



## Study on Levels of Glycosylated Haemoglobin and Urinary Microalbumin in Diabetic Cases and Healthy Controls

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### Abstract

*Diabetes is one of the most common endocrine disorders characterized by hyperglycaemia. Diabetic nephropathy is a consequence of long standing diabetes. The prevalence of microalbuminuria predicts progression to diabetic nephropathy. The present study was conducted to determine the prevalence of microalbuminuria in relation to HbA1c in Diabetic cases in comparison to healthy controls.*

**Objective of the study:** *Lots of studies have been conducted on the levels of microalbumin and HbA1c in a patient with diabetes mellitus. Since the role of these parameters are increasingly reviewed and evaluated, the need to carry out further such studies become obvious. The present study is thus designed to evaluate the role of microalbumin and glycated haemoglobin in predicting incipient nephropathy in diabetics so as to prevent the onset of overt nephropathy*

**Materials and Methods:** *This case-control descriptive study was carried out in a Silchar Medical College And Hospital from July 2013 to July 2014. One hundred known diabetic patients with age 21–90 years were included in the study. Informed consent and a structured questionnaire of each patient were recorded. Fasting venous blood and morning urine sample was collected for analysis of Fasting blood glucose, HbA1c and urinary microalbumin respectively. Statistical analysis was done using graph stat statistical software.*

*All p-values <0.05 were considered as statistically significant.*

**Results:** *Fasting blood glucose levels, urinary microalbumin, and blood HbA1c levels were very high in diabetic cases as compared to healthy controls (p-value 0.0). Also the urinary microalbumin levels were very high in the diabetic cases with poor glycaemic control.*

**Conclusion:** *The present study found higher level of HbA1c and urinary microalbumin level in diabetics. Also high level of microalbuminuria in the cases which could be due to poor glycaemic control (high HbA1c >7%). Screening for microalbuminuria and HbA1c test should be done in both newly and already diagnosed diabetic patients as an early marker of renal dysfunction and glycaemic control.*

### INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors

contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals

will have diabetes by the year 2030<sup>1, 2</sup> Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries, and 6 of the top 10 countries with the highest rates are in Asia<sup>1</sup>. Current diagnostic criteria for diabetes are based on the concept that microvascular complications such as retinopathy and nephropathy occur above a threshold level of hyperglycaemia that can be used to differentiate people with and without diabetes<sup>2,3</sup>. DM is classified on the basis of the pathogenic process that leads to hyperglycemia. The two broad categories of DM are designated as type 1 and type 2. Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progress. Type 1 diabetes is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production<sup>1</sup>.

Diabetic Nephropathy is a common consequence of long standing diabetes mellitus. Its pathogenesis appears to be complex interactions between genetic and environmental factors<sup>4</sup>. The patho-physiologic basis for elevated urinary albumin excretion entails the binding of glucose to proteins resulting in excessive protein glycosylation with the build up of advanced glycated end products. This leads to deposition of advanced glycated end products on the glomerulus

resulting in renal and glomerular hypertrophy, mesangial matrix accumulation and thickening of glomerular basement membrane<sup>5</sup>. This abnormality permits the leakage of low molecular weight proteins (albumin). This is the stage of microalbuminuria (Incipient Nephropathy) which could be reversible with good glycemic control. However, with persistent microalbuminuria, further leakage of protein in urine will result in overt diabetic nephropathy<sup>6</sup>.

Microalbuminuria (MA) is considered to be a risk factor for diabetic nephropathy (DN) and progressive renal insufficiency in diabetes<sup>7,8,9,10,11</sup>. Diabetic nephropathy is one of the most serious complications of insulin-dependent diabetes mellitus (IDDM)<sup>12</sup>. Early epidemiological studies suggested that up to 40% of patients with IDDM were at risk of developing overt diabetic nephropathy and patients with IDDM and nephropathy suffered a cumulative death rate of around 50–75% within 10 years<sup>13</sup>. The frequency of microalbuminuria increased with the increase in duration of diabetes<sup>14</sup>. Microalbuminuria is a risk factor for the development of overt nephropathy in type 1 and type 2 diabetic patients<sup>15,16</sup>. Importantly, improvement of glycemic control and early intervention with antihypertensive drugs can retard the development of microalbuminuria and possibly its progression towards overt nephropathy<sup>17,18</sup>. Microalbuminuria is defined as an increased excretion of albumin above the reference range for healthy nondiabetic subjects, but which is undetectable by the Albustix dipstick test<sup>19</sup>.

