ORIGINAL ARTICLE

# Association of HbA1c with Urinary ACR & eGFR in Type-2 Diabetes Mellitus

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## Abstract

### Background
Diabetic nephropathy is a chronic micro vascular complication of poorly controlled diabetes mellitus (DM), leading to end stage renal disease (ESRD). Control of DM is monitored by HbA1c. There are two early markers - to assess early renal impairment: Microalbuminuria (MA) & Glomerular Filtration Rate (GFR). Estimation of MA - needs 24 hours collection of urine. GFR is clinically assessed by creatinine clearance rate (CCR) at the same time for accurate estimation of GFR which also needs 24 hours urine collection. Faulty timing and non compliance for 24 hours urine collection - may give erroneous results. MA is better reflected by spot urine urinary albumin-creatinine ratio (ACR). Some formula based calculation of GFR, called estimated GFR (eGFR) are well correlated with CCR which needs only single blood sample for S.Creatinine (S.Cr). For example one such formula is Cockroft-Gault (C-G) formula.

### Objective
To evaluate the association of HbA1c with urinary ACR and eGFR in Type 2DM.

### Design
It was a cross sectional study carried out in the department of Biochemistry, Sylhet MAG Osmani Medical College, from July 2010 to June 2011.

### Methods
Fifty (50) known type 2 DM patients of 40-60 years age were evaluated dividing them on the basis of HbA1c (<8%,>8%), duration of DM (>5 years, <5 years), normotensives or hypertensives. FBS, S.Cr, Urinary Albumin & Creatinine were estimated. eGFR and urinary ACR were calculated. Results were expressed as mean ± SD. Data were analyzed with SPSS software version (12.0). Unpaired 't' test and Pearson's correlation tests were performed as tests of significance. Value of 'p'<0.05 was the level of significance.

### Results
Significant difference of S.Cr & HbA1c was found between study groups on HbA1c<8% and >8%. DBP was significantly raised in hypertensive type 2 DM. Duration of DM did not show significant correlation with renal functional parameters. Serum Creatinine & U.ACR had significant positive correlation with HbA1c>8% and only with ACR but not with S.Cr in study subjects having HbA1c<8%.

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**Conclusion**
Raised HbA1c is associated with urinary ACR. Urinary ACR should be estimated in monitoring risk assessment of Type 2DM in patients with raised HbA1c.

**Key words:** HbA1c, U. ACR (Albumin: Creatinine Ratio), eGFR, Type 2 DM.

## Introduction

Diabetes Mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin.[1] There are two clinically important types of DM (T1-IDDM & T2- NIDDM). Type 2DM comprises about 90% of diabetic population of any country. For all age groups worldwide, prevalence of DM was 2.8% in 2000.[2] Prevalence of Type 2DM was 2.3 % in 1999 & 6.8% in 2004 among rural population of Bangladesh.[3] In 2009, prevalence of DM was 7.8% in Dhaka city dwellers (20-79 years of age), which was 5.3% in 1985, increased by 47% over 24 years period.[4] According to a recent study prevalence of DM is 7.2% and IGR (IGT &/or IFG) 6.5% of rural population ≥25 years age.[5] Diabetic control is not reliably reflected by traditional blood glucose estimations only; due to wide fluctuations. HbA1c provides average glycemia over previous 6-8 weeks. Normal values of HbA1c are 5-7% of adult Hb.[6] Clinically, either S.Cr or Urea or both are estimated as the first line investigation, depending upon local choice. Though specific, serum creatinine may not exceed upper limit of reference range, until GFR (CCR) reduced by 60% of normal. Commonly CCR is a more sensitive indicator of early glomerular dysfunction than that of S.Cr conc.[7] MA is an early marker of reversible nephropathy, can identify very early stages of progressive glomerular disease. Early detection of diabetic nephropathy relies on tests for urinary excretion of albumin. There are two methods used to determine urinary albumin excretion: 24 hours AER (Albumin Excretion Rate) & spot urine ACR.[8] Spot urine ACR provides an equivalent result compared to 24 hours AER.[9] NKF (National Kidney Foundation) & NIDDK(National Institute of Diabetes, Digestive & Kidney Diseases) in USA - recommended for markers for renal impairment:1) S. Cr alone should not be used 2) GFR estimation should be done with standard prediction equations (eGFR): MDRD is better but CG formula may be preferable to S.Cr alone, 3) ACR(mg/gm, or μg/mg) on spot urine provides useful information, ≤ 30(normal), 30-300 (MA-Microalbuminuria), >300 (macroalbuminuria).[8] Albuminuria & eGFR have independent additive value. For this reason both are recommended to identify early risks for renal impairment at reversible stage.[10] It is uncertain the degree of hyperglycemia required to injure the vasculature directly or to permit injurious effects to other factors. Relation between hyperglycemia and MA is not linear. HbA1c 8.1% (average blood glucose 200mg/dl) is a threshold above which risk of MA increases logarithmically.[11] DM with MA is more resistant to insulin & likely to have poorer glycemic control.[12] Monitoring for glycemic control & Screening for MA and timely therapeutic intervention has become the standard of diabetic care worldwide. This study was designed to see the association of HbA1c

(marker for glycemic control) & two early markers of renal functional impairments: ACR (reflection of MA) and eGFR (reflection of CCR) in Type 2DM.

