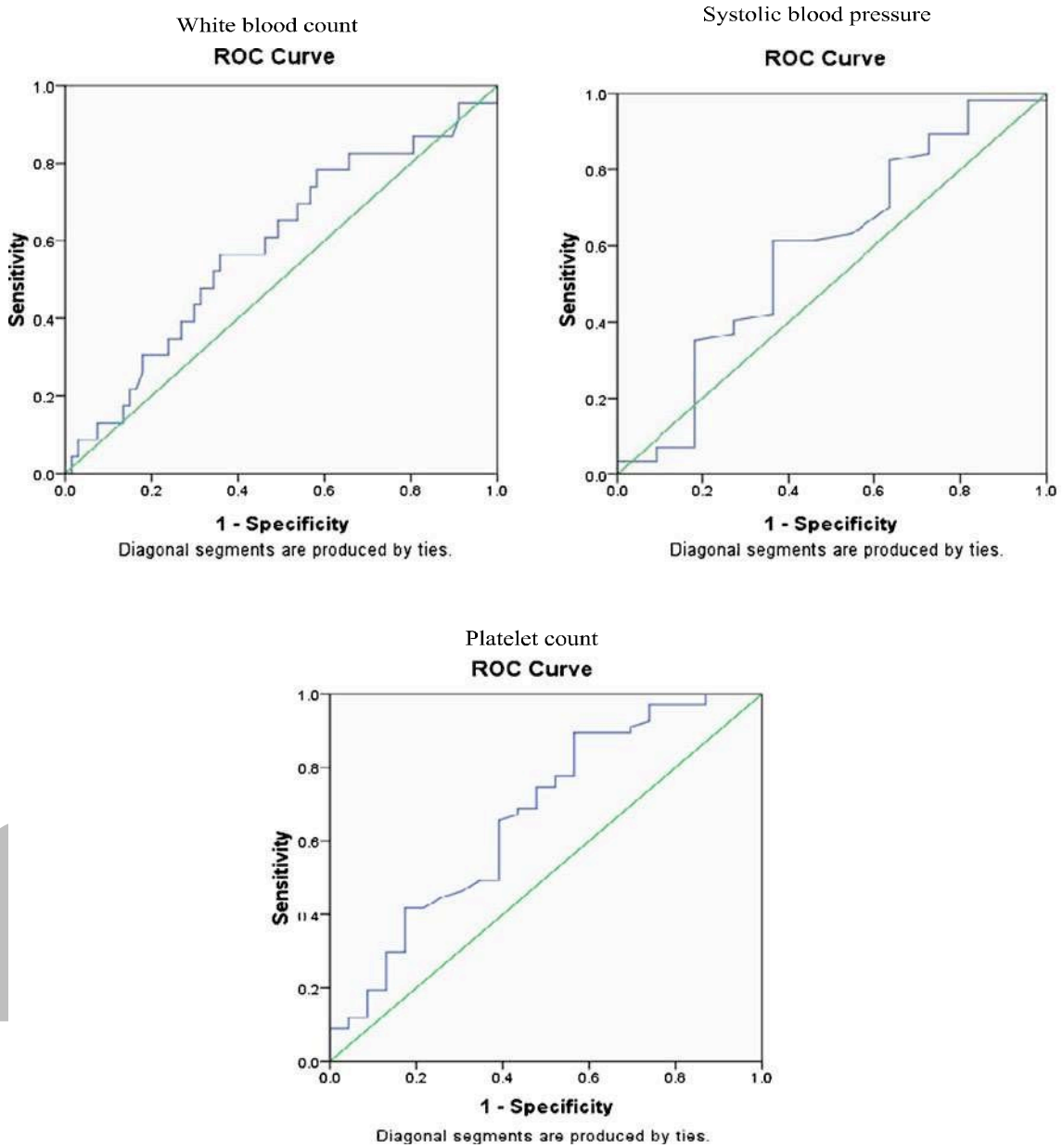

Fig. 3 ROC curves for white blood cells (WBC), systolic blood pressure (SBP) and platelets

mortality. Hypokalemia, acidosis, ketosis, anemia, random blood glucose and glycosylated haemoglobin were not associated with mortality in univariate analysis.

