Sensitivity and specificity of ischaemia modified albumin in detecting diabetic nephropathy in T2DM

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Background
Ischemia-modified albumin (IMA) is a novel marker of tissue ischemia and oxidative stress.
Aim
We assessed the level of IMA concentration in type 2 diabetes mellitus (T2DM) patients with diabetic nephropathy and its correlation with glycemia level, duration of diabetes, dyslipidemia, serum creatinine, and urinary albumin/creatinine ratio.
Patients and methods
This study included 91 patients who were divided into three groups: group A (33) included T2DM patients without nephropathy; group B (29) included T2DM patients with nephropathy; and group C (29) included healthy as control. Blood samples were analyzed manually for plasma IMA by spectrophotometric cobalt (II)-albumin-binding assay.
Results
Serum levels of IMA were significantly higher in group B in comparison to group A with a *P* value less than 0.001 and group C with a *P* value less than 0.0001. IMA positively correlated with blood pressure, duration of diabetes, fasting blood glucose, postprandial blood glucose, glycated hemoglobin, cholesterol, low-density lipoprotein, triglycerides, serum creatinine, and albumin/creatinine ratio, all with a *P* value less than 0.0001 and also with BMI ($P<0.003$). IMA was a significant discriminator for diabetic nephropathy ($P<0.001$) with 100% specificity and 100% sensitivity.
Conclusion
IMA could serve as an indicator of glycemic control and a sensitive marker of diabetic nephropathy.

Keywords:
diabetic nephropathy, ischemia-modified albumin, type 2 diabetes mellitus

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Introduction

Human serum albumin is one of the circulating antioxidants in the plasma. It has an important role in the direct protective effect on oxidative stress [1]. Many in-vivo studies showed that during hypoxia conditions the molecule of albumin is modified by changing the ability of the first three amino acids N-Asp-Ala-His to bind free metal ions. This abnormal molecule of human serum albumin is known as ischemia-modified albumin (IMA) [2,3]. Many studies used the new biochemical marker IMA for the diagnosis and evaluation of myocardial ischemia [4]. Also it was estimated in different states with ischemia of noncardiac origin such as systemic sclerosis and [5,6] peripheral vascular disease [7]. In diabetes, hyperglycemia and oxidative stress, both will induce chronic ischemia which may lead to necrosis of different tissues and lead to different diabetic complications [8,9].

The main goal of our study was the assessment of IMA concentration in type 2 diabetic patients complicated with diabetic nephropathy.

Patients and methods

This cross-sectional study included 62 Egyptian patients with type 2 diabetes, who were recruited from the outpatient clinic of Kasr Alainy Hospital, Cairo University and 29 healthy age-matched individuals as control. The study was performed from December 2015 to December 2016. The participants were divided into three groups. Group A: 33 type 2 diabetes mellitus (T2DM) patients

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without nephropathy, group B: 29 T2DM patients with nephropathy, and group C: 29 included healthy as control. We excluded patients with any renal disease other than diabetic nephropathy, congestive heart failure, coronary heart disease, cerebral infarction, cerebral hemorrhage, liver diseases, and type 1 diabetes.

The study protocol was approved by the institutional ethics committee and the review board. All the patients and controls provided written informed consents.

Procedures

All participants underwent a complete screening panel, including history taking and physical examination. Laboratory investigation included the following: fasting blood sugar, postprandial blood sugar, glycated hemoglobin (HbA1c), lipid profile, and serum creatinine. All laboratory investigations were carried out on Dimension RxL Max analyzer (Siemens Health GmbH- Henkestr, Erlangen, Germany) by colorimetric techniques. HbA1c was determined using the cation exchange resin. Albumin concentrations were measured in urine using a Mininephmicroalbumin kit based on the nephlometry method on Mininep-nephelometer (AD200) (The Binding Site, Birmingham, UK) [10]. Urinary creatinine was determined on Dimension RxL Max analyzer by the colorimetric technique and the ratio of urine albumin to creatinine was used to determine microalbuminuria.