## Materials and Methods

This cross sectional study was carried out in the department of Biochemistry, MAG Osmani Medical College, Sylhet, from July 2010 to June 2011. Fifty known Type 2DM patients of 40-60 years age were included consecutively from Outpatient Department of Sylhet Diabetic Hospital. Type 1DM, congestive cardiac failure, urinary tract infection, known nephropathy & pregnant women were excluded. Height, Weight & BMI were measured. BP was measured using Sphygmomanometer by auscultatory method. Patients were studied by dividing them according to various parameters: HbA1c<8% (n=16), >8% (n=34); Duration of DM - DOD <5 years (n=17), >5 years (n=33); Hypertensives (n=25), Normotensives (n=25). WHO (2000) criteria was used to diagnose DM1: FBG≥7.0 mmol/L. Two hour post glucose load, ≥11.1 mmol/L. Serum Creatinine was estimated to calculate- eGFR as per C-G

Formula[13] : $\frac{(140\text{-age}) \times \text{weight(kg)} \times k}{72 \times \text{serum creatinine (mg/dl)}}$

(K=0.85 for women & 1 for men)

BP≥140/90 mmHg was cut off point for hypertension.[14]

From each study subject 5 ml of fasting venous blood was drawn by disposable syringe with full aseptic precaution. One ml was transferred to an eppendorf with EDTA for HbA1c analysis and 4 ml of collected blood was taken in a properly cleaned & dried test tube without anticoagulant for FBG & S.Cr. Spot morning urine sample was collected to estimate urinary Albumin & Creatinine.

Results were expressed as mean ± SD. Data were analyzed with SPSS (version12.0). Independent 't' test & Pearson's Correlation test were done as tests of significance. $P<0.05$ was the level of significance.

Informed written consent was taken from each patient. Permission was taken from the ethical committee of Sylhet MAG Osmani Medical College.

## Results

Baseline characteristics were presented in Table-1.

**Table 1: Baseline characteristics of Total Study Subjects (n=50)**

| Parameters | Mean Values(±SD) |
|---|---|
| Age(Range 40—57 years) | 50.02±4.98 |
| Male | (n=23)50.65±4.89 |
| Female | (n=27)49.48±5.07 |
| DOD(Duration of Diabetes, years) | 6.36±1.65 |
| FBS (m mol/L) | 10.44±4.2 |
| HbA1c(%) | 9.42±3.04 |
| S.Cr(mg/dL) | 1.06±0.30 |
| ACR(mg/gm) | 241.54±189.66 |
| eGFR(ml/min) | 69.01±20.94 |
| BMI(Kg/M2) | 24.64±4.37 |

Baseline characteristics were compared between two groups, based on HbA1c (Table-II). Gr.I (n=16, HbA1c<8%), Gr.II (n=34, HbA1c>8%). There was significant difference of HbA1c and S.Cr between groups ('p'value <0.001 & 0.049) respectively.

**Table II: Comparison of baseline characteristics**

| Parameters | HbA1c<8% N=16, GrI | HbA1c>8% N=34, GrII | 't'value | 'p'value |
|---|---|---|---|---|
| DOD (years) | 6.31 ± 1.81 | 6.38 ± 1.59 | 0.132 | 0.896 |
| FBS (m mol/L) | 9.12 ± 3.45 | 11.06 ± 4.53 | 1.667 | 0.104 |
| S.Creatinine (mg/dl) | 0.96 ± 0.17 | 1.11 ± 0.34 | 3.019 | 0.049* |
| Urinary ACR(mg/gm) | 241.42± 192.20 | 241.60± 191.36 | 0.003 | 0.998 |
| eGFR (C-G formula) | 72.97 ± 21.75 | 67.15 ± 20.61 | 0.898 | 0.377 |
| HbA1c (%) | 5.96 ± 1.56 | 11.05 ± 2.03 | 9.69 | <0.001* |

