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Identification of potential biomarkers for early detection of nephrotic changes in type 2 diabetic patients: A comparative study of biomarkers

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***Abstract*---**Introduction: Diabetes mellitus (DM) is one of the most common endocrine disorders affecting almost 6% of the world's population. This chronic metabolic disorder affects the metabolism of carbohydrates, protein, fat, water, and electrolytes, leading to structural changes in tissues of many organ systems in the body, especially those of the vascular system. Nephropathy is a major cause of morbidity and mortality in patients with diabetes mellitus. Persistent albuminuria is the hallmark of diabetic nephropathy. 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. 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. Results In Table 3, the mean fasting blood glucose level in Group A was 131.71 ±12.04

mg/dl, in Group B was 148.29±13.06 mg/dl and in Group C was 161.92±15.11 mg/dl. In Table 4, in Group 'A' the mean of PPBG level was 203.43 ±18.54 mg/dl, in Group 'B' the mean of PPBG level was 228.64±18.43 mg/dl and Group 'C' the mean of PPBG level was 241.64±21.54 mg/dl. In Table 5, in Group 'A' the mean of HbA1c was 6.3 ±0.74%, in Group 'B' the mean of HbA1c was 188.43±18.43% and in Group 'C' the mean of HbA1c was 201.53±21.36%. Conclusion: Diabetic nephropathy is major microvascular complication leading to end stage renal failure and CVD associated death in diabetic patients, thus accounts for increased mortality and morbidity in these patients. Clinical definition of DN is presence of proteinuria over 0.5 g per 24h. It occurs in 15 – 30 % of type 1 diabetic patients after 20 years of diabetes duration, whereas prevalence in type 2 diabetes is more variable, ranging from 5 to 40 %. The fact that only subset of diabetic patients eventually develops DN despite long-term severe chronic hyperglycemia, together with the evidence of familial clustering of DN and various ethnic/racial prevalence of DN indicate hereditary predisposition to DN, independent form predisposition to diabetes mellitus.

***Keywords*---**diabetes mellitus, nephropathy, blood glucose level.

Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders affecting almost 6% of the world's population. This chronic metabolic disorder affects the metabolism of carbohydrates, protein, fat, water, and electrolytes, leading to structural changes in tissues of many organ systems in the body, especially those of the vascular system. (1) The prevalence of DM is on the increase and according to estimates the number of patients with DM will in 2025 reach 300 millions. (2) Two major forms of the disease are distinguished: type 1 and type 2. The pathophysiology of both forms is incompletely understood, but it has now been widely accepted that both genetic and environmental factors play a contributing role in the development of both forms of DM. (3)

The prevalence of nephropathy rises with prolonged duration of diabetes but levels out at around 15 years after diabetes diagnosis, after which it declines substantially, in contrast to diabetic retinopathy, where the incidence rate is linear (4). It is less easy to demonstrate it in type 2 diabetics, where the onset of the disease is not clearly defined. It is thus apparent that only subset of diabetic patients are at risk of having renal complications. After 25 years of diabetes duration, the lifetime risk for developing overt nephropathy and ESRD is low in the diabetic patients with normal UEA. This observation can be interpreted to imply that some genes either expose the subject to or protect the subject from diabetic nephropathy during a certain time-span (5).

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. (6) 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. (7) Several recent studies have also shown that patients with type 2 DM and overt nephropathy exhibit high levels of diverse acute phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A, fibrinogen, andIL-6. (8)

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, Fasting blood >126mg/dl. Fasting defined as no calorie intake for at least 8 hours. Or 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 blood glucose
- Post prandial blood glucose

- Glycosylated hemoglobin (HbA1c)
- Serum
- Serum hs-CRP

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

In both the groups, maximum number of patients were in the age group of 51-60 years and least number of patients were ≤40 years of age. Mean age in group A patients were 52.29±6.55, in Group B patients were 51.10±6.62 and 53.53±5.64 years. There was no statistically significant difference in mean age of patient from Group A, B and Group C patients

