CORRELATION OF HbA1c WITH URINARY ACR, eGFR AND SERUM CREATININE IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

BACKGROUND

Diabetes Mellitus (DM) is a metabolic syndrome characterised by hyperglycaemia due to absolute or relative deficiency of insulin. Type 2 DM comprises about 90% of diabetic population of any country. Diabetes has many complications. One such major chronic complication of poorly controlled diabetes is diabetic nephropathy which may lead to end stage renal disease (ESRD). Longterm control of diabetes is monitored by estimation of glycated haemoglobin (HbA1c). There are two important markers to assess renal impairment – glomerular filtration rate (GFR) & microalbuminuria. Microalbuminuria is better reflected by spot urine albumin-creatinine ratio (Urinary ACR). There are some formulae based calculation of GFR, called estimated GFR or eGFR. One such formula is MDRD (Modification of Diet in Renal Disease) study equation.

MATERIALS AND METHODS

In this hospital based cross sectional study, HbA1c, S. Creatinine, Urinary ACR and eGFR (by MDRD formula) were measured in selected 105 type 2 diabetic patients of 40-70 years of age (mean years of duration of diabetes is 10.01 ± 3.46), at a private medical college hospital in Kolkata, West Bengal, India. Data were analysed by appropriate statistical software and correlation ('r' value) of HbA1c with Urinary ACR, eGFR and S. Creatinine were calculated by Pearson's correlation test and respective 'p' value were calculated as level of significance.

RESULTS

This study shows that in total study subjects (n=105); HbA1c has significant positive correlation with Urinary ACR & with S. Creatinine ('r' value = 0.576 and 0.734 respectively; and 'p' value is <0.001 in both cases) and there is significant negative correlation of HbA1c with eGFR ('r' value is - 0.672 & 'p' value is < 0.05).

CONCLUSION

For early detection and treatment of diabetic nephropathy, both eGFR and urinary ACR should be measured along with tight glycaemic control so that progression to ESRD can be prevented.

KEYWORDS

HbA1c, Urinary ACR, eGFR, Type 2 Diabetes Mellitus, Diabetic Nephropathy, Microalbuminuria, MDRD Formula.

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BACKGROUND

Diabetes Mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in blood over a prolonged period. In other words, diabetes is a metabolic syndrome characterised by hyperglycaemia due to absolute or relative deficiency of insulin.^[1] There are many types of diabetes, of them, two clinically important types of diabetes are: Type 1 DM or Insulin dependent diabetes mellitus or IDDM and Type 2 DM

or Non-insulin dependent diabetes mellitus or NIDDM. Type 2 diabetes comprises about 90% of diabetic patients of any country.

According to International Diabetes Federation (IDF), in 2013, 382 million people had diabetes worldwide,^[2] of which Type 2 makes up about 90% of the cases.^[3] This is equal to 8.3% of the adult population^[3] with equal rates in both women and men.^[4] More than 80% of diabetic deaths occur in low and middle-income countries like Asia and Africa.^[5] The number of people with diabetes is expected to rise to 592 million by 2035.^[6]

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.^[7,8] According to Wild et al,^[9] the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with maximum increase in India. Type 2 diabetes mellitus (T2 DM) represents a significant global health problem. It is estimated that six (6) people die every minute from the disease worldwide, a figure that will soon make T2 DM one of the world's most prevalent cause of preventable mortality.^[9] This gave India the dubious distinction of the "diabetes capital of the world".^[10]

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Diabetes has many complications. One such major chronic complication of poorly controlled diabetes is diabetic nephropathy which may lead to end stage renal disease (ESRD).

Currently, diabetic nephropathy is the leading cause of chronic kidney disease (CKD) in United States and other Western Societies. Diabetes is responsible for 30-40% of all ESRD cases in United States. The estimated overall incidence rate of CKD and end-stage renal disease (ESRD) in India is currently 800 per million population (pmp) and 150-200 pmp, respectively.^[11,12] It has made observation that DM as the cause of CKD found in 31.2% of patients.

Long-term control of diabetes is monitored by estimation of glycated haemoglobin (HbA1c).

There are two important markers to assess renal impairment – glomerular filtration rate (GFR) & microalbuminuria (MA). Microalbuminuria is better reflected by spot urine albumin-creatinine ratio (Urinary ACR). There are some formula based calculation of GFR, called estimated GFR or eGFR. One such formula is MDRD (Modification of Diet in Renal Disease) study equation.

