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Evaluation of Biochemical Markers of Early Type-2 Diabetic Nephropathy

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Abstract:

Introduction: Micro-vascular complications in type-2 Diabetes Mellitus (DM) leading to diabetic nephropathy (DN) and end stage renal disease (ESRD) is a most common observation. With the improvement of treatment for DM increasing number of diabetic patients live a longer life span resulting in development of diabetic nephropathy (DN) and ESRD. Knowledge and evaluation of biochemical markers are essential for detection and treatment of DN as well as its prevention. Once the disease is diagnosed at an early stage measures can be undertaken for postponement of disease process. In the current study we have tried to evaluate the efficacy of some of the common biochemical parameters such as serum urea & creatinine, impaired glucose tolerance (IGT), Glycosylated haemoglobin (HbA1c), microalbuminuria, creatinine clearance test for early detection of DN.

Material and methods: This is a cross sectional study in which 50 patients (n-50) of known type-2 diabetes mellitus (KD), 50 patients (n-50) of newly diagnosed type-2 diabetes mellitus (NDD) with similar numbers (n-50) healthy controls (HC) were randomly selected. Blood and urine samples were collected from these cases sent for biochemical analysis which included glucose tolerance test (GTT), serum urea and creatinine, creatinine clearance test, urine for microalbumin and protein. Statistical analyses of each parameter were performed.

Results: Mean HbA1c, microalbuminuria and serum creatinine were highest in KD when compare with NDD and controls. Creatinine clearance value were low in KD and bears a significant correlation with duration of diabetes.GTT performed with these subjects showed IGT in NDD patients which is more evident in KD cases. Postprandial blood sugar (PPBS) after two hours showed marked IGT in both NDD and KD cases.

Keywords: diabetes, HbA1c, microalbuminuria, nephropathy

Introduction:

Micro-vascular complications in type-2 Diabetes Mellitus (DM) leading to diabetic nephropathy (DN) and end stage renal disease (ESRD) is a most common observation.[1,2] With the improvement of treatment for DM increasing number of diabetic patients live a longer life span resulting in development of diabetic nephropathy (DN) and ESRD. Due to these factors prevention of diabetic renal disease, or at least the postponement or slowing down of the disease process, has emerged

as a key issue.[3.4] DN has been defined as presence of proteinuria>0.5 gm/24 hours. This stage has been named as overt nephropathy where as presence of small amount of albumin in urine which cannot be detected by conventional methods has been termed as incipient nephropathy or microalbuminuria.[5]

Knowledge and evaluation of biochemical markers are essential for detection and treatment of DN as well as its prevention. Once the disease is diagnosed at an early stage measures can be undertaken for postponement of disease process. Urine is a biological fluid that has only recently begun to be explored by proteomic techniques.[5] As many as 1543 different proteins have been recently identified from a pool of normal urines.[6] Estimation of many glomerular and tubular markers have been under taken to find out early renal damage. These include a battery of expensive tests like urinary estimation of transferrin, fibronectin, and other components of glomerular extracellular matrix, and low molecular weight proteins (β_2 microglobulin, retinol binding protein, α_1 microglobulin, other proteins. Although more than 1500 identified urinary proteins represent a useful database for the identification of biomarkers for DN, these can hardly be of any utility in clinical practice because they do not give any added advantage over traditionally performed laboratory tests to assess early kidney damage.[7]

Aim and objective of the study:

In the current study we have tried to evaluate the efficacy of some of the common biochemical parameters keeping in mind of the risk factors those lead to DN such as hyperglycaemia, hypertension, old age, duration of diabetes, evidence of other micro-vascular involvement like retinopathy, smoking and genetic factors. Parameters include measurement of serum urea, creatinine, impaired glucose tolerance (IGT), glycosylated haemoglobin (HbA1c), microalbuminuria, creatinine clearance test. We have also tried to assess the efficacy of these tests to detect early nephropathy in type-2 DM.

