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Evaluation of Method for Glycated Hemoglobin and Its Correlation with Microalbuminuria as Early Markers of Nephropathy in Type II Diabetes Mellitus

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ABSTRACT
Diabetic nephropathy is the leading cause of end stage renal disease and mortality. The aim of the study was (i) to correlate between two methods of HbA1c estimation; the column chromatography and the immunoturbidimetric method and (ii) to evaluate microalbuminuria and glycated hemoglobin (HbA1c) as early risk markers of nephropathy in type II diabetes mellitus. The present study includes two parts; the first part consists of forty known diabetic patients in which the method comparison of HbA1c was studied. The second part of the study comprises of eighty-four known diabetic patients and thirty healthy control subjects in which the presence of HbA1c and microalbumin was estimated and correlated. Venous blood was obtained for HbA1c & plasma glucose for the first part of the study. For the second part, venous blood was obtained for HbA1c, and blood glucose, while their morning urine sample was obtained for detection of microalbuminuria. Statistical analysis was done using SPSS version 24. All p-values <0.05 were considered statistically significant. The two HbA1c methods were highly correlated (r=0.905). However, the mean of immunoturbidimetric method is slightly lower (5.23%) when compared with column chromatographic method (5.69%).Both the methods had good correlation with random blood sugar value. Random Blood Sugar (RBS), HbA1c and microalbumin were the highest in diabetic patients (240.4±43.7 mg%), (9.96±1.21%), and(108.5±36.2mg%) when compared with non-diabetic healthy control subjects (93.4±16.1mg%), (4.80±0.50%)and (8.1±2.4 mg%) respectively. Microalbuminuria and HbA1c had a significant correlation (r=0.626, p<0.01). Microalbuminuria also had a good correlation with duration of diabetes (0.764, p<0.01).
Keywords: *Random Blood Sugar--RBS, Glycated Hemoglobin---HbA1c, Microalbumin, Nephropathy, Diabetes Mellitus(DM).*

Introduction

Diabetes is a common endocrine disorder, characterized by persistent hyperglycemia as a result of inadequate insulin and/or insulin resistance [(1)]. Untreated hyperglycemia can cause a lot of complications like neuropathy, nephropathy and retinopathy. Insulin resistance is characterized by a subnormal response to a given concentration of insulin and can be measured indirectly by a fasting insulin level: the increased levels of insulin correspond to higher degrees of insulin resistance [(2)].

Diabetic nephropathy is a long term complication of (diabetes mellitus) DM and is the chief cause of morbidity and premature mortality. The pathophysiologic basis for elevated urinary albumin excretion entails the binding of glucose to proteins resulting in excessive protein glycosylation with the buildup of advanced glycated end products. This leads to the deposition of advanced glycated end product on the glomerulus resulting in renal and glomerular hypertrophy, mesangial matrix accumulation and thickening of glomerular basement membrane. This abnormality permits the leakage of low molecular weight proteins like albumin [(3)]

Glycated hemoglobin (HbA1c) is the presence of carbohydrate-protein linkage on the N-terminus of the β-chains of hemoglobin, predominantly HbA in adults[(4)]. The percentage of glycated hemoglobin in the plasma of a patient provides an estimate of blood glucose levels over a period of 120 days [(5)].

Microalbuminuria is defined as Urinary albumin excretion between 30-300mg/day. In the early stages of the disease, there is an increase in urinary albumin excretion, which progresses to overt albuminuria and then to renal failure [(6)].

In type II diabetic patients, the duration of diabetes and elevated glycated hemoglobin (HbA1c) value, predicted increased microalbumin excretion rate. As determination of microalbumin level in urine is an easy method of screening the diabetic patients, it may be useful to prevent the onset of future renal disease. A good glycemic control in the early stages reduces chances of microalbuminuria [(7)].

Material and Methods

Forty patients who had been diagnosed with type II diabetes mellitus with duration of more than one year were included in the study. The patients were receiving treatment and counselling on monthly basis at the diabetic clinic of Hospital. Their fasting blood glucose was being measured once in a month while HbA1c was checked once every three months. Informed consent was taken from the patients before including them into the study. Another group consisting of eighty-four known diabetic patients (56 females and 28 males), admitted as inpatients and attending outpatient clinics of the hospital, were also included in the present study. Thirty healthy subjects were included in the control group.

Inclusion criteria

Known diabetic patients, aged between 35 to 90 years, of either sex, were chosen for the study.

