Original Article

Urine Zinc-alpha-2-glycoprotein as a Potential Biomarker for Incipient Diabetic Nephropathy: A Pilot Study at a Tertiary Care Hospital

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Abstract

Introduction: Urine albumin-creatinine ratio (UACR) continues to be used as an indicator for detecting diabetic nephropathy (DN); however, damage starts much before that. Currently, no biomarkers are there to indicate incipient damage. As a result, researchers are looking for new biomarkers that could be used to detect DN threats sooner and perhaps hinder the development of end-stage renal disease. The present study intended to know if urine Zinc-alpha-2-glycoprotein (ZAG) levels correlate with glomerular filtration rate (GFR) in the study participants of type 2 diabetes mellitus (T2DM). **Materials and Methods:** The study included 68 participants with a known history of T2DM. Serum urea and creatinine levels, fasting plasma glucose, serum cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total protein, glycated hemoglobin (HbA1c) and urine ZAG were estimated and UACR along with estimated GFR (eGFR) were calculated for all individuals. The characteristics of the study participants in the microalbuminuric and normoalbuminuric groups were compared. **Results:** The levels of urine ZAG in the microalbuminuric and normoalbuminuric groups were not observed to be substantially different. The relationship between urine ZAG and diabetes mellitus duration was found to be highly significant in normoalbuminuric patients. Urinary ZAG and correlation with categories of HbA1c % (good <7, 7–9 moderate, >9 poor) among normoalbuminuric individuals were not found to be significant. **Conclusion:** Despite previous research, we were unable to find a positive relationship between urinary ZAG concentrations and eGFR in this study. Prospective studies with greater sample sizes and follow-up are required to fully comprehend the possible use of ZAG as a biomarker in diabetic nephropathy.

Keywords: Diabetic nephropathy, microalbuminuric, normoalbuminuric, urinary zinc-alpha-2-glycoprotein

Introduction

An irreversible microvascular complication of diabetic nephropathy (DN) affects 25%–40% of diabetic patients.[1] This progression to DN can be slowed down with appropriate medication and intervention. The progression occurs in stages, Stage 1 is the hyperfiltration-hypertrophy phase, followed by the silent or normoalbuminuric stage, which is not detected clinically. Microalbuminuria or incipient DN is stage 3, which can be detected clinically whereas Stage 4 is overt DN.[2]

It was found in a recent Indian study that a high percentage of type 2 diabetes patients (about 45.2%) present with normoalbuminuria despite being categorized as CKD 3 A using estimated glomerular filtration rate (eGFR).[3] Large studies like the DEMAND study have already established that DN was present in a whopping 17% of patients with normal urine albumin.[4] Since a considerable number of diabetic patients with normal urine albumin excretion also have significant renal damage, more research into novel markers that can predict DN progression in normoalbuminuria is needed. The presence of zinc-alpha-2-glycoprotein (ZAG), an adipocytokine[5] in urine is a new biomarker for incipient damage in DN with normal urinary albumin concentration.[6,7] The higher prevalence of

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Submitted: 31-May-2023 **Revised:** 12-Jun-2023
Accepted: 17-Jul-2023 **Published:** 22-Dec-2023

Access this article online

Quick Response Code:

Website: www.actamedicainternational.com

DOI: 10.4103/amit.amit_45_23

How to cite this article: Tamizhan S, Rajput S, Rabari K, Mamgain G, Naithani M. Urine zinc-alpha-2-glycoprotein as a potential biomarker for incipient diabetic nephropathy: A pilot study at a tertiary care hospital. Acta Med Int 2023;10:91-5.

diabetes in Uttarakhand[8] makes it imperative to quantify ZAG levels in the urine and see if they may be used as a risk marker for GFR decrease.