Ischemia-modified albumin determination

A measure of 2 ml of venous blood samples were collected from each patient participating in the study and was left to clot and then the serum was separated by centrifugation at 3000*g* for 10 min and IMA assay was performed immediately. IMA concentration was determined by addition of a known amount of cobalt (II) to a serum sample and measurement of the unbound cobalt (II) by the intensity of the colored complex formed after reacting with dithiothreitol (DTT) by a colorimeter. An inverse relationship thus exists between the level of albumin-bound cobalt and the intensity of the color formed. The preparations for the cobalt (II) albumin-binding protocol involved the addition of 200 µl of patient serum to 50 µl of a solution of 1 g/1 cobalt chloride, followed by vigorous mixing and 10-min incubation. DTT (50 µl of a 1.5 g/1 solution) was then added and mixed. After 2-min incubation, 1.0 ml of a 9.0 g/l solution of NaCl was added. The absorbance of the assay mixture was read at 470 nm. The blank was prepared similarly with the exclusion of DTT. The values are expressed in U/ml. Standard curve was prepared in the range 6.0–60.0 µg $CoCl_2$/ml. One IMA unit was defined as µg of free cobalt (II) in the reaction mixture per ml of serum sample [11].

Statistical analysis

Data were analyzed through the statistical package for the social science software program (version 20; IBM, Armonk, New York, USA). Numerical variables were presented as mean±SD, while categorical variables were presented as number percentage. χ^2 test was used for comparison between groups as regards qualitative demographic data and score variables. Independent *t* test and one-way analysis of variance with least-significance difference post-hoc tests were used for comparison and multiple comparison between groups as regards quantitive variables. Receiver operating characteristic (ROC) analysis was used to test the discriminatory ability of IMA measures to predict nephropathy and to derive the most suitable cutoff point with considerable sensitivity and specificity. *P* values equal to or less than 0.05 were considered statistically significant.

Results

Our patients had type 2 diabetes of 8–12 years duration. The demographic and laboratory data of our studied groups are shown in Table 1. The metabolic derangements were evident in group B (diabetic patients with diabetic nephropathy), as they have the highest statistical significance values of the HbA1c, postprandial blood glucose (PPBG), triglycerides, low-density lipoprotein (LDL), creatinine, and urinary albumin creatinine in the three groups with *P* value less than 0.001. As for high-density lipoprotein (HDL) level, it was statistically significantly lowest in group B in the three groups with *P* value less than 0.002.

Serum levels of IMA were statistically significantly different between all groups ($P<0.001$) and with the post-hoc Tukey's test for pairwise comparison, they were significantly higher in group B diabetic patients with nephropathy (125.43±8.4) in comparison to group A (81.01±10.9) with *P* value less than 0.001 and group C (49.4±5.5) with *P* value less than 0.0001.

IMA strongly positively correlated with blood pressure, fasting blood glucose (FBG), PPBG, HbA1c, cholesterol, LDL, triglycerides, serum creatinine ($P<0.0001$), duration of diabetes ($P<0.05$), and BMI ($P<0.003$). There was a significant negative correlation between IMA and HDL ($P<0.001$). There was no statistically significant correlation between IMA and the age of the patients (Table 2).

