



Original Article

The Pattern of Plasma Gas-6 Elevation among Type-II Diabetes Mellitus Patients in Relation to Microalbuminuria using Urinary Albumin Creatinine Ratio.

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ABSTRACT

Due to the global increase in diabetes, diabetic nephropathy is becoming a significant issue. Diagnosis and treatment in the early stages of nephropathy may avert this considerable complication, and new biomarkers other than microalbuminuria are required to detect diabetic nephropathy earlier in type 2 diabetes mellitus. Moreover, the pathogenesis of complications associated with diabetes as indicated in recent experimental studies showed that growth arrest-specific-6 protein (Gas-6) might have a role. We therefore, embarked on this study to determine the pattern of plasma Gas-6 elevation among type 2 diabetes mellitus patients and to describe any relationship with microalbuminuria using urinary albumin creatinine ratio. This research was a descriptive; a hospital-based cross-sectional study conducted at the Endocrinology Clinic, University College Hospital, Ibadan, Oyo State, Nigeria. We recruited 71 type-2 diabetic participants, and 71 apparently-healthy participants who served as the control. The study showed that the concentrations of Gas-6 protein and UACR in the diabetic participants were all significantly higher than in the healthy control participants ($p < 0.001$). There was also a positive correlation between Gas-6 protein and UACR value which was statistically significant ($\rho = 0.41$, $p = <0.001$). The sensitivity and specificity of Gas-6 was 75.0% and 19.1% respectively, with a PPV and NPV of 0.68 and 0.25 respectively. In conclusion, Plasma Gas-6 protein correlated with microalbuminuria. However, Gas-6 protein alone may be of limited diagnostic value in diabetic nephropathy.

Keywords: Diabetic nephropathy, Gas-6, Microalbuminuria, Type II Diabetes Mellitus

INTRODUCTION

In the year 2000, the number of individuals with diabetes, globally, was estimated to be 171 million

(2.8% of the world's population). This projected figure is to increase to 366 million by the year 2030 (6.5% of the world's population), and 298 million of this figure (366 million) are projected to live in developing countries.¹ The estimated prevalence of diabetes in Africa is 1% in rural

areas², up to 5% to 7% in urban sub-Saharan Africa, and between 8%² and 13% in more developed areas such as South Africa and in populations of Indian origin.² In Nigeria, the national prevalence of diabetes Mellitus is 4.9%³ and data from the World Health Organization (WHO) suggest that Nigeria has the highest number of people living with diabetes in Africa.¹

Diabetes mellitus is a significant cause of chronic kidney disease, accounting for about 45% of cases of end-stage renal disease.³ Diabetic nephropathy is a clinical syndrome characterized by persistent microalbuminuria of >300 mg per day or >200 µg per minute that is confirmed on at least two occasions of 3-6 months apart. This is associated with a progressive decline in the glomerular filtration rate (GFR) and elevated arterial blood pressure.⁴ It is also characterized by structural abnormalities that are reversible in the early onset.³ Three significant histologic changes occur in the glomeruli in diabetic nephropathy. First, there is direct induction of mesangial expansion by hyperglycaemia, perhaps through increased matrix production or glycation of matrix proteins. Secondly, the glomerular basement membrane (GBM) thickening occurs. Lastly, there is glomerular sclerosis caused by intraglomerular hypertension (induced by dilatation of the afferent renal artery or from ischaemic injury induced by hyaline narrowing of the vessels supplying the glomeruli).⁵ In diabetic nephropathy, the earliest morphologic abnormality is the thickening of the GBM and expansion of the mesangium due to accumulation of extracellular matrix.⁵

With a prevalence of microalbuminuria of 39%, diabetic nephropathy has become a global epidemic.⁶ In Sub-Saharan Africa, the prevalence is estimated to be 6–16%,⁷ and has risen from 19% in 1971⁸ to 28.4% in 2003⁹ in Nigeria.

