

Study of Ischemia-Modified Albumin as Marker of Vascular Injury in Diabetic Nephropathy

Abstract

Background: Diabetes mellitus (DM) is strongly correlated to ischemia caused by stress that is involved in dysfunction of endothelium leading to angiopathy, particularly diabetic nephropathy (DN). Immediately after occlusion of an artery, free radical production paired with reduced antioxidant defensive response and related ischemia in DM result in changes in the tertiary structure of human serum albumin; therefore, it is called ischemia-modified albumin (IMA). The aim of our work was to evaluate IMA as marker of vascular injury in DN.

Patients and Methods: This trial was conducted on 25 normal individuals as a control group (I) and 75 cases with type II DM. Cases have been subdivided into Group II: 25 diabetic cases with normal albumin in urine, Group III: 25 diabetic cases with albumin in urine and glomerular filtration rate >60 ml/min/1.73m² and Group IV: 25 diabetic cases with albuminuria and glomerular filtration rate <60 ml/min/1.73m². Medical history, clinical features and laboratory investigations which include the serum IMA have been carried out to all cases.

Results: Compared to the control group, considerably greater serum levels of IMA have been reported in Diabetic cases there had been a wide variation in IMA among the 4 groups. There were considerable +ve correlations among serum IMA and DM duration, eGFR, blood urea, serum creatinine, UACR, FBS, PPBS and HBA1c. A considerable -ve correlation among

serum IMA and eGFR was observed. IMA had an optimal cutoff value ≥ 90 ng/ml with 0.92 area under ROC curve, 96% sensitivity and 92% specificity (p-value < 0.001).

Conclusion: IMA could function as a glycemic control marker and a sensitive DNA indicator.

Keywords: Ischemia-Modified Albumin, Marker, Vascular Injury, Diabetic Nephropathy

UNDER PEER REVIEW

Introduction:

Diabetes mellitus (DM) is a common metabolic disorder characterized by increased blood glucose level due to abnormality in insulin function, release or both ^[1]. In DM cases, rigorous control of blood glucose level reduces the prevalence of adverse effects of diabetes that can affect one's life and the condition of such cases. The positive implications of rigorous control of blood glucose on coronary heart diseases, a major reason of mortality in Cases with diabetes, have been speculated by widely reported experimental researches. ^[2].

The prevalent clinical findings due to unmonitored high blood sugar involve vascular endothelium thickness, membrane lipid deposition and oxidation (cholesterol) and cell proliferation, leading to adverse effects blood vessels such as vascular narrowing and premature arteriosclerosis. Increasing prevalence, seriousness and impaired functioning from stress-induced ischemia involved in endothelial dysfunction are strongly correlated to adverse effects of diabetes, that might give rise to angiopathy, particularly diabetic nephropathy (DN) ^[3].

DN pathophysiology is a complicated procedure and is not clearly appreciated yet. An abnormality in tubule-interstitial cell wall filtration that results from renal endothelial dysfunction due to increased blood glucose, oxidative stress, decreased blood supply, and inflammation are being directly linked to the reason of elevated albuminuria, that is assumed to be an early warning sign of DN ^[4, 5].

Even so, many other researchers have described DN progression in 29 percent-61 percent of cases with Type 2 DM with normal levels of urine albumin, as micro albuminuria only occurs when there has already been considerable deterioration to glomerular system ^[6, 7].

Immediately after occlusion of arteries, generation of free radicals associated with reduced antioxidant defensive measure and accompanying ischemia in DM causes a change in the tertiary structure of human serum albumin; so even, it is called ischemia-modified albumin

(IMA). Conformational changes of the molecule of albumin may happen as a consequence of enhanced reactive oxygen species (ROS) production. IMA is a novel, helpful and sensitive biomarker for detecting early tissue ischemia and oxidative stress ^[8]. The aim of this study was to evaluate IMA as a marker of vascular injury in DN.

Subjects and Methods:

This study enrolled 25 healthy individuals as a control group and 75 cases with DM type two attending at Department of Internal Medicine of Tanta University Hospital and EL-Menshway General Hospital from June 2018 to the end of April 2019. Permission was taken from all cases after the risks and benefits were fully explained. The Ethical Committee of the Faculty of Medicine of Tanta University approved this trial.

Individuals were divided to: Group I: 25 normal cases as a control group. Group II: 25 diabetic cases with normal albumin in urine. Group III: 25 diabetic cases with albumin in urine and glomerular filtration rate >60 ml/min/1.73m². Group IV: 25 diabetic cases with albumin in urine and glomerular filtration rate <60 ml/min/1.73m².