Exclusion criteria

Diabetic patients who had macroalbuminuria were excluded from the study. Patients with mental illness or suffering from serious medical condit-ions like jaundice, anemia etc, were excluded.

A structured questionaire regarding the demographic data such as name, age, sex, duration of diabetes, presence or absence of hypertension, smoking habit was recorded from each patient.

The blood samples (1ml) were collected by the technicians at the Central Blood Collection Centre from patients, in EDTA containers for HbA1c and in oxalate and fluoride vials for glucose. The samples were sent to laboratory to measure HbA1c with column chromatography and immunoturbidimetric methods. The blood samples for HbA1c were kept in refrigerator (4^{o}C) until analyzed. Blood sample was analyzed for blood glucose using GOD-POD method. For microalbumin estimation, morning urine sample was taken. All urine samples were tested for the presence of albumin by pyrogallol red method.

Statistical analysis

All statistics in the study was performed using SPSS statistical software version 24

Results

A total of 40 samples were analyzed for HbA1c estimation. The mean age of the patients included in the study was 41.48±11.4years (range 25-48years). There were 32 (80%) females and 8 (20%) males. A comparison was made between two methods available in the laboratory for the estimation of HbA1c.. The results for both the techniques are shown in table1. The mean HbA1c

was slightly lower for immunoturbidimetric (5.23%) method than column chromatography (5.69%). The correlation analysis was also done between the results obtained by column chromatography and immunoturbidimetric method (Fig1) The correlation coefficient was found to be 0.905. The result showed a good correlation between both methods. A good correlation was observed between RBS & HbA1c by both the methods used in study. HbA1c value by Column chromatographic method and RBS had correlation coefficient of 0.70 and HbA1c value by immunoturbidimetric method and RBS had correlation coefficient of 0.78 (Table 11)

In the second part of the study on type II diabetic patients, microalbumin was estimated in all the diabetic and control cases in the spot urine sample. HbA1c and random blood sugar was also estimated for these subjects.

Eighty-four type II diabetes mellitus patients aged between 35-90 years were selected for this cross sectional study. The mean age of the patients were 58.64 years, out of which 56 (66.6%) were females and 28 (33.3%) males.

The value of Random blood sugar,HbA1c and Microalbumin was compared between diabetic cases and non diabetic healthy controls (Table 111).The RBS for Diabetic group was 240.40+/-43.70mg% compared to 93.4+/-16.1mg% for controls.HbA1c was 9.96+/- 1.21 % in diabetics compared to 4.8+/-0.5% for controls and Microalbumin was 108.5+/-36.22mg% in diabetic group compared to 8.1+/-2.4mg% in controls. All the results were statistically significant.

Microalbumin level in relation to duration of Type 11 diabetes was also compared. Its level was observed to be higher in diabetic subjects with duration of diabetes more than 10 years.(Table 1V).There was also good correlation between microalbumin and duration of diabetes (r=0.764, p<0.01)(Graph 2)

A correlation was also observed between microalbumin and HbA1c level in Type 11 diabetic patients(r= 0.626,p<.001)(Fig.3)

The result of the study shows that poor glycemic control was associated with higher proportion of microalbuminuria. Sixty of the cases i.e (71.4%) with poor glycemic control had microalbuminuria. The difference was statistically significant (P<0.001).

There was significant sex predominance, as 66.6% of females were found to have microalbuminuria compared to 33.3% of males (p=0.701).

Table 1: Comparison between column chromate-graphy and immunoturbidimetric method.

HbA1c (%)	Mean ± SD
Column chromatography	5.69 ± 1.99
Immunoturbidimetry	5.23± 1.95

Table 11 : Correlation of HbA1c with RBS

Method	RBS (mg%)	HbA1c (%)
Columnchromatography	119.7 ±30.2	5.69 ± 1.99
Immunoturbidimetry	119.7±30.2	5.23± 1.95