Table 1: Comparison of Mean Age in Groups

Age-Group	Group A		Group B		Group C	
	No	Percentage	No	Percentage	No	Percentage
≤40 year	03	6%	02	4%	01	2%
41--50	18	36%	16	32%	17	34%
51--60	29	58%	32	64%	32	64%
Total	50	100	50	100	50	100
Mean±SD	52.29±6.55 years		51.10±6.62 years		53.53±5.64 years	

Table 2: 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

The table 2 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 3: Comparison of Mean Fasting Blood Glucose level between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Fasting Blood Glucose (mg/dl)	131.71 ±12.04	148.29±13.06	161.92±15.11

In Table 3, the mean fasting blood glucose level in Group A was 131.71 ±12.04 mg/dl, in Group B was 148.29±13.06 mg/dl and in Group C was 161.92±15.11 mg/dl.

Table 4: Comparison of Mean Postprandial Blood Glucose between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Postprandial Blood Glucose (mg/dl)	203.43 ±18.54	228.64±18.43	241.64±21.54

In Table 4, in Group 'A' the mean of PPBG level was 203.43 ±18.54 mg/dl, in Group 'B' the mean of PPBG level was 228.64±18.43 mg/dl and Group 'C' the mean of PPBG level was 241.64±21.54 mg/dl.

Table 5: Comparison of Mean HbA1c between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
HbA1c (%)	6.3 ±0.74	7.7±0.73	8.9±0.78

In Table 5, in Group 'A' the mean of HbA1c was 6.3 ±0.74%, in Group 'B' the mean of HbA1c was 188.43±18.43% and in Group 'C' the mean of HbA1c was 201.53±21.36%.

Table 6: 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 6, 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 7: Comparison of Mean Serum h-CRP between Group A, B and C

	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD
Serum h-CRP (mg/L)	2.43 ±0.35	3.53±0.42	6.42±0.73

In Table 7, in Group 'A' the mean of Serum h-CRP was 2.43 ±0.35 mg/L, in Group 'B' the mean of Serum h-CRP was 3.53±0.42 mg/L and in Group 'C' the mean of Serum h-CRP was 6.42±0.73 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. [8] In our study, in Table 3, the mean fasting blood glucose level in Group A was 131.71 ±12.04 mg/dl, in Group B was 148.29±13.06 mg/dl and in Group C was 161.92±15.11 mg/dl. In Table 4, in Group 'A' the mean of PPBG level was 203.43 ±18.54 mg/dl, in Group 'B' the mean of PPBG level was 228.64±18.43 mg/dl and Group 'C' the mean of PPBG level was 241.64±21.54 mg/dl. In Table 8, in Group 'A' the mean of HbA1c was 6.3 ±0.74%, in Group 'B' the mean of HbA1c was 188.43±18.43% and in Group 'C' the mean of HbA1c was 201.53±21.36%.

DN is characterized by structural and functional changes. In glomeruli, there is mesangial expansion, thickening of the basement membrane, and, characteristically, nodular glomerulosclerosis (Kimmelstiel–Wilson nodules). [9] In early DN, tubular hypertrophy is present but eventually interstitial fibrosis with tubular atrophy develops, along with arteriolar hyalinosis. [10] In advanced cases, there is an infiltrate of macrophages and T-lymphocytes. Ultrastructurally, there is podocyte loss and reduced endothelial cell fenestration. Functionally, there is early glomerular hyperfiltration and increased albumin excretion; and with advancing nephropathy, increasing proteinuria and declining GFR. A brief description of the functional and cellular pathology is provided below. [11] Although it is conceptually easier to describe these pathways individually, these pathways overlap and interact with one another in vivo, and enhance one another's biophysiological effects. [12]