Table 1 Demographic, clinical, and laboratory data of the studied groups

Item ANOVA				*P* value	Pairwise comparison		
	Group A (*N*=33) (mean±SD)	Group B (*N*=29) (mean±SD)	Group C (*N*=29) (mean±SD)		A×B	A×C	B×C
Age (years)	51.9±6.54	50.5±5.56	51.5±4.5	<0.001	0.038	<0.001	0.023
Duration (years)	8.66±4.820	11.18±526		0.092	0.092	<0.001	<0.0001
BMI (weight/height)	27.39±3.992	27.93±4.317	24.48±2.148	<0.001	0.613	<0.0001	<0.0001
BP systolic (mmHg)	130.61±10.856	147.59±8.724	118.97±3.009	<0.0001	<0.0001	<0.0001	<0.0001
BP diastolic (mmHg)	86.97±5.855	91.38±4.411	78.62±5.158	<0.0001	0.002	<0.0001	<0.0001
HbA1c (%)	7.388±1.7980	8.990±1.3857	4.028±1.2265	<0.001	<0.0001	<0.0001	<0.0001
FBG (mg/dl)	203.66±20.274	208.36±32.95	86.90±7.993	<0.001	0.508	<0.0001	<0.0001
2HPP (mg/dl)	283.73±45.229	291.72±44.524	111.55±10.284	<0.001	0.487	<0.0001	<0.0001
Cholesterol (mg/dl)	195.86±28.150	218.267±43	138.52±30.264	<0.001	0.032	<0.0001	<0.0001
TG (mg/dl)	161.27±64.456	170.66±60.309	108.03±36.594	<0.001	0.558	<0.0001	<0.0001
LDL (mg/dl)	138±40.464	152.34±34.452	60.38±18.204	<0.001	0.141	<0.0001	<0.0001
HDL (mg/dl)	44.97±10.987	40.93±14.914	52.48±9.661	0.002	0.226	0.006	<0.001
VLDL (mg/dl)	37.97±18.360	28.41±17.904	17.62±5.220	<0.001	0.043	<0.0001	0.003
UAC (ratio) (μg/mg)	13.5±5.5	848.83±379.043	11.59±3.839	<0.001	<0.0001	<0.0001	<0.0001
CREAT (mg/dl)	0.808±0.1733	4.295±1.6295	0.793±1.223	<0.001	<0.0001	0.709	<0.0001
IMA (U/ml)	81.018±10.9490	125.434±8.4077	49.448±5.5295	<0.001	<0.001	<0.0001	<0.0001

2HPP, 2-h postprandial; ANOVA, analysis of variance; BP, blood pressure; CREAT: serum creatinine; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IMA, ischemia-modified albumin; LDL, low-density lipoprotein; TG, triglycerides; UAC, urinary albumin creatinine ratio; VLDL, very low-density lipoprotein. *P* value of less than 0.05 is significant and less than 0.001 is highly significant.

Table 2 Correlation between serum ischemia-modified albumin and other variables in the studied groups

Variables	*P* value	*r*
Age (years)	0.101	0.173
Duration of DM	0.056	0.244
BMI	0.003	0.706
BP systolic	0.0001	0.776
BP diastolic	0.0001	0.631
HbA1c	0.0001	0.795
FBG	0.0001	0.709
PPBG	0.0001	0.747
Cholesterol	0.0001	0.794
TG	0.0001	0.515
LDL	0.0001	0.646
HDL	0.6	-0.5
UAC ratio	0.0001	0.734
Serum creatinine	0.0001	0.765

BP, blood pressure; CREAT, serum creatinine; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IMA, ischemia-modified albumin; LDL, low-density lipoprotein; PPBG, postprandial blood glucose; *r*, Spearman's correlation coefficient; TG, serum triglycerides; UAC, urinary albumin creatinine ratio. *P* value of less than 0.05 is significant and less than 0.001 is highly significant.

ROC curve analysis has shown that IMA was a significant discriminator for diabetic nephropathy ($P<0.001$) with 100% specificity and 100% sensitivity (Table 3 and Figs 1–6).

