Urinary immunoglobulin G versus microalbuminuria as an indicator of diabetic nephropathy in type 2 diabetic patients

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Context
Diabetic nephropathy in type 2 diabetes mellitus is one of the most serious microvascular complications. Immunoglobulin G (IgG), with molecular weight of 150 kDa, is excreted in urine of normoalbuminuric diabetic patients.
Aims
The aim was to compare urinary IgG with microalbuminuria as an early indicator of diabetic nephropathy in patients with type 2 diabetes mellitus.
Settings and design
The study was conducted in Zagazig University Hospital.
Materials and methods
This case–control study was conducted on 88 type 2 diabetic adult patients who were divided into four groups. Group I: 22 patients with normal albumin creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR), group II: 21 patients with normal ACR (<20 mg/g creatinine) and eGFR (>120 ml/min/1.73 m^2), group III: 24 patients with ACR from 20 to 200 mg/g creatinine and eGFR ranging from 60.74 to 102.48 ml/min/1.73 m^2, and group IV: 21 patients with ACR more than 200 mg/g creatinine and eGFR ranging from 17.16 to 85.27 ml/min/1.73m^2. Patients were subjected to complete blood count, kidney function tests (KFT), urinary albumin/creatinine ratio, urinary IgG/creatinine ratio, random blood sugar, and estimation of GFR by modification of diet in renal disease equation.
Statistical analysis
The data were analyzed using statistical package for the social science (SPSS), windows version 17. Description of qualitative variables was done by frequency and percentage. Description of quantitative variables was in the form of mean±SD. χ^2-Test, Student's *t*-test, analysis of variance (*F*-test), and correlation analysis were used for analytical examination.
Results
High significant difference among the different groups was found regarding ACR ($P<0.001$) and immunoglobulin G creatinine ratio (IgGCR) ($P<0.001$). ACR and IgGCR are highly increased in groups III and IV in comparison with groups 1 and II. There was a significant positive correlation between IgGCR and age, diabetes mellitus (DM) duration, serum creatinine ($P<0.001$), blood urea nitrogen, ACR, and renal sonography, and negative correlation with eGFR ($P<0.001$), hemoglobin, and serum albumin.
Conclusion
IgG appears in urine in early stages of diabetic nephropathy even before microalbuminuria, so we recommend its use to define high-risk patients, allowing prompt interventions.

Keywords:
chronic kidney disease, diabetic nephropathy, Egypt, immunoglobulin G

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Introduction

More than 350 million people worldwide are affected by diabetes mellitus [1]. Diabetic nephropathy affects ~30% of all diabetic patients [2]. Before the appearance of microalbuminuria, many biomarkers either glomerular or tubular will appear [3] such as transferrin, type IV collagen, cystatin C, ceruloplasmin, immunoglobulin M (IgM), associated lipocalin, and IgG [4].

In type 2 diabetes mellitus (T2DM), there are four IgG subclasses, but IgG1 was found to be the most abundant IgG in blood and urine. It was observed to have anti-inflammatory activities and also the ability to release proinflammatory mediators. It was detected in urine in prediabetic stage [5].

So, this study aimed to assess urinary IgG in comparison with microalbuminuria as an early indicator of diabetic nephropathy in patients with T2DM.