Material and methods:

This study was conducted in the Department of Biochemistry, Hi-Tech Medical College & Hospital, Bhubaneswar, Odisha. Ethical, clearance obtained for the study from the hospital. It is a cross sectional study in which 50 patients (n-50) of known type-2 diabetes mellitus (KD), 50 patients (n-50) of newly diagnosed type-2 diabetes mellitus (NDD) with similar numbers (n-50) healthy controls (HC) were randomly selected. NDD case included the patients who have been detected since two years and KD cases included the patients who have been detected since ten years or more. The control group included non-diabetic healthy volunteers who were consistent with the patients according to the age and the exclusion criteria which included patients with secondary hyperglycemic states like hyperthyroidism, proteinuric conditions like congestive cardiac failure, renal failure and proven renal diseases, eye disorders before the onset of diabetes mellitus and pregnancy were excluded from study.

Blood and urine samples were collected from these cases sent for biochemical analysis which included glucose tolerance test (GTT), serum urea and creatinine, HbA1c, lipid profile, creatinine clearance test, urine for microalbumin and protein. Blood sugar was investigated by the glucose oxidase method, serum and urine creatinine by Jaffe's method, cholesterol by the oxidase/peroxidase (CHOD–POD) method, triglycerides by the enzymatic GPO–POD method, high density lipoprotein by phosphotungstate precipitation and CHOD–POD, glycosylated haemoglobin (HbA1c) by the cation exchange resin method and microalbumin levels in the urine sample by using the turbilatex method.

Statistical analysis of each parameters were performed by the Students 't' test by using microtab-2 software and the p values which were < 0.05 were considered as significant.

Results & Discussion:

Type-2 diabetes mellitus is a chronic condition which is a major public health problem because of micro-vascular complications leading to diabetic retinopathy and nephropathy.[8] With this background, a case-control study were conducted to assess the role of biochemical markers in the prediction of the micro-vascular complications and to outline the correlation between different commonly used parameters to assess the progression of the complications in Type-2 diabetic patients. The following findings were observed in this study.

The pathophysiologic mechanisms of diabetic nephropathy are incompletely understood but include glycosylation of circulating and intrarenal proteins, hypertension, and abnormal intrarenal hemodynamics. The earliest demonstrable abnormalities include impairment of glomerular filtration and microalbuminuria. Clinically, the most important screening tool for identifying early nephropathy is detection of microalbuminuria which is earliest manifestation of kidney pathology. At this stage appropriate interventions can retard the progress of the disease. For this microalbuminuria cannot be counted as a marker to assess the progress of the disease. In our study newly detected diabetes cases (NDD)and controls microalbumin is within normal limits, however in KD there is substantial rise of microalbuminuria (Table 2), impaired glycaemic control (Table 1 & 3)which indicates that IGT is an important factor for progress from microalbuminuria to overt albuminuria ultimately progressing to diabetic nephropathy.[9]

As regards IGT is concerned, aim of treatment should be to bring down a blood glucose level as close to normal (HbA_{1c} <7%) as possible without causing dangerous hypoglycemia.HbA1c closely correlates to diabetic control and in our study percentage is high in KD (Table-1). Diabetic nephropathy is associated with an altered lipid profile characterized by elevated triglyceride rich lipoproteins, present even in the earlier stages of the renal disease.[10] Patients with diabetic nephropathy often have multiple lipoprotein abnormalities.[11] In patients with DN, increased plasma levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and triglycerides are usually found. However, the plasma level of high-density lipoprotein (HDL) is lower than those patients with normoalbuminuria. Persistent rise in LDL level is more pathognomic for development of DN.[12] Our study also indicates abnormal lipid profile status with significant higher LDL levels in case of KD patients (Table1). It has been documented that hyperglycemia and derangement in lipidprofile status promotes kidney damage.[13,14] Our studies also revealed similar finding as documented in Table 1.

Creatinine clearance test has been performed in all the cases including controls and documented in Table 2. Creatinine clearance being a relatively easy procedure has been adopted by us to assess GFR.[15] In our study creatinine clearance gradually decreases with progress of diabetes and correlates with 24 hours protein excretion in urine (Table2). Estimation of urea and creatinine in serum seems to be of not much significance as a marker in early renal DN (Table1).

