Journal of Clinical Laboratory Analysis 31: e22058 (2017)

Evaluation of the Relationship Between Microalbuminuria and Urine Ischemia-Modified Albumin Levels in Patients with Diabetic Nephropathy

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Introduction: Ischemia-modified albumin (IMA) is a marker which can be associated with oxidative stress in various ischemic and non-ischemic processes. Oxidative stress plays roles in diabetes mellitus, its complications and pathogenesis. Serum IMA levels are examined in various clinical events. However, urine IMA levels have not yet been evaluated in diabetic patients. In this study, we aim to examine the relationship between metabolic features and urine microalbuminuria levels of diabetic patients and their urine IMA levels. *Materials and Methods:* There were totally 50 type 2 diabetic patients in the study at the Mevlana University Hospital. Patients with cerebrovascular disease, acute myocardial infarction, hemodialysis patients with end stage chronic renal failure, pulmonary embolism, and malignant disease were excluded from the study. Metabolic features, urine IMA levels and cardiological parameters of patients were evaluated. *Results:* Mean age of patients was 59 $\pm$ 9 years, 20 of them (40%) were male and 30 of them (60%) were female. There were six patients with albuminuria value of <0.03 mg/g (normal), there were 39 patients with microalbuminuria value of 0.03–0.3 mg/g and there were five patients with macroalbuminuria of >0.3 mg/g. According to the analysis of patients with microalbuminuria (n = 39), there was no correlation between IMA levels and numerical demographic data, albuminuria, glucose, HbA1c, lipid profile, creatinine, uric acid, hematological parameters. *Discussion:* Conclusively, there was no relationship between urine IMA levels and microalbuminuria related to the diabetic nephropathy. These findings can be associated with urinary excretion mechanisms of IMA. J. Clin. Lab. Anal. 31:e22058, 2017.

Key words: diabetic nephropathy; epicardial adipose tissue; ischemia-modified albumin; microalbuminuria

INTRODUCTION

Ischemia-Modified albumin (IMA), which was introduced as a novel serum biomarker of myocardial ischemia by Bar-Or et al., attracted researchers' attention and was evaluated in various ischemic and non-ischemic conditions and then suggested as an oxidative stress marker rather than being an ischemia marker (1–7). The test for detection of serum IMA levels is albumin cobalt binding test (ACB) and the test principle is based on decreased affinity of albumin to Co (II) ions due to the modifications at N-terminal region of the protein which is responsible for binding to transitional elements such as cobalt, nickel, and etc. Free radical damage, hypoxia, acidosis, sodium and calcium pump disruptions, and free iron and copper ion exposures are among suggested

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Received 16 June 2016; Accepted 31 July 2016
DOI 10.1002/jcla.22058
Published online in Wiley Online Library (wileyonlinelibrary.com).

underlying mechanisms of this modification in albumin structure. However, the exact biochemical mechanism is still unclear (8–10).

Oxidative stress has been suggested to be involved in pathogenesis of diabetes mellitus (DM) and diabetic complications. It has been previously reported that IMA levels increased in various types of diabetes (T1 DM, T2 DM), gestational diabetes and suggested to be related with impaired glucose tolerance, hyperlipidemia, and some other metabolic parameters (11–13). Diagnosis of the diabetic nephropathy (one of the complications of the diabetes mellitus) in the recognition of microalbuminuria stage is an important step in order to prevent this complication. Spot urine measurements are not sufficient sometimes in order to early diagnose the microalbuminuria. Even though it is albumin measurement in the 24 hr urine samples is the gold standard for the detection of microalbuminuria (14, 15). Therefore, sensitive and novel biomarkers should be found to diagnose the diabetic nephropathy in an early period.

Original ACB assay was developed for serum samples and serum levels of IMA were evaluated in all reports dealing with IMA except a recent study by Toker et al. in which they applied this test to salivary samples (14). But there is no report about determination of IMA in urine samples.

We aimed to evaluate urinary IMA levels and the relation between IMA levels and other metabolic parameters in diabetic patients with micro- or macroalbuminuria.

MATERIALS AND METHODS

Subjects

Mean age of patients was 59 ± 9 years, 20 of them (40%) were male and 30 of them (60%) were female. There were totally 50 type 2 diabetic patients in the study at the Mevlana University Hospital. Patients with cerebrovascular disease, acute myocardial infarction, hemodialysis patients with end stage chronic renal failure, pulmonary embolism and malignant disease were excluded from the study.

