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Potential biomarkers for early diagnosis of nephrotic changes in type 2 diabetic patients

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***Abstract*---**Introduction: Type 2 diabetes (T2D) is metabolic disease with chronically elevated glucose levels, characterized by two main abnormalities: impairment of insulin secretion and decrease in insulin sensitivity. (1) Undiagnosed or poorly controlled, leads to complications such are blindness, renal insufficiency, amputation of lower extremities, heart disease, or stroke. Approximately 150 million people worldwide are affected by type 2 diabetes, with expectancy to double in next 20 years. Two factors are participating on clinical picture of type 2 diabetes: environment and genetics. (2) Materials and Methods: This is a prospective study carried out in the Department of Biochemistry at Index Medical College, Hospital and Research center, among the people attending the OP/IP departments, based on random selection. Informed consent was taken from the patients. Institutional Ethical Clearance Certificate was obtained. Number of samples: 150 samples were collected from the study subjects, of which Group A: 50 Normo-albuminuria, Group B: 50 Microalbuminuria and Group C: 50 Microalbuminuria with type 2 diabetes. Results: In our study, in Group 'A' the mean of Total Cholesterol level was 173.43 ±15.54 mg/dl, in Group 'B' the mean of Total Cholesterol level was 188.43±18.43 mg/dl and in Group 'C' the mean of Total Cholesterol level was 201.53±21.36 mg/dl. In Group 'A' the mean of Triglycerides level was 153.65 ±13.35 mg/dl, in Group 'B' the mean of Triglycerides level was 163.36±16.85 mg/dl and in Group 'C' the mean of Triglycerides level was 184.36±17.64 mg/dl. In Group 'A' the mean of HDL level was 48.26±4.62 mg/dl, in Group 'B' the mean of HDL level

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was 42.63±4.59 mg/dl and in Group 'C' the mean of HDL level was 39.14±3.63 mg/dl. In Group 'A' the mean of LDL level was 94.44±8.25 mg/dl, in Group 'B' the mean of LDL level was 113.13±10.47 mg/dl and in Group 'C' the mean of LDL level was 125.52±14.21 mg/dl. In Group 'A' the mean of VLDL level was 30.73±2.67 mg/dl, in Group 'B' the mean of VLDL level was 32.67±3.37 mg/dl and in Group 'C' the mean of VLDL level was 36.87±3.52 mg/dl. Conclusion: The presence of a microvascular complication like diabetic nephropathy in newly detected type 2 diabetes mellitus patients shows the importance of early detection of diabetic mellitus as well as screen for its complications, to have a tight glycemic control as well as blood pressure to reduce morbidity and mortality in diabetes mellitus and also to have a good quality of life.

***Keywords*---**Diabetic nephropathy, Type 2 Diabetic patients, Lipid Profile.

Introduction

Type 2 diabetes (T2D) is metabolic disease with chronically elevated glucose levels, characterized by two main abnormalities: impairment of insulin secretion and decrease in insulin sensitivity. (1) Undiagnosed or poorly controlled, leads to complications such are blindness, renal insufficiency, amputation of lower extremities, heart disease, or stroke. Approximately 150 million people worldwide are affected by type 2 diabetes, with expectancy to double in next 20 years. Two factors are participating on clinical picture of type 2 diabetes: environment and genetics. (2)

A large body of studies refers about the role of environmental factors in the development of type 2 diabetes. It has been reviewed that ethnicity may play important role. The prevalence of type 2 diabetes differs in people with different ethnic origins in Africa or Asia (3). Other studies demonstrate the importance of residence on the prevalence of diabetes mellitus. The role of geographical location in the development of type 2 diabetes is supported for example by the fact that Japanese living in Brazil, or in Hawaii and Los Angeles have higher prevalence of type 2 diabetes compared to those in Japan (4). The difference between the prevalence of diabetes in the urban and rural setting may be due to influence of junk food supply in the rural versus urban setting, and possibilities of physical activity. These are another factors influencing insulin sensitivity and glucose tolerance in type 2 diabetes (5).

