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A CROSS SECTIONAL STUDY OF ASSOCIATION OF URINARY ALBUMIN WITH HBA1C LEVELS IN SUBJECTS OF TYPE 2 DIABETES MELLITUS

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Abstract

Introduction: Diabetes mellitus (DM) refers to a set of known metabolic conditions that can be caused by different genetic and environmental factors, which share the phenotype of hyperglycaemia. The prevalence of DM in India as of 2019 was 8.9% with an increase from 7.1% a decade before. It affects almost 8% of the Maharashtra population, according to 2016 data. The diagnosis of diabetes mellitus can be broadly classified into two categories Insulin-dependent diabetes mellitus (T1DM) and non Insulin dependent Diabetes Mellitus (T2DM).

Materials and methods: We included 100 diagnosed subjects of type 2 diabetes mellitus attending Outpatient Department of medicine in a tertiary care center in West Maharashtra. The sample size was based on purposive sampling method. Patients with fasting blood sugar level > 126 mg / dL and post meal blood sugar > 200 mg / dL were included and patients with history of hypertension, renal diseases, liver disease, valvular heart diseases and any infection were excluded.

Results: This hospital based cross sectional study is done at a tertiary care hospital. In this present study, subjects are selected with Type 2 Diabetes Mellitus (T2DM) with duration more than one year and age 30 years and above. Subjects were divided into two groups. Group 1 includes 62 subjects with normoalbuminuria (i.e., with Urine Albumin < 30 mg / 24 hr). Group 2 includes 38 subjects with microalbuminuria (i.e., with Urine Albumin > 30 to < 300 mg /24 hr). Glycosylated haemoglobin (HbA1c) showed significantly higher levels in group 2 subjects as compared to group 1.

Conclusion: It was concluded that in a developing country like India, there is a need of estimation of microalbuminuria and HbA1c testing in both newly diagnosed as well as those already diagnosed as an early marker of renal involment. The present study emphasizes on

education about strict glycaemic control and testing for microalbuminuria, which is an early indicator of diabetic nephropathy, and should be mandatory for all type 2 diabetes patients.

Key Words: Diabetes mellitus, hypertension, obesity, renal diseases, liver disease.

INTRODUCTION

Diabetes mellitus (DM) refers to a set of known metabolic conditions that can be caused by different genetic and environmental factors, which share the phenotype of hyperglycaemia. The prevalence of DM in India as of 2019 was 8.9% with an increase from 7.1% a decade before. It affects almost 8% of the Maharashtra population, according to 2016 data.[11] The diagnosis of diabetes mellitus can be broadly classified into two categories Insulin-dependent diabetes mellitus (T1DM) and non Insulin dependent Diabetes Mellitus (T2DM).[1]

T1DM is described by a near-total lack of insulin due to destruction of β-cells of the pancreas. β-cell destruction can be due to drugs, viruses, or auto-immunity. It usually develops in children, teenagers, and young adults; however, it can happen at any age.[2] T2DM is triggered by insulin resistance, and in previous literature, obesity, especially excessive abdominal fat called visceral fat, are the key reason for insulin resistance. In a person with insulin resistance, cells in their muscles and adipose tissue, don't respond well to normal or increased levels of insulin. This is characterized by a decrease in the target cell response to insulin. Inflammatory cytokines and inflammatory markers are excessively produced by adipocytes in T2DM due to obesity-induced dysregulation of adipocytes. Type 2 diabetes mellitus has emerged as one of the most critical chronic public health problems and is a rising reason of morbidity and mortality. T2DM is the leading cause of various micro and macro vascular complications.8Micro-vascular complications include neuropathy, retinopathy, and nephropathy. Macro-vascular complications consist of coronary artery disease (CAD), cardiomyopathy, cerebrovascular diseases, peripheral artery disease, etc.[3]

Glycosylated haemoglobin indicates average plasma glucose over the period of 3 months.[4] Quality of glycosylated haemoglobin of being tested at any time of the day without any special preparations, has made it the preferred test for assessment of glycaemic control in patients of diabetes mellitus. Likewise, estimating urinary albumin has become a gold standard for monitoring diabetic nephropathy progression.[5]

In the present study, we correlated association between urinary albumin excretion and HbA1c levels in subjects of type 2 diabetes mellitus.

MATERIALS AND METHODS

We included 100 diagnosed subjects of type 2 diabetes mellitus attending Outpatient Department of medicine in a tertiary care center in West Maharashtra. The sample size was based on purposive sampling method. Patients with fasting blood sugar level > 126 mg / dL and post meal

blood sugar > 200 mg / dL were included and patients with history of hypertension, renal diseases, liver disease, valvular heart diseases and any infection were excluded.

Sample was collected after obtaining written / informed consent in regional language from subject or subject's legal representative, attending a tertiary level rural hospital.

- Urine Sample - 24 hr. urine sample was collected for estimation of albumin in urine
- Blood Sample - 2 ml of blood sample was collected from antecubital vein and stored in EDTA (Ethylene-Diamine-Tetra-Acetic Acid) bulb. The samples were allowed to clot & then be centrifuged at 3000 rpm for at least 10 minutes to separate plasma. After separating plasma, buffy coat was removed, and the remaining erythrocytes were used to prepare the haemolysate. The haemolysate was stored at – 20° C in aliquots and used for was estimation of glycosylated haemoglobin.