Study protocol was approved by ethics committee of our institute 27/03/2015/26857650/116 and informed consent was obtained from all participants. All procedures were carried on in accordance to Helsinki declaration. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Sample Collection

A 5 ml of venous blood samples were obtained after overnight fasting into evacuated serum separator tubes with K2EDTA (Vacuette, Greiner BioOne, Kremsmünster, Austria) and tubes without any anticoagulants (Vacuette, Greiner BioOne) by antecubital venipuncture. Blood samples in tubes with K2EDTA were used for HbA1c analysis. Sera were separated, aliquoted and used for routine chemical tests. Random urine samples (>25 ml) were obtained from all participants into plastic disposable urine collection bottles, simultaneously.

Routine Biochemical Analysis

Glucose, blood urea nitrogen (BUN), creatinine, triglyceride, total-cholesterol, HDL- cholesterol, high sensitive C-reactive protein, sodium, potasium, serum albumin, serum uric acid, HbA1c, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) levels were determined on Cobas Integra 800 system (Roche Diagnostics, Rotkreuz, Switzerland) with the original Cobas reagents (Roche Diagnostics). Urinary albumin and creatinine concentrations were measured on the same analyzer using original Cobas reagents. After urine chemistry analysis, the remaining urine samples were aliquoted in polypropylene tubes with stoppers and stored at −70°C until analysis.

Modified ACB Assay in Urine Samples

At the time of analysis all the urine samples are thawed and mixed well. We have modified ACB test described by Bar-Or et al. (1) and used this modified method for estimation of urinary ischemia modified albumin levels. Test steps of the modified ACB method for urine samples are as follows: *Step 1*. Determination of the pH values of urine samples. *Step 2*. Pipetting 200 µl of urine samples into polypropylene tubes. *Step 3*. Addition of 200 µl citrate-phosphate buffer (0.2 M pH = 7) to the reaction tubes. *Step 4*. Addition of 100 µl 0.1% cobalt chloride solution. *Step 5*. After mixing with vortex for 10 sec, incubation of the tubes for 15 min at room temperature. *Step 6*. Addition of 100 µl dithiothreitol (DTT) 1.5 mg/ml H_2O to the reaction mixture as colorizing agent. Brownish color develops by means of the reaction of DTT with the unbound cobalt ions in the reaction mixture. *Step 7*. Quenching of the reaction 5 min later by addition of 1.0 ml NaCl (0.9%). *Step 8*. Preparation of the blank similarly with the exclusion of DTT. *Step 9*. Reading the absorbance values of sample and blank tubes at 470 nm using a spectrophotometer (Shimadzu UV 1800, Tokyo, Japan). *Step 10*. IMA values for urine samples were calculated by subtraction of the blank

absorbance values form the ones recorded for sample tubes. The results were reported as absorbance units (ABSU). Intra- and inter-assay CV values for this modified ACB test were <5% and <10%, respectively. All chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich, GmbH, Taufkirchen, Germany).

Since IMA values present strong negative correlations with albumin concentrations and many authors developed some formulas or adjustments for IMA values with albumin concentrations. We used Urinary IMA/Urinary Creatinine, Urinary IMA/Urinary albumin, Urinary IMA/Urinary albumin creatinine ratio values for adjusted urinary IMA values.

Measurement of Epicardial Adipose Tissue

Echocardiography data of all patients were taken by scanning the device. Scanning was performed by the same cardiologist who was unaware of the clinical data. Epicardial adipose tissue measurements were performed in the parasternal long axis view by measuring the layer between the echodens pericardium and echolucent space on right ventricular free wall, perpendicularly, at the end of diastole (16).

Statistical Analysis

SPSS 17.0 (SPSS for Windows, Chicago, IL) was used to analyze the data. Continuous variable distribution was evaluated by using the Kolmogorov Smirnov test. Categorical values were represented as percentage and frequency. Besides, continuous values which were normally distributed were represented as mean and standard deviation and continuous values which were not normally distributed were represented as percentage and frequency. Since the urine IMA values were normally distributed, spearman correlation analysis was used in order to determine the correlation between urine IMA levels and other parameters. Kruskal–Wallis test was used, when two groups of patients (which were grouped according to their HbA1c and BMI values) were compared to each other in terms of their IMA and other parameters, and when three groups of patients (which were grouped according to their albuminuria levels) were compared to each other in terms of their IMA and end organ damage markers. Statistical significance was accepted as $P < 0.05$.

RESULTS

Patient Characteristics

Mean age of patients was 59 ± 9 years, 20 of them (40%) were male and 30 of them (60%) were female. There were totally 50 type 2 diabetic patients in the study. Mean HbA1c level of patients was % 8.44 ± 1.52 and 14 patients (28%) had nine or higher HbA1c level. The median value of BMI value of patients was 30.5 kg/m^2 (20.5–52) whereas 27 of them (54%) were obese and 23 of them (46%) of them were non-obese. There were six patients with albuminuria value of <0.03 mg/g (normal), there were 39 patients with microalbuminuria value of 0.03–0.3 mg/g, and there were five patients with macroalbuminuria of >0.3 mg/g. Laboratory findings of patients can be seen in Table 1. Urine albumin and IMA values, IMA ratio of urine creatinine and microalbumin can be seen in Table 2. In the same table, ejection fraction and epicardial fat ratio can also be seen.

