Urinary and serum Zinc Alpha 2 Glycoprotein as Novel Biomarker of Early Diabetic Nephropathy

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Abstract: Objective: The aim of the study is to elucidate that urinary and serum ZAG could be used as early biomarkers for DN. **Subjects and Methods**: A total number of 88 participants will be included in this work and we classified them into four groups (each group include 22 subjects). Three patients groups and one control group. Patients groups will include patients with type2 D.M. Diabetic nephropathy notified by urinary albumin/creatnine ratio (UACR) into: normoalbumiuria (UACR < 30 mg/g, *n* = 22, which further divided according to eGFR to 2 sub-groups: normal eGFR group and increased eGFR group >120ml/min/$m^{2,}$ early DN group (*n* =22) with microalbminuria (UACR >30, but < 300 mg/g); overt DN group (DN, *n*=22) with macro albuminuria (UACR > 300 mg/g); and Control group, selected to be normal healthy volunteers, with no medical diseases (n = 22). Serum and urine concentrations of ZAG were determine linked imunosorbentassay. **Results:** The concentrations of ZAG in serum and urine were both of significantly higher in patient with T2DM compared with concentrations in healthy control subjects and also a statistical significance difference among studied groups in both urinary and serum ZAG is found. Serum ZAG concentration was positively correlated with eG.F.R. Urine ZAG concentration also, was positively corr. elated with UA.CR. Urine concentration of ZAG in the higher eGFR group was higher than that in the normal eGFR group. **Conclusion:** These preliminary findings suggest that urine and serum ZAG might be potentially useful biomarkers for early diagnosis of diabetic nephropathy in patents with T2DM.
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Key words: Zinc.alpha.2-glycoprotein, diabetic nephropathy, albumnuria, biomarker

1. Introduction

Diabetes mellitus is one of the most challenging health concerns of the 21st century [1]. Diabitic nephropathy (DN) has been the leading cause of chronic kidney disease in patents starting renal replacement therapy [2].

The syndrome " Diabetic nephropathy " was discovered by Britesh physcian Cliford Wilseon **(1906-1997)** and Germen-born American Paul Kimmelstiel **(1900-1970)** and was published for the first time in 1936 [3]. Diabetic nephropathy is a microvescular complication 1 and type 2 diabetes, which is associated renal disease (ESRD) [2].

The developmental clinical nephropathy is insidious, and albuminuria [urinary albumin excretion rate>30.0 mg/2.4 hours], mark contagion, is preceded by a phase of microalbuminuria (UA.E 30–300 mg/24 hours), which usually lasts 5 to 10 years. As al.buminuria worsens and blood pressure increases, there are decline in GFR and progress to ESRD [3]. Microalbumuria is considered as the best invasive valuable marker for DN risk at present, but which has in adequate spasticity and sensitivity [5]. Progress renal functions decline in diabetes is an early event that occurs in a proportion of patents with increased albumin excretion rate [6]. Moreover, microalbumnuria is not merely a predictor of diabetic nephropathy renal damage. Therefore, other biomarkers for estimation of renal function have been researched for that.

2. Materials and Methods

This case control study has been carried out in internal medicine department in collaboration of clinical pathology department, Faculty of Medicine, Zagazig University, during the period from February 2017 to February 2018. A total number of 88 subjects were included and were classified into four main groups (22 subjects, each). Three patients' groups included patients with type2 D.M, and divided according to urinary albumin/creatinine ratio (UACR) into: normo-albuminuria (UACR < 30 mg/g, *n* = 22, which further subdivided according to eGFR to 2 sub-groups: normal eGFR group patients and increased eGFR group >120ml/min/$m^{2,}$ early DN group (*n* =22) with microalbuminuria (UACR >30, but < 300 mg/g); overt DN group (DN, *n* = 22) with macro albuminuria (UACR > 300 mg/g); and Control group, selected to be normal healthy volunteers, with no history of D.M or other medical diseases (n = 22). All groups will match age, sex and body mass index. *Exclusion critieria*: all subjects were selected to be free from hepatic diseases, heart failure, thyroid disorders, autoimmune diseases, sepsis, inflammatory conditions, and malignancy, renal impairment of

known origin, urinary tract infection, past History of rapidly progressive renal failure, any type of Glomerulonephritis and patient with polycystic kidney.