Exclusion criteria were those suffering from acute infection, inflammatory diseases, type 1 DM, cases with serum albumin level <3 g/dl and >5.5 g/dl, renal diseases (other than DN), cases with GFR <15 ml/min/1.73m², pregnancy, cases with other endocrinal diseases, cardiac cases, cancer, cases with chronic liver diseases, HIV cases and cases on steroids or hormones. All cases underwent complete historical examination, complete clinical examination, laboratory investigations, including regular laboratory investigations [CBC, liver function tests, fasting and postprandial blood glucose, HbA1c, lipid profile (total cholesterol, triglycerides, LDL, and HDL), serum urea, serum creatinine, serum albumin, estimation of GFR and urine albumin to creatinine ratio (ACR) b) Specific laboratory investigations (serum IMA by ELIZA). 4) Pelvi-abdominal **sonography** 5) ECG

Serum IMA level was done by ELIZA: All blood sample was put at room temperature for 60 minutes or placed at 4 ° c overnight and centrifuged at approximately 1000 x g for 20 minutes, collected the supernatant and immediately carried out the assay. Balance the TMB substrate at 37 ° C for 30 minutes prior to actually incorporating reagents into wells. They should be prepared by mixing totally and uniformly when diluting specimens and reagents. For each test, it is advised to draw a standard curve. 500ng/ml 0.1ml liquor, 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml, 15.625ng/ml, 7.813ng/ml, standard solutions in standard wells. Into the control (zero) well, add 0.1 ml Sample/Standard Dilution Buffer. Into the test sample wells, add 0.1 ml of the correctly diluted sample (human serum, plasma, tissue homogenates and other biological fluids). Seal the sheet with a shield and allowed to stand for one and half hour at 37 ° C. Turn off the heat and dispose the contents of the plate and wash the plate with the Wash Buffer 2 times. At any time, the wells are not let to dry completely. Add 0.1 ml of biotin-labeled working antibody solution to the above wells (standard, test sample & zero wells). Apply the solution without hitting the sidewall at the bottom of each well. Enclose the sheet with a cover and inoculated it for 60 min at 37 ° C. Uncover and wash the plate with Wash Buffer 3 times, and each time let the wash buffer remain in the wells for 1 minute. Add 0.1 ml of SABC Working Solution to each well, cover the plate and cook for 30 minutes at 37 ° C. With the Wash Buffer, remove the cover and wash plate 5 times, and let the wash buffer remain in the wells for 1-2 minutes each time. Add 90µl of TMB substrate to each well, cover the plate and incubate for 15-30 minutes at 37 ° C in the dark. In the first 3-4 wells it will turn blue, the other wells do not show visible color. Add 50µ stop solution to each well and properly combine them. The color switches instantly to yellow. Read your O.D. 450 nm absorbance in the Microplate Reader immediately after the stop solution is applied.

Statistical analysis:

The data was evaluated using version 19 of SPSS (IBM Inc. Chicago, IL, USA). The range, mean and standard deviation were determined for the quantitative results, and the F (ANOVA) test was compared. The frequency and percentage of qualitative data were represented and compared using the Chi-square test (χ^2). Correlations among variables were assessed using the correlation coefficient of Pearson (r). To test the sensitivity and specificity of the serum IMA level in the studied weapons as a marker for DN, where the cut-off values were determined from the approximate ROC curve, the field under the Receiver Operating Characteristic (ROC) curve was illustrated. The standard of meaning was introduced at $p < 0.055$.

Results

No considerable difference was noted among the four groups as regarding age, sex and BMI but a considerable difference of statistical significance was noted among the mean of DM duration, **systolic and diastolic pressures** in 3 diabetic groups ($P < 0.001$). (Table 1)

A considerable difference of statistical significance was noted among the 4 groups in FBS, PPBS and HBA1C ($P < 0.001$). (Table 2)

A considerable difference of statistical significance was noted among the 4 groups in blood urea, serum creatinine, UACR, GFR and IMA ($P < 0.001$). (Table 3)

