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ORIGINAL ARTICLE

Study of Urinary Angiotensinogen as an Indicator of Severity of Diabetic Nephropathy in Type 2 Diabetes

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ABSTRACT

Background: Urinary angiotensinogen levels are higher in type 2 diabetes patients with nephropathy, a risk factor for developing renal and cardiovascular consequences. However, the potential relationship between serum urine angiotensinogen levels and albuminuria (in terms of its varying concentrations) is not yet established. As a result, we conduct this research. **Methods:** Sixty-six adults with type 2diabetes were split into three groups, each of which included 22 people based on their degree of albuminuria, in addition to an age- and gender-matched control group of 22 healthy volunteers. Routine laboratory tests and urine angiotensinogen were evaluated in the study groups and correlated to other study parameters. **Results:** The current study showed that urine angiotensinogen levels are more significant in diabetic patients with higher albuminuria levels, and it was also found to be associated with urinary albumin excretion in diabetic patients with different grades of albuminuria ($n = 66$, $r = 0.666$, $p<0.001$), and it had highly significant validity in the prediction of albuminuria among individuals with diabetes with sensitivity of 88.6%, specificity of 81.8% and accuracy of 86.4%. **Conclusions:** According to the findings of this study, increased urine angiotensinogen levels were associated with urinary albumin excretion, and elevated urinary angiotensinogen might be utilized as an effective marker for identifying the degree of albuminuria in diabetic individuals.

Keywords: albuminuria; angiotensinogen; nephropathy; diabetes mellitus.

INTRODUCTION

An important worldwide health issue is chronic kidney disease (CKD). It is spreading rapidly around the world [1].Type 2 diabetes mellitus is a public health issue, it affects at least 285 million people globally, with the figure estimated to rise to 438-530 million by 2030 [2]. Egypt is anticipated to be among the top ten countries in the world with the highest diabetes prevalence rates by 2025 [3]. Diabetic nephropathy (DN) is at the top of the list of causes that lead to end stage renal disease, together with hypertension [4].DN is a major diabetes-related complication that has been linked to adverse cardiovascular, renal outcomes with increased fatality. Early care can reduce the loss of kidney function and improve patient outcome, hence, diagnostic biomarkers to identify early DN are crucial. Even before microalbuminuria manifests in diabetic individuals, urine biomarkers may be elevated, and they can be employed as a valuable marker to identify nephropathy in patients with normo-albuminuria (early stages of DN) [5].

The cells of the proximal convoluted tubules produce angiotensinogen (AGT), which is then released from the apical cells into the lumen [6]. Urinary AGT excretion was considerably higher in T2DM patients with albuminuria as compared to

normoalbuminuric patients and control subjects, indicating that AGT first occurred before albuminuria developed. Additionally, there is a significant correlation between albuminuria in diabetic individuals, and AGT levels [7, 8]. According to studies, patients with albuminuria and high urine AGT levels exhibited a continuous deterioration in overall renal function as well as a substantial increase in cardiorenal endpoints. This indicated that a greater level of urine AGT in T2DM patients with diabetes and nephropathy make them at a high risk for deteriorating renal and cardiovascular consequences [9].

In summary, we may claim that the relationship between urine AGT and albuminuria is complex and still poorly understood. Albuminuria, nevertheless, is already recognized as a risk factor for chronic kidney disease [8]. Urinary AGT may be a valuable test for identifying nephropathy in people with normoalbuminuria and other levels of albuminuria. Our aim is to evaluate urinary angiotensinogen as an indicator of diabetic nephropathy and to correlate it with the magnitude of albuminuria in these patients.

METHODS

Study design

This case-control study was conducted in the inpatient unit of the internal medicine department, in collaboration with the clinical pathology department, Faculty of Medicine, at Zagazig University Hospitals between February 2021 and February 2022.

Study Population

Assuming the mean ±SD of urinary angiotensinogen in the diabetic patients' group is (34.6±57.6) and in the control group is (13.3±12.6) and the case: control ratio is 3:1. So the sample size was 88 (22 in each group) at a confidence level of 95% and power of 80% using the Open Epi program.

