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Early diagnostic predictors: useful in treatment and progression of diabetes associated nephropathy

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Abstract: Diabetic nephropathy (DN) is one of the major complications of type 2 diabetes mellitus (T2DM) characterized by frequent microalbuminuria, elevated arterial blood pressure, persistent decline in glomerular filtration rate and high risk of morbidity and mortality. It encompasses long-term duration of diabetes, which has an effect on the minute blood vessels of kidney. The biochemical parameters play a key role in the prediction of nephropathy in T2DM patients. Therefore, the present study was conducted to investigate the role of biochemical markers in the prediction of DN in T2DM patients. The aim of this study was addressed in case-control setting, 230 T2DM, 200 DN patients and 110 non diabetic healthy individuals were included in order to assess the biochemical parameters and risk of DN. Patients were recruited according to WHO's criteria from various hospitals of Karachi, Pakistan. After getting informed consent from patients and control subjects, clinical data was recorded. Five hundred and forty (n=540) samples were studied for their serum blood glucose, blood pressure, glycosylated hemoglobin (HbA1c), serum creatinine, serum urea, lipid profile and urinary albumin levels. The analysis showed that incidence and the progression of the DN increased with hyperglycemia, longer duration of diabetes, dyslipidemia, elevated level of serum urea, creatinine and urinary albumin levels in patients with T2DM. Therefore, these biochemical predictors can anticipate the occurrence of nephropathy in later stages of diabetes.

Key words: Biochemical parameters, diabetic nephropathy, serum urea, T2DM, urinary albumin
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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is considered as most prevalent form of diabetic cases worldwide[1]. Increasing rate of diabetes can be attributed to life style changes over the period. It is expected that the frequency of diabetic patients' worldwide, will be increased remarkably from 135 million to 300 million in 30 years time from 1995 to 2025[2]. Speculations from epidemiology and risk factor determination of diabetes in Pakistan have shown a high prevalence of T2DM in Pakistan. It represents a typical combination of genetic and environmental factors as the main contributor[3]. The impact of ethnic and racial differences on diabetes underscores the need for the identification of genetic aspects that contribute to differences in susceptibility and the pathology of the disease[4-6].

Diabetes-associated metabolic dysregulations can develop organ specific micro and macrovascular complications in T2DM patients[7]. Despite the commonly available tests for T2DM screening and diagnosis, the disease remains under diagnosed[8]. Almost 25% of the diabetic patients remain undiagnosed for microvascular complications upto 4-7 years[9,10]. Among all the microvascular complications, Diabetic nephropathy is one of the major complications worldwide and a major indicator for dialysis and transplantation[11]. However, around one third of diabetic patients develop diabetic nephropathy in later stages of life[12]. Micro albuminuria is an early clinical appearance of diabetic nephropathy, which generally appears 5-15 years after the onset of diabetes. By the passage of time albuminuria increases in many T2DM patients and this condition progress towards micro albuminuria. Consequently, kidney function gradually declines and end stage renal disease (ESDR) develops in a significant number of patients[13]. Although diverse factors may be involved in pathogenesis of DN, but earlier disease identification and the intensive management of hyperglycemia can lessen the risk for further progression[14,15]. Hence, several risk factors such as poor glycemic control, dyslipidemia, hypertension, obesity and cigarette smoking act synergistically to develop nephropathy in patients with T2DM[16,17]. Since progression to DN complications is likely to occur in a remarkable percentage of diabetic patients, the role of these risk factors needs to be further explored in our population. The present study was performed to investigate the role of the biochemical parameters in the prediction of the DN in T2DM patients.

MATERIALS AND METHODS

An ethical approval was taken from the institutional ethical committee. Five hundred and forty (n=540) individuals were selected for this study. After getting informed consent of patients, individuals were categorized into three groups; 110 healthy control subjects, 200 DN patients and 230 T2DM patients. The average duration of diabetes in T2DM patients selected was more than 5 years while in DN group average duration was more than 9

years. Spot urine samples were collected in sterile plastic bottle for the evaluation of protienuria. Urinary albumin was measured by immuno-turbidometry latex method. Fasting blood samples of patients and controls were drawn from cephalic vein in 10ml vacutainers containing gel and clot activator. The serum was separated by centrifuging blood at 2500 rpm for 15 minutes at 4°C temperature and stored at -80°C until analysis. These serum samples were analyzed for fasting blood glucose, serum creatinine, serum urea, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride and cholesterol levels, while blood samples were collected again after two hours postprandial for estimation of random blood glucose. Glycosylated hemoglobin (HbA1c) was estimated in blood samples,collected in EDTA vacutainers. Fasting and random blood glucose was investigated by the GOD PAP method, serum urea by Urease-GLDH (UV), serum creatinine by Jaffe's method, LDL and cholesterol by CHOD-PAP method, HDL by homogeneous enzymatic colorimetric method, triglyceride by GPO-PAP method and HbA1c by the high performance liquid chromatography (HPLC) method. Statistical analyses was performed by independent student's t- test using SPSS V.16 software.