Microalbuminuria is defined as a persistent elevation of albumin in the urine of either 30 - 300 mg per day, or 20 - 200 µg per minute, or 30-299 µg per mg of creatinine.¹⁰ It is currently used as a diagnostic marker to predict future overt nephropathy.¹¹ Nephropathy has been shown to be an independent risk factor for early death due to cardiovascular diseases in diabetic patients.^{12,13} To prevent development and progression of diabetic nephropathy, early screening and early renoprotective interventions are essential.^{1,6} According to current recommendations, early detection involves testing for markers of glomerular

pathology, specifically microalbuminuria.¹ Mesangial cell proliferation is an early occurrence in diabetic nephropathy. Some studies have shown elevation of glomerular markers even before the development of microalbuminuria.¹⁴⁻¹⁷ Growth arrest-specific 6 protein (Gas-6), a 75 kilo Dalton (kD) multimodular vitamin K-dependent protein, similar to plasma anticoagulant protein S, is produced by the mesangium and rises very early as a result of mesangial proliferation. Gas-6 and its receptor, Axl, have been shown to play an essential role in the pathogenesis of diabetic nephropathy at the level of glomerular hypertrophy and mesangial proliferation.¹⁸ This forms the basis for the hypothesis that there is a link between plasma Gas-6 concentrations and microalbuminuria in patients with type 2 diabetes. We therefore, embarked on this study to determine the pattern of plasma Gas-6 elevation among type 2 diabetes mellitus patients and to describe any relationship with microalbuminuria using urinary albumin creatinine ratio.

MATERIALS AND METHODS

The study was carried out at the Endocrinology clinic of the University College Hospital, Ibadan. All the participants in the study were recruited after granting written informed consent and Ethical approval obtained from the University of Ibadan/University College Hospital (UI/UCH) Health Research Ethics Committee. The research study was a descriptive cross-sectional study and the study population consisted of type 2 diabetic patients who were attending the Endocrinology clinic. Seventy-one (71) type 2 diabetic patients (21 males and 50 females) were recruited by simple random sampling method. The control group consisted of seventy-one (71) apparently healthy volunteers (22 males and 49 females) residing in Ibadan and its environment. All the control subjects were subjected to fasting plasma glucose to ensure none of them was diabetic. Urine samples were collected in the morning at 8:00 O'clock. At least 5 ml of urine was obtained from each subject to a sterile universal bottle. The urine samples were centrifuged at 1500 revolutions per minute for 10 minutes using Uniscope Laboratory centrifuge, model SM 112 (Surgifriend Medicals, England). Subsequently, the supernatant was decanted into plain sample bottles. From the antecubital fossa, under the strict aseptic procedure, 5ml of venous blood was obtained using pyrogen-free disposable needles

and syringes. The withdrawn blood was aliquoted into Lithium heparin sample bottles for Gas-6 estimation. The blood was centrifuged at 3000 g for 5 minutes using Uniscope Laboratory centrifuge, model SM 112 (Surgifriend Medicals, England) to obtain plasma that was decanted into plain bottles. Plasma Gas-6 was assayed by immunoassay technique by using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions on the stat fax 4200 awareness technology, provided by WKEA Med. Supplies, China; cat. no WH-1770. The Urinary Albumin Creatinine Ratio (UACR) was calculated using the following formula: $\text{UACR (mg/g)} = \frac{\text{Urinary albumin (mg/dl)}}{\text{Urinary creatinine (g/dl)}}$.

RESULTS

The UACR was significantly higher among the type 2 diabetic participants (median = 48.00, IQR = 28.00 – 90.00) compared with the healthy control participants (median = 27.00, IQR = 26.00 – 28.00), $U = 6.45$, $P =$

0.001. A total of 142 participants comprising of 71 type-2 diabetics and 71 apparently healthy controls were studied. The mean ages of the diabetic and control participants are 58.1 (SD 10.7) years and 53.8 (SD 12.2) years, respectively. Higher proportions (approximately 35%) of the participants were between 60 and 69 years of age in both the diabetic and control participants (**Table 1**).

The male and female ratios in the diabetic and study participants are 1:2.6 and 1:2.5, respectively. There was no significant statistical difference between the age of the study participants and controls. Hence, age and gender were matched for both diabetics and controls; $p = 0.305$ and 0.713 respectively. The mean age of the study participants increases with increasing duration of DM, as shown in **Table 2**. Participants who had DM <5 years have a mean age of 53.6 (SD 11.3) years, while those with a duration of DM between 5 – 10 years and >10 years have mean ages of 59.0 (SD 8.7) years and 64.3 (SD 8.9) years respectively. The relationship between the duration of diabetes and microalbuminuria (UACR) are shown in **Table 3**.