Discussion

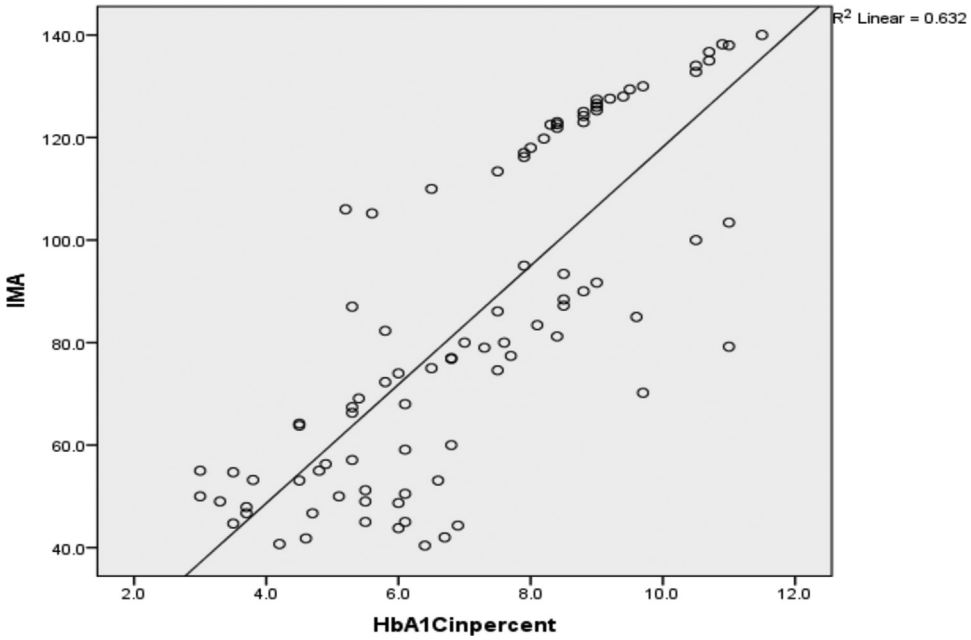
Diabetes hyperglycemia, through several mechanisms such as glucose autooxidation, stimulation of the polyol pathway, imbalance between the amount of reduced and oxidized coenzymes forms, nonenzymatic glycation and formation of advanced glycation end-products-AGEs, leads to several biochemical sequels leading to oxidative stress. This oxidative stress in type 2 diabetes is responsible for the destruction of the pancreatic islets. It is responsible for the oxidative protein damage and the formation of advanced oxidation products such as IMA [12–14]. Our study checked the level of serum IMA in type 2 diabetes and assessed if there was any difference in its level between type 2 diabetic patients with diabetic nephropathy and those without diabetic nephropathy. We tried to analyze how the level of glycemia and duration of diabetes influence IMA and if there were any correlations between the IMA level and the risk factors associated with diabetes such as obesity, blood pressure, and dyslipidemia. We found that the level of IMA was statistically significantly higher in type 2 diabetic patients when compared with the control group with *P* value less than 0.0001. The glycooxidation processes and disturbance of the oxidative–antioxidative balance can induce chronic ischemia which lead to necrosis of different tissues

Table 3 Discriminated ability of ischemia-modified albumin to predict nephropathy

	AUC	95% CI	*P* value	Cutoff	Sensitivity (%)	Specificity (%)
IMA	1.000	1.000–1.000	<0.001	105.6	100	100

AUC, area under the curve; CI, confidence interval; IMA, ischemia-modified albumin.

Figure 1



Correlation between serum level of IMA and HbA1c of the studied groups. HbA1c, glycated hemoglobin; IMA, ischemia-modified albumin.

and accelerate the development of diabetic complications [15].

