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Ischemia Modified Albumin Levels in Patients with Diabetic Nephropathy

Diyabetik Nefropatili Hastalarda İskemi Modifiye Albumin Düzeyleri

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Abstract

Objective: Diabetic nephropathy is a frequent complication of diabetes mellitus and is a common cause of the end-stage renal disease. Diabetic nephropathy should be detected and treated at the early microalbuminuria stage, which is potentially reversible. Microalbuminuria used as a gold standard to determine the degree of advancement of diabetic nephropathy has certain limitations; hence, additional markers are being sought for the early identification of diabetic complications. Ischemia-modified albumin, an oxidative stress marker, has been shown to be associated with diabetic complications.

The aim of the study: The present study is undertaken to evaluate ischemia-modified albumin as a useful marker in estimating kidney dysfunction in patients with diabetic nephropathy along with urine microalbumin and creatinine.

Material and Methods: This was a case-control study that enrolled 40 patients with diabetic nephropathy and 40 age- and gender-matched controls. All biochemical parameters were analyzed on a Beckman Coulter Unicel DXC 600 clinical chemistry auto analyzer, Galway, Ireland. Ischemia-modified albumin was estimated by the albumin cobalt binding test using a Perkin Elmer Lambda 25 spectrophotometer.

Results: Compared with controls, urine microalbumin and ischemia-modified albumin levels were significantly increased in patients with diabetic nephropathy ($p<0.001$). Ischemia-modified albumin levels showed a significant positive correlation between creatinine and microalbumin in patients with diabetic nephropathy. Ischemia-modified albumin showed the high sensitivity of 92.5% and specificity of 97.5% than microalbumin did.

Conclusion: The results suggest that simultaneous measurement of ischemia-modified albumin with other parameters such as creatinine and microalbumin might be useful in identifying early kidney dysfunction in patients with diabetes mellitus.

Keywords: Diabetic nephropathy; ischemia-modified albumin; kidney dysfunction; microalbumin; oxidative stress

Özet

Amaç: Diyabetik nefropati; diyabetin sık görülen bir komplikasyonu olup, son dönem böbrek hastalığının en sık nedenidir. Diyabetik nefropati geri dönüşümlü olabildiği erken mikroalbuminüri evresinde tespit ve tedavi edilmelidir. DN derecesini tespit etmede altın standart olarak kullanılan mikroalbüminürinin bazı kısıtlamaları olmasından dolayı diyabetik komplikasyonların erken tanınması için ek belirteçler aranmaktadır. Bir oksidatif stres belirteci olan iskemi modifiye albüminin diyabetik komplikasyonlarla ilişkili olduğu gösterilmiştir. Bu çalışma, diyabetik nefropati olan hastalarda böbrek fonksiyon bozukluğunun tahmini için iskemi modifiye albüminin idrarda mikroalbümin ve kreatinin ile birlikte faydalı bir belirteç olup olmadığını değerlendirmek için gerçekleştirilmiştir.

Gereç ve Yöntemler: Bu vaka-kontrol çalışmasına, 40 DN hastası ile yaş ve cinsiyet açısından eşleşen 40 sağlıklı kontrol alınmıştır. Tüm biyokimyasal parametreler Beckman Coulter Unicel DXC 600, Galway, İrlanda marka klinik kimya oto analizörü kullanılarak analiz edilmiştir. IMA tahmini, Perkin Elmer Lambda 25 spektrofotometre kullanılarak albümin kobalt bağlayıcı test ile yapılmıştır.

Bulgular: Kontrollerle kıyaslandığında, diyabetik nefropati hastalarında idrar mikroalbümin ve iskemi modifiye albümin düzeylerinin anlamlı olarak yükseldiği gözlenmiştir ($p<0.001$). Diyabetik nefropati hastalarında iskemi modifiye albümin düzeyleri ile kreatinin ve mikroalbümin arasında anlamlı pozitif korrelasyon gözlendi. Mikroalbümine kıyasla iskemi modifiye albümin daha yüksek duyarlılık ve özgüllüğe sahip idi (sırasıyla %92,5 ve %97,5).

Sonuç: Sonuçlar, iskemi modifiye albümin değerlerinin kreatinin ve mikroalbümin gibi diğer parametrelerle eş zamanlı ölçümünün diabetes mellitus hastalarında erken böbrek fonksiyon bozukluklarının belirlenmesinde faydalı olabileceğini göstermiştir.