Materials and methods

A case–control study was conducted on adult patients with type 2 diabetes who were randomly selected from the inpatient and outpatient clinics of Internal Medicine Department, Zagazig University. After informed consent and local ethical committee approval, the patients were recruited for the study from July 2016 to July 2017. This study was done on 88 participants who were divided into four groups: group I include 22 diabetic patients (11 males and 11 females) with normal albumin creatinine ratio (ACR) (<20 mg/g creatinine) and normal estimated glomerular filtration rate (eGFR) (90–120 ml/min/1.73 m^2); group II included 21 diabetic patients (11 males and 10 females) with normal ACR (<20 mg/g creatinine) and eGFR more than 120 ml/min/1.73 m^2; group III included 24 diabetic patients (13 males and 11 females), with ACR from 20 to 200 mg/g creatinine and eGFR ranging from 60.74 to 102.48 ml/min/1.73 m^2; and group IV included 21 diabetic patients (eight males and 13 females), with ACR more than 200 mg/g creatinine and eGFR ranging from 17.16 to 85.27 ml/min/1.73 m^2. Inclusion criteria were as follows: type 2 diabetic patients, age group above 30 years old, and no other associated medical diseases causing proteinuria. Exclusion criteria were as follows: diabetic patients with end-stage renal disease (eGFR<15 ml/min/1.73 m^2); diabetic patients with urinary tract infection, obstructive uropathy, diabetic foot, autoimmune diseases, and heart failure; and pregnant females.

All persons included in this study were subjected to the following assessment at the time of the study: complete clinical history and clinical examination and laboratory investigation like complete blood count, random blood sugar, glycated hemoglobin, serum albumin, serum creatinine, blood urea, and eGFR. Urinary IgG was measured by using human enzyme-linked immunosorbent assay kits (normal value is <8.8 mg/l). Urinary ACR measurement, immunoglobulin G creatinine ratio (IgGCR) in urine (normal value is <2.0 mg/g) measurement, and urine analysis were also done.

Data management

The collected data were revised, verified, and edited on a personal computer and then analyzed statistically using statistical package for the social science (SPSS; SPSS Inc., Chicago, Illinois, USA), windows version 17.

The following statistical tests were used: description of qualitative variables was done by frequency and percentage, and description of quantitative variables was done in the form of mean±SD. χ^2-Test was used for comparison of qualitative variables with each other. Comparison between quantitative variables was carried out using Student's *t*-test of two independent samples. For comparison of more than two quantitative groups, Kruskal–Wallis analysis of variance (*F*-test) was used for categorical data. Correlation analysis was done when appropriate. Multiple regression analysis was done to find the predictors that related to ACR and IgGCR.

Table 1 Demographic and clinical data of different groups

	Group I (mean±SD)	Group II (mean±SD)	Group III (mean±SD)	Group IV (mean±SD)	*P*
Age (years)	48.68±9.32	45.90±8.89	57.00±8.90$^{*,+}$	60.86±8.09$^{*,+}$	<0.001
BMI (kg/m)	31.9 27±2.79	31.44±2.99	32.63±4.22	31.49±1.97	0.548
Sex [*n* (%)]					
Male	11 (50.0)	11 (52.4)	13 (54.2)	8 (38.1)*	0.71
Female	11 (50.0)	10 (47.6)	11 (45.8)	13 (61.9)	
Duration of DM	5.36±2.11	5.57±2.40	9.88±3.22$^{*,+}$	21.29±5.75$^{*,+,\#}$	<0.001
Associated HTN [*n* (%)]	8 (36.4)	8 (38.1)	13 (54.2)	16 (76.2)$^{*,+,\#}$	0.033*
RBS	222.7±49.27	241.33±45.83	270.5±50.3*	226.5±59.99$^{\#}$	0.009
HbA1c%	6.78±0.46	8.02±0.34*	7.93±0.46*	7.45±0.95$^{*,+,\#}$	<0.001
Hb (g/dl)	12.45±1.80	13.78±1.54*	12.00±1.53$^{*,+}$	10.11±1.39$^{*,+,\#}$	<0.001
Serum albumin (g/dl)	4.16±0.35	4.45±0.44	3.88±0.24$^{*,+}$	3.18±0.24$^{*,+,\#}$	<0.001
Serum creatinine (g/dl)	0.80±0.1	0.70±0.08	0.93±012^{+}	1.5 0.75$^{*,+,\#}$	<0.001
Blood urea	27.09±5.98	19.33±3.34*	27.96±5.78^{+}	63.38±34.90$^{*,+,\#}$	<0.001
eGFR	124.30±4.7	95.59±4.40*	79.21±11.4$^{*,+}$	49.32±20.1$^{*,+,\#}$	<0.001
ACR (mg/g)	10.14±5.62	7.30±4.15	67.9±48.8$^{*,+}$	1209.9±604.5$^{*,+,\#}$	<0.001
IgGCR (mg/g)	3.78±1.86	89.58±42.51*	233.3±46.7$^{*,+}$	469.14±76.4$^{*,+,\#}$	<0.001