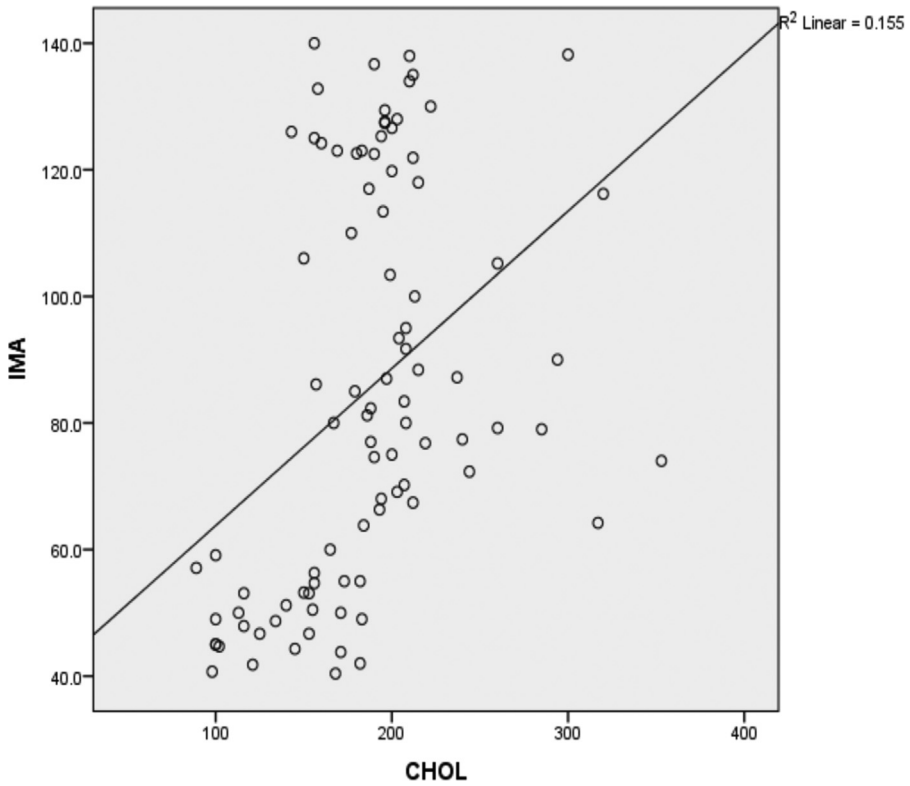
One of the most frequent complications of diabetes is diabetic nephropathy which leads to end-stage kidney disease and shortens human life [16]. Early detection and treatment of diabetic nephropathy especially at the microalbuminuric stage can potentially render it reversible [17]. Microalbuminuria, albumin concentration in 24-h urine sample, and albumin/creatinine ratio are all used as markers for prediction of diabetic nephropathy [18].

IMA is a novel marker of tissue ischemia and oxidative stress [19]. Its level is connected with diabetic complications and may be helpful in assessing the development of diabetic nephropathy, its severity, and even its response to treatment [20]. The increased plasma level of IMA in type 2 diabetes is connected with diabetic nephropathy and with chronic oxidative stress and protein oxidation, which cause kidney dysfunction [20]. Many authors pointed to the role of hypoxia in addition to the chronic oxidative stress and protein oxidation in the development of diabetic disturbance such as in renal medullary hypoxia by hypoxia-inducible factors contributing to the development of diabetic nephropathy [21]. In our study, the serum level of IMA was statistically significantly higher in type 2 diabetic patients with diabetic nephropathy compared with type 2 diabetic patients without nephropathy with a *P* value less than 0.001; also it was statistically significantly higher when compared with the control group with a *P* value less than 0.0001. IMA was a significant discriminator for diabetic nephropathy with a cutoff point of 105.6 U/Ml ($P<0.001$) with 100% specificity and 100% sensitivity using ROC curve analysis.

These findings were supported by Piwowar *et al.* [22], who reported significant higher plasma levels of IMA in patients with type 2 diabetes in comparison with healthy individuals and that IMA levels were higher in diabetic patients with complications and was related to the level of HbA1c. Moreover, Ukinc *et al.* [23] reported that plasma IMA increases in type 2 diabetic patients, either with or without cardiovascular diseases.