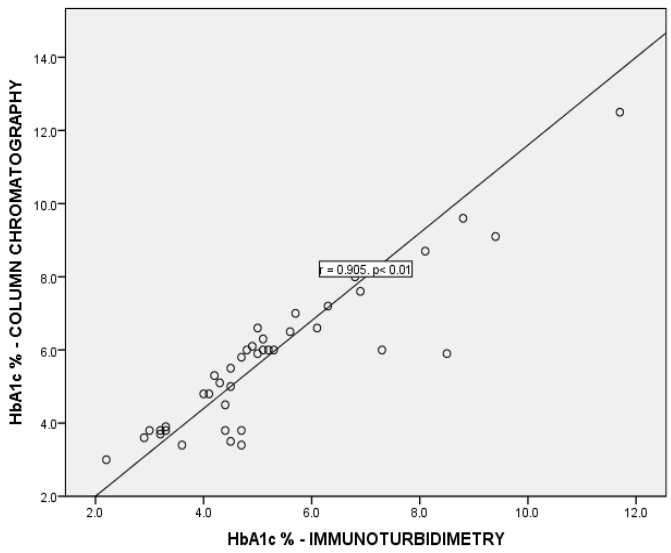
Table 111--Mean level of biochemical parameters in controls and Type 11 Diabetic patients

Parameter	Controls	Diabetic cases	P value
RBS(mg%)	93.1 +/- 16.1	240.4+/- 43.7	<0.001
HbA1c(%)	4.30 +/- 1.21	9.96+/- 1.21	<0.001
Microalbumin(mg%)	8.10 +/- 2,4	108.5 +/- 36.22	<0.001

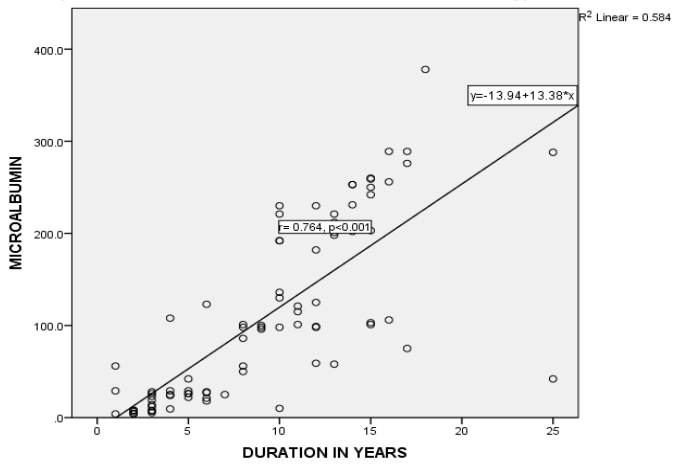
Table 1V----Microalbumin in relation to duration of diabetes (in years)

Duration of Diabetic in years	Microalbumin(mg/dl)Mean+/- S.D	P value
1-------4	25.5 +/- 10.9	<0.001
5------10	82.7 +/- 26.9	<0.001
>10 years	187.5 +/- 36.7	<0.001

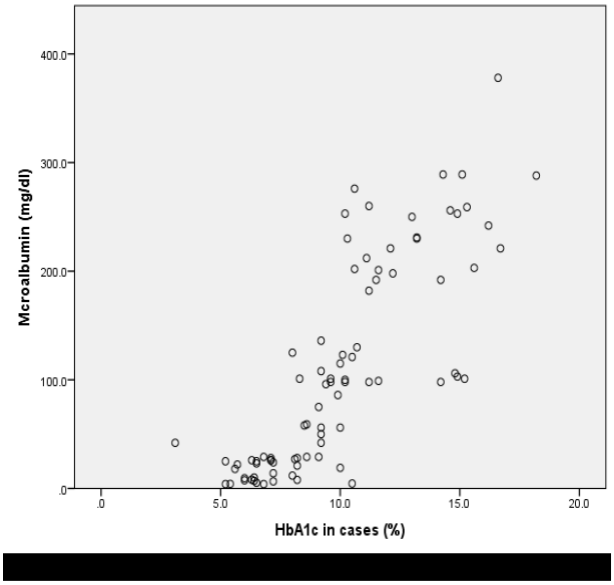
Graph 1. Correlation between column chromatography and immunoturbidimetric method of HbA1c estimation.



Graph 2. Correlation of microalbumin levels to duration of type 2 diabetes



Graph 3: Correlation of microalbumin levels to HbA1c in diabetic cases



Discussion

The prevalence of diabetes is increasing worldwide at an alarming rate. The patient's long-term glycemia is associated with various complications [8] and can be measured using glycated hemoglobin[9]. Therefore, glycated hemoglobin measurement by laboratories should be precise and accurate. When glycated hemoglobin was measured by different methods, important differences were observed [10]

In the present study, it was found that the two methods are strongly correlated. The correlation coefficient r; was 0.905(Fig1). However, immonuturibidimetric method has lower mean of HbA1c (5.23± 1.95%) compared to column chromatography (5.65±1.99%)(Table1). Since immunoturbidimetric method was faster and easier to handle, it was chosen for further study.

