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Association of glycosylated hemoglobin with microalbuminuria in patients with type 2 diabetes mellitus

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Abstract

Background: Type 2 diabetes mellitus (DM) is a complex heterogenous disease characterized by abnormal glucose homeostasis. Microalbuminuria is the initial stage of nephropathy which is revesible with good glycemic control. High levels of HbA1C indicate a poor glycemic control.

Objectives:

The present study was planned to assess the association of microalbuminuria with glycosylated hemoglobin in type 2 DM patients.

Methodology: 100 patients diagnosed for type 2 DM, age 40-60 years with no

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evidence of any other pathology involving proteinuria were selected and fasting blood sugar (FBS), HbA1C and 24 hr urine microalbumin were estimated in the above patients.

Result: The patients were grouped as good glycemic control (HbA1C < 8.0%) n= 40 and poor glycemic control (HbA1C > 8.0%) n= 60. Statistically high levels of urinary microalbumin were observed in patients with poor glycemic control. A positive correlation was observed between microalbuminuria and HbA1C and FBS. However, no significant correlation was observed between microalbuminuria and duration of diabetes.

Conclusion: Microalbuminuria exhibits a strong association with HbA1c and hence with the glycemic control. Patients with a poor glycemic control are at risk of developing nephropathy. This situation can be averted by adopting lifestyle changes, frequent monitoring.

Keywords

Microalbuminuria, glycemic control, Diabetes Mellitus, microvascular, macrovascular, Glycated hemoglobin, serum urea

INTRODUCTION

Diabetes Mellitus has become a major health problem in India. It has been estimated that the burden of Type 2DM for India is projected to increase to 87 million by the year 2030. The impacts of Type 2DM are considerable (as a lifelong disease). It increases morbidity and mortality and decreases the quality of life. At the same time, the disease and its complications cause a heavy economic burden for diabetic patients themselves, their families and society [Ramchandran A et al., 2009[1]; American Diabetes Association, 2011] [2].

Microalbuminuria is decisive indicator of early stages of DN. Microalbuminuria is common and is a well-established risk factor for macrovascular diseases in type 2 diabetics. Microalbuminuria represents the simplest and most sensitive prognostic factor to evaluate the risk of overt nephropathy in diabetes, representing the first stage of progressive diabetic renal disease [Tobe SW, et.al. 2002][3].

Simultaneously, microalbuminuria has turned out to be an analytical marker for cardiovascular disease (CVD) and the finding of microalbuminuria is a sign of screening for possible vascular disease and aggressive intervention to decrease all cardiovascular risk factors [Morrish NJ et al.2001[4]; Cec-Calvo L et al.2006][5]. Epidemiological and research statistics show that microalbuminuria is related with an amplified risk for all cause of cardiovascular mortality, cardiac abnormalities and cerebrovascular disease [Keller C et al.1996[6]; Charpentier G et al.2003[7]]. Endothelial

function and chronic inflammation have been suggested as potential candidates to clarify the associations between microalbuminuria and cardiovascular disease [Guerci B et al.2001][8].

The underlying risk factors for microalbuminuria are raised blood pressure and poor glycemic control. Some studies have revealed duration of diabetes, male sex, and pre-existing retinopathy as major risk factors for microalbuminuria [Allawi J et al.1988[9]; Gatling W et al.1988[10]; Marshall SM et al. 1989[11]; John L et al.1991[12];

Glycosylated hemoglobin is a blood glucose control marker in diabetic patients. HbA1c results from post-translation changes in the hemoglobin molecule, and their levels correlate well with glycemic levels over the previous six to ten weeks. Glycosylation of hemoglobin takes place under physiological condition by a reaction between glucose and N-terminal valine of beta chain of molecules [Kareem I et.al.2004][13].

Currently, India leads the world with the largest number of diabetic subjects and this is expected to further rise in the coming years [Ramachandran A et al. 1997[14]; King H et al. 1998][15]. Therefore, studies on diabetes related complications are vital to assess the burden of diabetes.

OBJECTIVES

- To determine correlation among microalbuminuria level in type 2 diabetics with glycosylated hemoglobin

MATERIAL & METHOD

The study was conducted in the Out-Patient Department of Tertiary care Hospital, Jaipur, Rajasthan. Approval from the Institutional Ethics Committee was obtained to conduct the study. It was a single center observational study. A total of 100 patients, fulfilling inclusion and exclusion criteria as listed below were enrolled in the study after obtaining informed consent in writing.