Methods: All subjects in the study were subjected to the following; Full history and thorough clinical examination and Abdominal ultrasound. Routine investigations: Were done included: Urine analysis, complete blood picture. Chemical investigations; Fasting plasma glucose level, bilirubin (total and direct), s. albumin, ALT, AST, serum creatinine, urea, uric acid, serum total cholesterol, serum triglycerides, HDL- cholesterol and LDL-cholesterol calcium and phosphorus. serum sodium, potassium. HbA1c. Calculation of eGFR using MDRD equation: eGFR (mL/min/1.73 m2) = 175 x $(Scr)^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female) [7]. UACR: The urine creatinine value was divided by 100 to convert mg/dL to g/L and then divide the urine albumin value by the urine creatinine value to ACR (mg/g) = Urine albumin (mg/L) x100/Creatinine in urine (mg/dl). Express ACR as (mg albumin/g creatinine) [8]. Specific investigations: Included Measurement of urinary and serum Zinc-alpha-2-glycoprotein by human AZGp1 (Zinc-alpha-2-glycoprotein) ELISA Kit (Spanbiotec, Bantian industrial park, Guandong, China). For the quantitative determination of human α2GP concentrations. Principle of the Assay: is a sandwich ELISA technique, anti-Zα2GP antibody coat microtiter plate, make solid-phase antibody, then add α 2GP to wells, and a bioten conjugated detection were added to the wells, after wash, HRP-Streptavidin was added and excess conjugate was removed by wash, then TMB substrate was used to visualize the reaction. The concentration of α 2GP in the samples is measured by the standard curve.

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0.

3. Result

Table (1): comparison among studied groups regarding demographic data and investigations.

Variable	Group I (n=22)		Group II (n=22)		Group III (n=22)		Group IV (n=22)		F	p
Age (years) Mean ± SD Range	51 ± 6.91 40 – 62		51 ± 6.29 42 – 64		51 ± 5.76 43 – 62		51 ± 6.62 40 – 61		0.01	0.99 NS
Weight (Kg): Mean ± SD Range	79.59 ± 9.42 62 – 95		81.18 ± 9.12 62 – 95		80.95 ± 6.59 71 – 93		80.36 ± 7.65 68 – 94		0.16	0.92 NS
Variable	**No**	**%**	**No**	**%**	**No**	**%**	**N**	**%**	**χ^2**	**P**
Sex Male Female	11 11	50 50	12 10	54.5 45.5	13 9	59.1 40.9	13 9	59.1 40.9	0.51	0.92 NS
Smoking: No Yes	17 5	77.3 22.7	15 7	68.2 31.8	14 8	63.6 36.4	16 6	72.7 27.3	1.09	0.78 NS
Duration (years) Mean ± SD Range	a 4.59 ± 0.95 3 – 6		b 7.91 ± 0.97 6 – 10		c 12.68 ± 2.82 9 – 20		-------		**111.62**	**<0.001****
SBP (mmHg): Mean ± SD Range	a 119.05 ± 7.5 100 – 130		a 120.45 ± 5.75 110 - 130		a 123.85 ± 5.01 120 – 140		a 119.09 ± 7.26 110 – 130		2.86	0.052 NS
DBP (mmHg): Mean ± SD Range	a 78.45 ± 7.75 70 – 90		a 79.55 ± 4.75 70 – 90		a 82.09 ± 3.84 80 – 100		a 77.18 ± 6.95 70 – 80		2.63	0.06 NS
Hb: (gm/dl) Mean ± SD Range	a 12.64 ± 0.94 11 – 15		a 12.11 ± 2.64 1 – 14		a 12.61 ± 1.07 11 – 14.5		a 12.66 ± 1.1 11 – 14.5		F 0.59	0.62 NS
WBCs: ($\times10^3$/ul) Mean ± SD Range	a 6.46 ± 1.63 4 – 10		a 7.28 ± 1.51 4.8 – 10		a 6.98 ± 2.12 4 – 10.5		a 7.26 ± 1.69 5.9 – 11		F 1.13	0.34 NS
Platelets: ($\times10^3$/ul) Mean ± SD Range	a 276.36±59.65 150 – 350		a 277.73 ± 72.5 170 – 420		a 313.64 ± 76.67 175 – 430		a 309.86±80.27 185 – 430		F 1.68	0.18 NS
ESR: (mm/Hr) Mean ± SD Range	14.77 ± 4.96 5 – 23		14.95 ± 5.92 5 – 24		18.14 ± 4.42 11 – 25		16.59 ± 6.04 7 – 25		K 1.89	0.14 NS
Albumin: (g/dL) Mean ± SD Range	a 4.15 ± 0.54 3.1 – 5.1		a 4.19 ± 0.58 3.4 – 5.2		b 3.31 ± 0.08 3.2 – 3.4		c 4.53 ± 0.66 3.5 – 5.6		F 22.06	<0.001**
T.protein: (g/dL) Mean ± SD Range	a 7.12 ± 0.52 6.5 – 8.2		a 7.21 ± 0.54 6.5 – 8.2		b 6.39 ± 0.09 6.3 – 6.5		a 7.26 ± 0.55 6.3 – 8.1		F 16.85	<0.001**
AST: (u/L)	a		a		a		a		K	0.9