Anahtar kelimeler: Diyabetik nefropati; iskemi-modifiye albümin; böbrek fonksiyon bozukluğu; mikroalbümin; oksidatif stres

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Introduction

The characteristic features of diabetic nephropathy (DN) are a persistent loss of albumin in urine, progressive fall in glomerular filtration rate, and increase in blood pressure leading to an increase in cardiovascular morbidity and mortality (1). Microalbuminuria is usually characterized by the albumin excretion rate in urine (between 30 and 299 mg/24 h), which is the earliest index for the evaluation of DN. The cause for increased albumin excretion in urine could be attributed to the damage in the renal endothelium because of hyperglycemia, oxid ative stress (OS), inflammation, and ischemia, further resulting in defective glomerular membrane filtration (2). Several studies have reported that patients with type 2 diabetes mellitus progressed to the development of DN even with normal urine albumin levels, as microalbuminuria starts only when the glomerular function has been damaged substantially (3, 4). Because of this important observation, the use of microalbuminuria as a better predictive marker is limited and, therefore, a marker with high sensitivity and specificity for the early detection of DN is needed (5). The major cause for vascular and biochemical alterations in patients with DN is chronic hyperglycemia. This leads to increased oxidative stress in the vascular and cellular environment resulting in endothelial dysfunction, an early consequence of chronic hyperglycemia. Oxidative stress markers, such as reactive aldehydes and other aldehydes, advanced glycosylation end products, malondialdehyde, lipid hydroperoxides, oxidized low-density lipoproteins, advanced lipoxidation end products, arachidonic acid derivatives, F2 isoprostanes, have been evaluated in patients with DN. An increased generation of reactive oxygen species (ROS) leads to the alteration in the structure of the albumin molecule. In patients with type 2 diabetes mellitus, within a few minutes of arterial occlusion, increased generation of free radicals and decreased antioxidant production might lead to the structural modifications in albumin. This structurally altered form of albumin is termed as ischemia-modified albumin (IMA). IMA was first described as a marker of myocardial ischemia; it was later shown to be associated with OS-associated conditions progressing to sudden ischemic changes and hypoxia in the endothelial cells (6, 7). The diagnostic role of IMA has been reported in cardiac and noncardiac disorders (8). IMA, an oxidative stress marker, has been shown to be associated with diabetic complications (9). Hence, the present study evaluated the utility of IMA in comparison with microalbuminuria in assessing renal dysfunction in patients with DN.

Material and Methods

A case-control observational study was conducted involving 80 age- and gender-matched individuals. Data were collected prospectively. In the present study, 40 patients with DN visiting the outpatient departments of nephrology and endocrinology at Sri Venkateswara Institute of Medical Sciences, Tirupati, India from March 2012 to February 2013 were included. Patients with type 2 diabetes mellitus with urinary albumin excretion (UAE) of 30-299 mg/g creatinine on two occasions were included in group 2. Patients with macrovascular complications such as cardiovascular, cerebrovascular, and peripheral vascular diseases; acute infections; inflammatory disorders; known cases of liver, renal, and thyroid disorders; cancer; human immunodeficiency virus infection; patients on hormonal medication; and pregnant and lactating women were excluded. Forty age- and gender-matched healthy individuals with normoalbuminuria (UAE<30 mg/g creatinine) from the hospital staff were taken as controls (group 1). Subjects with diabetes mellitus, pregnant and lactating women, history of smoking and alcoholism, active or chronic persistent infection, inflammation, and those not willing to participate in the study were excluded as controls. The data of family history and personal history were collected through a standard questionnaire. The study was conducted after obtaining approval from the institutional ethics committee and written informed consent from the participants in the study was taken before sample collection.

Sample collection

Four milliliters of fasting venous sample were collected. From that sample, 3 mL was collected in additive-free tubes and 1 mL

was transferred to sodium fluoride vials. The collected samples were centrifuged at 3000 revolutions per min for 15 min, and the centrifuged samples were stored at −80 °C until further analysis. Random spot urine samples were collected and the urine samples were centrifuged at 3000 revolutions per min for 10 min. The centrifuged urine samples were stored at −80 °C for microalbumin (MA) analysis.

Laboratory analysis of biochemical parameters

Fasting plasma glucose (FPG) and postprandial blood sugar (PPBS) were measured by the glucose oxidase-peroxidase method. Serum urea and creatinine levels were estimated by using UV-kinetic and Modified Jaffe's rate kinetic methods, respectively, on a Beckman system pack (10). Serum albumin was estimated by the bromocresol green method using commercial kits. Urine MA was estimated by the turbidimetry method (11). All the above parameters were analyzed on a Beckman Coulter Unicel DXC 600 clinical chemistry autoanalyzer, Galway, Ireland. IMA was estimated by the albumin cobalt binding Test on a Perkin Elmer Lambda 25 spectrophotometer (12).

Statistical analysis

The distribution of data was tested by using the Kolmogorov-Smirnov test. For the data, normally distributed values were expressed as mean±standard deviation (SD). The unpaired t-test was used for comparing means between controls and cases. Correlation between the studied parameters was assessed using Pearson's correlation analysis. To assess the utility of IMA, receiver operating characteristic (ROC) curve analysis was used to calculate the area under the curve (AUC). Data analysis was performed using Microsoft Excel spreadsheets and statistical software SPSS version 22.0. A two-tailed p-value of <0.05 was considered significant.