Table 1 Socio-demographic and Clinical characteristics of study participants

Characteristic	Participants Diabetics (n - 71)	Control (n - 71)	p-value
Age			
30 – 39	5 (7.0%)	9 (11.3%)	0.305
40 – 49	9 (12.7%)	16 (22.5%)	
50 – 59	20 (28.2%)	20 (28.2%)	
60 – 69	27 (38.0%)	22 (31.0%)	
70 – 79	10 (14.1%)	5 (7.0%)	
Mean age (Mean, SD)	58.1 (10.7)	53.8 (12.2)	0.021
Gender			
Male	20 (28.2%)	22 (31.0%)	0.713
Female	51 (71.8%)	56 (69.0%)	
Duration of DM			
<5 years	23 (16.2%)		
5-10 years	26 (18.3%)		
>10 years	22 (14.8%)		

Values for categorical variables are n (%)

Table 2: Duration DM illness in the study participants

Variable	Frequency	Mean age	M:F
Duration of DM illness			
<5 years	23 (16.2%)	53.6 (11.3)	1:3.6
5-10 years	26 (18.3%)	59.0 (8.7)	1:2.3
>10 years	22 (14.8%)	64.3 (8.9)	1:2.5

The prevalence of microalbuminuria (UACR) in the diabetic participants was 64.1% and that of the control participants was 21.8%. Fifty (50) diabetic and 21 control participants had microalbuminuria respectively. The plasma Gas-6 levels and comparison between groups are shown in **Table 4** show that Gas-6 was significantly higher among the type 2 diabetic participants (median = 5.90, IQR = 4.00 – 12.00) compared with the healthy control participants (median = 0.20, IQR = 0.00 - 6.50), $U = 2.33$, $P = 0.002$. In addition, the association of Gas-6 with UACR in the study

participants as shown in correlation analyses in Figure 1 revealed a moderate, positive correlation between Gas-6 and UACR value which was statistically significant ($\rho = 0.41$, $p < 0.001$). The predictive values of plasma Gas-6 are summarized in Table 5. About 36 (67.2%) of the study participants with elevated Gas-6 also had elevated UACR, while 4 (30.0%) of those with normal Gas-6 were normoalbuminuric. Using a cut-off of 4.1 ng/ml, sensitivity and specificity of Gas-6 was 75.0% and 19.1% respectively, with a PPV and NPV of 0.68 and 0.25 respectively.

Table 3: Relationship between the duration of diabetes and microalbuminuria

	<5 years	5-10 years	>10 years	F-score
Normoalbuminuria (<30 mg/g)	9 (39.1%)	7 (26.9%)	5 (22.7%)	4.63
Micro/macro-albuminuria (>30 mg/g)	14 (60.9%)	19 (73.1%)	17 (77.3%)	4.63

The median and interquartile ranges for Gas-6 and UACR in both the diabetic and the control participants are summarized in Table 4. The concentration of Gas-6 and UACR in the diabetic group were all significantly higher than in the healthy control participants, $p = 0.001$.

Table 4: Biochemical parameters of the study participants (diabetic and non-diabetic participants)

Variable	Participants		p-value
	Diabetics (n - 71) Median (IQR)	Control (n - 71) Median (IQR)	
Urinary albumin (mg/dl)	5.0 (3.3 – 6.0)	3.9 (3.3 – 5.9)	<0.031
Urinary creatinine (g/dl)	59.8 (51.9 – 92.1)	119.1 (86.4 – 135.3)	<0.001
UACR (mg/g)	48 (28 - 90)	27 (26 - 28)	<0.001
Gas-6 (ng/ml)	5.9 (4.0 – 12.0)	0.2 (0 – 6.5)	<0.002

P value is significant at < 0.05 . Values for categorical variables are n (%)