Inclusion Criteria

i. Diagnosed cases of type 2 diabetes mellitus.

ii. Age 40 to 60 years, either gender.

iii. Patients who are willing to participate and sign consent document.

Exclusion Criteria

i. Pregnant or lactating women.

ii. Patients with alcohol or drug dependence.

iii. Patients who had any major surgery within 4 weeks of screening.

iv. Patients with acute illness, fever and urinary tract infection.

Study Design and Methodology:-

It was a single center observational study. The study was conducted in Department of Biochemistry in association with other Clinical Departments of Tertiary care Hospital, Jaipur Rajasthan, Jaipur.

Total 100 subjects with type 2 DM were enrolled. Patients enrolled in the study were recommended not to have heavy exercise at least 24 hours before examination. This study was one shot visit; no follow ups had been done.

Each enrolled patient was subjected to the detailed medical history, general physical examination and biometrics. Fasting blood samples were collected by venipuncture for biochemical analysis and 24 hours urine samples were also collected for estimation of microalbuminuria. In view of measuring urinary albumin concentration correctly, patients were given necessary instructions regarding the collection of urine samples. When no evidence of infection and/or haematuria was found in the urinalysis, urine samples were examined for microalbuminuria.

Biochemical Investigations:

All tests were performed in Randox Daytona auto analyzer. All parameters were calibrated by use of saline and Randox calibration serum level 3 (Cal 3). Quality control was performed by using Randox assayed multisera, level 2 and level 3.

The major investigations were included:-

- Blood Glucose (Fasting)
- Blood Urea
- Serum Creatinine
- HbA1c
- Urinary Microalbuminuria

Diagnostic Criteria:-

· **Blood glucose**

Fasting blood glucose was estimated by Glucose Oxidase method.

Sample Type: Venous blood was taken in fluoride vial for plasma seperation.

Normal Value (Fasting Plasma Glucose)	75-115 mg /dl

- **HbA1c**

Glycated hemoglobin [HbA1c] is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. Measurement of HbA1c is used to determine average glycemic control over an 8-12 week period, and HbA1c level has been linked to development of microvascular complications [Jesudason DR et al.2003][16].

- **Serum Creatinine**

Serum creatinine was estimated by Jaff's method by colorimetrically.

Sample Type: Venous blood was taken in plain vial for serum seperation.

	Normal values
Male	**0.6 – 1.1 mg/dl**
Female	**0.5 – 0.9 mg/dl**

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Microalbumin in urine

Immunoturbidimetric assay for urinary albumin liquid stable

	Albumin level (mg/day)
Normal	**2-20**
Microalbuminuria	**20-300**
Macroalbuminuria	**>300**

Statistical Analysis

The present study was planned to assess the association of levels of HbA1c with hyperuricemia and microalbuminuria in type 2 diabetic patients. The study was conducted on 100 T2DM patients. Blood samples were collected and analyzed for fasting sugar, urea, creatinine, uric acid and glycosylated hemoglobin (HbA1c). 24 hr urine samples were also collected for analysis of microalbumin.

Based on the level of HbA1c, the patients were distributed in two groups viz:

Group I (good glycemic control i.e. HbA1c $\leq$ 8.0)	**n = 40**
Group II (Poor glycemic control i.e. HbA1c > 8.0).	**n = 60**

The results obtained were presented as Mean $\pm$ SD and subjected to statistical analysis. The values of different parameters in the two groups were compared by applying **z-test**.

Further, to assess the correlation of HbA1c and microalbuminuria with serum urea, creatinine and also with the age and duration of diabetes,

Pearson's correlation was applied. XY scatter plots were plotted for all the correlations.

A p-value of $\leq$ 0.05 was considered to be statistically significant.