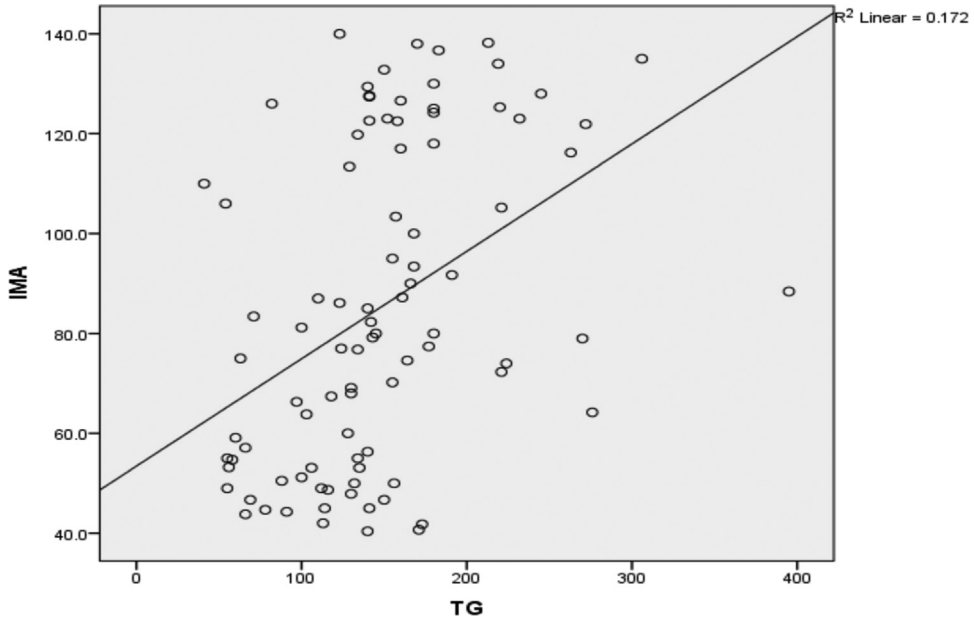
Dash and colleagues registered a high plasma IMA level in DM cases compared with controls, more prominent in the group of diabetic patients associated with complications. They also documented a positive correlation between IMA and malondialdehyde as well as IMA with hsCRP. Thus, it was apparent that

Figure 2



Correlation between serum level of IMA and serum cholesterol of the studied groups. IMA, ischemia-modified albumin.

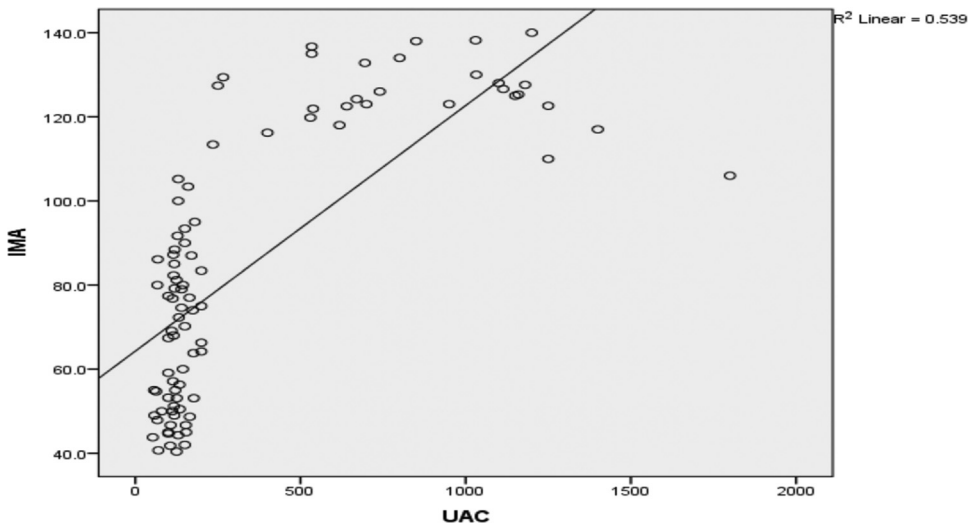
Figure 3



Correlation between serum level of IMA and serum level of triglycerides of the studied groups. IMA, ischemia-modified albumin.