**Table1:** Showing microalbuminuria in various urine samples

Definitions of microalbuminuria<sup>20</sup>

	Individual	Lower limit	Upper limit	Unit
24h urine collection		30	300	mg/24h (milligram albumin per 24 hours)
Short-time urine collection		20	200	µg/min (microgram albumin per minute)
Spot urine albumin sample		30	300	mg/L (milligram albumin per liter of urine)

Measurement of glycated hemoglobin is the standard method for assessing long-term glycemic

control. When plasma glucose is consistently elevated, there is an increase in nonenzymatic

glycation of hemoglobin; this alteration reflects the glycemic history over the previous 2–3 months, since erythrocytes have an average life span of 120 days (glycemic level in the preceding month contributes about 50% to the HbA1c value)<sup>1</sup>. Randomised controlled trials and observational studies have shown that glycated haemoglobin or haemoglobin A1c is also a good predictor of microvascular complications<sup>21,22,23,24,25</sup>, is highly correlated with FPG (Fasting Plasma Glucose)<sup>26</sup> and does not require measurement in the fasting state. Thus, the use of HbA1c as a diagnostic test for diabetes has been proposed<sup>27,28</sup>.

The present study was carried out to assess the level of urinary microalbumin level with HbA1c in known diabetic cases and normal healthy controls. Microalbuminuria and HbA1c were measured as a marker of renal damage and glycaemic control respectively.

## REVIEW OF LITERATURE

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals will have diabetes by the year 2030<sup>1,2</sup>. Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries, and 6 of the top 10 countries with the highest rates are in Asia<sup>1</sup>. Current diagnostic criteria for diabetes are based on the concept that microvascular complications such as retinopathy and nephropathy occur above a threshold level of hyperglycaemia that can be used to differentiate people with and without diabetes<sup>2,3</sup>. Microalbuminuria (MA) is considered to be a risk factor for diabetic nephropathy (DN) and progressive renal insufficiency in diabetes<sup>7,8,9</sup>. Diabetic nephropathy is one of the most serious complications of insulin-dependent diabetes mellitus (IDDM)<sup>7</sup>. Microalbuminuria is defined as persistent

proteinuria that is below detection by routine reagent strips but greater than normal. It can be diagnosed from a 24-hour urine collection (between 30–300 mg/24 hours) or, more commonly, from elevated concentrations in a spot sample (30 to 300 mg/L)<sup>1</sup>. Urine albumin-creatinine ratio (UACR) is a ratio between two measured substances. Both the urine albumin (mg/dl) and the urine creatinine (g/dl) are measured values with both substances measured by mass. Microalbuminuria is defined as UACR  $\geq$  3.5 mg/mmol (female) or  $\geq$  2.5 mg/mmol (male), as a UACR between 30 and 300  $\mu$ gm albumin /mg creatinine. An alternative definition of microalbuminuria is a UACR on a random urine sample of more than 30 mg (but less than 300 mg) of albumin per gram of creatinine<sup>1</sup>.

Measurement of glycated hemoglobin is the standard method for assessing long-term glycemic control. When plasma glucose is consistently elevated, there is an increase in nonenzymatic glycation of hemoglobin; this alteration reflects the glycemic history over the previous 2–3 months, since erythrocytes have an average life span of 120 days (glycemic level in the preceding month contributes about 50% to the HbA1c value)<sup>1</sup>. Randomised controlled trials and observational studies have shown that glycated haemoglobin or haemoglobin A1c is also a good predictor of microvascular complications<sup>22,23,24,25</sup>, is highly correlated with FPG (Fasting Plasma Glucose)<sup>26</sup> and does not require measurement in the fasting state. Thus, the use of HbA1c as a diagnostic test for diabetes has been proposed<sup>27,28</sup>.