Materials and Methods

Study design

The present study was a cross-sectional study conducted in collaboration with the Department of Internal Medicine and the Department of Biochemistry at AIIMS Rishikesh from December 2017 to August 2018. Ethical approval was granted by the Ethical Committee of AIIMS Rishikesh. After getting written informed consent, 68 individuals who met the inclusion criteria were included in the study. The study was conducted as an Intramural project funded by AIIMS Rishikesh (27/IM/2016) and had Institutional ethics clearance (IEC number: ATIMS/IEC/|6/70).

Study settings and sample collection

A thorough medical history, along with a clinical examination, was conducted for all study participants. Fasting samples were collected. A serum sample was used in the analysis of fasting plasma glucose levels, serum creatinine, urea, serum cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total protein, and glycated hemoglobin (HbA1c). The eGFR was calculated using the Cockcroft–Gault formula. A urine sample was also simultaneously collected. Urinary ZAG measurement was performed using a human ELISA kit (Spanbiotec, Guandong, China). Based on the UACR ratio, patients were split into two subgroups: normoalbuminuric (<30 mg/g, $n = 54$) and microalbuminuric (30–300 mg/g, $n = 14$).

Sample size

This was conducted as a pilot study, so the sample size was kept at 70; two patients had high values of ZAG and were excluded from the analysis.

Inclusion criteria

Patients of AIIMS Rishikesh OPD with diabetes type 2 having normal blood pressure (BP) of ≤20/80 mm Hg were included in the study.

Exclusion criteria

1. Patients who are prescribed antihypertensive agents at baseline
2. Patients having smoking habit
3. Patients having diabetes mellitus complications such as peripheral vascular disease, coronary artery disease, retinopathy, and neuropathy suspected on clinical grounds were excluded.

Statistical analysis

The SPSS program (version 25.0) was used to statistically analyze the collected data. For the presentation of qualitative data, frequencies, and relative percentages were used, whereas for quantitative data, mean ± standard deviation was used. To compare variations between two quantitative groups, an unpaired *t*-test was used. ANOVA was used for comparison between quantitative variables and correlation was seen with the help of the Pearson correlation coefficient. Statistical significance cutoff was kept at $P < 0.05$.

Results

A total of 68 participants (48 males and 20 females; mean 55.85 ± 10.4 years) enrolled and consented to the study. The clinical characteristics and demographic features of the two groups (microalbuminuric and normoalbuminuric group) after estimating UACR levels are presented in Table 1. Between the microalbuminuric and normoalbuminuric groups, urine ZAG values were not having a statistically significant difference [Table 1]. The average eGFR was found to be 110.08 ± 41 ml/min per 1.73 m^2.

Our findings concerning age, weight, gender, BP, and duration of diabetes mellitus are statistically very similar in both groups. We have observed significant differences in serum urea and creatinine levels; however, fasting plasma glucose, total serum cholesterol, serum triglycerides, LDL, HDL, total protein, HbA1c, and urine ZAG were all found to be having no significant difference.

We attempted a correlation between urine ZAG among normoalbuminuric individuals ($n = 54$) with eGFR, HbA1c, duration of diabetes, and urinary albumin concentration. Correlation with eGFR was found to be statistically insignificant. However, urine ZAG significantly correlated only with the duration of diabetes mellitus and not with HbA1c and urine albumin levels [Table 2].

Urinary ZAG and its correlation with HbA1c categories (HBA1c <7% as good control, HBA1c 7%–9% as moderate control, and more than 9% as poor control) were not found to be significant in normoalbuminuric individuals. The comparison was attempted between eGFR categories (low, normal, and high) with Urine ZAG-2 protein, Hba1c, and duration of DM using one-way ANOVA. This did not yield any significant results [Table 3].