The history of the patient was combined with laboratory results (either the fasting plasma glucose (FPG) value of 126 mg/dL or the 2-hour plasma glucose during the oral glucose tolerance test (OGTT) of 200 mg/dL or the hemoglobin A1C criterion of 6.5%) to determine the diagnosis of diabetes [10]. We included 88 individuals, which were then divided into two main groups: Control Group (I): Include 22 healthy volunteers with normal renal function and normal urinary albumin excretion. Another group included individuals with T2DM: a total of 66 individuals were divided into three sub-groups according to the state of albuminuria according to Kidney Disease Improving Global Outcome [(11)]: Group (II): included 22 individuals with T2DM with (normal to mildly increased ACR < 30 mg/g). Group (III): included 22 individuals with T2DM with (moderately increased albuminuria ACR 30 - 300 mg/g). Group (IV): included 22 individuals with T2DM with (Severely increased albuminuria ACR >300 mg/g).

Inclusion criteria:

Individuals with T2DM with albuminuria, age > 18 years old, both genders. Also, we included healthy individuals without diabetes of the same age and sex as control subjects.

Exclusion criteria:

Individuals with: type 1 diabetes mellitus (T1DM) or gestational diabetes, active malignancy. severe heart, lungs, or liver disease, stroke. chronic infection, e.g., tuberculosis, within 1 year of starting the study. Albuminuria and CKD due to any cause other than diabetes. Pregnant women. Used drugs that affect RAS. Individuals with acutely increased blood pressure or uncontrolled hypertension. Any immunological or inflammatory disorders.

Ethical clearance:

The study was submitted for approval by the Institution Research Board (IRB) number **(IRB#:6351-24-8-2020)** and the Ethical Committee of the Internal Medicine Department in the Faculty of Medicine before the start of the study. All the methods employed in the current investigation adhered to the Helsinki Declaration's current revision. All participants received information about the study's different aspects, and they were only enrolled after giving a signed consent form. All study participants underwent a comprehensive clinical examination as well as a full history. A comprehensive cardiovascular examination is performed together with a general and local assessment of other systems. A complete blood count (CBC), liver function tests, renal function tests, hemoglobin A1c, and lipid profile were performed routinely in accordance with the clinical pathology and hospital laboratory protocols of Zagazig University Hospital.

Human AGT ELISA kits (Angiotensinogen ELISA Kit; Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan) were used to assess AGT concentrations in the urine. The urine albumin creatinine ratio was used to express the urinary albumin and creatinine concentrations (UACR). By detecting UACR in at least two of the most recent three urine samples, the presence of diabetic nephropathy was identified. According to the previously mentioned levels of UACR and urine protein, patients were classified into three groups.

Statistical analysis:

The collected data were organized, tabulated, and statistically analyzed using the SPSS program (Statistical Package for Social Science) version 27.0 (IBM, 2020). For quantitative normally distributed data, the range, mean and standard deviation were calculated. For quantitative non-normally distributed data, the median was added. In order to present qualitative data, numbers and percentages were used. For qualitative data, the comparison between two groups and more were done using Chi-square test ($\chi 2$) and Fisher Exact test (FE) if appropriate. For comparison between means of the studied groups, ANOVA test was used with Tukey post hock. It was replaced by a non-parametric test, Kruskal-Wallis teat for non-normally distributed data. Spearman's correlation coefficient was used to assess the relationship between the variables. Reliability data were calculated using Sensitivity, Specificity, Positive predicted value, negative predicted value, and Accuracy. The most sensitive and specific cut-off values for several factors were determined using receiver operating characteristic (ROC) curve analysis to predict the outcome. The area under the ROC curve serves as a measure of accuracy. P value of ≤ 0.05 indicates significant results. P value of <0.001 indicates highly significant results.

RESULTS

We included eighty-eight individuals' [30 male (34.1%) and 58 female (65.9%)] in the current study. The mean age of the study participants was 55.87 ± 7.47 years. Patients and controls were age and sex matched (p=0.23 and 0.24, respectively) as mentioned in table 1. As regards individuals with diabetes (group II through IV), the mean diabetes duration was higher in group IV compared to other groups **(table 1).** Twenty-eight (42.42%) patients in the groups with diabetes were on insulin therapy. There was a statistically significant increase in HbA1c among Group IV compared to other groups and among Group II and III compared to Group I. As regard other laboratory data between case and control groups it is summarized in **(table 2 & S table 1)**.