## MATERIALS AND METHODS

A quantitative estimation of urinary microalbumin levels in diabetes mellitus with assessment of glycaemic control (HbA1C), was carried out in the laboratory of the Department of Biochemistry, Silchar Medical College & Hospital, Silchar, Assam, during the period of study. For the purpose Systemic Random Sampling technique will be used. As it is a hospital based study and the number of cases may be limited, every third

case will be taken as sample, keeping exclusion and inclusion criteria in view.

**Duration of study:** one(1) year

**Type of study :** case-control study

**No. of cases:** One hundred (100) Group 1

**No. of controls:** One hundred (100) Group 2

**Selection of cases:** This study will include persons who have diabetes mellitus and have no other concomitant disease including those condition that results in microalbuminuria and HbA1c levels.

**Selection of controls:** The control group will include normal healthy persons who have no diagnosed diseases and who do not have diabetes mellitus or take any drug or addictive substances that may cause transient or sustained elevation of blood glucose level.

**Exclusion criteria:** Patients who are suffering from some pre-existing diseases that causes microalbuminuria and alter urine albumin-creatinine ratio and levels of glycated haemoglobin.

The patients will be selected from the outdoor and indoor wards, Silchar Medical College. These patients will be compared to non diabetic volunteers of either sex from almost same age group. Strict adherence to exclusion criteria will be followed.

The detailed clinical history as well as dietary history, drug history, history of consumption of alcohol, smoking as well as any other symptoms related with diabetes mellitus will be noted. Blood glucose level (fasting) will also be noted.

**Collection of blood sample:** 5ml blood sample was collected under total aseptic precautions from the subjects. The blood was collected from a suitable vein (preferably fr antecubital vein) with the help of vacutainer method. EDTA vials were used for HbA1C estimation and NaF vials were used for fasting glucose estimation.

**Collection of urine sample:** Urine sample will be collected in a sterilized urine pot taking into consideration all the aseptic measures. Morning sample will be preferred. An amount of 5ml was sufficient.

The following laboratory investigations will be carried out:

a) Blood glucose level(Fasting)

b) Microalbumin in urine

c) Glycated haemoglobin (HbA1c) level in blood

Fasting blood glucose estimation was done using Hexokinase method (Beckman-Coulter AU systems). HbA1c was estimated by Boronate affinity chromatography which separates total glycated haemoglobin by binding to solid-phase dihydroxyborate<sup>13</sup> using QdxA1C kit (Piramal Health Care). Microalbumin was estimated by Immunoturbidimetric Assay method (Beckman-Coulter AU Systems). All statistical analysis was done by using graph pad stat statistical software . Pearson correlation was applied to observe association of microalbuminuria with different parameters. All *p* values <0.05 considered as statistically significant.

## RESULTS

### Analysis of Biochemical Parameters among Cases and Controls Urinary Microalbumin:

Table 2: Showing the Distribution of Urinary Microalbumin Levels in the Studied Groups