Independent 't' test was done, p<0.05 was the level of significance Renal functional parameters (S.Cr, ACR & eGFR), HbA1c & BP parameters were compared between two groups based on the presence or absence of hypertension- (Normotensives 25; hypertensives 25). No significant difference of HbA1c, ACR, eGFR, S.Cr & SBP was seen between normotensive and hypertensive patients. Only DBP was significantly raised in Hypertensive patients (p= 0.018). No significant difference of HbA1c & renal functional parameters was noted between two groups based on DOD (<5years: n=17; >5years: n=33). Association of HbA1c was assessed with FBS, S.Cr, ACR & eGFR among total study subjects (n=50), Gr.I, HbA1c < 8% (n=16) & Gr.II HbA1c, >8% (n=34), (Table-III)

**Table-III: Correlation of HbA1c with renal functional parameters &FBS**

| Study Subjects | Correlation Parameters | | 'r' value | 'p'value |
|---|---|---|---|---|
| Total Study Subjects | HbA1c | FBS | 0.159 | 0.270 |
| n=50 | | S.Cr | 0.368 | 0.008* |
| | | ACR | 0.532 | <0.001* |
| | | eGFR | 0.202 | 0.158 |
| Gr.I, | HbA1c | FBS | 0.209 | 0.438 |
| HbA1c<8%, | | S.Cr | 0.135 | 0.617 |
| n=16 | | ACR | 0.654 | 0.006* |
| | | eGFR | 0.039 | 0.887 |
| Gr.II, HbA1c>8%, | HbA1c | FBS | 0.038 | 0.832 |
| n=34 | | S.Cr | 0.384 | 0.04* |
| | | ACR | 0.940 | <0.001* |
| | | eGFR | 0.239 | 0.174 |

Pearson's correlation test was done. There was significant positive correlation of HbA1c with S.Cr & ACR in total study subjects (P=0.008&, <0.001 respectively), with ACR only in Gr.I,

HbA1c<8% (p=0.006). Significant correlation of HbA1c remained with S.Cr and ACR in Gr.II, HbA1c>8% (p=0.04 & <0.001) respectively.

**Discussion**

Diabetic nephropathy is a chronic micro vascular complication in uncontrolled Type 2DM. There is a spectrum of changes in CKD, with well defined functional progression from hyperfiltration to micro to macro albuminuria to renal failure.[15]

In early renal impairment, classical markers (Urea & Creatinine) may be normal, but there are early glomerular changes- thickening of basement membrane, accumulation of matrix material in the mesangium, subsequently nodular deposits with consequent MA. At this stage, glomerular pathological changes can be reversed by pharmacological intervention.[1] So, newly detected or known T2DM patients need monitoring for glycemic control, with simultaneous monitoring for early reversible nephropathy, MA.

There were 50 known Type 2DM patients (40-57 years age). Mean BMI was 24.64. Age distribution was similar to Sheikh et al (2009)[16] & Mogensen et al (1984).[17] In contrast to Venugopal & Lyer (2010)[18] where majority of subjects were overweight or obese, majority of subjects in this study were with normal BMI. Overweight or obesity was not an associated complication in this study.

Baseline characteristics were compared between two study groups based on HbA1c. There was significantly raised HbA1c in Gr. II with HbA1c>8%, implicative of poor control of diabetes. No significant difference of DOD, FBS, ACR & eGFR was found between study groups. There was significant difference of S.Cr, p=0.049 (but, within normal reference range), might be related to gradual changes in glomerular membrane in uncontrolled DM with a trend to impaired renal function.

Ardekani, Modarresi & Amirchaghmaghi (2008)[19] found 7.3% & 28.1% prevalence of MA, in pts with DOD≤10 years & >10 years respectively. Duration of diabetes (DOD) showed no significant effect in any study param eters, including, HbA1c, ACR, eGFR & S.Cr. All study subjects in this study had DOD<10 years. Though not significant, MA in this study increased with increasing DOD from 5 years onwards: mean ACR 254.78 mg/gm in DOD>5years, in contrast to 234.72 in diabetic subjects<5 years. Probably longer duration is needed for any significant change in glomerular structure.

There was 50% diabetic hypertensives with significantly raised DBP in this study, p=0.018. Venugopal & Lyer (2010)[18] also found nearly 50% of the diabetic subjects with hypertension and significantly higher MA compared to normotensives. In this study, there was no significant difference of U.ACR & eGFR between normotensives and hypertensives, but ACR was raised in hypertensives (mean ACR 236.88 & 246.21 in normotensives and hypertensives respectively). Hypertension is common among patients with T2DM and may precede the onset of diabetes, the root cause may be insulin resistance for hidden years before clinical appearance of DM. Hypertension can cause MA and can accelerate the progression of diabetic nephropathy.