Variable	Group I (n=22)	Group II (n=22)	Group III (n=22)	Group IV (n=22)	F	p
Mean ± SD Range	26.55 ± 7.33 15 – 38	27.41 ± 7.58 17 – 40	27.59 ± 7.53 15 – 38	25.95 ± 9.44 11 – 39	0.2	NS
ALT: (u/L) Mean ± SD Range	a 29.91 ± 10.53 11 – 45	a 31.23 ± 12.77 10 – 52	a 29.23 ± 15.08 2 – 53	a 35.45 ± 10.17 19 – 50	K 1.14	0.34 NS
T.bilir.: (mg/dl) Mean ± SD Range	a 0.88 ± 0.18 0.5 – 1.1	a 0.88 ± 0.19 0.4 – 1.2	a 0.88 ± 0.19 0.5 – 1.2	a 0.85 ± 0.20 0.4 – 1.2	F 0.19	0.9 NS
FBS: (mg/dl) Mean ± SD Range	83.14±18.79 8 – 100	87.91±8.77 73 – 100	85.64±8.08 75 – 100	88.41±9.26 73 – 100	F 0.88	0.45 NS
PPBS: (mg/dl) Mean ± SD Range	126.5±7.65 115 – 140	126.14±7.7 111 – 140	126.27±9.4 112 – 140	128.27±10.99 110 – 146	F 0.27	0.85 NS
HbA1c: (%) Mean ± SD Range	4.75 ± 0.86 3.5 – 6.5	4.73 ± 0.98 2.5 – 6	4.64 ± 0.97 3 – 6	4.8 ± 1.03 3 – 6	F 0.11	0.96 NS
Cholesterol: (mg/dL) Mean ± SD Range	124 ± 24.3 80 – 172	142.95 ± 28.29 88 – 185	141.14 ± 29.17 86 – 198	137.27±33.14 83 – 184	F 1.96	0.13 NS
TG: (mg/dL) Mean ± SD Range	121.64±17.71 90 – 150	121.41 ± 15.25 90 – 148	129.32 ± 14.76 93 – 150	128.09±16.53 99 – 150	F 1.48	0.23 NS
HDL: (mg/dL) Mean ± SD Range	65.81 ± 14.54 6 – 79	66.45 ± 5.51 60 – 80	69.45 ± 6.92 60 – 82	69.73 ± 7.55 60 – 86	F 1	0.4 NS
LDL: (mg/dL) Mean ± SD Range	97.14 ± 21.38 64 – 130	102.27 ± 21.19 64 – 135	103.23 ± 24 63 – 139	106.82 ± 21.2 64 – 133	F 0.73	0.54 NS
CRP: (mg/dl) Mean ± SD Range	5.09 ± 2.29 2 – 9	5.68 ± 2.15 2 – 9	5.32 ± 2.44 2 – 9	5.32 ± 2.17 2 – 9	K 0.26	0.86 NS
GFR: (ml/min) Mean ± SD Range	a 124.09±11.23 100 – 137	b 98.86 ± 3.85 90 – 106	c 82.36 ± 4.87 74 – 91	d 108.73 ± 7.11 96 – 118	**125.43**	**<0.001****