	MEAN	SD	SEM	95% C I	p VALUE
CASES	118.35	105.5 5	10.5 5	97.40 - 139.29	0.0
CONTROLS	15.08	4.33	0.43	14.23 - 15.95	

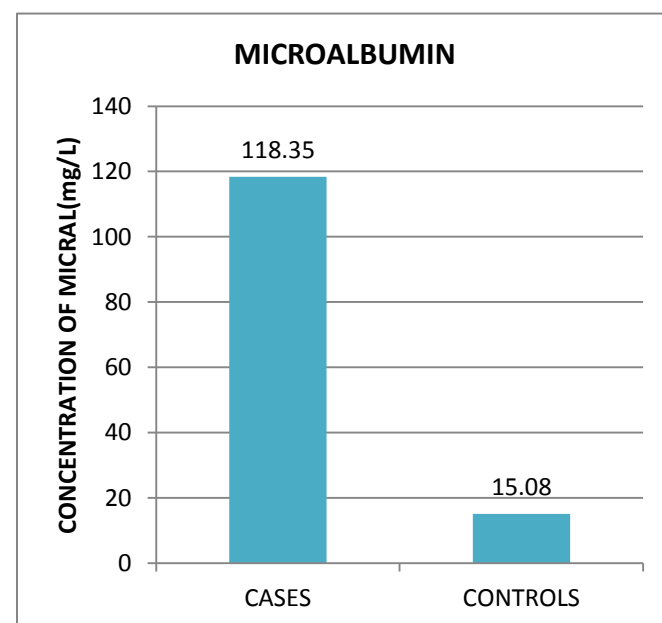


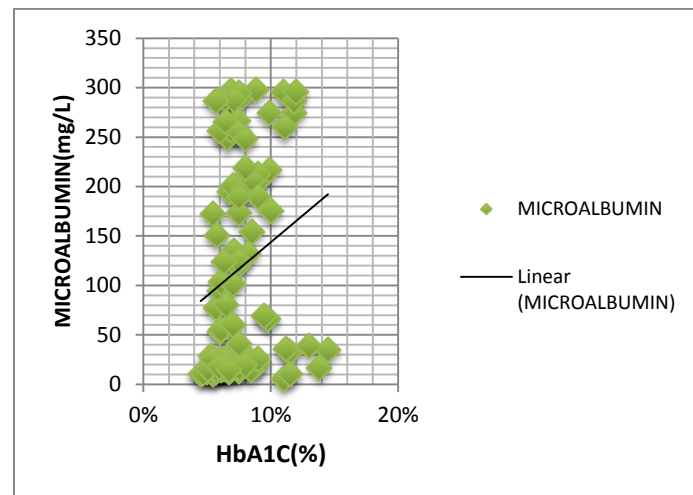
Figure1: Showing the Distribution of Urinary Microalbumin Levels in the Studied Groups

The mean urinary levels of MICROALBUMIN in diabetic cases is found to be  $118.35 \pm 105.55$  and in controls  $15.08 \pm 4.33$ . In the Unpaired t test between case and control groups, the two-tailed P value is 0.0 (extremely significant).

**Glycated Haemoglobin (HbA1c):**

**Table 3 :** Showing the Distribution of Blood Glycosylated Haemoglobin(HbA1c) Levels in the Studied Groups

	MEAN	SD	SEM	95% C I	P VALUE
CASES	7.67	2.05	0.205	7.26-8.07	0.0
CONTROLS	4.75	0.446	0.044	4.66-4.83	



Pearson’s Correlation coefficient  
 (r) = 0.2101  
 Coefficient of determination  
 (r squared) = 0.0441  
 The two-tailed P value is 0.03, considered significant.

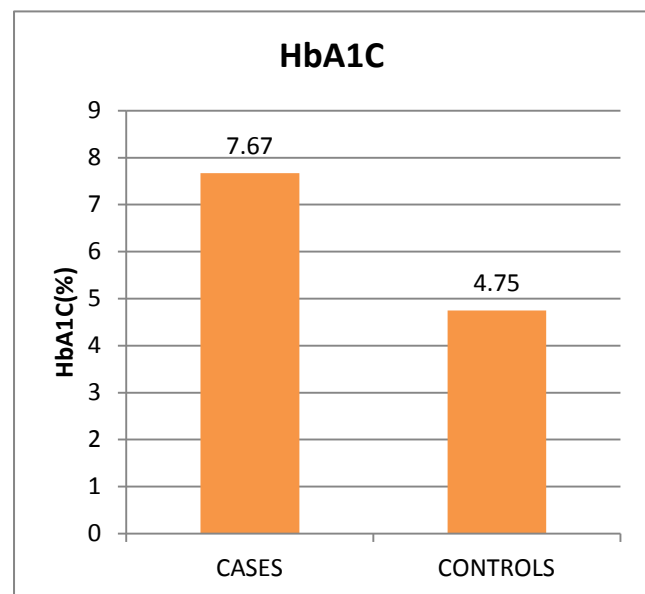
**Figure 3:** Showing Correlation Of HbA1c And Microalbumin In Case Group

As can be seen from the above, there is significant correlation between blood HbA1c and urinary microalbumin in the case group .

