Uromodulin- Glycated Hemoglobin Ratio Plays The Trump In Diabetic Nephropathy Detection- A Case Control Study

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Abstract: Background: To investigate urine uromodulin as a marker for Diabetic nephropathy (DN) and its relation with glycaemic control. Material & Methods: a cross-sectional comparative study on 180 healthy controls (Group I), 205 patients of Diabetes Mellitus were classified as Group II without microalbuminuria and those with microalbuminuria as Group III**.**Urineuromodulin, albumin and creatinine was estimated along with routine biochemistry. Results: The FBS, PPBS, HbA1c and serum creatinine were lowest in Group I as compared to groups II and III ($p < 0.01$, <0.01, <0.01, 0.008 respectively). There was no difference in urine uromodulin levels among the three groups ($p= 0.609$) but the Uromodulin HbA1c Ratio (UHR) showed a significant difference ($p <0.01$). UHR showed a statistically significant negative correlation with FBS, PPBS, HbA1c and urine ($p=$ <0.01, <0.01, <0.01 and 0.004 respectively). The Odds of having DN with UHR > 8.6 was 0.49 (95% CI is 0.308- 0.78). Conclusion: The non-occurrence of a diabetic complication that is nephropathy in our study group is favourable to those diabetic patients with a higher UHR (>8.6). Estimation of urine uromodulin will be beneficial along with albuminuria in detecting DN. [Sahu S Natl J Integr Res Med, 2021; 12(1):07-11]

Key Words: urine uromodulin, glycemic control, HbA1c, uromodulin: HbA1c ratio, type 2 diabetes mellitus, tubulo-protective.

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Introduction: "Uromodulin is the most abundant protein in normal urine" is often stated in the urine analysis classes for medical students. And our graduates remained status quo in their knowledge about this enigmatic product from the tubules. There has been a lot of development in our knowledge and understanding of uromodulin metabolism in the recent past. It was earlier known as Tamm Horsfall protein, and is synthesized by the ascending loop of Henle cells[1].

It is rich in cysteine residues which help in forming inter molecular crosslinks leading to a gel like consistency[2] and its glycosylated residues anchor to epithelial cell surface till it is enzymatically cleaved[3]. Diabetic nephropathy (DN) is one of the leading causes of chronic kidney disease (CKD) and if it is not treated, it can lead to end stage renal disease, thereby, considerably reducing the quality of life and survival[4,5]. The gold standard test to diagnose DN is detection of microalbumin in urine [6]. But there has been reports of urine albumin being modified by hyperglycaemia and dyslipidaemia in blood and by lysosomal degradation during its passage through the renal tubules in the diabetic patients[7,8]. Hence, the question arises whether urine albumin is satisfactory as a marker or do we need to investigate more biomarkers. This study was designed to explore the possibilities of urine uromodulin as a marker for DN and its relation with glycaemic control.

Material and Methods: Study Design: It was a cross-sectional comparative study on ambulatory Type 2 Diabetes mellitus (T2DM) subjects conducted in a tertiary care centre in Eastern India after the approval of the Institutional Ethics Committee.

Sample Size: 205 patients of T2DM and 180 controls

Inclusion Criteria: Controls were taken as group I: 180 non diabetic healthy subjects were recruited after taking their written consent. The 205T2DM patients recruited were classified into two groups according to their urine microalbuminuria report.

Group II had urine albumin creatinine ratio (UACR) < 30 mg/gm and Group III had 30- 300 mg/gm.

Exclusion Criteria: Subjects unwilling to participate, or those having hypertension, protein urea, or other co morbidities like thyroid disorders, disorders necessitating the need for long term use of anti-inflammatory drugs or steroids, pregnancy and Type 1 Diabetes mellitus were excluded.