BUN: (mg/dl) Mean ± SD Range	a 12.36 ± 3.85 7 – 20	a 14.86 ± 4 8 – 20	a 13 ± 4.17 7 – 20	a 14.05 ± 4.19 7 – 20	1.64	0.19 NS
S.Uric acid: (mg/dl) Mean ± SD Range	a 4.63 ± 1.03 3 – 6.5	a 4.71 ± 1.07 3.4 – 6.7	a 4.97 ± 1.17 3.4 – 7	a 5.28 ± 1.09 3.5 – 7.1	1.59	0.2 NS
S.Creatinine: (mg/dL) Mean ± SD Range	a 0.85 ± 0.11 0.6 – 1	b 0.91 ± 0.12 0.8 – 1.1	c 0.93 ± 0.11 0.8 – 1.1	b 0.86 ± 0.12 0.7 – 1	2.48	007 NS
Albu.creat.ratio: (mg/g) Mean ± SD Range	a 20.72 ± 5.37 10.8 – 28	b 77.74 ± 28.93 40 – 146	c 383.55 ± 61.59 314.9 – 530	a 20.4 ± 5.11 10.8 – 29.1	**569.05**	**<0.001****
S.Na: (mEq/L) Mean ± SD Range	a 138.18 ± 2.17 135 – 142	a 137.27± 2.57 134 – 142	a 137.59 ± 3.17 134 – 143	a 138.41 ± 2.65 135 – 144	0.85	0.47 NS
S.K: (mmol/L) Mean ± SD Range	a 4.05 ± 0.42 3 – 4.8	a 4.11 ± 0.48 3.5 – 4.9	a 4.03 ± 0.43 3.4 – 4.8	a 4.05 ± 0.49 3.4 – 5	0.12	0.95 NS
S.Ca: (mg/dl) Mean ± SD Range	a 9.41 ± 0.37 8.9 – 10	a 9.5 ± 0.38 8.9 – 10.1	a 9.47 ± 0.36 8.9 – 10	a 9.32 ± 0.33 8.9 – 10	1.11	0.35 NS
S.Po4: (mg/dl) Mean ± SD Range	a 3.63 ± 0.64 2.6 – 4.6	a 3.46 ± 0.7 2.5 – 4.7	a 3.51 ± 0.64 2.6 – 4.6	a 3.58 ± 0.68 2.5 – 4.6	0.28	0.84 NS
Variable	**No** **%**	**No** **%**	**No** **%**	**No** **%**	**χ^2**	**p**
eGFR: <90 90 – 120 > 120	0 0 8 36.4 14 63.6	0 0 22 100 0 0	19 86.4 3 13.6 0 0	0 0 22 100 0 0	**119.7**	**<0.001****
Urinary ZAG: (mg/g) Mean ± SD Range	a 36.86 ± 3.76 28 – 41	b 46.09 ± 2.31 42 – 50	c 56.73 ± 2.62 54 – 63	d 26.91 ± 2.41 19 – 30	444.93	<0.001**
Serum ZAG: (mg/l) Mean ± SD Range	a 24.55 ± 1.68 22 – 28	b 32.23 ± 2.11 29 – 36	c 40.82 ± 1.89 38 – 44	d 20.27 ± 1.52 18 – 23	545.43	<0.001**