Observations

Table 1: Level of HbA1c in T2D patients in the groups based on Glycemic control

Group	No. of cases (n)	Age (years)	P-value
I	40	49.31 $\pm$ 6.70	0.000
II	60	50.51 $\pm$ 6.76	

- Values presented as Mean $\pm$ SD
- P-value as obtained on applying **z-test**

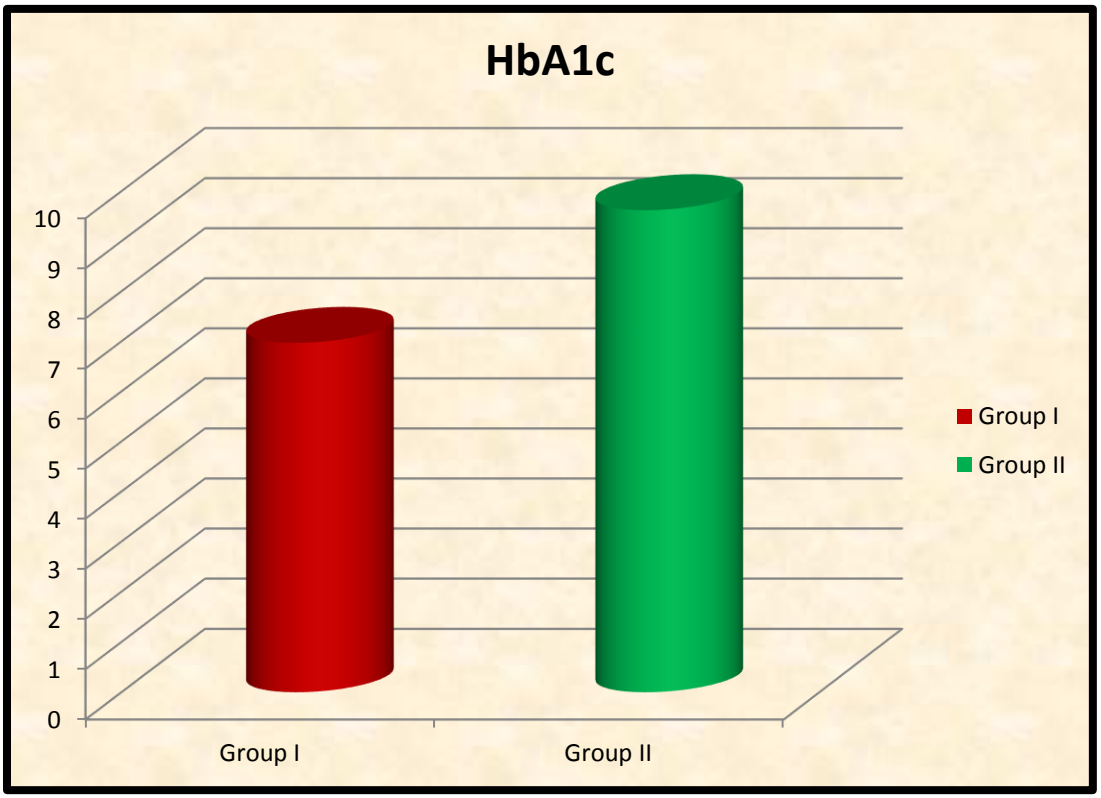

Figure 1: Level of HbA1c in T2D patients in the groups based on Glycemic control

Table 2: Level of urinary microalbumin in T2D patients in the groups based on Glycemic control

Group	No. of cases (n)	U. Microalbumin (mg/24 hr)	P-value
I	40	40.92 ± 24.03	0.000
II	60	120.9 ± 88.26	