Sample Collection And Biochemical Assay: The urine samples from both cases and controls were collected as a midstream portion of a mid-morning void, and were stored in 3 separate 1ml aliquots at −20 °C till assay of the following: Urine uromodulin by ELISA; urine albumin by immunoturbidimetric method and urine creatinine by Jaffe's kinetic method in autoanalyzer from Beckman Coulter Chemistry Analyzer AU5800 (Beckman Coulter, Brea, USA).

The urine samples of controls (Group I) were checked for qualitative proteinuria using dipstick, before storage. 5ml of blood was collected in different vacutainers for estimation of the following: serum creatinine, glycated haemoglobin (HbA1c**)**, fasting (FBS) and post prandial blood sugar (PPBS). All the colorimetric estimations were done the same day using the Beckman Coulter Chemistry Analyzer AU5800 (Beckman Coulter, Brea, USA).

Calculated Parameters: The degree of early DN was determined using the urinary albumin-to-creatinine ratio (UACR) and expressed as mg/gm; microalbuminuria range for UACR: 30–300 mg/g is considered as a marker for DN; < 30 is normal and > 300 as overt proteinuria.

Results: Clinical Characteristics: The comparison of the means of the variables (Table 1) was done by One-way ANOVA to see the overall difference between the three groups and Post hoc Bonferroni test was used to find the difference between specific groups. It was seen that there was no difference in age of subjects, their urine uromodulin and creatinine among the three groups. The FBS, PPBS, HbA1c and serum creatinine were lowest in Group I as compared to groups II and III ($p < 0.01$, <0.01, <0.01, 0.008 respectively) but the difference was not statistically significant between the latter two diabetic groups.

The urine microalbumin and its derivative ACR were high in Group III followed by Group II ($p<0.01$, <0.01) and Group I ($p<0.01$, <0.01) but was similar in groups I and II. Though there was no difference in urine uromodulin levels among the three groups ($p= 0.609$) (Table 1), there was a negative correlation observed of HbA1c with uromodulin, hence on calculating the Uromodulin HbA1c Ratio (UHR) and comparing in the same among the groups showed a significant difference ($p<0.01$).

Table 1: General Characteristics Of The Three Groups In The Study.

Group →	**I**	**II**	**III**	
N →	**180**	**103**	**102**	**P***
Age (years)[a]	49.4 (11.3)[b]	52.4 (9.5)	50.2 (11.3)	0.073
FBS (mg/dl)	93.2 (11.4)	159.0 (69.1)	173.5 (61.8)	<0.01
PPBS (mg/dl)	127.1 (65.6)	238.8 (95.1)	259.9 (86.7)	<0.01
HbA1c (%)	5.8 (0.5)	8.3 (2.0)	8.7 (1.7)	<0.01
Serum Creatinine (mg/dl)	0.9 (0.2)	1.0 (0.2)	1.0 (0.4)	0.002
Urine microalbumin (mg/L)	7.2 (6.7)	14.8 (7.5)	94.6 (63.9)	<0.01
Albumin Creatinine Ratio (ACR)	10.7 (18.1)	29.1 (32.2)	121.2 (206.0)	<0.01
Urine Creatinine (mg/L)	1.5 (2.3)	1.4 (2.0)	1.8 (2.4)	0.404
Uromodulin (ng/ml)	72.3 (56.3)	77.0 (79.6)	68.1 (59.7)	0.609
Uromodulin HbA1c Ratio	12.6 (9.7)	9.6 (10.0)	8.3 (7.8)	<0.01

P* was significant for levels <0.05 for the One- way ANOVA , The units of measurement are given in brackets & The values in the columns are Mean (Standard Deviation),

Table 2: Correlation Of Glycaemic Control Markers With UHR.

	r*	**P**
FBS	-0.207	<0.01
PPBS	-0.221	<0.01
HbA1c	-0.242	<0.01
Urine microalbumin	-0.148	0.004