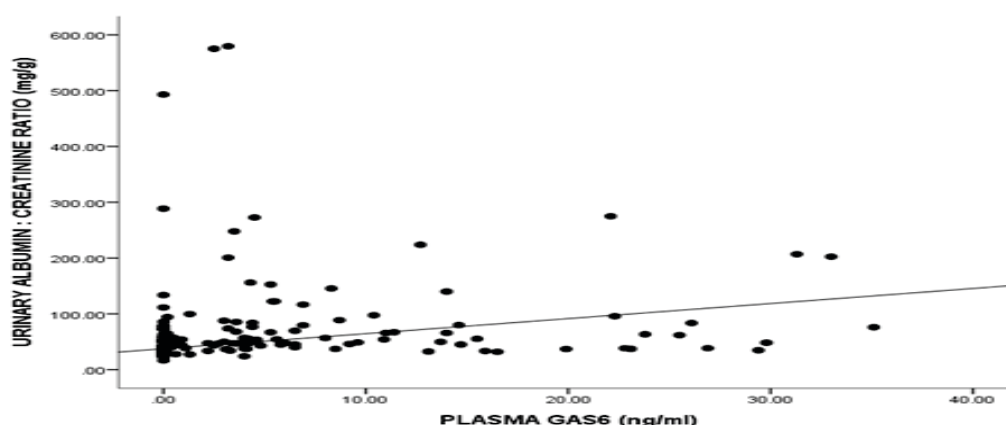


Figure 1: Correlation analysis between Gas-6 and urinary albumin creatinine ratio (UACR).

Table 5: Predictive values of Gas 6 among the study participants

	Normal Gas-6	Elevated Gas-6
Normoalbuminuria	4 (30.0%)	17 (32.8%)
Elevated Albuminuria	12 (70.0%)	36 (67.2%)

Sensitivity: 75.0%, Specificity: 19.1%, PPV: 0.68, NPV: 0.25, p-value: 0.001

P value is significant at < 0.05

Values for categorical variables are n (%)

PPV – Positive predictive value

NPV – Negative predictive value

Sensitivity = True Positive / (True Positive + False Negative) × 100
 $= 36 / (36 + 12) \times 100$
 $= 75.0\%$

Specificity = True Negative / (True Negative + False Positive) × 100
 $= 4 / (4 + 17) \times 100$
 $= 19.1\%$

Positive Predictive Value = True Positive / (True Positive + False Positive)
 $= 36 / (36 + 17)$
 $= 0.68$

Negative Predictive Value = True Negative / (True Negative + False Negative)
 $= 4 / (4 + 12)$
 $= 0.25$

DISCUSSION

This study showed that the urinary albumin creatinine ratio (UACR) was high in diabetes mellitus. The prevalence of microalbuminuria is 64.1%, agreeing with the previous study at Ibadan, Nigeria²⁰, where the prevalence was reported as 60% in type 2 diabetic patients attending the medical out-patient department. However, some studies conducted in other parts of the world recorded much lower figures which range from 20.8 – 34%.²¹⁻²³ There are several possibilities that could be used to explain the higher incidence of microalbuminuria in diabetics in this environment compared to other regions. The possibility of co-existing nondiabetic glomerulonephritis should be considered, especially as this study is hospital-based. In a study done in Denmark²⁴, twenty-seven per cent (27%) of type 2 diabetic patients' studied were found to have a variety of nondiabetic glomerulonephrites such as mesangioproliferative glomerulonephritis and minimal lesion nephropathy on kidney biopsy.²⁴

Physiologic causes of proteinuria, especially exercise proteinuria,

co-existing with diabetic nephropathy are also a likely explanation. The type of proteinuria seen during exercise has been discovered to be largely albumin and has been shown to result from enhanced glomerular permeability.²⁵ Proteinuria has been found to be most significant during the first 20 – 30 minutes after stopping the exercise.²⁶

Recurrent urinary tract infections treated in the past, especially among the elderly, cannot be excluded. Antibiotics used to treat urinary tract infection such as aminoglycosides and cephalosporins can lead to kidney damage.²⁷

This study demonstrated that the increasing age of the participants occurred with increasing duration of DM. This is in keeping with the survey done in Indore (India)²⁸, where HbA1c (haemoglobin A1c) levels showed a significant increase with the duration of diabetes. A possible explanation to this is the increasing damage to the glomerular mesangial cells that occurs with increasing duration of DM.²⁹ Also, microalbuminuria was found to increase with increasing duration of DM. This agrees with the previous study where the prevalence of microalbuminuria was found to increase with increasing age.³⁰ This could be due to the fact that previous studies have demonstrated that in diabetes mellitus, increasing damage to the glomerular mesangial cells correlated with increasing duration of DM.²⁹ Diabetic nephropathy (characterized by microalbuminuria) does not occur until about 7 – 15 years after the onset of diabetes.³¹ Proteinuria develops in only 4% of type 1 diabetic patients within ten years of diabetes. The result of the disease is much more likely to be due to early-onset proteinuria other than diabetic nephropathy.³² In type 2 diabetic patients, proteinuria can be present at diagnosis in as many as 8%³³, and the number presenting with proteinuria increases with increasing age at presentation. Therefore, there is the possibility of diabetes having been present earlier than the duration volunteered by diabetic participants. Some of these patients could be a reflection of the 8% population that presents with proteinuria at diagnosis.