Considerable correlations of statistical significance were noted among serum IMA and DM duration ($r = 0.947$, $p\text{-value} < 0.001$), blood urea ($r = 0.880$, $p\text{-value} < 0.001$), serum creatinine ($r = 0.787$, $p\text{-value} < 0.001$), UACR ($r = 0.839$, $p\text{-value} < 0.001$), eGFR ($r = - 0.870$, $p\text{-value} < 0.001$), FBS ($r = 0.642$, $p\text{-value} < 0.001$), PPBS ($r = 0.853$, $p\text{-value} < 0.001$) and HBA1c ($r = 0.922$, $p\text{-value} < 0.001$) in studied groups. (Table 4)

Serum IMA had an optimal cutoff value ≥ 90 ng/ml with area under ROC curve at 0.92 sensitivity of 96%, specificity of 92%, + ve predictive value of 89%, -ve predictive value of 97%, accuracy of 95% ($p\text{-value} < 0.001$) This showed that IMA in serum can be regarded as a

marker in cases with type 2 diabetes for early identification of DKD (diabetic kidney disease). (Figure 1)

Discussion

Complex situations that play a part in the progression and advancement of atherosclerosis are endothelial dysfunction and low-grade systemic inflammation. One of frequent microvascular adverse events of DM is DKD. DKD is now the largest contributor of end stage renal disease that affecting the standard of the cases' lives and survival.^[9] About 20%-30% of cases with T2DM, complicated with renal insufficiency showed normo-albuminuria which is a condition referred to now as non-protein-uric DKD so the need for new biomarkers for earlier detection of DKD is necessary^[1].

There is scientific proof that vascular alterations in DM are started in the form of oxidative disruption and sub-endothelial low level chronic inflammation prior to the appearance of overt DM. These alterations are progressing progressively towards peripheral vascular dysfunction, nephropathy, retinopathy and neuropathy. Oxidative stress and the resulting chronic ischemia are related to sub-endothelial inflammation as an underlying etiology. Ischemia refers to a conformational shift in the N-terminus of albumin, including hypoxia and free oxygen radicals, and this molecule is called IMA^[10].

In our study, serum creatinine and blood urea were considerably higher in group III and IV than group II and control group (p-value<0.001). Our results were matched with Wu et al.,^[11] who reported that there were considerable differences of statistical significance in serum creatinine and blood urea among studied groups. This was supported by Ghosh et al.,^[12], who revealed considerable difference was noted among creatinine in serum in studied groups. In current trial, there was a statistically considerable difference among the studied groups regarding UACR (p-value<0.001). It was considerably higher in group III and IV than group II and control group. These data agreed with Zeng et al.,^[13] which reported that UACR was

higher in cases with diabetes with nephropathy than in cases with diabetes without nephropathy. Our results disagreed with results of Kocak et al.,^[14] who reported that there was no considerable difference of statistical significance among the two studied groups in UACR as it has been reported that a decline in the renal function of cases with DM was not always accompanied by an increased UACR.

This study showed that, there were considerable differences of statistical significance among the 3 groups in FBS, PPBS and HbA1C (p-value<0.001). Shao-gang et al., {Ma, 2011 #49} examined HbA1c-related peripheral arterial disorders and IMA in T2DM. They noted that HbA1c is commonly linked to diabetic and peripheral arterial disorders complications.

This agreed with Mahmoud et al.,^[15] who found that, As a glycemic control marker, HbA1c was substantially greater in Group B cases with DN than in Group A cases without nephropathy, which may indicate the correlation between low blood sugar control and DM length and the occurrence of diabetic adverse effects such as nephropathy.

An elevated HbA1c was related to complications in type 2 DM^[15]. This was proved by Ahmed et al.,^[16] who found considerable difference regarding FPG, two-hour PPPG, and HbA1c in the studied groups.

In our study, the serum levels of IMA were considerably high in diabetic cases when compared with control group. Furthermore, IMA was considerably increased in cases with albuminuria than in cases with normal albuminuria. This agreed with El Said et al.,^[17] who found that, the serum level of IMA was considerably increased in cases with type 2 diabetes with DN in comparison with type 2 diabetic cases with no nephropathy and control group (p-value <0.001). These findings were supported by Piwowar et al.,^[18], who reported considerable higher plasma levels of IMA in cases with type 2 DM than healthy individuals and that in diabetic cases with adverse effects, IMA levels were increased and were linked to the value of HbA1c.

In addition, Ukinc et al. {Ukinc, 2009 #50} observed an increase of IMA in diabetic cases than in stable healthy controls. Compared to cases with diabetes without nephropathy, IMA levels have increased in patents with DN.

The same research also identified the role of persistent ischemia in cases with diabetes and emphasized that the associated subclinical cardiovascular condition may represent increased IMA values.