Monitoring for blood sugar control and screening for microalbuminuria and timely treatment has become the standard care of diabetic patients all over the world. We have done this study to see the association of HbA1c (which is a marker of long-term glycaemic control) with the two important markers of renal impairments: urinary ACR (which reflects microalbuminuria) and eGFR (marker of creatinine clearance rate or CCR) in Type 2 diabetic patients.

In this hospital based cross sectional study, HbA1c, S. Creatinine, Urinary ACR and eGFR (by MDRD formula) were measured in selected 105 type 2 diabetic patients of 40-70 years of age (mean years of duration of diabetes is 10.01 ± 3.46), at a private medical college hospital in Kolkata, West Bengal, India. Data were analysed by appropriate statistical software and correlation ('r' value) of HbA1c with Urinary ACR, eGFR and S. Creatinine were calculated by Pearson's correlation test and respective 'p' value were calculated as level of significance.

MATERIALS AND METHODS

Study Area

Type- 2 diabetic patients in out-patient clinic of Department of Medicine and in Diabetic clinic in KPC Medical College & Hospital, Jadavpur, Kolkata.

Study Population

Type-2 diabetic individuals with/without family history of diabetes and with or without co-existence of hypertension (HTN).

Study Period

A period of one year from inception (July, 2015-July, 2016).

Study Design

Cross-sectional, descriptive and observational study in diabetic patients in Indian reference population.

Sample Size

Initially 126 patients with type 2 diabetes were selected, but due to incomplete data and unclear history 21 patients were excluded from the study. So, 105 patients with type 2 diabetes who met all the inclusion criteria in this study are

taken irrespective of sex, nutritional status, socioeconomic status.

Inclusion Criteria

Type 2 diabetic patients with - 1) Family history of diabetes (with or without), 2) With or without co-existing HTN, 3) Both old cases and newly diagnosed cases of type 2 DM, 4) Type 2 diabetic patients with or without treatment, 5) Age ≥ 40 years and ≤ 70 years.

Exclusion Criteria

1) Acute myocardial infarction (AMI), 2) Acute Renal Failure, 3) Severe trauma, 4) Hyperglycaemic crisis (DKA, HONK), 5) Type 1 DM, Gestational Diabetes and other rare type of DM, 6) Hypertensive crisis, hypertensive emergencies, 7) CKD patients of Stage 5 (eGFR <15 mL/min/1.73m² or on Dialysis), 8) Patients with Acute infection or Sepsis, 9) Age < 40 years and Age > 70 years.

Data Collection

The Study protocol, Informed Consent and Case Record Form were submitted to the Ethical Committee of K.P.C. Medical College & Hospital for approval.

Informed consents were taken from all participants before inclusion in the study in a language of their own understanding. Illiterate individuals had given their finger-print (left-thumb impression) instead of signature.

Necessary clearances were obtained from Institutional Ethics Committee.

After obtaining permission of Head of the Departments of Medicine and Biochemistry and appropriate authority, data collection is started by using pre-designed and pre-tested schedule, interviewing the participants, performing clinical examinations, laboratory investigations and record analysis. In this way all eligible subjects are included in the study for a period of one year.

Study Technique

- A. Detailed assessment of history of the patients under study.
- B. Thorough general physical examination.
- C. Biochemical Estimations-
 - a. Sample Collection and Storage: - 10 mL of fasting venous blood was collected from the antecubital vein of each patient/study subject 5 mL dispensed in clotted vial for estimation of serum creatinine; 2 mL in ethylenediaminetetraacetic acid (EDTA) vial for estimation of HbA1c by High Performance Liquid Chromatography (HPLC); 3 mL in fluoride vial for estimation of fasting plasma glucose (FPG). Written consent was taken from each patient before the procedure, and each patient was counselled separately about the procedure and purpose of the study. Twenty (20) mL morning urine sample was collected from each study subject for the estimation of spot urine ACR.
 - b. Fasting plasma glucose (FPG) was estimated by Glucose oxidase-peroxidase method.^[13]

- Glycated haemoglobin or HbA1c was estimated by using HPLC technique^[14] and the obtained value is expressed as – percentage (%).
- Urinary microalbumin (Immunoturbidimetric method) & urinary creatinine (Modified Jaffe's method) were estimated by Mindray Autoanalyser using supplied respective reagent kit and their ratio i.e., Urinary ACR was calculated and expressed in mg/g (µg/mg) unit.^[15,16]
- And serum creatinine (mg/dL) is measured by Modified Jaffe's Method.^[17,18]
- eGFR is calculated by using MDRD formula. This equation does not require patient's weight.^[19]

MDRD formula = $eGFR \text{ (mL/min/1.73 m}^2\text{)} = 186 \times (S_{cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$

[Here, serum creatinine is not IDMS traceable. So, we have used the value, 186 instead of 175.]