Our results recorded that no difference between age and weight among studied groups. Also, there were no difference among studied groups regarding sex distribution and frequency of smoking. There was statistical significance differences between the studied groups in duration of DM also there was an increase in both SBP and DBP in group III compared to other groups but with no statistical significance. There were no statistical significance differences among studied groups regarding CBC (Hb, WBCs, platelets) and ESR.

There were statistical significance differences among studied groups regarding albumin and protein level. Regarding serum albumin Group III are statistically decreased as compared to other groups. Group IV are statistically higher than other groups. also Group III show statistical significance decrease in protein level as compared to other groups. There were no statistical significance differences among studied groups regarding FBS, PPBS, HbA1C, lipid profile & CRP.

Table (2): Correlation between urinary and serum ZAG and age, body weight, BP, duration of DM and lab. Investigation of the three cases groups included in the study.

Variable	Urinary ZAG (n=66)		Serum ZAG (n=66)	
	r	P	r	P
Age (years)	0.06	0.63 NS	0.002	0.98 NS
Weight	0.13	0.31 NS	0.07	0.56 NS
Duration of DM (years)	**0.88**	**<0.001****	**0.86**	**<0.001****
SBP (mmHg)	0.23	0.21 NS	0.20	0.26 NS
DPB (mmHg)	0.27	0.18 NS	0.26	0.19 NS
Hb (g/dL)	0.1	0.42 NS	0.09	0.48 NS
WBCs ($\times 10^3$/uL)	0.12	0.33 NS	0.16	0.2 NS
Platlets ($\times 10^3$/uL)	0.19	0.34 NS	0.21	0.29 NS
Albumin (g/dL)	**-0.51**	**<0.001****	**-0.52**	**<0.001****
Total protein (g/dL)	**-0.49**	**<0.001****	**-0.47**	**<0.001****
AST (u/L)	0.04	0.77 NS	0.15	0.23 NS
ALT (u/L)	0.005	0.97 NS	-0.04	0.75 NS
Total bilirubin (mg/dL)	0.08	0.52 NS	0.04	0.77 NS
FBS: (mg/dl)	0.04	0.74 NS	0.03	0.84 NS
PPBS: (mg/dl)	-0.02	0.9 NS	-0.02	0.99 NS
HbA1c: (%)	-0.08	0.51 NS	-0.02	0.86 NS
Cholesterol: (mg/dL)	0.25	0.06 NS	0.24	0.06 NS
TG: (mg/dL)	0.19	0.13 NS	0.22	0.07 NS
HDL: (mg/dL)	0.21	0.09 NS	0.18	0.15 NS
LDL: (mg/dL)	0.19	0.13 NS	0.12	0.34 NS
ESR: (mm/Hr)	0.27	0.03 NS	0.27	0.03 NS
CRP: (mg/dl)	0.08	0.52 NS	0.008	0.95 NS
GFR: (ml/min)	**-0.78**	**<0.001****	**-0.87**	**<0.001****
BUN: (mg/dl)	0.1	0.41 NS	0.02	0.9 NS
Uric acid: (mg/dl)	0.16	0.2 NS	0.12	0.34 NS
Creatinine: (mg/dL)	0.15	0.23 NS	0.08	0.46 NS
Albu.creat.ratio: (mg/g)	**0.86**	**<0.001****	**0.89**	**<0.001****
Na: (mEq/L)	-0.02	0.86 NS	-0.03	0.8 NS
K: (mmol/L)	0.01	0.92 NS	-0.03	0.82 NS
Ca: (mg/dl)	0.14	0.27 NS	0.07	0.56 NS
Po4: (mg/dl)	-0.08	0.54 NS	-0.04	0.77 NS
Urinary ZAG: (mg/g)	-----	------	**0.93**	**<0.001****
Serum ZAG: (mg/l)	**0.93**	**<0.001****	------	-----

There were statistical significance differences among studied groups regarding estimated GFR and albumin/creatinine ratio. Regarding eGFR There were statistical significance differences among all groups. Regarding Albumin creatinine ratio, Group III and Group II show statistical significance increase as

compared to other groups. Also there were statistical significance differences among studied groups in urine analysis with increase concentration of albumin in urine among Group II & III as compared to other groups.

There were statistical significance differences among all studied groups regarding both urinary and serum ZAG. There was a statistical significance positive correlation between both urinary and serum ZAG and duration of DM, albumincreatinine ratio and with each other. But –ve significant correlation found between both urinary and serum ZAG and (albumin, total protein and eGFR).

There was a statistical significance increase in serum and urinary ZAG in cases with eGFR >120 ml/min in Group I. There was a statistical significance increase in Urinary ZAG in cases with eGFR > 120 ml/min. The accuracy of urinary ZAG was 95.5%, and that of serum ZAG was 90.9% and both of them was 95.5%. The accuracy of urinary and serum ZAG and both of them was 100%.

Table (3): Validity of Urinary and serum ZAG in prediction of albuminuria:

Variable	Cutoff	AUC	Sens.	Spec.	+PV	-PV	Accuracy	p-value
Urinary ZAG	≥28.5	0.99	98.5	86.4	95.6	95	95.5	<0.001**
Serum ZAG	≥22.5	0.99	97	72.7	91.4	88.9	90.9	<0.001**
Both	--	0.99	97	90.9	96.9	90.9	95.5	<0.001**