- Values presented as Mean ± SD
- P-value as obtained on applying **z-test**

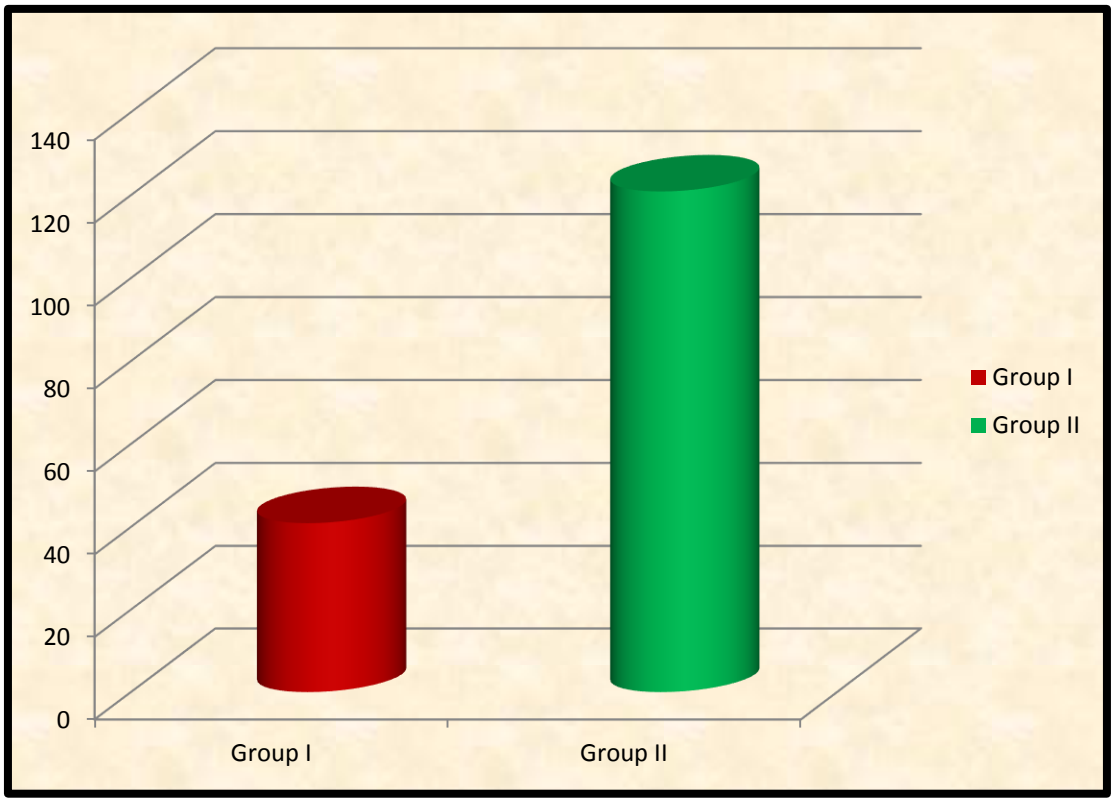

Figure 2: Level of urinary microalbumin in T2D patients in the groups based on Glycemic control

Table 3: Level of B. Urea in T2D patients in the groups based on Glycemic control

Group	No. of cases (n)	B. Urea (mg/dl)	P-value
I	40	33.03 ± 13.94	NS
II	60	31.49 ± 8.71	

- Values presented as Mean ± SD
- P-value as obtained on applying **z-test**

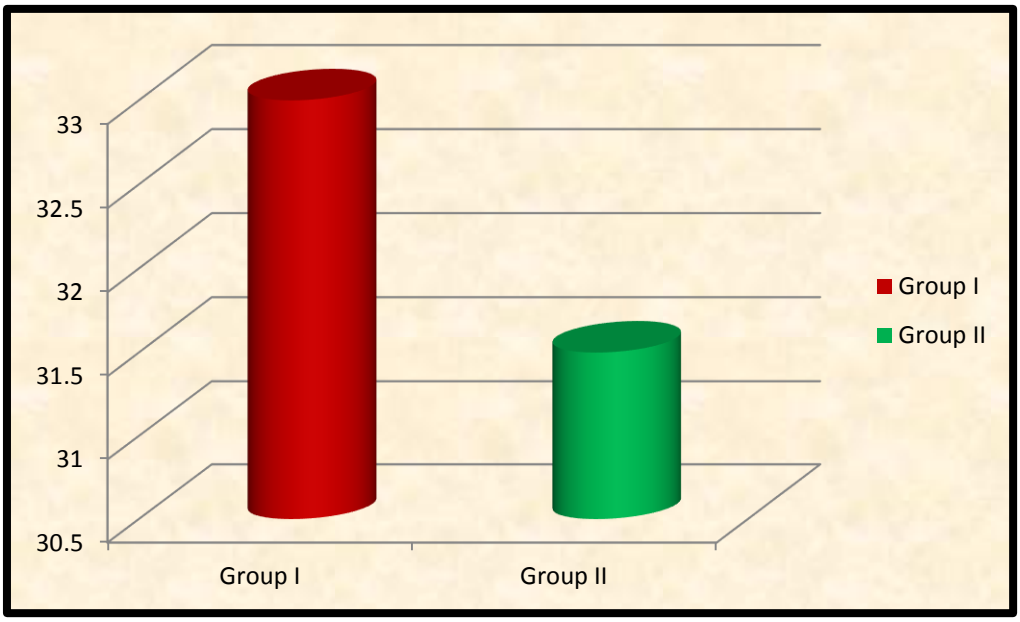

Figure 3: Level of B. Urea in T2D patients in the groups based on Glycemic control

Table 4: The correlation coefficient for different factors.