Through another research, elevations of IMA in DM cases were also documented by Kaefer et al. {Kaefer, 2010 #51}, and elevated inflammation and hyperglycemia in people with diabetes led to the development of IMA.

Dash et al. {Dash, 2014 #52} registered a high plasma IMA level in DM cases compared with controls, more prominent in the group of diabetic cases associated with complications.

Dayanand et al.,^[19] Piwowar et al.,^[20] and Dash et al. {Dash, 2014 #52} showed that elevated IMA levels might indicate underlying subclinical disease or vascular dysfunction in diabetic kidney and may be used for early detection of renal dysfunction.

Such findings show that albumin alteration develops at the beginning of the disease, potentially triggered by stress induced by increased blood glucose level, and is implicated in the pathophysiology of diabetic complication.

This agreed with Ahmad et al.,^[16] who found that, a considerable difference of statistical significance was noted in the values of IMA among cases with early DN (154 ng/mL), DM without nephropathy (109.4 ng/mL), and normal cases (45.7 ng/mL), with highest values in early DN cases.

Supporting this statement, the Sharada et al. {Sharada, 2012 #53} also reported considerable correlation of IMA and AOPP with HbA1c, UACR, and serum creatinine in DN cases (micro- and macro-albuminuria cases) as compared with MDA. Also in Saleh et al., {Mahmoud Saleh, 2019 #54} who found that, the serum IMA were higher among diabetic

cases with nephropathy than those without nephropathy and control group with P value<0.005 which is statistically considerable.

The subsequent tissue hypoxia may have resulted in greater albumin alteration due to the greater IMA amount. Serum level of IMA positively associated with HbA1c, a glycemic regulation marker and a predictor of complication growth.

This agreed with Mahmoud et al., ^[15] who found that, serum level of IMA was positively correlated with HbA1c, a marker of glycemic control and **an indicator of development of complications.**

This finding is supported by Sowjanya et al., ^[21] who reported that the +ve association of raised plasma IMA with HbA1c.

Blood pressure, dyslipidemia, obesity, DM duration, glycemia level, and urinary albumin creatinine ratio are all risk factors associated with DM and contribute to the development of DM complications ^[17].

In our study, we found that there were statistically considerable +ve correlations among serum IMA and blood urea (r=0.880, p-value<0.001), serum creatinine (r=0.787, p-value<0.001), UACR (r=0.839, p-value<0.001).

This were supported and agreed by Ahmed et al., ^[16], who found a +ve correlations among serum IMA and UAC ratio (p value<0.05, r=0.734) also it showed +ve correlations among serum IMA, and serum creatinine (p<0.05, r=0.765). The +ve correlations among IMA and the UAC ratio implies that IMA increased progressively with the degree of albuminuria.

This agreed also with Mahmoud et al., ^[15] who found that, there were statistically considerable +ve correlations among serum IMA and blood urea, serum creatinine and UACR. This was supported by Ahmad et al., ^[16], Krzysek- Krzystek-Korpicka et al., ^[22], and who reported that plasma levels of IMA considerably correlated with the UACR (P<0.001) and plasma creatinine concentration (P<0.05).

Sowjanya et al.,^[21] concluded that there was an increase in the value of serum IMA in cases with dyslipidemia, so they concluded that there were +ve correlations among serum IMA and dyslipidemia in general.

In this study, serum IMA had an optimal cutoff value ≥ 90 ng/ml with area under ROC curve at 0.92. Serum IMA had Sensitivity of 96% and Specificity of 92%, +ve predictive value of 89%, -ve predictive value of 97% and Accuracy of 95.00% which proved that serum IMA could be considered as biomarker for early detection of DKD in type 2 diabetic cases.

Similarly, Titan et al.,^[23] who found that serum IMA levels of ≥ 99 ng/mL are ideal for differentiating these cases because of its high value of specificity, +ve predictive value, and +ve likelihood ratio. Also, in El Said et al.,^[17] who found IMA was a considerable discriminator for DN ($P < 0.001$) with 100% specificity and 100% sensitivity. IMA could serve as an indicator of glycemic control and a sensitive marker of DN.

This was supported and agreed by Mahmoud et al.,^[15] who reported that, the ability of serum IMA to detect DN with 95% confidence interval equals 1 with (p value < 0.001) with ($AUC > 0.7$) with 100% specificity and 100% sensitivity.