RESULTS

T2DM is considered as one of the major leading cause of nephropathy in most of developing as well as developed countries. Hyperglycemic condition and prolong duration of diabetes can progress the disease rapidly[7]. With this background, this case-control study was performed to assess the part of biochemical parameters in the prediction of nephropathy in T2DM patients. The comparison of the biochemical parameters between controls and T2DM patients is shown in Table1. Fasting blood glucose (at the $p<0.001$ level), Random blood glucose (at the $p<0.001$ level), HbA1c (at the $p<0.001$ level), triglycerides (at the $p<0.001$ level) and urine albumin (at the $p<0.01$ level) values were significantly higher in T2DM patients as compared to controls subjects. Whereas the value of HDL (at the $p<0.001$ level) was significantly higher in controls as compared to T2DM patients. There was no significant elevation in serum urea, serum creatinine, LDL and cholesterol levels.

Table-2 shows significantly higher values of fasting blood glucose (at the $p<0.001$ level), random blood glucose (at the $p<0.001$ level), serum urea (at the $p<0.001$ level) serum creatinine (at the $p<0.05$ level), LDL (at the $p<0.001$ level), HDL (at the $p<0.001$ level), cholesterol (at the $p<0.001$ level), triglycerides (at the $p<0.001$ level) and urinary albumin (at the $p<0.001$ level) in DN patients compared to their respective controls. Whereas, value of HDL (at the $p<0.001$ level) was significantly higher in controls subjects.

In table 3, fasting blood glucose, random blood glucose, serum urea, serum creatinine, HbA1c, LDL, cholesterol, triglycerides and urine albumin values were significantly higher ($p<0.001$ level) in DN group with respect to their T2DM group. No significant difference was observed in random blood glucose level. In case of HDL, significantly higher values was obtained at the $p<0.001$ in T2DM patients as compared to DN subjects.

Table 1: Comparison of biochemical parameters between T2DM patients and control group.

Parameters (mg/dl)	Controls (n=110)	T2DM Patients (n=230)	p-value
Fasting Blood Glucose	82.88±1.02	170.15±1.98	<0.001
Random Blood Glucose	127.58±0.80	256.42±5.15	<0.001
Serum Urea	30.899±0.59	29.65±0.41	N.S
Serum Creatinine	0.94±0.06	1.07±0.05	N.S
HbA1c (%)	5.38±0.81	8.00±0.09	<0.001
LDL	98.80±1.49	102.8±2.09	N.S
HDL	52.47±0.62	47.80±0.63	<0.001
Triglyceride	86.40±1.45	105.23±3.81	<0.001
Cholesterol	187.61±2.05	185.43±2.14	N.S
Urine albumin	8.14± 0.49	12.5±1.34	<0.01

Values are mean±SEM, statistical significances between controls and T2DM patients were analyzed by independent student't' test, $p<0.01$ and $p<0.001$ were considered significant. "n" represents the number of samples.

DISCUSSION

Diabetes is one of the most prevalent diseases in Pakistan and has been ranked by WHO as 6th in number in the prevalence list[6]. All T2DM patients have twenty-nine time greater risk to develop microvascular complications, especially nephropathy[18]. Diabetic nephropathy is a progressive disorder that occurs due to angiopathy of capillaries in the kidney glomeruli. One third or more of the T2DM patients develop DN with progressive deterioration of renal structure and function[19, 20]. Early diagnosis as well as timely interventions may

be useful in reducing the progression of the disease and associated complications[21]. The study was conducted to provide the association of biochemical parameters with T2DM and DN.

Table 2: Comparison of biochemical parameters between DN patients and control group.