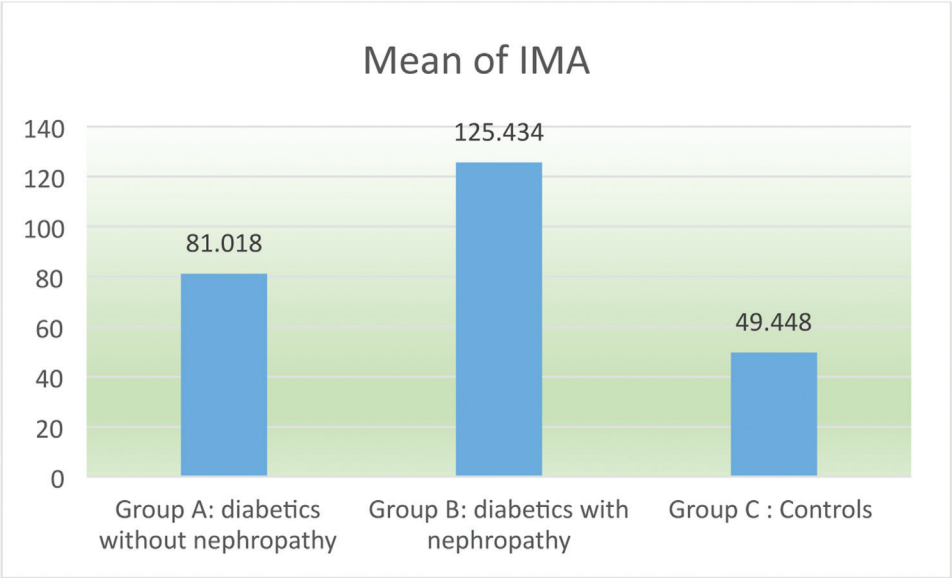
plasma IMA level increased in the presence of increased oxidative stress as indicated by the level of malondialdehyde, which is an established marker of oxidative damage. Also they found that there was a positive correlation between the level of IMA and hsCRP which was also found to be increased with advancement of the diseases suggesting that an underlying chronic inflammatory process continues in the presence of increased oxidative damage which is probably responsible for the increased endothelial damage and vascular pathology leading to hypoxia [24]. Dayanand *et al.* [25] studied the use of IMA as

Figure 4



Correlation between serum level of IMA and urinary albumin excretion ratio of the studied groups. IMA, ischemia-modified albumin.

Figure 5



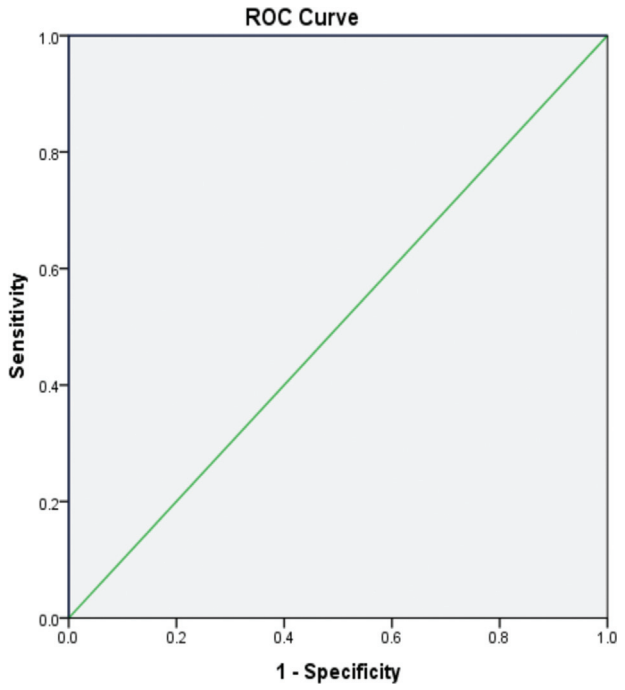
Mean values of IMA of the three groups. IMA, ischemia-modified albumin.

a marker of ischemic changes in T2DM patients and conclude that elevated IMA levels might indicate the underlying subclinical vascular disease in these patients.