Statistical Methods

The data were arranged in a master chart and analysed by using R -Studio Software. 'Shapiro-Wilk' normality test was done for the normal distribution of data. All data were normally distributed here. Then, Statistical Mean and Standard Deviation of the parameters under study were calculated and their respective 'p' values were obtained from Unpaired Student's t-test by running a logistic regression in R-Studio software to know the level of significance. Pearson's Correlation tests were done to know the association of HbA1c with serum creatinine, urinary ACR & eGFR and the respective 'r' values were calculated and the respective 'p' values were again obtained from Unpaired Student's t-test. A 'p' value < 0.05 is the level of significance. Scatter Plots were used for the graphical representation of HbA1c vs. ACR, eGFR & serum creatinine.

RESULTS

Results were presented in the form of tables and graphs. The significance of results was discussed.

Parameters	Mean Value (+/- SD)	'p' value
Age (range- 40 – 70 years)	58.88 (± 7.71)	0.965
Male (age in years)	61.38 (± 7.10)	NA
Female in (age in years)	56.19 (± 7.22)	NA
DOD (Duration of diabetes in years)	10.01 (± 3.46)	0.967
FPG (mg/dL)	122.05 (± 21.32)	0.070
HbA1c (%)	7.68 (± 1.31)	0.155
Serum Creatinine(mg/dL)	1.07 (± 0.40)	0.945
eGFR (mL/min/1.73 sq. m)	72.77 (± 20.60)	0.120
Urinary ACR (mg/g)	54.48 (± 90.16)	0.063

Table 1. Baseline Characteristics of Total Study Subjects (n=105)

[*at 95% confidence interval. The 'p' values have been obtained by running a logistic regression. All data are normally distributed. (NA= Not Applicable)]

Study Subjects	Correlation Parameters	'r' value	'p' value
Total Study Subjects (n=105)	HbA1c	S. Creatinine 0.734 Urinary ACR 0.576 eGFR -0.672	<0.001* <0.001* <0.036*
Gr. I, HbA1c<8%, n= 71	HbA1c	S. Creatinine 0.380 Urinary ACR 0.227 eGFR -0.327	0.001* 0.057 0.005*
Gr. II; HbA1c ≥ 8%; n = 34	HbA1c	S. Creatinine 0.657 Urinary ACR 0.543 eGFR -0.614	<0.001* <0.001* <0.001*

Table 2. Shows Correlation (Pearson's) of HbA1c with and Renal parameters (S. Creatinine, ACR and eGFR)

* Statistically significant, 95% confidence interval, $\alpha=0.05$. For n=105 corresponding df=103, n=71 corresponding df=69 and for n=34, df=32.

From the table above, it is quite evident that patients with HbA1c greater than 8% exhibit somewhat similar to total study.

[HbA1c ≥ 8% suggests poor glycaemic control. Formula for estimated average glucose (eAG) in plasma (mg/dL) = $(28.7 \times \text{HbA1c}) - 46.7$. So, if HbA1c = 8%, then eAG = 182.9 mg/dL. And renal threshold for glucose is 180 mg/dL]

A 'p' value < 0.05 is the level of significance.

Figure 1. Shows scatter plot representation of HbA1c with ACR, eGFR & Serum Creatinine-

Scatter Plot: HbA1c vs. Urinary ACR

Scatter plot: HbA1c vs. eGFR

Scatter Plot: HbA1c vs. S. Creatinine (S. Cr.)

Association of HbA1c was assessed with Serum Creatinine, Urinary ACR & eGFR among total study subjects (n=105); Gr I with HbA1c < 8% (n=71) & Gr. II with HbA1c $\geq$ 8% (n=34) [Table -2]. Pearson Correlation test was done.

There is significant positive correlation of HbA1c with Urinary ACR & S. Creatinine in total study subjects ('r' value= 0.576 & 0.734 respectively; and 'p' value = <0.001 in both cases); and only with S. Creatinine in Gr. I, HbA1c < 8% ('p' value =0.001).

Significant positive correlation of HbA1c remained with S. Creatinine and ACR in Gr. II, HbA1c $\geq$ 8% ('p' value is <0.001 in both cases).