A Kruskal-Wallis H test showed that there was a statistically significant difference in post test scores between the groups, X2(3) = 54.04, p<0.001, with a mean rank AGT of 16.16 for group I, 36.43 for group II, 56.70 for group III, and 68.70 for group IV **(table 3, figure 1).** The correlation between urine AGT, urinary albumin excretion, and other study parameters was tested using suitable correlation analysis. A positive correlation between urinary albumin excretion and urinary AGT in patients with diabetes (n= 66, r = 0.66, P < 0.001), also between serum creatinine and urinary AGT (n= 66, r = 0.626, P < 0.001), a negative correlation between urinary AGT and eGFR (n= 66, r = -0.462, P < 0.001) were determined **(figures 2 and 3).** Other correlation analyses were summarized in **(S table 2).** For the prediction of albuminuria in patients with diabetes, at the best cut-off value of urinary AGT (> 99.87), the sensitivity and specificity were 90.9% and 81.8%, respectively, with an AUC of 86.5% **(Figure 4).**

Table (1): Demographic data among the studied groups

Variable	Group I (n = 22	Group II (n = 22)	Group III (n = 22)	Group IV (n = 22)	Sign.	P value
Age (yr) *Mean ± SD* *Range*	56.86±8.38 42 – 78	54.73±6.21 45 – 67	58.05±7.87 45 – 70	53.82±7.41 40 - 67	***F*** = 1.46	0.23
Sex (No, %) *Male* Female	11 (50%) 11 (50%)	5 (22.7%) 17 (77.3%)	8 (36.4%) 14 (63.6%)	6 (27.3%) 16 (72.7%)	χ^2= 2.25	0.24
Duration (yr) *Mean ± SD* *Median* (IQR) *Range*	——	3.52±1.52[a] 3 (1-7)	7.09±1.93[b] 8 4 – 12	16.05±5.87[c] 16 8 – 28	***KW***= 52.22	**<0.001****

Group I = Control, Group II = T2DM + Normoalbuminuria, Group III = T2DM + Microalbuminuria, Group IV = T2DM + Macroalbuminuria
SD: Stander deviation, KW: Kruskal-Wallis Test, $\chi 2$: Chi square test. F: ANOVA test
NS: Non-significant (P>0.05) *: Significant (P<0.05) **: highly significant (P<0.001)

Table (2): Laboratory findings among the studied groups

Variable	Group I (n = 22	Group II (n = 22)	Group III (n = 22)	Group IV (n = 22)	F/KW	P value
HbA1C: (%)						
Mean ± SD	5.03±0.37[a]	6.61±0.2[b]	6.79±0.32[b]	8.06±0.43[c]	**290.2**	**<0.001** **
Range	4.5-5.7	6.3-6.96	6.1-7.3	7.3-8.8		
FBG: (mg/dl)						
Mean ± SD	101.18±8.4[a]	117±33.06[a]	135.73±44.60[b]	261.14±58.99[c]	**70.64**	**<0.001** **
Range	89-124	87-220	87-202	169-386		
WBC: ($x10^3/mm^3$)						
Mean ± SD	6.86±1.38	6.93±1.37	6.93±1.43	5.71±1.49	2.9	0.07 NS
Range	4.8-11	4.8-10.3	4.8-10.4	3.8-8.9		
HB: (gm/dl)						
Mean ± SD	12.49±1.5[a]	12.78±1.78[a]	12.12±1.33[a]	10.47±1.18[b]	**10.98**	**<0.001** **
Range	8.9-14.6	6.2-14.5	7.7-13.7	8.1-13.4		
Na: (mEq/L)						
Mean ± SD	138.54±4.37	138.96±3.45	138.11±3.51	140.50±4.41	1.52	0.22 NS
Range	134-149.6	133.4-145.4	135-145.7	133.4-145.8		
K: (mmol/L)						
Mean ± SD	3.96±0.42	4.2±0.39	3.84±0.81	4.02±1.07	0.94	0.43 NS
Range	3.5-4.7	3.5-5.1	2-5.2	2.6-8		

SD: Stander deviation, **F**: ANOVA test KW: Kruskal-Wallis test. **NS**: Non significant (P>0.05)
**: Highly significant (P<0.001) Post-hoc: Tukey (Groups with different letters are statistically significant (P<0.05))

