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COMPARISON OF BLOOD SORBITOL LEVEL AND MICROALBUMINURIA IN EARLY DETECTION OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES

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ABSTRACT

Type 2 DM is a global disease and one of its resultant complication is diabetic nephropathy. An accelerated polyol pathway in diabetes contributes to the development of cataract and nephropathy. Microalbuminuria an early indicator of diabetic nephropathy is an independent risk factor for cardiovascular morbidity and mortality. The main aim of this study was to compare the blood sorbitol levels with microalbuminuria in the early prediction of diabetic nephropathy. The study group included 3 groups of subjects aged between 55-68yrs, Group1-Non diabetic senile cataract patients, Group 2-Diabetic cataract patients without nephropathy, Group3- Diabetic cataract patients with nephropathy. The biochemical parameters estimated included sorbitol, glucose and creatinine in blood; albumin and creatinine in urine. A significant increase in blood sorbitol and glucose along with urine albumin creatinine ratio (UACR) was observed in group 2 and 3. Group 3 showed highly significant increase in blood sorbitol ($p < 0.01$) and UACR ($p < 0.05$) as compared to group 2. Blood sorbitol levels could be used as alternative to MA in the early prediction of diabetic nephropathy.

KEY WORDS

Diabetes Mellitus (DM), Microalbuminuria (MA), Urinary Albumin Creatinine Ratio (UACR).

INTRODUCTION

Diabetes mellitus is a clinical syndrome leading to complications, of which, cataract formation and nephropathy are common. Microalbuminuria present at the time of diagnosis of diabetes mellitus reflects the long asymptomatic period prior to diagnosis. It has also been reported that MA is an important predictor of cardiovascular events and early renal damage in patients with essential hypertension (1, 2). MA as a predictor of diabetic nephropathy is affected by various other conditions other than diabetes such as hypertension, congestive cardiac failure, prostate disease and urinary tract infections etc (2). Invariably hypertension accompanies overt nephropathy in type 2 diabetes. The polyol pathway has an important role in development

of diabetic cataract and nephropathy (3). Accumulation of excessive sorbitol causes increased tissue osmolarity and consequent hydration because of water influx from aqueous humor into the cells. But definite diagnosis of nephropathy requires a kidney biopsy which is not possible in all patients (4). Chronic elevation of blood glucose in diabetics presents a severe risk factor for development of cataract as one of earliest secondary complications of DM (5). Many investigators have determined sorbitol levels in various tissues, such as erythrocytes, lens, renal tissues etc & analyzed the correlations between sorbitol levels & complications (6-9). An elevated level of urinary sorbitol has also been observed in diabetic patients (10, 11).

The main objective of this study was to compare sorbitol levels with MA in early prediction of diabetic nephropathy.

MATERIALS AND METHODS

The present study design included 60 subjects between age group of 55- 68 years admitted in Government Wenlock hospital, Mangalore. The study subjects included 3 groups. Group 1: senile cataract patients without diabetes, Group 2: diabetic subjects with cataract, Group 3: diabetic subjects with cataract and nephropathy.

Inclusion criteria: Hospitalized diabetic subjects with cataract or h/o cataract and nephropathy.

Exclusion criteria: Subjects with type 1 diabetes, pregnant with type 2 diabetes and patients with urinary tract infection were excluded from the study. The study was approved by the Institutional Time Bound Research committee. A written informed consent was taken from the subjects.

Sample collection
Blood samples were collected during pre-operative period from both senile and diabetic cataract patients. Six ml of venous blood and early morning mid stream urine was collected

under aseptic precautions. Age, sex, duration of diabetes and blood pressure were noted. The blood was analyzed for glucose, sorbitol and creatinine. Assessment of microalbuminuria was done by UACR.

Biochemical estimations
Sorbitol in blood was estimated by the modified spectrophotometric method (11). Sorbitol in blood is converted to fructose in presence of enzyme sorbitol dehydrogenase with simultaneous reduction of NAD^+ to NADH. The NADH formed was measured at 340nm. Plasma glucose was estimated by GOD – POD method (12). Estimation of creatinine was done by the modified Jaffe's method (13-15). Albumin in urine was estimated by Turbidimetric method (16) and creatinine was determined by alkaline picrate method (17). The presence of MA in early morning mid stream urine samples was defined as urinary albumin excretion in the range of 30-300 mg/G creatinine.

Statistical analysis
Results were subjected to statistical analysis by Analysis of variance (ANOVA)(Kruskal wallis test) and the values expressed as mean ± SD. Since the data showed a skewed distribution median & inter quartile range were given. Wherever the Kruskal wallis test was significant pair wise comparison was done using mann whitney test adjusting for type 1 error.

RESULTS

The results of the study are shown in table 1 and 2. There was no significant difference in the age and weight of the subjects. A significant increase in the systolic blood pressure is observed in the group 2 &3 subjects as compared to group 1(Table 1). The levels of blood sorbitol, glucose and UACR were significantly increased in group 2 & 3 as compared to control subjects. However there was no significant change in the serum creatinine levels. Pair wise comparison

between group 2 & 3, showed a significant increase in blood sorbitol ($p < 0.001$) and UACR ($p < 0.05$) in group 3. The correlation between the groups showed no significance.