In a recent study performed on an African American population in the United States, was observed that the prevalence of diabetes increases with the degree of inactivity and obesity (9). Obesity is present in about 80% of type 2 diabetic patients and has been implicated as one of the risk factors for type 2 diabetes (6). Body mass index is directly associated with increased risk of type 2 diabetes in many ethnic groups (7). Severe and prolonged stress associated with modern life style may also be one of the environmental factors associated with glucose intolerance and may hence increase the risk of type 2 diabetes by activation of

the adrenal hormones, notably the glucocorticoids, that have been observed to cause glucose intolerance (8). Also drugs such as corticosteroids and some oral contraceptive steroids may cause glucose intolerance and type 2 diabetes in susceptible individuals (9).

Nephropathy is a major cause of morbidity and mortality in patients with diabetes mellitus. Persistent albuminuria is the hallmark of diabetic nephropathy. According to Mogensen staging system, diabetic nephropathy consists of five stages 2 which include microalbuminuria as stage 3 known as incipient nephropathy. (10) Control of Microalbuminuria in patients with Type 2 DM is an important indicator for renal and cardiovascular risk reduction. The most important risk factor for development of diabetic nephropathy is poor glycaemic control. Inpatients with poor glycaemic control, uncontrolled Hypertension may predispose them further as was noted by studies that showed those with HbA1C >12 % and uncontrolled hypertension were at higher risk for developing nephropathy when followed up for 20 years. (11)

Obesity, smoking, age, gender, dyslipidemia, degree of proteinuria at diagnosis, family history of diabetes and kidney diseases have also been suggested as possible contributing factors. Individuals who develop type 2 DM after the age of 50 years are considered more prone for nephropathy, so are family history of hypertension and cardiovascular events in first degree relatives. Primary prevention of diabetic nephropathy is possible with vigorous glucose and blood pressure control. Screening for diabetic nephropathy falls within scope of secondary prevention. (12)

Materials and Methods

This is a prospective study carried out in the Department of Biochemistry at Index Medical College, Hospital and Research center, among the people attending the OP/IP departments, based on random selection. Informed consent was taken from the patients. Institutional Ethical Clearance Certificate was obtained.

Number of samples: 150 samples were collected from the study subjects, of which Group A: 50 Normo-albuminuria, Group B: 50 Microalbuminuria and Group C: 50 Microalbuminuria with type 2 diabetes.

Inclusion criteria:
Type 2 diabetic patients with age group of 40 years and above are included in the study.
Patients were considered to be diabetic based on WHO criteria for diagnosis of diabetes mellitus. Which is,

1) Fasting blood >126mg/dl. Fasting defined as no calorie intake for at least 8 hours.
2) Or
3) Two hours post prandial glucose > 200mg/dl

Exclusion criteria:
Patients with type 1 diabetes,
Hypertension,

Congestive cardiac failure,
Renal insufficiency
Who are not willing to give informed consent are excluded from the study.

Method of data collection
The following data is collected from all the patients. Name, Age, Sex, Weight, Height, family history, drug history.

Investigations include-
Fasting lipid profile
Serum creatinine
Serum electrolytes
Urine transferrin
Urine cystatin C

First morning mid-stream urine sample collected in sterile container was used for analysis. Urine samples with hematuria, urinary tract infection, and vaginal fluid contamination are excluded before analysis.

Statistical analysis

Statistical analyses were performed using SPSS software packages. Data distribution was assessed by manual inspection of histograms and the Kolmogorov-Smirnov test. Data that followed a normal distribution were expressed as mean ±standard deviation (SD), otherwise they were summarised using median and interquartile range. For continuous data differences between the 3 study categories were determined by one-way analysis of variance (ANOVA) if the data were normally distributed, or by Kruskall-Wallis test if not normally distributed. Tukey's family error rate was used to account for multiple comparisons. Two sample t-tests and Mann Whitney U-tests were used for sub-group analysis on appropriately distributed data. P-values <0.05 were considered statistically significant

Results

The table 1 and graph 2 reflects that Group A: 29 were male (58%) while 21 were female patients (42%). In Group B consisted of 31 male patients (62%) and 19 female patients (38%). In Group C consisted of 27 male patients (54%) and 23 female patients (46%). There was no statistically significant difference in number of patient from Group A, B and Group C patients.