Table (3): Angiotensinogen levels among the studied groups

Variable	Group I (n = 22)	Group II (n = 22)	Group III (n = 22)	Group IV (n = 22)	KW	P value
Angiotensinogen**: (ng/mL)**						
Mean ± SD	56.77 ± 23.02 [a]	90.16±29.13 [b]	125.56±30.83 [c]	162.66 ±61.03[d]	**54.04**	**<0.001 ****
Median (IQR)	54.43	86.61	125.64	154.1		
Range	8.1-105.53	37.3-172.27	31.39-216.07	46.83-313.2		

SD: Stander deviation, **KW**: Kruskal-Wallis test.
**: Highly significant (P<0.001)Post-hoc: Tukey (Groups with different letters are statistically significant (P<0.05))

Figure (1): Angiotensinogen level among the studied groups

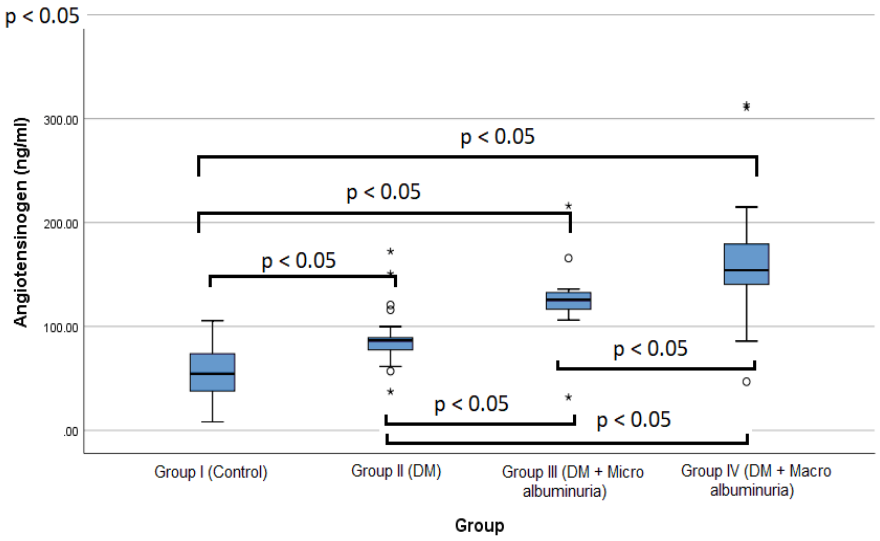


Figure (2): Correlation between angiotensinogen level and eGFR among Cases groups

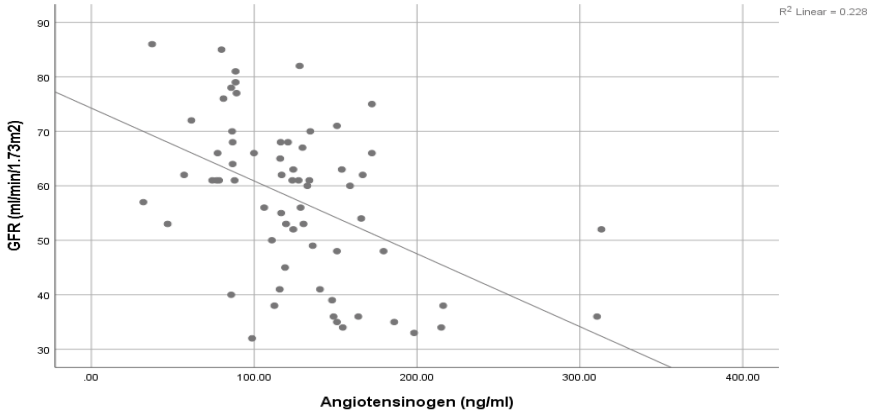


Figure (3): Correlation between angiotensinogen level and uACR among Cases groups