Association of HbA1c with FBS, S.Cr, ACR & eGFR was assessed by Pearson's Correlation test. There was significant correlation of HbA1c with S.Cr & ACR in total study subjects ('p' value 0.008 & <0.001 respectively). This correlation remained significant for group-II (HbA1c>8%) and only with ACR in Gr-I having HbA1c<8%. There were studies with similar significant association. Sheik et al (2009)[16] found significant positive correlation of HbA1c with MA (p<0.05) & S.Cr (p<0.001).

Venugopal & Lyer (2010)[18] found significant correlation of HbA1c with MA.

S.Cr is widely used clinically as an index of renal function. Correlation of raised HbA1c with S.Cr & ACR might be related to poorly controlled DM leading to renal impairment. There was no significant correlation of HbA1c with eGFR, indicating that MA (reflected by ACR) is a better marker for early diabetic nephropathy than eGFR. In this study it is proved that, HbA1c is associated with ACR.

The message from this study might be that, raised HbA1c in monitoring DM calls attention for Renal Function Tests. For early diagnosis of preventable renal impairment- MA as diagnosed by 24 hours urinary albumin is well reflected by spot urine ACR.

**References**


1. Frier M, Fisher BM. Diabetes Mellitus. In Colledge NR, Walker BR, Ralston SH (eds). Davidson's Principles & Practice of Medicine. 21st edn. Printed in China, Churchil Livingstone. 2010;793-830.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27:1047-53.
3. Rahim MA, Hussain A, Khan AKA. Rising prevalence of T2DM in rural Bangladesh: a population based study. Diabetes Research & Clinical Practice. 2007;77(2):300-305.
4. Diabetes Atlas. IDF, Belgium, 2009.
5. Akhter A, Fatema K, Afroj A, Bhowmik B, Ali L, Hussain A. Prevalence of DM & its associated risk Indicators in a rural Bangladeshi population. Open Diabetes Journal. 2011;4:6-13.
6. Kilpatrick ES. HbA1c in the diagnosis & monitoring of DM. Journal of Clinical Pathology. 2008;61:977-982.
7. Mayne PD. Clinical Chemistry in Diagnosis & Treatment. 6th edn. ELBS, Lloyd-Luke (Medical Books) Ltd. Glasgow, UK. 1994;18-19.
8. Eknoyan G, Hostter T, Barkis GL. Proteinuria & other markers of chronic kidney diseases: a position statement of the national kidney foundation (NKF), and the national institute of diabetes & kidney disease (NIDDK).AJKD. 2003;42(4):617-622.
9. Suma MN, Prasant V, Akila P, Dhar M, Anjali BS. Prediction MA by using spot urine samples & regression analysis to convert spot microalbumin values to 24 hours microalbuminuria. International Research Journal of Biochemistry. 2011;3:71-75.
10. Hiddo J, Heerspink L, Holtkamp FA, Zeeuw D, Ravid M. Monitoring kidney function and albuminuria in patients with diabetes. Diabetes Care. 2011;

    34(2):5325-5329.
11. Krolewski AS, Quinn M, Lori MB, James H, Warram. Glycosylated Hb & the risk of microalbuminuria in patients with IDDM. The NEJM. 1995;332:1251-1255.
12. Carlo G. A Glycemic threshold for diabetic complications. The NEJM. 1995;332:1293-1294.
13. Newman DJ, Price CP. Non protein nitrogen metabolites. In Burtis CA, Ashwood ER(eds). Teitz Fundamentals of Clinical Chemistry. 5th edn. Harcourt (India) Private Limited. 2001;419.
14. Chobanian AV, Bakris GL, Black HR. Seventh report of the joint national committee on prevention, detection, evaluation & treatment of high blood pressure. Hypertension. 2003;42:1206-252.
15. Zachary T, Bloomgarden. Diabetic Nephropathy. Diabetes Care. 2005;28(3):745-51.
16. Sheik S, Beig JA, Iqbal T, Kazmi T. Prevalence of microalbuminuria with relation to glycemic control in T2 diabetic patients in Karachi. J Ayub Med Coll. Abbottabad. 2009;21(3):83-86.
17. Mogensen CE. Microalbuminuria predicts clinical proteinuria & early mortality in maturity onset diabetes. N Eng J Med. 1984;310:356-360.
18. Venugopal S, Layer UM. Risk factor analysis and prevalence of microalbuminuria among T2DM subjects: the need for screening and monitoring microalbumin. Asian J Exp Biol Sci. 2010;1(3):652-659.
19. Ardekani MA, Modarresi M, Amirchaghmaghi E. Prevalence of microalbuminuria and its risk factors in type 2 diabetic patients. Indian Journal of Nephrology. 2008;18(3):112-117.