Discussion

The first clinical manifestation of DN is thought to be microalbuminuria.[6] Both the cellular elements in the renal tubular interstitium and glomeruli are impacted by DN.[9] This is because proteinuria is typically brought on by glomerular damage, which brings a lot of focus on glomerular damage in type 2 diabetes mellitus (T2DM) patients.[10] Despite the absence of significant albuminuria, some diabetic patients with decreased GFR progress to end-stage renal disease.[11] Treatment effectiveness is lower in patients with microalbuminuria having advanced renal pathology.[10,11] Albuminuria has a low sensitivity and specificity for detecting the early stages of DN, and there is only little correlation between albuminuria and eGFR.[11] Many new markers of tubular damage such as kidney injury molecule-1 a

Table 1: Baseline characteristics among microalbuminuric and normoalbuminuric groups of study participants and using unpaired *t*-test

Characteristics of participants	Microalbuminuric group (*n*=14)	Normoalbuminuric group (*n*=54)	*P*
Age (year)	56.84±11.02	55.58±10.35	0.40
Sex (male/female), % (*n*)	71 (10)/29 (4)	70 (38)/30 (16)	0.95
Weight (kg)	68.69±10.89	67.24±11.31	0.85
Height (m)	159.56±7.97	162.33±6.63	0.22
Systolic blood pressure (mmHg)	143.50±14.66	140.40±25.61	0.34
Diastolic blood pressure (mmHg)	87.50±6.69	87.06±15.27	0.91
Duration of DM (year)	12.45±5.22	6.49±2.97	0.67
Fasting plasma glucose (mg/dL)	196.33±74.37	163.28±61.65	0.50
Total cholesterol (mg/dL)	203.46±72.84	183.50±50.85	0.45
Serum triglycerides (mg/dL)	185.15±129.01	173.72±131.49	0.81
LDL-C (mg/dL)	135.92±58.53	115.51±35.84	0.25
HDL-C (mg/dL)	41.30±10.37	43.18±10.11	0.62
Urea (mg/dL)	59.30±56.62	27.70±10.87	≤0.05
Serum creatinine (mg/dL)	1.68±0.69	0.81±0.33	≤0.05
Total protein (g/dL)	7.56±0.63	7.63±0.58	0.87
HbA1c (%)	8.63±2.22	8.39±1.95	0.60
Urine ZAG	0.21±0.02	0.22±0.40	0.90

*$P \leq 0.05$ statistically significant. LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, DM: Diabetes mellitus, HbA1c: Glycated hemoglobin, ZAG: Zincalpha-2-glycoprotein

Table 2: Urine zinc-alpha-2-glycoprotein correlation with various variables among normoalbuminuric individuals (*n*=54)

Analytes/variables	Pearson correlation coefficient (*r*)	*P*
e-GFR	−0.12	0.35
Urine albumin concentration	−0.07	0.84
HbA1c	−0.03	0.85
Duration of diabetes mellitus	−0.56	0.0001*

*$P \leq 0.05$ statistically significant. HbA1c: Glycated hemoglobin, e-GFR: Estimated glomerular filtration rate

transmembrane protein, interleukin-18 a cytokine, or proteins such as lipocalin-2, liver fatty acid binding protein, and many metabolomics studies have identified urine metabolites such as lactic acid, allantoin and hippuric acid which have been investigated to forecast renal damage in DN patients.[12,13] As a potential biomarker to help with the early detection of DN, the current study seeks to determine whether urine ZAG levels correlate with GFR in people with diabetes.

In the present study, the normoalbuminuric and microalbuminuric groups of study participants had significantly different serum urea and creatinine values. However, no statistically significant differences were found in values of fasting plasma glucose, cholesterol, triglycerides, LDL, HDL, total protein, HbA1c, and urine ZAG. Our findings are in contrast to a study published by Elsheikh *et al*., which states there is a significant difference among groups with respect to fasting or random blood glucose, HbA1C, and serum creatinine was recorded. In Elsheikh *et al*. study results, no significant correlation with urea was found.[13]

Urine ZAG in normoalbuminuric diabetics has shown a significant correlation with the duration of diabetic mellitus, which is supported by other studies.[14] There was no significant correlation between HBA1c and urine albumin levels. This is in contrast with results by Elsheikh *et al*., which state that the urine ZAG levels positively correlated with urine albumin–globulin ratio. They also found urine ZAG levels and eGFR had a negative correlation which in our study was not found significant.