Parameters (mg/dl)	Controls (n=110)	DN Patients (n=200)	p-value
Fasting Blood Glucose	82.88±1.02	177.96±1.45	<0.001
Random Blood Glucose	127.58±0.80	268.46±3.77	<0.001
Serum Urea	30.899±0.59	54.61±0.79	<0.001
Serum Creatinine	0.94±0.06	3.01±0.23	<0.001
HbA1c (%)	5.38±0.81	10.92±0.48	<0.001
LDL	98.80±1.49	116.2±2.8	<0.001
HDL	52.47±0.62	42.80±0.88	<0.001
Triglyceride	86.40±1.45	245.04±5.26	<0.001
Cholesterol	187.61±2.05	205.12±3.7	<0.001
Urine albumin	8.14± 0.49	139.00±7.68	<0.001

Values are mean±SEM, statistical significances between controls and DN patients were analyzed by independent student't' test, $p<0.001$ was considered significant. "n" represents the number of samples.

An elevated fasting and random blood glucose levels were observed in T2DM and DN patients, resulting in atherosclerosis. Untreated patients could lead to high blood pressure and consequently progression towards vascular damage, stroke, heart attack, or impaired kidney function[22]. An important observation of the present investigation is that there is a strong relationship between fasting and random glucose and HbA1c levels in T2DM and DN patients. This could serves as a marker for average blood glucose level over the past three months prior to the measurement. Higher level of HbA1c found in patients with persistent elevated blood glucose level indicating poorer glycemic control[23].

This persistent and prolong blood glucose level can also damage other organs specially kidney by filtering increase amount of blood. All this extra work is hard on the nephrons and cause impairment of kidney function, which is a progressive disorder[24]. In the present study, an increase in serum creatinine and urea levels was found when DN patients were compared with control subjects. These findings reveal that there may be a direct relationship of blood glucose with serum creatinine and urea levels[25]. Raised serum creatinine and urea levels may indicate a pre-renal problem and may be due to impaired function of the nephrons, consequently may disturb filtering capacity of the kidney thus leading to accumulation of waste products within the system and develop nephropathy in the later stages of life[26-28]. Furthermore, clinically significant elevation in urinary albumin level is observed in DN patients as compared to controls and may be associated with increase trend in the duration of disease, high blood pressure, poor glycemic control and disturbed kidney function[29]. Moreover, decreased level of observed triglyceride level in this study can be associated with increase in HDL level in T2DM patients, which has previously been well established[30,31]. On the same note, lipid profile is more disturbed in DN patients as compared to T2DM patients studied. Observed lipid profile abnormalities may be due to resistance to insulin and high blood glucose levels.

Table 3: Comparison of biochemical parameters between DN and T2DM patients.

Parameters (mg/dl)	T2DM Patients (n=230)	DN Patients (n=200)	p-value
Fasting Blood Glucose	170.15±1.98	177.96±1.45	<0.01
Random Blood Glucose	256.42±5.15	268.46±3.77	N.S
Serum Urea	29.65±0.41	54.61±0.79	<0.001
Serum Creatinine	1.07±0.05	3.01±0.23	<0.001
HbA1c (%)	8.00±0.09	10.92±0.48	<0.001
LDL	102.8±2.09	116.2±2.8	<0.01
HDL	47.80±0.63	42.80±0.88	<0.001
Triglyceride	105.23±3.81	245.04±5.26	<0.001
Cholesterol	185.43±2.14	205.12±3.7	<0.001
Urine albumin	12.5±1.34	139.00±7.68	<0.001

Values are mean ± SEM, statistical significances between DN and T2DM patients were analyzed by independent student't' test, $p<0.01$ and $p<0.001$ were considered significant. "n" represents the number of samples.

Postprandial elevated blood glucose and lipid levels might be the risk factors for vascular diseases in T2DM patients[32]. Insulin is involved in the regulation of most of the body functions, specifically lipid and carbohydrate metabolism and these functions are compromised in T2DM patients, as it is associated with insufficient amount of harmon insulin[33]. In order to reduce the other risks associated with T2DM, general practitioners must recommend

early and effective lipid-lowering therapy to control the progression of disease[34,35]. Control in both lipid profile and glycemic condition at this time point may help improve the overall management of diabetes and could delay the progression of nephropathy in T2DM patients[36].

CONCLUSION

Elevated blood glucose levels, serum urea, serum creatinine, urinary albumin and disturb lipid profile in T2DM patients should be considered as risk factors for late diabetic complications, particularly for nephropathy. Future studies on large database will be helpful in establishing the reported facts.

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