Factors	Correlation coefficient (r)	P-value
HbA1c vs Fasting Sugar	0.766	0.000
HbA1c vs Uric Acid	0.211	0.032
HbA1c vs MA	0.381	0.000
HbA1c vs Creatinine	0.278	0.004
HbA1c vs Duration	0.250	0.011
MA vs Fasting sugar	0.379	0.000
MA vs Uric Acid	0.340	0.000
MA vs Creatinine	0.357	0.000
MA vs Duration	0.037	NS

- **r** and **P**-value as obtained on applying **Pearson correlation**
- **MA - Microalbuminuria**

RESULT & DISCUSSION

Despite advances in the care of patients, diabetese remains one of the most common causes of end stage renal diseases [Jalal DI et al. 2010[17]; Baradaran A et al. 2012[18]; Rahimi Z. et al. 2012[19]; Rouhi H et al. 2013[20]; Tolouian R etal. 2013][21].

Development of DN in Type 2 Diabetic patients is associated with several factors which include age, duration of diabetes, hypertension and poor glycemic control [Hovind P etal. 2009[22]; Jalal DI et al. 2010[23]; Baradaran A et al. 2012[18]; Gheissari A et al. 2012[24]; Nasri H et al. 2012[25]; Rahimi Z. et al. 2012[19]; Rouhi H et al. 2013[20]; Tavafi M et al. 2013[26]; Unsal A et al. 2012][27].

Glycosylated Hb in Type 2 DM is said to be an indicator of poor glycemic control which in turn is a risk factor for progression of DN.

The present study was therefore planned to assess the association of HbA1c with serum uric acid levels, microalbuminuria and hence with progression of DN in Type 2 patients.

The results obtained in the two groups were expressed as mean ± SD. HbA1c levels in the two groups were 6.97 ± 0.75 in group 1 and 9.62 ± 1.50 in group 2. The results were subjected to statistical analysis to compare the levels of serum urea, microalbuminuria etc. (Table 1, figure 1)

In a recent study by Naveen et al 2012[28], urinary microalbuminuria levels were reported to be 121.0 ± 49.89 mg/24 hrs. in poor glycemic control group as compared to 47.14 ± 39.15mg/24 hrs. in the good glycemic control group. The results of the present study were quite close to the above mentioned finding. Diabetic Nephropathy is said to be a common consequences of long standing DM. Elevated glucose levels in blood lead to binding of glucose to protein resulting in excessive protein glycosylation which in turn leads to elevated glycated end products. Incresaed deposition of these glycated end products on the glomerulus resulting in renal & glomerulohypertrophy and thickening of glomerular basement membrane. This allows leakage of low molecular weight protein [Albumin] [Naveen et al.][28]. This condition is turned as incipient nephropathy [microalbuminuria].

Further, in the present study a linear correlation was observed between glycemic controls [HbA1c levels] and microalbuminuria [r = 0.381] (Table 4). This above finding was similar to the finding of Naveen et al. 2012[28] and Kundu et al. 2013[29].

Table 3, shows the level of serum urea in poor & good glycemiccontrol groups. No significant variation was observed in the urea levels in the two groups. As such no study has quoted a significant association in the serum urea levels with hyperuricemia, proteinuria or HbA1c levels (figure 13).

A positive correlation of serum uric acid and microalbuminuria in type 2 DM was shown in a study conducted by Fukui et al [Fukui M et al. 2008][30]. Similarly Fu et al reported that hyperuricemia was significantly associated with abnormal albuminuria in chinese diabetics.

CONCLUSION

The present study reported that a poor glycemic control in type 2 diabetics may lead to development of microalbuminuria and hyperuricemia which in turn may bring about changes resulting in progressive renal disease and also cardiovascular complications. The situation can be averted by maintaining a good glycemic control and adopting a healthy lifestyle. The study suggests a regular screening of HbA1c, microalbuminuria and Sr. uric acid in type 2 diabetic patients for identification and timely management of patients at risk.

The study further proposes assessment of the association of microalbuminuria and hyperuricemia with other cardiovascular risk factors such as components of lipid profile and blood pressure etc. The effect of uric acid lowering drugs and HbA1c variability on risk factors of DN and other cardiovascular complications may be interesting to explore further.

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