Single radial Immuno Diffusion for estimation of microalbuminuria:

Monospecific human serum antibody 150 µL (as standardized by trial-and-error method) was incorporated into 15 ml of molten agarose gel (1 %) at 560 C taken in small glass beaker and mixed thoroughly by gentle rotation. The agarose gel was poured onto the glass slide of 12.5 × 7.5 cm size. It was allowed to solidify for half an hour at 40 C in a humid chamber. Wells were punched out at 1.5 cm apart each other using the gel cutter and the water suction pump. The individual well was charged with 5 µL of the urine / standard with micropipette. The loaded gel was allowed to diffuse for 24 hrs. at 40 C in a moist chamber. The resulting ring diameters of the circular immunoprecipitates were noted. Damp filter paper was kept over the slide for drying at room temperature. The dried plate was put into Coomassie brilliant blue R stain for 30 minutes.

Then washed under the tap and destained with destaining solution till background was clear. Standard curve was plotted taking the square of the diameter on y axis against the concentration of standards on x axis. The concentration of the unknown samples was read from the graph by measuring diameters of the rings.

Estimation of Glycosylated Haemoglobin:

As the amount of HbA1c also depends on total quality of haemoglobin the reported HbA1c value is indicated as a percentage of the total haemoglobin concentration.

Total Hb and HbA1c in haemolyzed blood bind with the same affinity to particles in R1. The amount of binding is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2a) binds to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R2b) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to particles, which in turn is proportional to the percentage of HbA1c in the sample.

Statistical Analysis:

The biochemical analysis was carried out at our Central Clinical Biochemistry Laboratory. The samples were analysed by Erba EM 360 (Random Access Analyser). Data were analysed using descriptive and inferential statistics using student unpaired t-test and software used were SPSS version 24.0 and GraphPad prism 7.0 version. $P < 0.05$ is considered as level of significance. Pearson correlation coefficient was used to find the linear relation between HbA1c and urinary albumin. All values were expressed as Mean ± SD. P value was obtained using students unpaired t test.

RESULTS

This hospital based cross sectional study was done at a tertiary care hospital. In this present study, subjects with Type 2 Diabetes Mellitus (T2DM) disease with duration more than one year and age 30 years and above. Subjects were divided into two groups. Group 1 includes 62 Type 2 DM subjects with normoalbuminuria (i.e., with Urine Albumin < 30 mg / 24 hr). Group 2 includes 38 Type 2 DM subjects with microalbuminuria (i.e., with Urine Albumin > 30 to < 300 mg /24 hr).

Gender	Group 1 (n=62)	Group 2 (n = 38)	R-value	P Value
Male	35 (56.91%)	25 (64.94%)	0.255	0.24
Female	27 (43.09%)	13 (35.06%)		
Average Age wise Distribution				
Age (years)	43.75 ± 5.95 (Range=34-62 years)	51.06 ± 7.94 (Range = 34 - 64 yrs.)	0.417	0.0001

Table 1: Gender and Age Wise Distribution

Parameter	Group 1	Group 2	R-value	P-value
HbA1c (%)	7.06 ± 0.56	8.27 ± 1.30	0.210	0.0001

Table 2: Comparison of HbA1c Levels in Group 1 & Group 2 Subjects

Glycosylated haemoglobin (HbA1c) showed significantly higher levels in group 2 subjects as compared to group 1 as seen in table No. 2.

Variables	Mean	SD	P-value
Urinary Albumin (mg / 24 hr.)	14.68	6.63	-
HbA1c (%)	7.05	0.55	0.004

Table 3: Correlation of Urinary Albumin with HbA1c Levels in Group 1

Variables	Mean	SD	P-value
Urinary Albumin (mg / 24 hr.)	60.76	18.73	-
HbA1c (%)	8.27	1.29	0.0001

Table 4: Correlation of Urinary Albumin with HbA1c Levels in Group 2

DISCUSSION

We enrolled a total 100 diagnosed subjects of type 2 diabetes mellitus with average age of 43.75 ± 5.95 years in group 1 (n =62) and 51.06 ± 7.94 years in group 2 (n = 38). Most of the subjects were clustered between 30 to 55 years of age. Other studies done in developing countries have found that majority of patients with diabetes are in the age group of 45-64 years, whereas in developed countries, diabetes mellitus is diagnosed at higher age (>65 years). Beginning of diabetes mellitus at lower age hints that these patients may develop diabetes in the high yielding years of their life and have a higher chance of starting complications.[6] The prevalence of diabetes in the young Indian people may be explained by both environmental and genetic factors.[7]

Different ways of estimating microalbuminuria, racial differences in the study populations, method of urine collection may be credited for the differences in prevalence of albuminuria.[8]

In our study, we found that the mean age of the patients with normoalbuminuria (43.75 ± 5.95 years) was significantly lower than microalbuminuria patients (51.06 ± 7.94 years). This showed that prevalence of microalbuminuria rises as the age increases. Similar studies from India shows that the mean age of patients with normoalbuminuria is lower as compared to mean age of patients with microalbuminuria.[9]

We found that there is positive correlation of urine albumin with HbA1c levels in both group 1 (r = 0.255; p value = 0.004) and group 2 (r = 0.0001; p value =0.001). Our study is consistent with other researchers proving support for complete screening for diabetic complications especially microalbuminuria at the time of diagnosis of type 2 diabetes mellitus. Increased albumin excretion and other microvascular complications take place with substantial frequency before diabetes is diagnosed clinically. The correlation between the development of microalbuminuria and degree of hyperglycaemia shows that early action towards obtaining glycaemic control might serve to help prevent the development of diabetic nephropathy.[10]

CONCLUSION

It was concluded that in a developing country like India, there is a need of estimation of microalbuminuria and HbA1c testing in both newly diagnosed as well as those already diagnosed as an early marker of renal involment. The present study emphasizes on education about strict glycaemic control and testing for microalbuminuria, which is an early indicator of diabetic nephropathy, and should be mandatory for all type 2 diabetes patients.

LIMITATION OF THE STUDY

Limitation of the study is about duration of diabetes mellitus should have been elaborated in data connection to establish temporal relationship with microalbuminuria.

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