ROC analysis has been utilized for IMA, and was accepted by Ahmad et al. {Ahmad, 2016 #55}, who assessed the unequal influence of the measures and their predictive usefulness. The capability to differentiate persons with no renal diseases (normo-albuminuria) from others with upper normal albuminuria, represented by both micro- and macro-albuminuria, was tested. The result shows the maximum and identical average AUC for IMA because of these parameters (0.930). In an attempt to pick reasonable cut - off points from the ROC plot for the optimal indicator of the three variables, it was noticed that IMA had 97.5 percent sensitivity and 78 percent accuracy. This suggests that to identify persons with upper normal albuminuria or early DN, IMA may be used with greater precision.

This suggests that oxidative stress-induced protein alteration and inflammatory components are more frequently aligned with endothelial dysfunction and ischemia inducing DN above a certain amount of time than oxidative altered lipids ^[24].

The present research involved only those participants with a serum albumin level of between 3 and 5.5 g/dl in order to invalidate the impact of serum albumin on IMA levels. The cross-sectional model by which the causal difference could not be defined may be a possible constraint. Large-scale prospective observational research are needed to add to established theories and to assess the importance of IMA as an early indicator of oxidative stress that caused vascular damage in DNN pathophysiology.

Further large studies are needed to investigate the role and the difference of IMA to DKD in T2DM cases.

Conclusion:

The levels of serum IMA were considerably high in diabetic cases in comparison to control group, furthermore; IMA was considerably higher in cases with albuminuria than in cases with normal albuminuria. IMA increased early before albuminuria in T2DM and is increased considerably with progression of DKD. Thus, IMA can be considered as early marker of DKD before the development of albuminuria in T2DM.

Disclaimer regarding Consent and Ethical Approval:

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

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Table (1): Participant characteristics among the studied groups (n = 100)

Variables	Group I (Control group) (n=25)		The studied type 2 diabetic cases (n=75)						Test	P value
			Group II (With normal albumin in urine) (n=25)		Group III (With albuminuria & GFR>60) (n=25)		Group IV (With albuminuria & GFR <60) (n=25)			
	N	%	N	%	n	%	n	%		
Age years:										
Range	38-65		33-78		40-67		40-67		F = 0.556	0.645
Mean ± SD	54.04±7.92		55.44±9.76		53.48±7.72		56.24±8.37			
Sex:										
Males	13	52.0	15	60.0	15	60.0	12	48.0	$\chi^2 = 1.091$	0.779
Females	12	48.0	10	40.0	10	40.0	13	52.0		
Body mass index (BMI) kg/m²:										
Range	26.00-31.00		26.00-31.00		25.00-32.00		27.00-31.00		F = 0.745	0.528
Mean ± SD	28.78±1.56		28.60±1.19		29.33±1.47		29.03±1.01			
Duration of disease (years):										
Range	----		3.00-5.50		8.00-11.00		12.00-16.50		F = 65.982	<0.001*
Mean ± SD	----		4.22±0.79		9.76±1.00		14.34±1.39			
Systolic BP (mmHg):										
Range	100-130		130-150		135-155		140-155		F=74.905	<0.001*
Mean ± SD	119.20±10.17		143.00±7.50		144.80±5.49		146.40±5.50			
Diastolic BP (mmHg):										
Range	60-80		70-90		70-90		80-95		F=21.450	<0.001*
Mean ± SD	75.20±5.49		83.60±6.21		84.80±5.49		86.80±4.76			

P=probability value, N=number t=student's test χ^2 value of Kruskal Wallis test,

Considerable: p<0.05

Table (2): Diabetes markers among the studied type 2 diabetic cases and control group (n=100).

Diabetes markers	Group I (Control group) (n=25)	The studied type 2 diabetic cases (n=75)			Test	P
		Group II (With normal albumin in urine) (n=25)	Group III (With albuminuria & GFR>60) (n=25)	Group IV (With albuminuria & GFR <60) (n=25)		
Fasting blood sugar (FBS) (mg/dl):						
Range	70-89	128-220	158.00-208.00	180-210	F= 208.827	<0.001*
Mean ± SD	80.24±5.11	164.90±30.81	181.64±15.48	196.74±9.13		
Postprandial blood sugar (PPBS) (mg/dl):						
Range	119-139	210-287	238-298	270 - 400	F= 676.237	<0.001*
Mean ± SD	127.34±5.58	251.00±21.36	278.96±15.35	367.24±27.01		
Glycated hemoglobin (HbA1c) %:						
Range	4.30-5.30	6.90-8.30	8.30-9.50	9.30-13.20	F= 538.412	<0.001*
Mean ± SD	4.83±0.29	7.62±0.44	8.94±0.31	11.89±1.10		