Table 1: Gender difference between Group A, B and C

	Group A		Group B		Group C	
	n=50	(%)	n=50	(%)	n=50	(%)
Male	29	58	31	62	27	54
Female	21	42	19	38	23	46
Total	50	100	50	100	50	100

Table 2: Comparison of Mean Lipid Profile between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Total Cholesterol (mg/dl)	173.43 ±15.54	188.43±18.43	201.53±21.36
Triglycerides (mg/dl)	153.65 ±13.35	163.36±16.85	184.36±17.64
HDL (mg/dl)	48.26±4.62	42.63±4.59	39.14±3.63
LDL (mg/dl)	94.44±8.25	113.13±10.47	125.52±14.21
VLDL (mg/dl)	30.73±2.67	32.67±3.37	36.87±3.52

In **Table 2,** in **Group 'A'** the mean of **Total Cholesterol** level was 173.43 ±15.54 **mg/dl,** in **Group 'B'** the mean of **Total Cholesterol** level was 188.43±18.43 **mg/dl and** in **Group 'C'** the mean of **Total Cholesterol** level was 201.53±21.36 **mg/dl**.

In **Group 'A'** the mean of **Triglycerides** level was 153.65 ±13.35 **mg/dl,** in **Group 'B'** the mean of **Triglycerides** level was 163.36±16.85 **mg/dl and** in **Group 'C'** the mean of **Triglycerides** level was 184.36±17.64 **mg/dl**.

In **Group 'A'** the mean of **HDL** level was 48.26±4.62 **mg/dl,** in **Group 'B'** the mean of **HDL** level was 42.63±4.59 **mg/dl and** in **Group 'C'** the mean of **HDL** level was 39.14±3.63 **mg/dl**.

In **Group 'A'** the mean of **LDL** level was 94.44±8.25 **mg/dl,** in **Group 'B'** the mean of **LDL** level was 113.13±10.47 **mg/dl and** in **Group 'C'** the mean of **LDL** level was 125.52±14.21 **mg/dl**.

In **Group 'A'** the mean of **VLDL** level was 30.73±2.67 **mg/dl,** in **Group 'B'** the mean of **VLDL** level was 32.67±3.37 **mg/dl and** in **Group 'C'** the mean of **VLDL** level was 36.87±3.52 **mg/dl**.

Table 3: Comparison of Mean Serum Creatinine between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Serum Creatinine (mg/dl)	0.7 ±0.04	0.9±0.06	1.92±0.11

In **Table 3,** in **Group 'A'** the mean of **Serum Creatinine** level was 0.7 ±0.04 **mg/dl,** in **Group 'B'** the mean of **Serum Creatinine** level was 0.9±0.06 **mg/dl and** in **Group 'C'** the mean of **Serum Creatinine** level was 1.92±0.11 **mg/dl**.

Table 4: Comparison of Mean Electrolytes between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Sodium (mEq/L)	143.34 ±5.65	146.75±5.73	152.36±6.37

Potassium (mEq/L)	4.13±0.51	4.4±0.43	4.9±0.45
Chloride (mmol/L)	93.36±1.42	96.38±1.64	99.53±1.63

In **Table 4,** in **Group 'A'** the mean of Sodium was 173.43 ±15.54 mEq/L**,** in **Group 'B'** the mean of Sodium was 188.43±18.43 mEq/L **and** in **Group 'C'** the mean of Sodium was 201.53±21.36 mEq/L.

In **Group 'A'** the mean of Potassium was 4.13±0.51 mEq/L**,** in **Group 'B'** the mean of Potassium was 4.4±0.43 mEq/L **and** in **Group 'C'** the mean of Potassium was 4.9±0.45 mEq/L.

In **Group 'C'** the mean of Chloride was 93.36±1.42 mEq/L**,** in **Group 'B'** the mean of Chloride was 96.38±1.64 mEq/L **and** in **Group 'C'** the mean of Chloride was 99.53±1.63 mEq/L.

Table 5: Comparison of Mean Urine micro albumin between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Urine microalbumin (mg/dl)	7.2 ±0.62	31.53±3.64	33.65±3.94

In **Table 5,** in **Group 'A'** the mean of Urine microalbumin was 7.2 ±0.62 mg/dl**,** in **Group 'B'** the mean of Urine microalbumin was 31.53±3.64 mg/dl **and** in **Group 'C'** the mean of Urine microalbumin was 33.65±3.94 mg/dl.

Table 6: Comparison of Mean Urine transferrin between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Urine transferrin (mg)	2.9 ±0.25	13.53±2.37	315.73±29.42

In **Table 6,** in **Group 'A'** the mean of Urine transferrin was 2.9 ±0.25 mg**,** in **Group 'B'** the mean of Urine transferrin was 13.53±2.37 mg **and** in **Group 'C'** the mean of Urine transferrin was 315.73±29.42 mg.