There is also significant negative correlation is found between HbA1c and eGFR in all cases i.e. in total study subjects (n=105), in Gr. I & in Gr. II (p values are 0.036, 0.005 & <0.001 respectively).

['p' value <0.05 is the level of significance.]

DISCUSSION

Among the many complications of diabetes; diabetic nephropathy is a major chronic complication in both uncontrolled Type 1 and Type 2 diabetes. There is a spectrum of changes in CKD (Chronic Kidney Disease), which progress from hyperfiltration to micro to macroalbuminuria and finally renal failure.^[20]

In early stage of renal disease, classical markers like serum urea and serum creatinine may be normal, but early glomerular changes like thickening of basement membrane of glomerulus, deposition of matrix materials in the renal mesangium and subsequent nodular deposits with consequent microalbuminuria (MA) occurs. At this stage, pathological changes in kidney can be reversed by pharmacological drugs.^[1] So, newly detected or known type 2 DM patients need strict monitoring for HbA1c, with simultaneous monitoring for glomerular filtration rate and microalbuminuria (MA).^[21]

In this hospital based, cross-sectional study, there are 105 known type 2 diabetic patients of age range between 40 - 70 years (mean age 58.88 ± 7.71) & mean duration of diabetes (in years) is 10.01 ± 3.36 . Association of HbA1c with Urinary ACR, eGFR & S. Creatinine is assessed by Pearson's Correlation test among total study subjects (n=105); Gr I with

HbA1c <8% (n=71) & among Gr. II patients where HbA1c $\geq$ 8% (n=34) [Table -2].

There was significant positive correlation of HbA1c with Urinary ACR & S. Creatinine in total 105 study subjects ('r' value= 0.576 & 0.734 respectively; and 'p' value = <0.001 in both cases).

In Gr. I (HbA1c < 8%, n=71) patients: we had also found significant positive correlation between HbA1c and S. Creatinine ('r' value =0.380 & 'p' value = 0.001). In this group, we had found only a positive correlation between HbA1c and Urinary ACR ('r' value = 0.227), but this correlation is statistically not significant as the 'p' value is 0.057 in this case.

In Gr. II patients (HbA1c $\geq$ 8%, n=34); we had found significant positive correlation of HbA1c with both Urinary ACR & S. Creatinine ('r' value =0.543 & 0.657 respectively; and 'p' value = <0.001 & <0.001 respectively).

Significant negative correlation was found between HbA1c and eGFR in all groups i.e. among the total study subjects (n=105), in Gr. I & Gr. II ('r' values = - 0.672, -0.327 & -0.614 and 'p' values are 0.036, 0.005 & <0.001 respectively.) [A 'p' value < 0.05 is the level of statistical significance.]

There are previous studies with similar type of significant association. Sheik et al (2009)^[22] found significant positive correlation of HbA1c with microalbuminuria (MA) ['p' < 0.05] & with S. Creatinine ('p' < 0.001) in type 2 diabetes. Venugopal & Layer (2010)^[23] had also found significant correlation of HbA1c with microalbuminuria (MA) in type 2 diabetes. Haque et al (2011)^[21] also found significant positive correlation of HbA1c with S. Creatinine and urinary ACR in type 2 diabetic patients ('p' values are 0.008 and <0.001 respectively. These positive correlations remained significant when HbA1c $\geq$ 8% (p values are 0.04 and <0.001) and when HbA1c <8%, they found only significant positive correlation between HbA1c and ACR ('p' value= 0.006) but not between HbA1c and S. Creatinine where p value is 0.617.

In contrast to our study, Haque et al, found positive correlation of HbA1c with eGFR although this positive correlation is not statistically significant ('p' value =0.158). But in our study, we got significant negative correlation between HbA1c & eGFR. [Table-2].

Possible explanation is that- in their study, total number of subjects were 50 (fifty) whereas in our study total subjects were 105. Mean years of duration of diabetes was 6.36 ± 1.65 in their study, whereas in our study the mean years of duration of diabetes was 10.01 ± 3.46 . Glomerular hyperperfusion and renal hypertrophy occur in the first years after the onset of DM and are associated with an increase of the GFR. During the first 5 years of DM, thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial volume expansion occurs as the GFR returns to normal.^[24]

CONCLUSION

In this study as we have found a significant correlation of HbA1c with both Urinary ACR, eGFR & S. Creatinine; it can be said that for early detection and treatment of nephropathy in Type 2 DM, both eGFR and urinary ACR should be measured along with tight glycaemic control so that progression to ESRD can be prevented.

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