Harris *et. al* (2003) compared four points -of -care methods with the Roche tinaqant and obtained the Pearson correlation of over 0.9 for all the four methods: DCA 2000, Nycocard, Diastat and D55. Diastat and DCA 2000 showed the best function among all the four methods. It was concluded that these two methods can be an appropriate replacement for each other, and also for the Roche method [11].

The first noticeable sign of diabetic renal disease is the presence of microalbuminuria [12], the early detection and intervention of which can delay the onset of overt nephropathy in diabetic patients [13]. In order to prevent the development of microalbuminuria, blood pressure should be maintained at less than 130/80mmHg and glycemic control (HbA1c) should be maintained below 7%. HbA1c level is an important factor in the transition from normoalbuminuria to microalbuminuria and then to overt diabetic nephropathy [14]. Diabetic nephropathy has been linked to elevated levels of advanced glycation end products [15].

The present study was conducted on 84 diabetic patients. Age of patients in this study ranged between 35-90 years with the mean of 58.64 years. A similar mean age was observed in several

studies by Chowta et.al (2009) & Mohammad et.al (2012) [14, 15].

In the present study values of Random blood sugar,HbA1c and microalbumin were higher in diabetic patients compared to controls(Table 111)There was also observed correlation between microalbuminuria and duration of diabetes (Table 1V). A correlation was also observed between HbA1c and microalbuminuria in diabetic patients. (Fig 111).Similar results have been reported byVarghese et al(2001).A good glycemic control in early stages can reduce chances of microalbuminuria [7]. A good glycemic control in early stages reduces chances of microalbuminuria.

It was found that sixty out of 84 diabetic patients (71.4%) had early nephropathy. The result is found to be low compared to other studies. A study by Omar *et. al* (2015) showed that 31 out of 40 (77.5%) diabetic patients had early nephropathy [16]. Other results were found to be higher than those reported by American Diabetes Association (ADA) (20-40%) [17].

In another study, it was shown that 82.7% had poor glycemic control; however, there was no correlation between HbA1c and microalbuminuria) [18]. A study by Vanelli *et. al* (2005), it was reported that higher HbA1c was associated with increasing age[19]. Difference in ethnic susceptibility to nephropathy might be the likely reason for this variation.

Being a developing country, there is a great need that microalbumin and HbA1c testing should be done in both, newly diagnosed as well as already diagnosed type II diabetic patients as early markers of nephropathy. This is because the prevalence of microalbuminuria in diabetic patients is found to be high. Therefore, patients and physicians should give very high priority to improving glycemic control sufficiently to maintain glycated hemoglobin values below 7%. If this can be achieved, the number of patients in whom microalbuminuria develops would decline substantially, and in turn, lower the number of patients who develop end-stage renal disease.

Conclusion

In the present study, two methods for estimating glycated hemoglobin were compared and relationship between microalbumin and glycated hemoglobin with duration of diabetes was evaluated.

- The present study was divided into two parts.
- In the first part of the study, a comparison was made between two methods available in the laboratory (immunoturbidimetric and column chromatography) for the estimation of HbA1c.The mean HbA1c was slightly lower for immunoturbi-dimetric (5.23%) method than column chromatography (5.60%) (Table 1).Correlation analysis between two methods was also made. The correlation coefficient was found to be 0.905 suggesting a good correlation between two methods (Graph 1).
- Correlation analysis between column chromatography and RBS and between immunoturbidimetric method and RBS was also done. The correlation coefficient was found to be 0.70 and 0.78 for the two methods suggesting a good correlation between HbA1c and RBS value for both the methods. In the second part of the study, 84 patients aged between 35 to 90 suffering from type II diabetes were selected along with 30 controls.
- HbA1c, random blood sugar and urinary microalbumin was estimated in these Diabetic patients and in controls.
- Levels of RBS, microalbumin, HbA1c were found to be higher in diabetic cases compared with controls ($p<0.001$) (Table 111).
- Microalbumin level in relation to duration of type II diabetes was also compared. Its level was observed to be higher in diabetic subjects with duration of diabetes more than 10 years (Table 1V) and there was good correlation between microalbumin

and duration of diabetes (r= 0.764) (Graph 2).

- A correlation was also observed between microalbumin and HbA1c in type II diabetic patients (r=0.626, Graph 3).
- Microalbumin was found to be higher in Diabetic cases (71.4%) compared with those having poor glycemic control (28.5%).

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