A study by Wang Y *et al*. with 80 T2DM patients and 20 controls, found that the urine ZAG levels correlated with the presence of albuminuria. However, they reported no correlation between urine ZAG levels and eGFR, BMI, age, serum creatinine, and hsCRP.[14] The present study also found similar results.

A study by Rao PV *et al*. also has shown that ZAG is the second-most abundant protein excreted in the urine of patients with DN with a progressive elevation from normoalbuminuria to macroalbuminuria.[15] This indicates that the increase will have some correlation with falling eGFR. The difference in concentration of urine ZAG was not found to be statistically significant in our present study. However, the study by Rao PV *et al*. looked at fold increase and not actual quantification.

However, urine ZAG significantly correlated with the duration of diabetes mellitus, HbA1c, and urine albumin levels. However, urine ZAG concentration was not found to be correlated with HbA1c categories (good <7, 7–9 moderate, >9 poor) in the present study. Most studies have seen serum ZAG correlation with HBA1c and Sonkar SK *et al*.[16] found serum ZAG levels to be negatively correlated with HbA1c.[17] A recent meta-analysis links serum ZAG with dysglycemia but

Table 3: Evaluation of estimated glomerular filtration rate categories (low, normal, and high) with urine zinc-alpha 2-glycoprotein, glycated hemoglobin, and duration of diabetes mellitus using one-way ANOVA

Variable	Mean±SD			*P*
	eGFR cat1 (low: <90) (*n*=17)	eGFR cat2 (normal: 90–120) (*n*=24)	eGFR cat3 High: >120) (*n*=13)	
Urine ZAG protein	0.23±0.04	0.22±0.04	0.22±0.04	0.55
Duration of DM	11.24±3.91	11.03±10.29	8.76±3.54	0.61
Urine albumin concentration	8.17±9.05	6.22±5.80	5.78±8.47	0.64
HbA1c	8.85±2.25	7.91±1.58	8.58±2.05	0.29

ZAG: Zinc-alpha-2-glycoprotein, DM: Diabetes mellitus, HbA1c: Glycated hemoglobin, e-GFR: Estimated glomerular filtration rate, SD: Standard deviation

also points to significant heterogeneity in included studies and that influence of adiposity cannot be excluded.[18] In our study, the weight of groups (microalbuminuric vs. normoalbuminuric) was not statistically different.

Jeon YK *et al*.[19] showed that declining eGFR and increased urinary albumin excretion resulted in DN progression. During the early stage of the disease increased renin-angiotensin system RAS activity results in increased angiotensin 2 levels leading to constriction of efferent arterioles resulting in proinflammatory and profibrotic molecules production which explains the pathogenesis.[10,20] The increase in urinary ZAG is due to the proximal tubules being particularly more vulnerable to diabetes-related injury, as they are exposed to a variety of metabolic and hemodynamic disturbances for long periods.[21] Since ZAG is primarily produced in the proximal convoluted tubules PCT and straight tubules,[22] this study's observations of variations in ZAG urine concentrations could indicate that tubular damage already exists in the early stages of DN, even before microalbuminuria appears.

A study by Wang *et al*. stated that ZAG concentrations were higher in urine than in serum, particularly in T2DM patients, suggesting that tubular epithelial cell secretion was the primary cause of the increased ZAG concentrations in urine.[15] Other studies revealed that in normoalbuminuric and microalbuminuric diabetics, urine ZAG levels rose over time, suggesting that it is connected to the development of nephropathy. The limitation of this study includes a small sample size and not performing a simultaneous serum ZAG concentration study.

Conclusion

In the present study, urine ZAG levels significantly correlated with the duration of DM, indicating a potential role in early diagnosis. Prospective studies with larger sample sizes and follow-ups are required to fully comprehend the use of ZAG as an early biomarker in DN.

Abbreviations

Urine albumin-creatinine ratio (UACR), Diabetic nephropathy (DN)

Financial support and sponsorship

This work was funded as an Intramural Fund by AIIMS Rishikesh.

Conflicts of interest

There are no conflicts of interest.

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