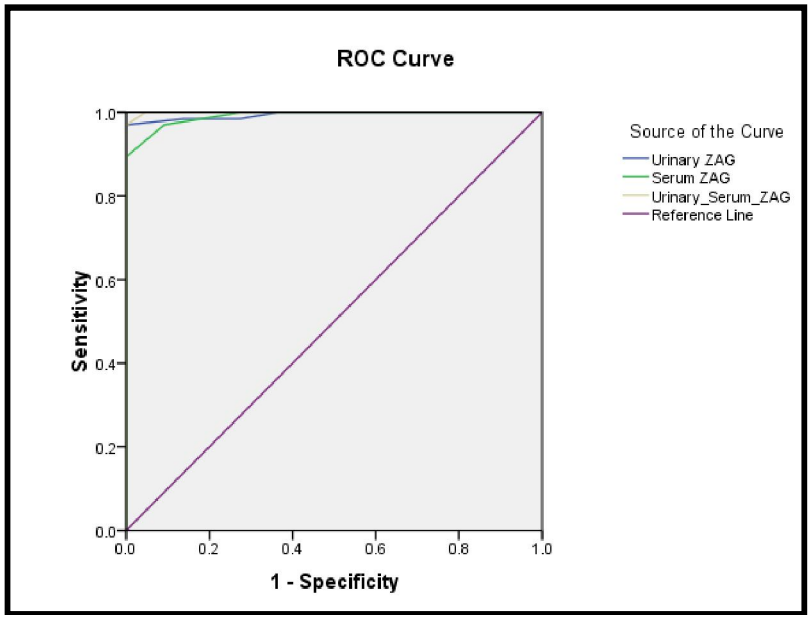

Figure (1): Roc curve for Validity of Urinary and serum ZAG in prediction of albuminuria.

Table (4): Relation between urinary and serum ZAG and eGFR in Group I and Group III:

Variable	Group I		t	p
	GFR 90 - 120 (n=8)	GFR >120 (n=14)		
Urinary ZAG: (mg/g) Mean ± SD Range	 32.63 ± 2.77 28 – 36	 39.29 ± 1.14 37 – 41	7.99	<0.001**
Variable	**Group III**		**t**	**p**
	GFR <90 (n=19)	**GFR 90 - 120 (n=3)**		
Urinary ZAG: (mg/g) Mean ± SD Range	 56.63 ± 2.69 54 - 63	 57.33 ± 2.52 55 - 60	0.42	0.78 NS

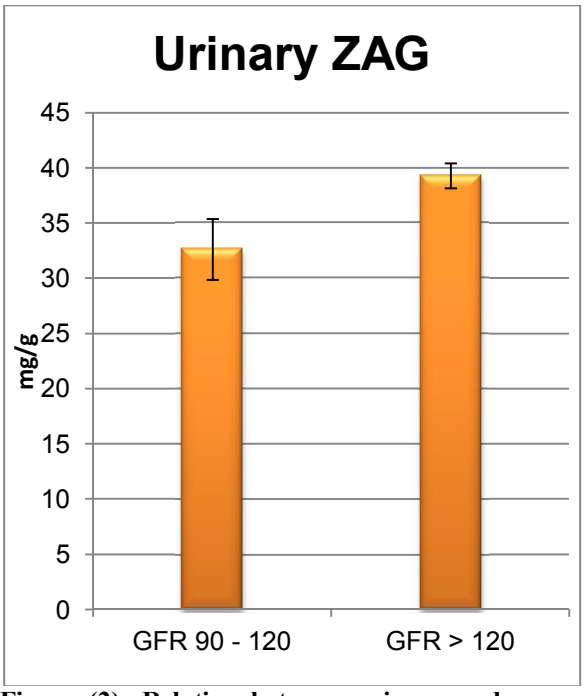

Figure (2): Relation between urinary and serum ZAG and GFR in Group I.

4. Discussion

A Diabetes is a major cause of chronic kidney disease (CKD) [9]. The routine evaluation of diabetic nephrophy includes appearance of microalbumnuria, decreased creatine clearance and increased serum creatine [10]. But, it has been reported that a decline in renal function of patents with diabetes was not always accompanied by an increased ACR [11].

Moreover, tubular involvement may precedeglomerular involvement before the appearance of microalbumuria and a rise in serum creatinine [12].

Zinc alpha2 glycoprotein (ZAG) is a 41–43 kDa glycoprotein assigned to the major I family of proteins [13,14]. ZAG is present in epithelia and is secreted into the body [15].

Our study aimed at detection of plasma level of zinc alpha 2 glycoprotein and urinary soluble ZAG at early stages and in combination for early detection of diabetic nephropathy in type 2 diabetic patients.

In this study there was no significant differ between groups regarding age and body mass index, which might exclude the effect of these factors on the result of urinary and serum ZAG in our study.

There were differences between the studied regarding duration of DM, which harmony with Kundu et al., [16]; Assal et al., [17] and Al-Agha et al., [18] who found a significant difference between normo and microalbuinuric group of type 2 diabetes regarding duration of diabetes. There was no statistical significance in both SBP and DBP between studied groups, as we select patients with controlled blood pressure.