Ischemia-Modified Albumin and Diabetes Regulation and Metabolic Parameters

When the correlation between IMA levels and age, findings related to diabetes, lipid levels, urine albumin levels, urine albumin/creatinine levels was evaluated, there was a weak and negative correlation between IMA levels and age (Table 3). Patients were classified into two groups according to their HbA1c levels (HbA1c levels below or above 9) and their urine IMA and albuminuria levels were compared to each other. According to the results, it was found that only urine albumin levels were different in both groups (9.4 (0.89–90); 10.5 (2.86–4.53), Mann–Whitney U test, $P = 0.026$). Besides, urine IMA levels, urine albumin/creatinin, urine IMA/creatinin levels were similar in both groups (Mann–Whitney U test, for all parameters, $P > 0.05$). Similarly, when these parameters were compared in obese and non-obese diabetic patients, all findings obtained from urine and serum were found as similar in both groups.

Relationship Between IMA, Albuminuria and Cardiovascular Risk Factors

When patients with albuminuria and patients without albuminuria were compared to each other, age,

TABLE 1. Laboratory Parameters of Patients

Glucose (mg/dl)	186 (88–330)
Creatinine (mg/dl)	0.71 ± 0.20
Total cholesterol (mg/dl)	194.5 (129–571)
Triglycerides (mg/dl)	170 (56–1073)
HDL cholesterol (mg/dl)	43 (26–114)
LDL cholesterol (mg/dl)	114 (49–319)
Uric acid (mg/dl)	5.02 ± 1.52
White blood cells (/mm^3)	7602 ± 2069
Hemoglobin (g/dl)	13.91 ± 1.84
Platelets (×1000/mm^3)	242 ± 56

TABLE 2. Urine Albumin, Creatinine, Ejection Fraction and IMA Values, Epicardial Fat Rate

Urine albuminuria (mg/dl)	9.8 (0.89–90)
Creatinine in urine (mg/dl)	111.2 (11.8–508)
Urine albumin/creatinine rate (gr/mg)	0.1375 (0.0082–0.8050)
IMA in urine	0.232 (0.157–0.552)
IMA/urine creatinine	0.00216 (0.0004–0.0186)
IMA/albuminuria	0.0233 (0.0024–0.2664)
(IMA)/(Microalbuminuria/creatinine)	2.99 (0.03–31.00)
Ejection fraction (%)	70 (50–75)
Epicardial fat (mm)	0.59 (0.25–9.28)

IMA, Ischemia-modified albumin.

TABLE 3. Correlation Between IMA and Demographic Data, Lipid Levels and Atherosclerosis-Related Parameters

	rho	*P*
Age	**−0.300**	**0.034**
HbA1c	0.002	0.986
BMI	−0.264	0.064
Cholesterol	0.178	0.216
Triglycerides	0.125	0.387
LDL-cholesterol	0.132	0.366
HDL-cholesterol	0.103	0.476
Urine albumin/Creatinine ratio	0.062	0.668
Left ventricular diameter	−0.031	0.828
Ejection fraction	0.150	0.298
Epicardial fat	**−0.302**	**0.033**
Systolic blood pressure	−0.192	0.181
Diastolic blood pressure	−0.073	0.616

Spearman Correlation Analysis.
Bold values are statistically significant.

HbA1c, glucose, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol levels were similar in normal, microalbuminuria patients and macroalbuminuria patients (Kruskal–allis test, $P > 0.05$, for all comparisons). End organ damage markers (such as creatinine, ejection fraction, left ventricular diameters, epicardial adipose tissue and blood pressure values) in albuminuria groups were also similar to each other (Kruskal–Wallis test, $P > 0.05$, for all comparisons). Similarly, when three groups were compared to each other in terms of their IMA levels, it was found that IMA levels were high in patients with microalbuminuria ($n = 39$) whereas the lowest IMA levels were shown in patients with macroalbuminuria ($n = 5$). Even though IMA levels of patients with microalbuminuria and macroalbuminuria were different, the difference was not statistically significant (Kruskal–Wallis test, $P = 0.071$) (Table 4).