Uromodulin: Glycated haemoglobin ratio (UHR)*r is for Pearson's correlation. The correlation of the UHR with the markers of glycaemic control showed (Table2) a statistically significant negative correlation with FBS, PPBS, HbA1c and

urine (p = <0.01, <0.01, <0.01 and 0.004 respectively). These observations suggest that there was a decrease in the tubular protein with uncontrolled diabetes. The median cut off for urine uromodulin was taken as 8.6 and using the same, it was seen that lower values of the ratio (< 8.6) had a risk for developing DN (33.3%) (Table3). The Odds of having DN with UHR > 8.6 was 0.49 (95% CI is 0.308- 0.78) (Table 4), thereby being tubule-protective. On the other hand, the risk of developing nephropathy with a lower UHR is the reciprocal of Odds, 1/0.49, that is 2.04, which is clinically relevant. The non-occurrence of a diabetic complication that is nephropathy in our study group is favourable to those diabetic patients with a higher UHR.

Table 3: Association Of Diabetic Nephropathy With UHR In Both Cases And Controls

Median UHR Cut-Off Of 8.6	Diabetic Nephropathy Present		Total
	Yes	No	
>8.6	38	155	193
(Low Risk)	19.70%	80.30%	100.00%
<8.6	64	128	192
(High Risk)	33.30%	66.70%	100.00%
Total	102	283	385
	26.50%	73.50%	100.00%

Table 4: Risk Of Nephropathy In Diabetics And Non-Diabetics.

Risk Estimate	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio of UHR > 8.61	0.49	0.308	0.78
For cohort DN = nephropathy	0.591	0.417	0.837
For cohort DN = No nephropathy	1.205	1.066	1.361
N of Valid Cases	385		

Discussion: Diabetes mellitus progressing to nephropathy occurs in half of the patients who are genetically predisposed for the same. The genetic predisposition is a non-modifiable factor but, DN progression to its chronic state and subsequently to its ESRD can be prevented by early detection, good glycaemic control and nephro protective treatment[9]. The most commonly used test in clinical practice to detect DN is urine albumin, but it is not absolute.

A pertinent weakness of the same is that, in about 30- 45% cases of DN, albuminuria is not detected[10,11]. This makes it important to add another marker for detection. In our study, we estimated urine uromodulin.

Though the pattern of urine uromodulin was not of a distinct trend in the three groups, there was a statistically significant decrease in UHR among diabetics and further decrease in the presence of DN. Similar results were reported by Zylka[9] and with serum uromodulin by Leiherer[12]. In another review article, it was reported that all the 11 studies observed a decline in uromodulin with development of DN[6]. The physiological action of uromodulin is to protect the tubules and inhibit inflammation in tubules caused by kidney injury mediators from the proximal tubules. This is explained by the Toll like receptor 4 (TLR 4) overexpression in proximal tubules in kidney injury[13]which further causes damage to distal tubules by inflammatory infiltrates and chemokines leading to fibrosis.

As a consequence, there is a decrease in the normal kidney parenchyma mass and function[14]. These effects of TLR 4 are annulled by the presence of uromodulin in normal amounts. Hence reduced levels of uromodulin limits tubular repair and causes progression to nephropathy in diabetics.

As prevention is better, aggressive glycaemic control cannot be substituted for screening tests for DN. Though in clinical practice, the detection of albuminuria is one of the significant predictors of renal function decline, other tubular injury markers like urine uromodulin, neutrophil gelatinase-associated lipocalin (NGAL), transferrin, and IgG and serum cystatin are recommended[15-17].

Our previous research has shown strong association of NGAL, ACR and eGFR in DN. As there are not reports on the UHR, and ours being limited by being a cross-sectional study, more research questions should be triggered and further studies prompted on studying the rate of UHR decline in DN.

Conclusion: In summary, the findings of our present study have clinical insinuations. An UHR of > 8.6 was tubule-protective. A decrease in the UHR, irrespective of the discrete levels of HbA1c or urine uromodulin may suggest tubular toxicity and that can hint on DN development before the detection of albuminuria. However, further studies and cohorts need to be conducted to confirm the conclusions.

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