Also in our study it is found that there were no differences between the studied groups as regard hemoglobin, WBCs, platelets and ESR, which may exclude the effect of changes in hemoglobin, WBCs, platelets and ESR on the result of urinary and serum ZAG.

In the pathophysiology, Proteinuria, a marker and potential contributor to renal injury, accompanies diabetic nephropathy. Increased glomerular permeability will allow plasma proteins to escape into the urine, Viswanathan. [19] found that Serum albumin was significantly lower in macroalbuminuric group of diabetic nephropathy.

But the current work didn't find any statistical significance difference among studied groups regarding lipid profile. Also there were no statistical significance differences among studied groups regarding Blood sugar parameters, as they we select patients controlled on medication. Also no statistical difference found regarding CRP.

Our study showed no significant difference between all groups regarding serum creatinine, but there were statistical significance differences regarding eGFR and albumin/creatinine ratio. Albumin creatinine ratio in Group III and Group II show a statistical significance increase compared to other groups. This result came in agreement with Chae et al. [20] who documented a significant difference between both diabetic groups as regarding previous parameters. Jeon et al., [21] reported that progression of diabetic nephropathy accompanied by declining GFR and increasing urinary albumin excretion.

It is found that a significant difference between control and normo-albuminuric as regard glomerular filtration rate (ml/min/1.73m²) by MDRD. These results are supported by a lot of studies as Murussi et al., [22]; Tidman et al., [23] and Gunzler et al., [24]. This can be explained by the pathogenesis of diabetic nephropathy as there is hyperfiltration in stage 1 due to an imbalance in afferent and efferent arteriolar resistance, resulting in increased glomerular hydrostatic pressure and hyperfiltration. [25.26].

As regard GFR, Lu et al., [27] and Dwyer et al., [28] support our study as they found significant difference between control and normo-albuminuric as regard GFR by MDRD being less in the later group. On the other hand our study found significant differences among different groups of the study regarding GFR by MDRD between control and micro-albuminuric groups and between normo-albuminuric and micro-albuminuric groups, which come in agreement with Lorenzo et al who reported that albuminuria is the strongest risk factor for faster annual eGFR decline in

153 Caucasian patients with type 2 diabetes with a baseline eGFR <50 ml/min per 1.73 m^2 during a 2.5-year follow-up [29].

The decline in GFR in macro-albuminuric group could be explained by American Diabetes Association [30] as albuminuria has long been regarded as a marker of the extent of glomerular damage; however, experimental and clinical studies suggest that albuminuria might also contribute to the development and progression of glomerular and tubule-interstitial lesions.

There were statistical significance differences among studied groups regarding urine analysis with increase concentration of albumin in urine among Group II & III as compared to other groups.

In the present study a statistical significance differences among studied groups in both urinary and serum ZAG is found. Regarding urinary ZAG, we can explain its increase in diabetic groups by the fact that The subjected to prolonged exposure to various metabolic and haemodynaic perturbations [31]. In chronic cases of diabetic nephropathy, renal function better the degree of tubulointstitial injury than glomerular lesions [31,32].

As ZAG is mainly expressed in the proximal convoluted [33]. In the present study might be indicative of the tubular damage. The ZAG expression is increased in the proximal tubular cells of aged mice [34].

It is unclear in the urine or actively secreted by the tubular epithelial cells. Wang et al., [35] found that the concentration of ZAG in urine was higher than that inserum, especially in patients with T2DM. Recent studies performed by Rao et al. demonstrated that urine ZAG levels were progressively increased across three categories of diabetic patients with normomcro and macroalbuminria by a robust 2-D DIGE approach coupled with LCMS/MS in Indian T2DM patents, indicating that it is positively related with diabetes nephropathy progression [36]

Moreover, studies performed by Jain et al. also indicated the appearance of ZAG in the albumin negative urine samples subsequently preceded the appearance of albumin in T2DM patients of South Asian Indians, suggesting that ZAG may be an earlier novel urinary biomarker useful for the screening of nonalbuminuric diabetic nephropathy [37].

Interestingly, a study performed on Singapore Chinese T2DM patients using the urinary proteomics approaches found that urinary ZAG levels were significantly higher in T2DM patients with persistently normal renal function (eGFR > 60 ml/min 1.73m2 and urinary albumin-creatinine ratio < 30 mg/g) than those with mildly renal dysfunction (consistently, eGFR ≤ 60mls/min 1.73m2 and urinary albumin-creatinine ratio < 30 mg/g) (arbitrary unit ± standard error, 12.7 ± 1.5 versus 6.6 ± 1.3, P = 0 009), implying that urinary ZAG level is also increased in diabetes patients with normoalbuminuria when they only have eGFR less than 60 ml/min 1.73m2[38].. Also supported by Wang et al., [35] who documented that ZAG might be a potentially useful biomarker for early diagnosis of diabetic nephropathy in patients with T2DM. All of these studies suggest that urine ZAG levels not only would be an earlier biomarker for diabetic nephropathy but also are associated with the renal dysfunction.