Ischemia-Modified Albumin and Microalbuminuria

According to analysis which was performed by considering patients who had only microalbuminuria, there was no correlation between IMA levels and numerical demographic data, albuminuria, glucose, HbA1c, lipid levels, creatinine, uric acid, and hematological parameters (Spearman Correlation Analysis, for all correlations, $P > 0.05$). There was no difference between patients with 9 or higher HbA1c levels ($n = 13$) and patients with lower than 9 HbA1c levels ($n = 26$) in terms of their IMA levels (0.251 (0.176–0.509) vs. 0.234 (0.157–0.552) (Mann–Whitney U test, $P = 0.965$). Similarly, there was no similarity between obese ($n = 17$) and non-obese ($n = 22$) patients (0.249 (0.176–0.509) vs. 0.239 (0.157–0.552) (Mann–Whitney U test, $P = 0.944$). There was a positive correlation between serum creatinine and uric acid levels and epicardial adipose tissue in patients with microalbuminuria (rho = 0.445, $P = 0.005$; rho = 0.340, $P = 0.034$, Spearman Correlation Analysis respectively).

DISCUSSION

As a result of our study, we could not find relationship between urine IMA levels and microalbuminuria related to the diabetic nephropathy. The relationship between serum IMA levels and kidney functions and diabetes mellitus is not valid for the relationship between urinary IMA levels and kidney functions and diabetes mellitus. The relationship between serum IMA levels and diabetes and its complications are discussed in the literature whereas urinary IMA levels and these complications are not associated to each other.

Urinary albumin/creatinine ratios are important markers in the evaluation of the albuminuria. However, definitive diagnosis is not always possible due to some technical issues. Recognition of the nephropathy in early stages is very important since early diagnosis is the reversible step of this complication (14, 15). Oxidative stress is one of the development mechanisms of diabetic complications. Recently, there have been various studies performed on diabetic complications and serum IMA levels and close relationship has been determined (11, 17). Therefore, we examined the urinary IMA levels and microalbuminuria due to the possible pathogenesis. However, there is no relationship between urinary IMA and microalbuminuria.

In the body, IMA formation occurs not only acutely but also due to the chronic oxidative damage (11, 18). By considering this, we did not detect a relationship between urinary IMA levels and diabetic microalbuminuria most probably due to two mechanisms. Primarily, urinary IMA levels may not be affected in the early steps of diabetic nephropathy. Secondarily, it is possible that we did not find any relationship due to the urinary excretion mechanisms of IMA.

TABLE 4. Comparison Between IMA and End Organ Damage in Albuminuria Group

	Normoalbuminuria (n = 6)	Microalbuminuria (n = 39)	Macroalbuminuria (n = 5)	P
IMA in urine	0.221 (0.181–0.255)	0.244 (0.157–0.552)	0.205 (0.161–0.227)	0.071
Left ventricular diameter (mm)	10.5 (1–12)	10 (1–15)	8.25 (1–14)	0.760
Epicardial adipose tissue (mm)	0.63 (0.25–9.28)	0.58 (0.33–1.32)	0.74 (0.40–0.81)	0.792
Ejection fraction (%)	67.5 (55–75)	70 (55–75)	70 (50–70)	0.952
Serum creatinine (%)	0.7 (0.4–0.9)	0.8 (0.4–1.2)	0.6 (0.3–1.0)	0.533

Kruskal–Wallis test.

In the process starts from the early stages of diabetic nephropathy to end-stage renal disease (ESRD), cardiovascular diseases progressively develop. Furthermore, cardiovascular diseases are the most important reason of the mortality (19, 20). There is a close relationship between serum IMA levels and classical atherosclerotic risk factors (11, 21). Epicardial fat thickness which is one of the cardiovascular risk markers have been recently examined (22). The relationship between serum IMA levels with epicardial fat and serum IMA levels was examined only by Baysal et al. in obese children population and the relationship was significant (23). In our study, we did not detect any association between urinary IMA levels and epicardial fat. Since serum IMA levels were not measured in our patient group, we cannot compare our findings with results of Baysal et al.

Serum IMA levels are examined in the literature and relationships between obesity, ESRD, hyperlipidemia, inflammation (except in diabetes mellitus) are shown (1, 3, 11, 23–25). In our study, there was no association between these parameters and urinary IMA levels.

Conclusively, urinary IMA levels are not as valuable as serum IMA levels in the detection of diabetes mellitus and related metabolic diseases. In order to use urinary IMA levels as the diagnostic marker, there should be further studies performed about urinary excretion mechanisms of IMA.

LIMITATIONS

Our study has some limitations. The single-center nature of this investigation may limit its applicability to the general population. Because of relatively limited size of sample, the interpretation of results should be cautious. Another limitation of our study is that urine IMA levels were not evaluated even though serum IMA levels were assessed.

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