In logistic regression model, SBP <90 mm/hg, WBC >10×10^6/L, and platelet count <150×10^6/L were the only significant predictors of in-hospital mortality (Table 3). Figure 1 depicts diagrams of observed groups and predicted probabilities in logistic regression model. The probabilities of survival for SBP <90 mm/hg, WBC >10×10^6/L, and platelet count <150×10^6 /L were 77.5%, 40% and 70% respectively. Table 3 shows that the odds of dying when SBP <90 mm/Hg was 13 times higher than when SBP ≥ 90 mm/hg, 17 times higher with WBC >10×10^6/L than WBC ≤ 10×10^6/L, 12 times higher with platelet count <150×10^6/L than platelet count ≥ 150×10^6/L. However, when hospitalization duration was taken into consideration to predict non-survival (mortality) using Cox's proportional hazard regression analysis, the odds to predict mortality for SBP <90 mm/hg, WBC >10×10^6/L and platelet count <150×10^6/L were two times lower than those observed in the logistic regression model respectively (Table 4). Figure 2 displays Kaplan-Meir curves with the probability of dying in hyperglycemic crisis stratified for SBP, WBC and platelet count. From the log rank test, Kaplan Meir curves and 95% CI of Hazard Ratios (Relative Risk) in Cox's regression as well as from the AUC in the ROC curves (Fig. 3), the highest probability of dying and discriminatory power between death and survival was observed for both WBC >10×10^6/L and platelet count <150×10^6/L (Table 5).

We analyzed and compared mortality rates in all admissions (N=219) that had complete data for systolic blood pressure, white cell and platelet counts. The mortality rate was 4.8% (N=3/62) in admissions with SBP ≥ 90 mm/hg, WBC <10×10^6/L and platelet count ≥ 150×10^6/L; 17.1% (N=19/111) in patients with any one of SBP <90 mm/hg, WBC >10×10^6/L and platelet count <150×10^6/L; 52.2% (N=12/23) with any two of SBP <90 mm/hg, WBC >10×10^6/L and platelet count <150×10^6/L and 66.7% (N=2/3) where all 3 of SBP <90 mm/hg, WBC >10×10^6/L and platelet count <150×10^6/L were present.

Discussion

The present study was conducted to develop a simple and valid tool to estimate risk of dying in patients admitted with hyperglycaemic crisis and managed in the general wards of a limited resource setting. Although several demographic, clinical and laboratory factors were associated with mortality in univariate analysis (Tables 1 and 2), only hypotension (SBP <90 mm/hg), leukocytosis (WBC >10×10^6 /mm^3) and thrombocytopenia (platelet count <150×10^6/mm^3) (Table 3) remained independently associated with mortality in multivariate analysis. Colinearity of several factors that were associated with mortality in univariate analysis may explain the scenario where only 3 of them remained significant in multivariate analysis. The imprecision in the odd's ratio obtained as indicated by the wide confidence intervals is likely related to their being only 18 deaths out of the 107 admissions that were entered into multivariate analysis

The relative risk for dying conferred by SBP <90 mmHg, WBC >10×10^6/L and platelet count <150×10^6/L was more precise (narrow 95% CI), robust ($p<0.0001$) and two-fold lower than the odds ratios obtained with logistic regression analysis without considering the duration of admission. The hospitalization time related management may explain the lack of discriminatory power of SBP <90 mmHg cut point demonstrated by its ROC curve and AUC. The relationship between SBP and survival in these hyperglycemic admissions could be U shaped in quadratic regression as many diabetics are hypertensive. Thus, the best risk-score of mortality may be based on the present logistic regression equation at presentation to guide in triaging patients with hyperglycemic crisis to high care unit: Y=4.8+(2.6×1) for SBP <90 mm/Hg + (2.8×1) for WBC >10×10^6/L + (2.5×1) for platelet count <150×10^6/L=12.7. SBP ≥ 90 mm/Hg, WBC ≤ 10×10^6/L, and platelet count ≥ 150×10^6/L were considered zero (0). Allotting 3 points for the presence of each independent predictor+5 for constant, the total score will range from a minimum of 5 points where all three independent predictors are absent to a maximum 14 points where all three independent predictors are present. The lowest mortality rate of 4.8% in admissions was seen where all of SBP, WBC and platelet counts were favorable [9, 10] and hyperglycemic crisis is managed in the high care or intensive care unit. Hence, patients presenting with such profiles may be triaged for general medical ward admission in our resource constrained setting. However, patients presenting with any one or more of SBP <90 mm/hg, WBC >10×10^6/L and platelet count <150×10^6/L (total score of 8 to 14) are better cared for in the high care unit as mortality rates in these admissions were very high with a range of 17.1% to 66.7%.