Blood pressure, dyslipidemia, obesity, diabetes duration, glycemia level, and urinary albumin creatinine ratio are all risk factors associated with diabetes and contribute to the development of diabetes complications. In our study, we found that there were statistically significant positive correlations between serum level of IMA and blood pressure, duration of diabetes, BMI, FBG, 2-h postprandial, HbA1c, serum creatinine, and urinary albumin creatinine (UAC) ratio. This was in agreement with Joha *et al.* [26] who found a positive correlation between serum IMA and BMI in obese patients above 30 kg/m [2] and with Arslan *et al.* [27] study which positively correlated serum IMA with obesity and metabolic syndrome. On the contrary, Yigitbasi *et al.* [28] found no significant correlation between IMA and BMI.

Serum level of IMA positively correlated with HbA1c, a marker of glycemic control and an indicator of development of complications. This finding is supported by Chawla and colleagues, Sowjanya and colleagues, and Patil and colleagues, who reported that the positive association of raised plasma IMA with HbA1c may be an effect of increased oxidative stress and free radical generation leading to widespread

Figure 6



Receiver operating characteristic curves for IMA for discriminating diabetic nephropathy. IMA, ischemia-modified albumin.

inflammation of the vascular endothelium. The resultant tissue hypoxia might have contributed to the increased modification of albumin attributing towards raised IMA level [29–31].

The positive correlation between IMA and the UAC ratio implies that IMA increased progressively with the degree of albuminuria. This was supported by Ahmad *et al.* [32], Krzysek-Korpacka *et al.* [33], and who reported that plasma levels of IMA significantly correlated with the urinary albumin/creatinine ratio ($P<0.001$) and plasma creatinine concentration ($P<0.05$) [20].

Dyslipidemia is a major contributing factor to vascular complications in diabetes. Measuring serum IMA in diabetic patients with dyslipidemia could provide an index of ischemia due to the structural modification of circulating albumin in the serum [34]. In our study, we found that there were significant positive correlations between the level of IMA and total chlolesterol, LDL, triglycerides with a *P* value less than 0.0001 and with very low-density lipoprotein with *P* value less than 0.051. As for HDL cholesterol which plays a protective role against the development of oxidation-induced atherosclerosis, we found that there was a significant negative correlation between the level of IMA and HDL with a *P* value less than 0.001. Our findings were supported by Refaat and colleagues who evaluated the relationship between serum IMA and lipid profile in type 2 Egyptian diabetic patients. The study showed a significant positive correlation of serum IMA to serum triglycerides, total cholesterol, LDL, very low-density lipoprotein, HbA1c of type 2 diabetic patients with dyslipidemia and a nonsignificant correlation of serum IMA to all lipids of the patients with type 2 diabetes but without dyslipidemia [34]. Sowjanya *et al.* [30] concluded that there was an increase in the value of serum IMA in patients with dyslipidemia, so they concluded that there were positive correlations between serum IMA and dyslipidemia in general.Kaefer *et al.* [35] have shown that the levels of triglycerides and IMA are higher in type 2 diabetic patients with a significant correlation of IMA to triglycerides in those patients. Piwowar and colleagues had shown a weak correlation between LDL cholesterol and IMA. On the other side, Rajinder and colleagues found that those patients with higher LDL cholesterol showed lower IMA levels. Chawla *et al.* [29] found that triglycerides did not appear to affect serum IMA.

In our study serum level of IMA and duration of diabetes mellitus showed a statistically significant correlation ($P<0.05$). On the contrary, Chawla *et al.* [29] reported no significant correlation between the duration of diabetes mellitus and serum IMA. He supported the positive correlations between IMA and markers of severity of diabetes mellitus such as HbA1c, FBG, and PPBG as found in our study.

Conclusion

IMA measurement is a simple technique that it can be measured at regular intervals in every diabetes mellitus patient along with other routine parameters like HbA1c, serum creatinine, and microalbuminuria. This study optimized and validated that IMA is a sensitive and a specific alternative new biomarker for the estimation of diabetic nephropathy and demonstrated the correlations of IMA with the level of glycemia, dyslipidemia, BMI, serum creatinine, and UAC. Therefore, we recommend that IMA could serve as an indicator of glycemic control and a sensitive marker of diabetic nephropathy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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