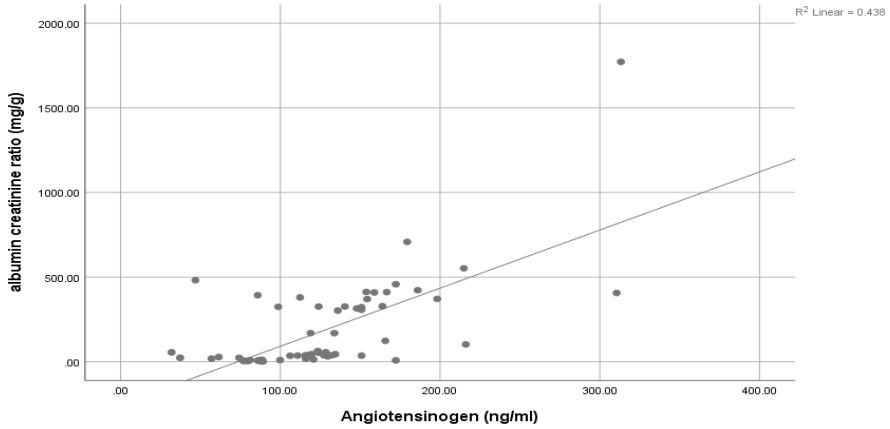
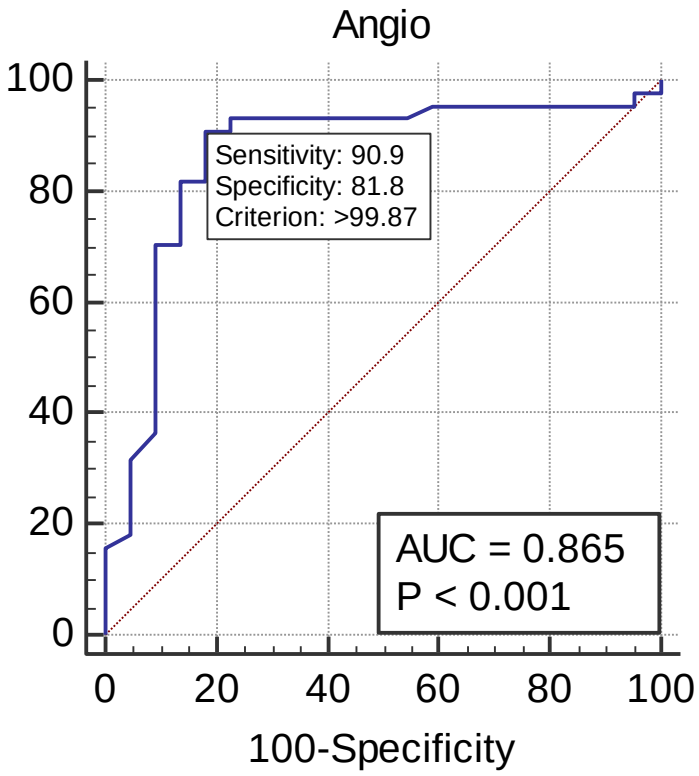


Figure (4): Roc curve for the validity of urinary angiotensinogen in the prediction of albuminuria among the studied case groups



DISCUSSION

A frequent and serious consequence of diabetes mellitus is diabetic nephropathy (DN). Its early detection may allow for rapid interventions and a better prognosis [12]. End-stage renal disease (ESRD) is most frequently caused by diabetic nephropathy (DN), which accounts for 30–47% of those who require long-term dialysis. The presence of albuminuria and/or a decline in eGFR in diabetics define this complex disease [13]. An albumin-to-creatinine ratio (ACR) of more than 30 mg/g is considered pathological albuminuria, according to the American Diabetes Association (ADA) [14]. Due to its high molecular weight, circulating angiotensinogen (AGT), which is mostly produced by the liver, could not pass past the glomerular basement membrane. Therefore, renal proximal tubular cells are the primary source of urine AGT (UAGT) [15, 16].

In experimental type 1 diabetic rats, urine albumin (UALB) increased later than UAGT. Thus, we evaluated the value of elevated UAGT levels as an early indicator of albuminuria in patients with T2DM and whether they could be elevated before the development of albuminuria [17]. According to Kidney Disease Improving Global Outcome [11], we studied the UAGT levels in T2DM patients with or without albuminuria and divided them into: normoalbuminuria (normal to mildly elevated ACR 30 mg/g), microalbuminuria (moderately increased albuminuria ACR 30 - 300 mg/g), and macroalbuminuria (severely increased albuminuria ACR >300 mg/g). This study includes 88 participants, 66 patients with T2DM and 22 healthy volunteers (Group I) enrolled in the study. Our patients were subdivided according to their urinary albumin-creatinine ratio (UACR) into three groups: Group II (n = 22) patients with normoalbuminuria, and Group III (n = 22) patients with microalbuminuria and Group IV (n = 22) patients with macroalbuminuria.