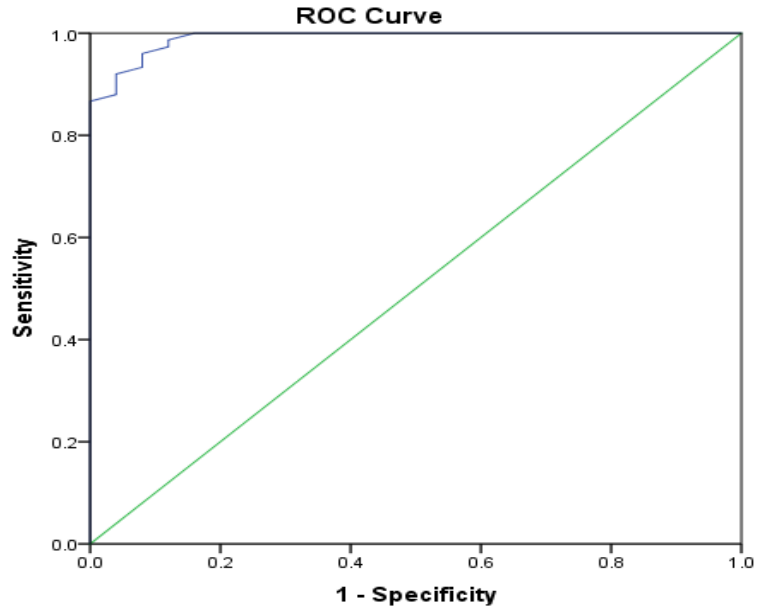
Table (3): Correlation among Ischemia-Modified Albumin (IMA) marker and other markers and variables among the studied type 2 diabetic cases (n=75).

Variables and markers	Serum IMA marker among The studied type 2 diabetic cases (n=75)	
	r	P
•Duration of the disease	0.947	<0.001*
•Blood urea (mg/dl)	0.880	<0.001*
•Serum creatinine (mg/dl)	0.787	<0.001*
•UACR	0.839	<0.001*
•eGFR	-0.870	<0.001*
•Fasting blood sugar (FBS) (mg/dl)	0.642	<0.001*
•Postprandial blood sugar (PPBS) (mg/dl)	0.853	<0.001*
•Glycated hemoglobin (HbA1c)	0.922	<0.001*

r= Correlation Coefficient, *Considerable P<0.05

Table (4): Kidney function markers and serum Ischemia-Modified Albumin (IMA) marker among the studied type 2 diabetic cases and the control group (n=100).

Kidney function markers	Group I (Control group) (n=25)	The studied type 2 diabetic cases (n=75)			Test	P
		Group II (With normal albumin in urine) (n=25)	Group III (With albuminuria & GFR>60) (n=25)	Group IV (With albuminuria & GFR <60) (n=25)		
Blood urea (mg/dl):						
Range	18.00-43.00	20.00-43.00	74.00-120.00	89.00-225.00	F=82.869	<0.001*
Mean ± SD	31.56±8.38	31.72±6.52	91.96±11.72	160.92±32.43		
Serum creatinine (mg/dl):						
Range	0.68-1.00	0.70-1.09	1.10-1.50	1.48-3.00	F= 83.771	<0.001*
Mean ± SD	0.83±0.10	0.88±0.11	1.33±0.15	2.02±0.41		
Urine albumin/ creatinine ratio (UACR) mg/gm:						
Range	14.00-20.50	16.20-25.00	120 -163	307.00-580.00	F=86.150	<0.001*
Mean ± SD	17.81±1.88	20.08±2.19	138.16±12.14	499.04±57.40		
Glomerular filtration rate (eGFR) (ml/min/1.73m²):						
Range	91.00-125.00	90.00-120.00	63.00-85.00	30.00-58.00	F=277.354	<0.001*
Mean ± SD	106.04±11.9	104.06±10.25	72.24±7.65	38.40±8.01		
Serum IMA marker:						
Range	34.00-98.00	85.00-135.00	88.00-170.00	150.00-215.00	F=336.926	<0.001*
Mean ± SD	54.08±17.21	106.56±11.56	153.56±18.85	188.52±15.03		



Diagonal segments are produced by ties.

Figure (1): ROC Curve for detection of serum IMA levels cutoff regarding the studied groups.