Although microalbuminuria is used currently as a diagnostic marker to predict future overt nephropathy, neither the sensitivity nor the specificity of microalbuminuria is high enough to detect it.³⁴ Microalbuminuria concentrations are used to indicate diabetic nephropathy, but it is not always found to correspond to matrix expansion of mesangial cells during the early stage.³⁴ Another difficulty with microalbuminuria is the false-positive results which occur due to factors like urinary tract infection, strenuous exercise within 24 hours, congestive heart failure, infectious disease, marked hypertension, fever, marked hyperglycaemia, pregnancy, and haematuria.³⁴ Hence, in the early phase of kidney injury, a new indicator would be helpful to predict diabetic nephropathy, which is reversible at this phase.

However, plasma Gas-6 value was found to be high in diabetics (5.9 ng/ml). In a study done in Turkey in 2014, 20.9 ng/ml of Gas-6 was found in type 2 diabetic patients with microalbuminuria.³ In another study done, among acute coronary syndrome patients, in Wuhan, China, in 2009, 10.65 ng/ml of Gas-6 was found.³⁵ In yet another study carried out in Novara, Italy, in 2009, among patients suffering from a pulmonary embolism, 18.2 ng/ml was reported.³⁶ No reviews were found to compare the value of Gas-6 among the Africans, as seen in this study. Studies have shown varying glomerular findings on histology between Africans and Caucasians. It is also known that Africans are more prone to fibrotic processes and also have a difference in renal pathogenesis based on genomics.³⁰ The presence of multiple etiologic factors in the African population, especially infective processes could imply multiple pathways to nephropathy among African diabetics.³⁷

In this study, plasma Gas-6 levels were significantly higher in microalbuminuric than in normoalbuminuric diabetic participants. This is supported by a study done in Turkey³ where plasma Gas-6 concentrations were found to be higher in participants with micro or macroalbuminuria compared to participants with normoalbuminuria.

The diagnostic value of Gas-6 as a marker for nephropathy has not been assessed as far as we know. However, the results from this study showed a moderately increased sensitivity and a very low specificity. The positive predictive value (PPV) and the negative predictive value (NPV) is also somewhat increased and very low, respectively. These results suggest that the role of Gas-6 in predicting the presence of nephropathy is limited. The low

NPV indicates that Gas-6 is weaker in ruling out diabetic nephropathy. This is surprising as inflammatory changes in the glomerulus usually stimulate Gas-6, which is an essential molecule for the full expression of glomerular injury.³ The presence of mesangial hypertrophy, a common finding in diabetic nephropathy, is brought about by the autocrine function of the Gas-6 molecule.³⁸ Also, hypercoagulability in glomeruli which worsen the renal function is implicated by the Gas-6/Axl pathway reported in diabetic nephropathy.³⁸ However, the humoral response that takes place in glomerular injury is independent of Gas-6³⁸, hence the reduced diagnostic accuracy of the Gas-6 molecule. It is also known that the deleterious effect of diabetes on the kidneys is not only via glomerular inflammation⁴⁰; the presence of advanced glycosylated end-products³⁹ and activation of the sorbitol pathway has not been shown to involve the Gas-6 molecules which would limit the diagnostic value of the test.

Finally, it is worthy to note that the prevalence proportion may be affected by the method used to collect the urine, the screening test and the precision and sensitivity of the analysis used to measure microalbuminuria.

In conclusion, plasma Gas-6 protein correlates with microalbuminuria. However, in view of the low predictive values shown in this study, Gas-6 protein may be of limited diagnostic value in assessing any patient with a background diabetic nephropathy. Plasma Gas-6 protein level is lower than the amount found in the Caucasians.

CONCLUSION

Plasma Gas-6 protein correlated with microalbuminuria. However, Gas-6 protein alone may be of limited diagnostic value in assessing any patient with a background diabetic nephropathy.

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