Regarding the assessment of urinary angiotensinogen (UAGT) level, the current study revealed that the mean UAGT levels was 162.66±61.03 ng/mL with a median of 154.1 ng/mL and ranged from 46.93 to 313.2 ng/mL in Group IV versus 125.56±30.83 ng/mL

with median 125.65 ng/mL and ranged from 31.39 to 216.07 ng/mL in Group III versus 90.16±29.13 ng/mL with median 86.61 ng/mL and ranged from 37.3 to 172.27 ng/mL in Group II versus 56.77 ± 23.02 ng/mL with median 54.43 ng/mL and ranged from 8.1 to 105.5 ng/mL in Group I respectively. Statistically, there was a statistically significant increase in UAGT among Group IV compared to other groups, among Group III, compared to Group I and Group II, and among Group II compared to Group I. These findings were in accordance with Lee et al. [18].

According to a study by Satirapoj et al., normoalbuminuric T2DM patients had UAGT levels that were significantly higher than those of healthy controls (7.42 ng/mL, P = 0.048), microalbuminuric T2DM patients (39 ng/mL, P 0.001), and macroalbuminuric T2DM patients (306.42 ng/mL, P 0.001), respectively. Additionally, there were noticeable changes between the UAGT values in each degree of albuminuria. The authors came to the conclusion that AGT might be one of the renal biomarkers for DN diagnosis [19].

A Pearson product-moment correlation coefficient was computed to assess the relationship between UAGT and age, UAGT and HbA1c FBS, ESR, hs-CRP, S. creatinine, S. albumin BUN, GFR and Hb, UAGT and duration of diabetes. The current study demonstrated a positive correlation between HbA1c, FBS, ESR, hs-CRP, S. creatinine, BUN, and UAGT. A positive correlation was also established between the duration of diabetes and UAGT. However, there was a statistically significant negative correlation between S. albumin, GFR, Hb, and UAGT among the studied groups. Moreover, there was no statistically significant correlation between UAGT level and any of the laboratory findings among the healthy control group. HbA1c and UAGT were found to be positively correlated in Sana et al. study (r=0.292, p=0.001). The duration of diabetes and UAGT also showed a positive correlation (r=0.183, p=0.035), demonstrating that UAGT was greater in patients with uncontrolled diabetes (measured by a higher HbA1c value) and diabetes that had been present for a longer period of time [20]. The outcomes reported by Amini et al., and Showail et al. were likewise comparable to this [21, 22].

However, Satirapoj et al. found no association between UAGT levels and age, fasting plasma glucose, or HbA1C. Moreover, UAGT levels were negatively correlated with estimated GFR but positively correlated with significance for the duration of diabetes, BUN, serum creatinine, and urine ACR. After several regression analyses, the only meaningful associations between increased UAGT levels and urine albumin were found. An increase in urine albumin, BUN, and serum creatinine as well as a reduction in estimated GFR often reflect how severe or aggravated renal function is in people with diabetic nephropathy [19].

Regarding the validity of UAGT in the prediction of albuminuria, UAGT had highly significant validity in the prediction of albuminuria among DM cases with a sensitivity of 90.9%, specificity of 81.8%, and accuracy of 86.5%. In general, the increase in urine albumin, the increase in serum creatinine, and the reduction in estimated GFR represent markers for diabetic kidney disease, the presence of such associations with urinary AGT support the hypothesis that its levels are associated with the severity of renal affection in patients with diabetes.

There are some limitations in our study. Our study is retrospective it may represent an inferior level of evidence compared with prospective studies. Second, it was performed at a single institution. However, the relatively large sample size and the inclusion of a control group may represent the strength points.

CONCLUSIONS

The results of this study suggested that elevated urine angiotensinogen levels were related to urinary albumin excretion and might be used as a reliable marker for detecting the degree of urinary albumin excretion in diabetic individuals.