Table 5 Accuracy of risk-score models developed by different statistical analysis for hyperglycemic crisis

Independent variables	AUC (95%CI)	Sensitivity %
SBP <90 mmHg	0.573 (0.456- 0.691) P=0.163	93
WBC >10×10^6/L	0.642 (0.557- 0.727) P<0.001	83
Platelets <150×10^6/L	0.651 (0.567- 0.741) P<0.001	93

AUC Area under curve, CI Confidence interval, SBP Systolic blood pressure, WBC White blood count

The factors that have been reported to be independently predictive of hyperglycemic crisis include advanced age [11], altered level of consciousness [12], and infection [13]. This is the first report to our knowledge of hypotension, leukocytosis and thrombocytopenia as independent predictors of mortality in hyperglycemic crisis. Hypotension at presentation in hyperglycemic crisis will usually be attributable to severe dehydration. The caregiver begins to entertain concern where hypotension persists despite adequate initial fluids resuscitation. Patients with hypotension at presentation may have included those with such co-morbidities as cardiogenic and septic shock. Cardiac enzymes and blood cultures were not done in all patients with hypotension and cases of myocardial infarction and sepsis may have been missed as both conditions may be atypical in diabetes [14, 15]. Thrombocytopenia in this study may be related to infection as suggested by the finding that leukocytosis was more prevalent in admissions with thrombocytopenia than those without thrombocytopenia (37.5% vs 21%, p00.04). Although retroviral disease is prevalent in our community [16], and is the commonest cause of thrombocytopenia in our medical wards, data on HIV status was not available for most patients as HIV testing is not routine in our center. Thrombocytopenia may also be related to factors as viraemia, disseminated intravascular coagulation and thrombotic thrombocytopenic purpura. Leukocytosis in hyperglycemic crisis is usually related to ketosis [17]. We however, cannot provide an obvious reason for the indepen- dent association of leukocytosis and mortality. Factors such as ketosis, infection at presentation and cerebrovascular disease were comparable in patients with and without leukocytosis. Leukocytosis may however, be a marker of an unidentified comorbidity.

The risk score model developed in this study is proposed to identify cases of hyperglycemic crisis at the highest risk of dying, who are likely to benefit from management in a high care unit. Importantly, this risk-score model was based on commonly available clinical parameters. The measurements of SBP, WBC and platelet count can all be readily assessed in daily practice at primary, secondary and tertiary health levels of most developing countries. A useful mortality prediction model has been provided in Greece [18], which also includes simple variables such as severe co-exiting disease, acidosis, insulin requirements, depressed mental status and fever. The present tool may be validated in other settings to demonstrate its external validity by risk stratification on hospital admission.

The present study may be limited to some degree because of its retrospective design and the non-availability of data for all variables as only 107 of 268 admissions had complete data for multivariate analysis. Nevertheless, the strength of this mortality prediction model was based on excluding cofounding risk factors using logistic regression model and Cox regression analysis. Moreover, patterns in probability to die (or to survive) over hospitalization time was predicted for individual admissions using Cox's proportional regression analysis.

In Conclusion, in this resource contrained province of South Africa, the suggested risk score utilizing systolic blood pressure, white cell and platelet counts can help guide admission and management into the general or high care unit.

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