In type 2 diabetics, more patients have DN at the time of diagnosis of diabetes as type 2 diabetes can go unrecognized for years. The AusDiab study of diabetic Australians showed that albuminuria is common among patients with established diabetes, is present before the onset of diabetes, and becomes more prevalent with worsening glucose tolerance. [13] About 20%–40% of type 2 diabetics with microalbuminuria progress to overt nephropathy; and about 20% will develop ESRD after the development of overt nephropathy. [14] Of obvious importance are the mechanisms by which IR can lead to DN: either indirectly through

hyperglycemia, hyperinsulinemia, hyperlipidemia, etc., or directly due to renal IR per se. Both prior and current research has centered on the mesangium. More recently, the critical role of the podocyte in DN has become apparent, recognizing normal insulin signaling as being necessary for proper functioning. [15]

Podocytes express insulin receptors and require normal insulin signaling for sustained viability. In vitro, podocytes respond to insulin at physiologic levels by translocating the glucose transporters GLUT1 and GLUT4 to the cell membrane, with a resultant doubling of glucose uptake. [16] This response is dependent on both a normally functioning actin cytoskeleton and nephrin. Reduced nephrin expression occurs in both early and advanced DN, and is required for normal podocyte insulin signaling. [17]

As in our patient, pathological evidence of DN in the absence of overt DM has occurred before. Some of the reported patients had impaired glucose metabolism that was below the threshold to diagnose DM, and they would be classified as having impaired fasting glucose or impaired glucose tolerance. Others had glucose levels diagnostic of DM either previously, concurrent, or subsequent to their renal biopsy. [18]

In our study, 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. Incipient nephropathy is the initial presence of low but abnormal amounts of urine albumin, referred to as microalbuminuria (persistent albuminuria at level 30–299 mg/24 hours). Overt nephropathy or macroalbuminuria (persistent albuminuria at level ≥300 mg/24 hours) develops after many years in type 1 diabetes but may be present at the time of diagnosis of type 2 diabetes. Patients who progress to macroalbuminuria are more likely to develop ESRD. [19]

Irrespective of these discrepancies, the data reviewed in this study showed that albuminuria, serum creatinine/eGFR, or the combination of both, were robust predictors of adverse outcomes in persons with T2DM. Of the three biomarkers, albuminuria was the most frequently evaluated, with the majority of studies identified in this review displaying an association with kidney decline and related outcomes, which is fitting with prior investigations. [20] Additional evidence in patients with T2DM has indicated that baseline micro- and macro-albuminuria as well as increasing albuminuria carry higher risks of declining kidney function and associated outcomes, beyond other existing renal biomarkers. [21]

In this study, in Group 'A' the mean of Serum h-CRP was 2.43 ±0.35 mg/L, in Group 'B' the mean of Serum h-CRP was 3.53±0.42 mg/L and in Group 'C' the mean of Serum h-CRP was 6.42±0.73 mg/L. In this study we found elevated hs-CRP levels were significantly associated with a higher incidence of DN, irrespective of a diagnosis of DM at baseline. Previous cohort studies have demonstrated a strong association between baseline hs-CRP concentration and prevalence of DN. [21] By contrast, Hayashino et al. [22] reported an independent association between serum baseline hs-CRP and the development but not progression of DN in 2,518 Japanese patients with DM at baseline followed for 1 year. Wang et al. [23] followed 4,213 Japanese civil servants for 6 years (~15% with

DM at baseline) and found an increase in incident cases of DM in the two quartiles with the highest CRP. However, they did not examine these study participants for development of DN.

Conclusion

Diabetic nephropathy is major microvascular complication leading to end stage renal failure and CVD associated death in diabetic patients, thus accounts for increased mortality and morbidity in these patients. Clinical definition of DN is presence of proteinuria over 0.5 g per 24h. It occurs in 15 – 30 % of type 1 diabetic patients after 20 years of diabetes duration, whereas prevalence in type 2 diabetes is more variable, ranging from 5 to 40 %. The fact that only subset of diabetic patients eventually develops DN despite long-term severe chronic hyperglycemia, together with the evidence of familial clustering of DN and various ethnic/racial prevalence of DN indicate hereditary predisposition to DN, independent form predisposition to diabetes mellitus.

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