To summaries mean HbA1c, microalbuminuria and serum creatinine were highest in KD when compared with NDD and controls. Creatinine clearance value was low in KD and bears a significant correlation with duration of diabetes. GTT performed with these subjects showed IGT in NDD patients which is more evident in KD cases. Postprandial blood sugar (PPBS) after two hours showed marked IGT in both NDD and KD cases. However it may be pointed out that though serum creatinine level increases with progress of nephropathy, it is not a reliable marker of early nephropathy because rise in creatinine level in serum becomes evident, only after when more than 60% of nephrons are destroyed.

Table 1: Comparison between different biochemical parameters in HC, NND and KD group of patients:

Biochemical Parameters	HC N=50	NDD N=50	KD N=50	P-Value
FBS mg/dl	90.2 ± 5.59	115.48 ± 1.05	145.2 ± 1.52	<0.0001
PPBS mg/dl	106.02 ± 0.97	135.08 ± 1.45	192.7 ± 2.58	<0.0001
HbA1c %	5.4 ± 0.40	7.6 ± 0.11	8.50 ± 0.12	<0.0001
Urea mg/dl	15.4 ± 0.92	19.6 ± 1.01	38.2 ± 0.58	<0.0001
Creatinine mg/dl	0.73 ± 0.06	0.96 ± 0.14	1.2 ± 0.07	<0.0001
TC mg/dl	144.2 ± 1.62	176.26 ± 1.19	195.6 ± 1.16	<0.0001
TG mg/dl	140.8 ± 3.82	157.22 ± 11.86	186.58 ± 14.36	<0.0001
HDL mg/dl	46.6 ± 5.02	38.42 ± 5.81	35.86 ± 5.56	<0.0001
LDL mg/dl	102.5 ± 11.22	115.76 ± 29.24	150.34 ± 14.78	<0.0001

Statistically Significant, P Value <0.0001 (TC= Total Cholesterol, TG=Triglycerides, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein)

Table 2: Comparison of different parameters in urine (Sample type- 24 hours urine)

Tests performed	HC	NDD	KD	P-Value
Microalbumin mg/dl	20.0±2.23	22.82±3.90	113.74±6.11	<0.0001
Creatine clearance ml/minute	135.1± 7.41	131.22±4.05	90.4±5.39	<0.0001

Statistically Significant, P Value <0.0001

Table 3: Estimation of glucose tolerance test

Test type GTT, Sample type- serum, Unit- mg/dl

Parameters	HC	NDD	KD	P-Value
FBS	90.4 ± 5.39	115.48 ± 1.05	145.2 ± 1.52	<0.0001
1/2hr PPBS	111.5 ± 4.56	152.22 ± 8.03	185.8 ± 11.26	<0.0001
1 hr PPBS	121.8 ± 2.84	172.08 ± 7.31	247.2 ± 15.58	<0.0001
1 & 1/2hr PPBS	111.3 ± 5.74	175.8 ± 8.10	257.1 ± 21.31	<0.0001
2 hr PPBS	94.82 ± 3.04	157.3 ± 11.90	255.8 ± 21.97	<0.0001

Statistically Significant, P Value <0.0001

Conclusion:

Type-2 diabetic patients at risk for the development of nephropathy could benefit from early intervention, if diagnosed at an early stage. We have tried to evaluate some biochemical markers which are economical and can even be performed in laboratories at district and primary health center level. Microalbuminuria, predicts clinical nephropathy to a lesser extent than originally described. Thus, the need exists for additional markers that either alone or in combination with microalbuminuria will identify early the individuals susceptible to clinical nephropathy, HbA1c, serum creatine, creatinine clearance, GTT are useful markers to assess the effective renal function. Microalbuminuria increases with KD and poor glycaemic control. Persistent increase of HbA1c, microalbuminuria decrease of creatinine clearance, IGT is important markers DN.

Abbreviations: DN-Diabetic Nephropathy, ESRD-End stage renal disease, IGT-Impaired glucose tolerance, DM-Diabetes mellitus, HbA1c-Glycosylated haemoglobin, NDD-Newly diagnosed type-2 diabetes mellitus, HC-Healthy controls, KD-Known type-2 diabetes mellitus, GTT-Glucose tolerance test, FBS-Fasting blood sugar, PPBS-Post prandial blood sugar.

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