**DISCUSSIONS**

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, >360 million individuals will have diabetes by the year 2030<sup>1</sup>. Microalbuminuria (MA) is considered to be a risk factor for diabetic nephropathy (DN) and progressive renal insufficiency in diabetes<sup>7,8,9,10,11</sup>.

To analyse the role of MICROALBUMIN and HbA1c as a predictive marker in diabetic nephropathy, this study was undertaken among the patients presenting with diabetes mellitus in Silchar Medical College & Hospital, Silchar. It was conducted on 100 Diabetic patients came to Silchar Medical College and Hospital, who constituted the Case group. Another 100 apparently healthy, age- and sex-matched, random individuals constituted the normal control group. Strict inclusion and exclusion criteria were



**Figure 2 :** Showing the Distribution of Blood Glycosylated Haemoglobin(HbA1c) Levels in the Studied Groups

The mean blood levels of HbA1c in diabetic cases is found to be  $7.67 \pm 2.05$  and in controls  $4.75 \pm 0.446$ . In the Unpaired t test between case and control groups, the two-tailed P value is 0.0 (extremely significant).

followed in selecting study groups to avoid confounding factors.

In the present study, the majority of the patients were males, who constituted 64% of the total patients. The Male: Female ratio was 64: 36. Male preponderance was found more in the studied population. In the present study among the cases the literate groups constituted 67% of cases whereas illiterates constituted 33% of total cases. This may be due to reason that illiterates does not have that health knowledge to come for check up. It was found that among the cases 57% cases were from non- BPL category and 43% cases were from BPL category , since there is no study confirms the association of diabetes mellitus with the economic status.

In the present study, the average Urinary Microalbumin level in patients was  $118.35 \pm 105.55$  mg/L, which was extremely significantly high as compared to that in normal age and sex-matched controls ( $p < 0.0001$ ). This suggests that urinary microalbumin level is increased in diabetic cases which is helpful in predicting onset of diabetic nephropathy.

Various epidemiological and cross sectional studies have reported marked variation in the prevalence of microalbuminuria<sup>29</sup>. *Gupta et al*<sup>29</sup> reported a prevalence of 26.6% in 65 north Indian non-proteinuric patients, while *John et al*<sup>30</sup> reported a prevalence of 19.7% from a tertiary hospital in vellore, south India. Studies in the white UK population revealed a prevalence of microalbuminuria of 7% - 9%. *Newman et al*<sup>31</sup> reported that urine albumin testing for early detection of diabetic complications.

. In the present study, the average Blood HbA1C level in patients was  $7.67\% \pm 2.05$  , which was extremely significantly high as compared to that in normal age and sex-matched controls ( $p < 0.0001$ ). This suggests that HbA1C level is increased in uncontrolled diabetes mellitus, which tells about the glycaemic control of the patients. *Kumar PR, Bhansali A, Ravikiran M et al*<sup>32</sup> and *Carson AP, Reynolds K, Fonseca VA et al*<sup>33</sup>

reported that HbA1C is used as a diagnostic marker for diabetes mellitus.

In the present study urinary microalbumin level was compared between the Literates and Illiterates and p value was found equal to 0.05 which is a significant finding and no any other study has been done previously suggesting any significant association. It signifies the importance of education in development of microalbuminuria, illiterates have a high urinary microalbumin level. In the present study urinary microalbumin level of cases was compared between the BPL and the NON-BPL and p value was found  $< 0.05$  which is a significant finding and no any other study has been done previously suggesting any significant association. It signifies that diabetics from low socioeconomic group are prone to have higher urinary microalbumin level.

As a positive PEARSONS correlation between Blood HbA1C level and Urinary microalbumin level, is seen in this study, so the diabetic cases should be screened for blood HbA1C , Urinary microalbumin, to detect the risk of development of incipient nephropathy and also to prevent overt nephropathy in diabetic patients and also to assess their glycaemic control.

## CONCLUSION

The screening for microalbuminuria is not yet consistently done in India. Being a developing country; there is a dire need that microalbuminuria and HbA1c testing should be done in both, newly diagnosed as well as already diagnosed diabetic patients as an early marker of renal risk factor. Strict glycaemic control, having a healthy lifestyle, literacy state and economic condition is especially important for diabetic patients.

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