Conflict(s) of interest: None

Financial Disclosures: None

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Supplementary file

Supplementary table (1) Liver and kidney function findings among the studied groups

Variable	Group I (n = 22	Group II (n = 22)	Group III (n = 22)	Group IV (n = 22)	F/KW	P value
AST: (U/L)						
Mean ± SD	24.09±4.78	27.09±6.58	27.64±5.43	26.59±4.80	1.82	0.15
Range	15-35	18-45	20-40	20-40		NS
ALT: (U/L)						
Mean ± SD	35.86±5.34	38.36±5.17	39.41±5.3	37.64±5.16	1.78	0.16
Range	25-45	29-50	25-45	29-45		NS
S. Albu: (mg/dl)						
Mean ± SD	2.68±0.44[a]	2.95±0.33[a]	2.30±0.33[b]	1.56±0.41[c]	**56.22**	**<0.001 ****
Range	1.8-3.6	2.08-3.3	2-2.9	1.05-2.09		
S. Creat: (mg/dl)						
Mean ± SD	1.14±0.16[a]	1.28±0.09[b]	1.53±0.12[c]	1.86±0.11[d]	**144.5**	**<0.001 ****
Range	0.8-1.35	1.04-1.36	1.4-1.8	1.7-2.1		
BUN: (mg/dl)						
Mean ± SD	56.82±12.49[a]	71.82±13.68[b]	70.91±14.45[b]	89.09±10.19[c]	**23.34**	**<0.001 ****
Range	40-80	50-110	50-90	80-110		
PT: (second)						
Mean ± SD	14.15±0.58	14.22±0.62	14.96±0.63	14.5±0.59	1.79	0.36
Range	13-15	13.2-15.6	13.8-15.9	13.3-15.8		NS
GFR: (ml/min/1.73m^2)						
Mean ± SD	78.41±14.70[a]	70.59±8[a]	57.36±9.88[b]	44.23±11.06[c]	**39.81**	**<0.001 ****
Range	60-104	61-86	38-82	32-66		
ACR: (mg/g)						
Mean ± SD	11.13±6.75[a]	11.39±9.16[a]	71.95±66[b]	459.33±307.53[c]	**73.09**	**<0.001 ****
Median (IQR)	13.35	7.85	43.9	386.15		
Range	1.1-25	4.3-18	31.4-300	306.6-1771.6		

SD: Stander deviation, **F**: ANOVA test KW: Kruskal-Wallis test. **NS**: Non significant (P>0.05) ******: Highly significant (P<0.001)
Post-hoc: Tukey (Groups with different letters are statistically significant (P<0.05))
AST: Aspartate transaminase **ALT**: Alanine transaminase **BUN**: blood urea nitrogen
PT: Prothrombin time **GFR**: Glomerular filtration rate **ACR**: albumin/Creatinine ratio

Supplementary table (2) Correlation between angiotensinogen levels and age, duration of disease and laboratory findings among studied groups:

Variable	Angiotensinogen (ng/ml) (n = 66)	
	r	***P***
Age (yrs)	0.081	0.518 (NS)
Duration of disease	**0.587**	**<0.001**** (HS)
HbA1C (%)	**0.514**	**<0.001**** (HS)
FBS (mg/dl)	**0.514**	**<0.001**** (HS)
WBCs	-0.128	0.306 (NS)
Hb	**-0.545**	**<0.001**** (HS)
K	0.015	0.903 (NS)
Na	-0.086	0.492 (NS)
ESR (mm/h)	**0.380**	**0.002*** (S)
hs-CRP (mg/dl)	**0.371**	**0.002*** (S)
ALT	0.042	0.738 (NS)
AST	0.029	0.817 (NS)
PT	0.238	0.054 (NS)
S. albumin (mg/dl)	**-0.496**	**<0.001**** (HS)
S. Creatinine (mg/dl)	**0.626**	**<0.001**** (HS)
BUN (mg/dl)	**0.280**	**0.023*** (S)
GFR (ml/min/1.73m^3)	**-0.462**	**<0.001**** (HS)
Albumin/creatinine ratio (mg/g)	**0.666**	**<0.001**** (HS)

r: Spearman's correlation coefficient. NS: Non significant (P>0.05) *S: Significant
**HS: Highly significant

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