Table 1
Age, weight and blood pressure of control and diabetic subjects with and without nephropathy

	GROUP 1 (n=27)	GROUP 2 (n=20)	GROUP 3 (n=13)
Age (yrs)	58.52±13.61	61.90±10.54	67.25±7.15
Weight (Kgs)	49.89±9.48	49.00±16.20	49.38±11.22
Diastolic blood pressure (mm/Hg)	80.52±8.3	81.50±8.13	86.25±5.18
Systolic blood pressure (mm/Hg)	127.41± 15	132.60 ± 13.95	144.50 ± 16.62*

* =   Significant ($p < 0.05$)
** =   Highly Significant ($p < 0.01$)
*** =   Very Highly Significant ($p < 0.001$)

Table 2
Blood and urine parameters of subjects

	GROUP1 (n=27)	GROUP 2 (n=20)	GROUP 3 (n=13)
Glucose (mg/dl)	91.50 [a***] (79.59-99.48)	142.17 [b***] (103.2-168.6)	174.53 [c*] (168.55-197.51)
Creatinine (mg/dl)	0.78 (0.7-0.9)	1.02 (0.68-1.39)	0.87 (0.63-1.37)
Sorbitol (nmol/L)	2218.00 [a***] (1298.7-2844.3)	5990.00 [b***] (3609.1-9570.5)	27103.10 [c***] (12815.3-34464.5)
UACR (mg/g)	51.61 [a***] (36.38-72.10)	86.76 [b***] (58.11-186.90)	249.72 [c*] (180.49-418.62)

* =   Significant ($p < 0.05$) ,** =   Highly Significant ($p < 0.01$),*** =   Very Highly Significant ($p < 0.001$)
a= Comparison between group 1 & 3 ,b= Comparison between group 1 & 2,c= Comparison between group 2 & 3

DISCUSSION

A significant increase in UACR in the test groups was an important finding of this study. The increase being 5 fold in group 3 and 1.6 fold in group 2 as compared to the subjects with group 1. Also the increase in group 3 was 2.8 fold as compared to group 2. Studies have shown that the presence of microalbuminuria in type 1 & type 2 diabetic patients is an important predictor of progressive renal failure. Several epidemiological studies have indicated the presence of microalbuminuria as an independent predictor of cardio vascular morbidity & mortality in patients with essential hypertension. Albumin to creatinine ratio (UACR) in the morning urine sample is a reliable estimate of the 24hrs albumin excretion rate (AER) and better than albumin

concentration alone (18-20), as noted in the study by Ravijt et al (21) in diabetic subjects and in the study by Sheth et al (24) in hypertensive subjects. The Random dipstick test is reliable only when definite proteinuria is present (23). It does not correlate well when proteinuria is in the lower range. UACR offers good reliability as a test for classifying degrees of proteinuria and accurately predicting nephrotic and physiological range proteinuria. Esjoh et al (19) have noted that importance of 24 hr urine sample for the prediction of microalbuminuria. The findings of the present study strongly suggests that UACR in the early morning midstream urine sample is an reliable indicator of albumin excretion as a 24 hr urine sample for albumin estimation in diabetics .

In the group 1 subjects we found slightly higher levels of UACR than the expected, this may be due to the age of subjects and proper evaluation was not possible because they admitted on the day of surgery.

Parving et al (24) have shown that rise in BP occurs in proteinuric insulin dependent diabetes. Wiseman et al (25) have reported high arterial pressure in microalbuminuric subjects with IDDM. The SBP was significantly higher in group 3 subjects indicating that blood pressure and UACR are related even type 2 diabetic patients. There was no significant difference in the serum creatinine levels. However this does not rule out the renal changes in diabetic patients, because studies have shown that estimation of serum creatinine may falsely indicate that the renal function is normal (26,27).

Significant increase in the whole blood sorbitol levels in group 2 and 3 (2.7 and 12 fold) as compared to group 1 subjects . Likewise the increase was 4.5 fold as compared to group 2. Study by Nakano et al (11) in diabetic patients have reported higher whole blood sorbitol levels as compared to the normal subjects.

The results of the present study strongly suggests that whole blood sorbitol estimation could be used as a marker for diabetic renal changes as compared to microalbuminuria, because albumin excretion is influenced by other conditions like hypertension and acute hyperglycemia, whereas sorbitol accumulation is seen only in diabetes mellitus. Thus, measurement of sorbitol in blood may serve as an early indicator of diabetic nephropathy.

However there is a need to determine the cutoff level of sorbitol which can predispose to chronic complications this was a limitation of this study

Further studies have to be done in larger population to confirm the role of sorbitol as a marker of diabetic complication. Also the excretion of sorbitol in urine needs to be studied.

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