Table 7: Comparison of Urine cystatin C between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Urine cystatin C (mg/L)	0.06 ±0.03	0.09±0.08	0.14±0.09

In **Table 7,** in **Group 'A'** the mean of Urine cystatin C was 0.06 ±0.03 mg/L**,** in **Group 'B'** the mean of Urine cystatin C was 0.09±0.08 mg/L **and** in **Group 'C'** the mean of Urine cystatin C was 0.14±0.09 mg/L.

Discussion

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics. [13]

In our study, table 6, in Group 'A' the mean of Total Cholesterol level was 173.43 ±15.54 mg/dl, in Group 'B' the mean of Total Cholesterol level was 188.43±18.43 mg/dl and in Group 'C' the mean of Total Cholesterol level was 201.53±21.36 mg/dl. In Group 'A' the mean of Triglycerides level was 153.65 ±13.35 mg/dl, in Group 'B' the mean of Triglycerides level was 163.36±16.85 mg/dl and in Group 'C' the mean of Triglycerides level was 184.36±17.64 mg/dl. In Group 'A' the mean of HDL level was 48.26±4.62 mg/dl, in Group 'B' the mean of HDL level was 42.63±4.59 mg/dl and in Group 'C' the mean of HDL level was 39.14±3.63 mg/dl. *Post hoc* analysis of large scale trials such as the Diabetes Control and Complications Trial (DCCT) revealed that albuminuria is associated with higher levels of TC, TG, and LDL-C. [14] The results of our study show that TC, TG, and LDL-C levels were significantly higher among the nephropathy patients. A study among related South Indian population has also shown that TC, TG, HDL-C, and LDL-C were significantly different between diabetic and diabetic nephropathy patients. [15] A study conducted even in a different ethnic population has observed results similar to the present study. [16] Another study showed that dyslipidemia associated with diabetic nephropathy is not limited to T2DM subjects, but is present among type 1 patients as well. [17] Since sustained hyperglycemia has profound effects on lipid metabolism, dyslipidemia among subjects of this study might be related to their poor glycemic control also. [18]

In our study in Group 'A' the mean of Urine transferrin was 2.9 ±0.25 mg, in Group 'B' the mean of Urine transferrin was 13.53±2.37 mg and in Group 'C' the mean of Urine transferrin was 315.73±29.42 mg. In addition, our results suggest that either the urinary transferrin or the urinary/index transferrin ratio are useful to identify patients with diabetic nephropathy at early risk for ED/vascular damage. These fndings suggess the dynamic participation of the whole transferrin metabolism as related with early renal and vascular damages. The clinical usefulness of urine transferrin and plasma/ urine transferrin ratio in the evaluation of early renal damage and subclinical atherogenesis, as a part of a routine test to identify high risk population with t2DM, are to be developed, and deserves further evaluation. [19]

In our study, in Group 'A' the mean of Urine cystatin C was 0.06 ±0.03 mg/L, in Group 'B' the mean of Urine cystatin C was 0.09±0.08 mg/L and in Group 'C' the mean of Urine cystatin C was 0.14±0.09 mg/L. In our study, urinary cystatin C was a predictor of renal impairment independent of serum cystatin C. In this study, urinary cystatin C and NAP, both clinical tubular damage markers, positively correlated with each other at baseline. Both markers were significantly associated with the annual decline in eGFR in type 2 diabetic nephropathy. In particular, both tubular damage makers affected a decline in eGFR at the early stage of nephropathy in type 2 diabetic patients (eGFR $60 mL/min/1.73 m2). Urinary NAP affected eGFR decline in patients with both eGFR $60 mL/min/1.73 m2 and normoalbuminuria, although urinary cystatin C did not reach statistical

significance. In addition, the increased levels of the two markers were also associated with the progression of CKD stage 3 or greater at the last follow-up. In general, unlike healthy subjects, diabetic patients are continuously exposed to the various metabolic and hemodynamic risks associated with this disease. [20]

Conclusion

The presence of a microvascular complication like diabetic nephropathy in newly detected type 2 diabetes mellitus patients shows the importance of early detection of diabetic mellitus as well as screen for its complications, to have a tight glycemic control as well as blood pressure to reduce morbidity and mortality in diabetes mellitus and also to have a good quality of life.

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