Xu et al., [39] firstly found high serum ZAG levels was higher in patients with mildly decreased eGFR (<90 ml/min) than ZAG levels, and the probability of the eGFR < 90 ml/min in patients with the high ZAG levels was 94% higher than ZAG levels after adjusting for age gender, and education, This phenomenon was more likely to happen in female patients with higher uACR (≥2.7mg/mmol) and bigger waist circumference (≥90cm for men or ≥85cm for women).

This result suggests that serum ZAG levels (in addition to urine ZAG levels) are more likely to be increased in female T2DM patients with diabetic nephropathy even if they only have mildly decreased eGFR (<90 ml/min), an earlier predictor than what is reported in previous report (eGFR less than 60 ml/min 1.73m2) [38]

Few studies showed that serum ZAG levels were evidently elevated in chronic hemodialysis (CH) patients, suggesting a decrease of its renal clearance [40,41].

A study performed in the adult patients with chronic kidney disease (CKD) also demonstrated that ZAG concentration sharply increased in CKD 5, CH, and peritoneal dialysis patients, implying that the kidney could play an important role in the maintenance of serum ZAG levels [42].

Normally, a fragment of serum ZAG passes through the glomerular membrane after which it is completely reabsorbed at the tubular level due to its rather small molecular weight (43 kDa) and size (Stokes radius 3.24 nm). Therefore, renal function impairment with decreased GFR results in a rise in ZAG/creatinine clearance ratio [43]., as what we observed in our present investigation in T2DM with mildly eGFR decrease. Another explanation for the increased serum ZAG concentration may come from the increased secretion and production of ZAG from epithelia cells from liver, kidney, breast, sweat glands, and gastrointestinal tract, as well as white adipose tissue (WAT) [39].

Gohda et al. reported that the ZAG mRNA levels in the liver and kidney were significantly increased in KK/Ta mouse, a spontaneous animal model of type 2 diabetes [44]. Selva et al. demonstrated that there was a significant positive correlation between ZAG serum

levels and mRNA levels of ZAG in subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and liver, implying that both adipose tissue and liver seem to be the important contributors to ZAG systemic levels [45].

Taking all of these studies into consideration, serum ZAG concentration seems to be influenced by both the renal elimination and the secretion from organism tissues. Furthermore, markers of renal function such as eGFR should be considered in studies investigating the physiology and regulation of ZAG. The physiologic significance of increased ZAG serum concentrations in T2DM with mildly eGFR decrease remains to be elucidated.

In our study there was statistically significant positive correlation between both urinary and serum ZAG and duration of DM, albumin creatinine ratio and with each other. Also –ve significant correlation between both urinary and serum ZAG and albumin, total protein and GFR, in contrary to Pelletier et al., [42]. who found an inverse relationship between ZAG levels and plasma protein. Also it is found a statistical significance increase in urinary ZAG in cases with GFR >120 ml/min in Group I. This was supported by Wang et al., [35] who documented the same results. We can explain this result, ZAG may be easily filtered, increase GFR will lead to increase filtration of ZAG thus increase its concentration in urine this may explain hyperfilteration stage in first stage of diabetic nephropathy. The serum ZAG concentration was positively correlated with eGFR but not with glucose, cholesterol, triglycrides, hsCRP or BMI which come in agreement with Wang et al., [35] who reported the same result.

The urine ZAG concentration was positively correlated with albuminuria and negatively correlated with eGFR. There was no relationship between ZAG conc. serum creatine, BMI, hsCRP and age and this was supported by Wang et al., [35]. The urine concentrations of ZAG were significantly increased in patents with T2DM compared with healthy control subjects.

This present study had some limitations, the absence of renal biopsies prevented the accurate diagnosis of diabetic nephropathy and the immunohistochmical evaluation of the levels of ZAG in the kidney.

5. Conclusion:

Our preliminary information suggested that ZAG may be a urinary and serum biomarker for the nonalbuminuric variant of diabetic nephropathy.

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