ACR, albumin creatinine ratio; DM, diabetic mellitus; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; HTN, hypertension; IgGCR, immunoglobulin G creatinine ratio; RBS, random blood sugar. $^{*}P<0.05$ when compare with group I. $^{+}P<0.05$ when compare with group II. $^{\#}P<0.05$ when compare with group III.

Results

Tables 1 and 2 showed that there were high statistical significant differences between different groups regarding all demographic and clinical data, whereas there were no statistical significances regarding sex and BMI.

Table 3 showed that there were high significant positive correlations of IgGCR with age, duration of diabetes mellitus (DM), serum creatinine, blood urea, ACR, and glycated hemoglobin. There were statistically high significant negative correlations of IgGCR with hemoglobin, eGFR, and serum albumin.

Table 4 shows the factors predicting IgGCR were studied, and the only significant predicting progression factors of diabetic nephropathy (DN) were duration of DM, urinary ACR, and eGFR.

Discussion

Diabetic nephropathy is more prevalent among Asians, Native Americans, and African Americans than among whites. Hypertension, impaired control of blood sugar, duration of diabetes, and smoking are the main risk factors for DN [6].

IgG is the most common type of antibody found in the circulation. Approximately 75% is synthesized and secreted by plasma cells. It has a molecular weight of 150 kDa [7]. Level of urinary IgG represents the extent of serious lesions in the kidney, and it has been suggested that urinary IgG/creatinine ratio might be helpful for selecting patients who have a higher risk of DN and is more sensitive than albuminuria as a marker of diabetic nephropathy [8].

IgGCR showed significant increase in normoalbuminuric diabetics with increased eGFR than group I diabetics, whereas there was no significant difference between both groups regarding ACR. This is in agreement with Narita *et al.* [9], who found that in early stages of diabetic nephropathy, increased intraglomerular hydraulic pressure causes parallel increase in some urinary proteins such as IgG, ceruloplasmin, and transferrin but does not cause increase in albuminuria. Moreover, Mistry and Kalia [10] found significant increase of U-IgGCR in diabetic patients with no diabetic complications in comparison with healthy participants, and there was further increase in DN. Previously, microalbuminuria has been approved to be a useful biomarker for diagnosis of DN, monitoring its progression, and assessment of its associated conditions as cardiovascular complications [11]. Pure albuminuria may occur if only charge selectivity is lost, as is proposed to be the case in minimal change nephropathy. This condition is not associated with this progressive renal damage [12]. However, increased urinary excretion of larger proteins, like IgG, imply a worse kidney outcome

Table 2 Simple Pearson's correlation between albumin creatinine ratio and the studied parameters

Parameters	ACR	
	r	*P*
Age (years)	0.339	<0.05
Duration (years)	0.748	<0.001*
Hb (g/dl)	–0.556	<0.001*
Serum creatinine (mg/dl)	0.787	<0.001*
eGFR (ml/min/1.73 m^2)	–0.765	<0.001*
Blood urea (mg/dl)	0.721	<0.001*
IgGCR (mg/g creatinine)	0.818	<0.001*
Serum albumin (g/dl)	–0.725	<0.001*

ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; IgGCR, immunoglobulin G creatinine ratio.

Table 3 Simple Pearson's correlation between immunoglobulin G creatinine ratio and the studied parameters

Parameters	IgGCR	
	r	*P*
Age (years)	0.504	<0.001
DM duration (years)	0.825	<0.001
Hb (g/dl)	–0.542	<0.001
Serum creatinine (mg/dl)	0.651	<0.001
eGFR (ml/min/1.73 m^2)	–0.782	<0.001
Blood urea (mg/dl)	0.623	<0.001
Serum albumin (g/dl)	–0.762	<0.001
ACR	0.818	<0.001
HbA1c	0.75	<0.001

ACR, albumin creatinine ratio; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; IgGCR, immunoglobulin G creatinine ratio.

Table 4 Multiple regression analysis for factors predicting immunoglobulin G creatinine ratio

	Unstandardized coefficients		Significance	95% confidence interval for B	
	B	SE		Lower bound	Upper bound
ACR	0.135	0.031	0.000	0.074	0.196
eGFR	1.571	0.733	0.035	3.031	0.112
Duration	8.278	2.505	0.001	3.290	13.265

ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate.

[13] There was a statistically high significant negative correlation of IgGCR and ACR with eGFR. This was supported by Mohan *et al.* [8], who found that high U-IgGCR is significantly associated with decreased eGFR.

Moreover, there was a statistically high significant negative correlation between hemoglobin and both ACR and IgGCR in macroalbumenuric stage. This can be explained by inadequate erythropoietin production and accumulation of uremic toxins in the later stages of diabetic nephropathy, which suppress red blood cell production. This result was supported by Amin *et al.* [14] who found significant decrease in hemoglobin in macroalbuminuric diabetic patients with renal impairment.

The results of the studied population had important statistically high significant negative correlation of IgGCR and ACR with serum albumin. This is owing to albumin loss through the kidney. Amin *et al.* [14] agreed with us as they found significant decrease of serum albumin in macroalbuminuric patients.

This study shows that there was a significant positive correlation between IgGCR and ACR and serum creatinine and blood urea, which goes in harmony with Kundu *et al.* [15] who had found higher serum creatinine in microalbuminuric type 2 diabetics in comparison with normoalbuminuric, and this increase correlated positively with the degree of albuminuria, and Yadav *et al.* [16] who approved that urinary excretion of IgG has linear correlation with UAE.

Regarding the age, there was also significant increase of IgGCR and ACR with increase in age, regarding the DM duration, there was also significant increase of IgGCR and ACR with increase in the duration of DM in macroalbuminuric in comparison with microalbuminuric and normoalbuminuric type 2 diabetic patients. This indicates that longer duration of diabetes has been considered a major risk factor for DN development and progression. This result is supported by Assal *et al.* [17] who found a significant difference between normoalbuminuric and microalbuminuric group regarding duration of diabetes.

In this study, there was no significant difference between four groups of type 2 diabetic patients regarding sex and BMI, which goes in harmony with Aly *et al.* [18] who found no significant difference between type 2 diabetic patients at different stages of diabetic nephropathy regarding BMI. However, Amin *et al.* [14] showed disagreement with our results, as they found significant increase of BMI in macroalbuminuric patients with T2DM than micoalbuminuric and normoalbuminuric patients with T2DM. Their patients were prone to uncontrolled hyperglycemia. This indicates the effect of obesity in development of microvascular complications, and this might be attributed to different study design, number of the patients, or their characteristics.In this work, there was a significant positive correlation between IgGCR and ACR and glycosylated hemoglobin. Moreover, it is significantly increased in type 2 diabetic patients with microalbuminuria compared with patients with normoalbuminuria, which goes in harmony with Kundu *et al.* [15] who studied fifty patients with T2DM and fifty healthy controls and found that diabetics with bad control of blood sugar had higher microalbuminuria in comparison with diabetics with good glycemic control.

Multiple regression analysis for factors correlated with ACR showed that significant factors were serum creatinine and duration of DM only. Multiple regression analysis for factors correlated with IgGCR showed that significant factors were ACR, eGFR, and duration of DM only.

So urinary IgG can be used as a biomarker for early detection of DN in T2DM in normoalbuminuric patients.

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Conflicts of interest
There are no conflicts of interest.

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