Results

The mean±SD of the biochemical parameters of 40 controls and 40 patients with DN by using the unpaired t-test are given in Table 1. Compared with the controls, the patients with DN had significantly elevated FPG, PPBS, urea, creatinine, potassium, MA and IMA levels and significantly decreased sodium levels (p<0.001). However, serum albumin levels between the two groups were similar. The bivariate Pearson's correlations between IMA with other biochemical parameters are given in Table 2. A statistically significant positive correlation was found between IMA and creatinine (r=0.345, p=0.002) and between IMA and MA (r=0.530, p<0.001). Figure 1 shows the ROC curve analysis for IMA, MA, and creatinine. The AUC for IMA was highly significant (AUC=0.987) compared with AUCs for MA and creatinine (AUC's=0. 851 and 0.947, respectively). The ROC curve findings also found that at the best cut-off value, IMA was the better marker in diagnosing end-stage renal disease (ESRD) with

Table 1. Mean and SD of the biochemical parameters of the study and control groups.

Parameter	Group 1 (n=40)	Group 2 (n=40)	p-value
FPG (mg/dL)	90.27±11.39	144.60±48.38	<0.001*
PPBG (mg/dL)	108.02±3.57	198.05±23.8	<0.001*
Urea (mg/dL)	20.65±5.26	38.57±15.36	<0.001*
Creatinine (mg/dL)	0.66±0.16	1.69±1.34	<0.001*
Sodium (mmol/L)	141.10±4.41	135.10±4.37	<0.001*
Potassium (mmol/l)	3.91±0.41	4.39±0.40	<0.001*
Albumin (g/dL)	3.76 ±0.29	3.73±0. 88	0.752†
IMA (ABSU)	0.66±0.15	1.15±0.14	<0.001*
MA (mg/dL)	0.56±0.44	10.37±8.53	<0.001*

Group 1: Controls, Group 2: diabetic nephropathy; n = number of patients and controls, FPG=fasting Plasma glucose, PPBG=postprandial blood glucose. IMA=ischemia modified albumin, ABSU= Absorbance units, MA=microalbumin. * Significant at the 0.05 probability level. † NS- Not significant at the 0.05 probability level.

Table 1. Pearson's correlation analysis of ischemia-modified albumin vs. other biochemical parameters in patients of diabetic nephropathy.

Parameter	r-value	p-value
FBG (mg/dL)	0.519	<0.001*
PPBG (mg/dL)	0.543	<0.001*
Urea (mg/dL)	0.468	<0.001*
Creatinine (mg/dL)	0.345	0.002*
Sodium (mmol/L)	–0.441	<0.001*
Potassium (mmol/l)	0.374	<0.001*
Albumin (g/dL)	- 0.029	0.798 †
MA (mg/dL)	0.530	<0.001*

r – Correlation coefficient * Significant at the 0.05 probability level. † NS- Not significant at the 0.05 probability level.

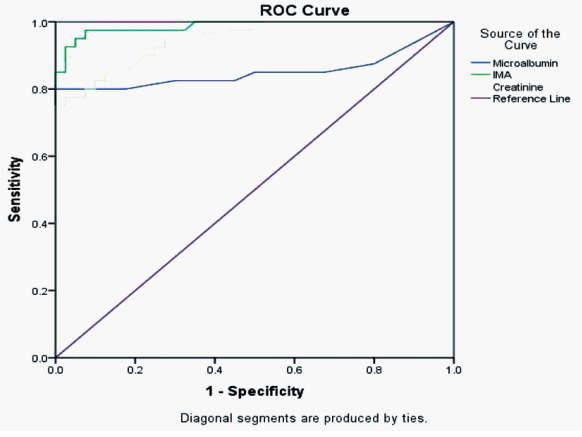

Figure 1: ROC Curve analysis of ischemia-modified albumin in patients with diabetic nephropathy.

high sensitivity of 92.5% and specificity of 97.5% compared with other parameters.

Discussion

The major microvascular complication of diabetes mellitus is DN; it is the most common cause of ESRD, which decreases the quality of life in patients with diabetes mellitus. The prevalence of diabetes mellitus and DN is increasing progressively. Biomarkers play a major role in the early detection of DN. In the present study, compared with controls, urinary albumin levels were significantly increased in patients with DN. These findings are consistent with those of Jafar et al. who reported that MA is a predictor of diabetic complications as well as an independent risk factor for cardiovascular disease (13). Piwowar et al. suggested the need for additional markers to identify patients in the early stage, as multiple mechanisms contribute to nephropathy development and its progression (14). IMA was used to assess the function of albumin. Under oxidative stress conditions, the structure and function of albumin might be altered. This impairs albumin's ability to bind the transition metal cobalt, which is a measure of IMA. In our study, statistically significant increased IMA levels were observed in patients with DN than in healthy controls. Our results are consistent with those of Piwowar et al. who showed an association of IMA in diabetes for the first time and reported a 75% increased value of IMA in patients with diabetes compared with controls (15). Similar findings were also reported by Ukinc et al. (16). Piwowar et al. also suggested that the measurement of IMA in patients with diabetes mellitus might be helpful in the diagnosis and monitoring of the course of the disease, especially with different stages of renal disorders (17). However, conflicting results have been reported with respect to IMA levels in patients with type 2 diabetes mellitus without complications. Although a few studies have reported elevated levels of IMA in patients with type 2 diabetes (18), others have not reported differences in the IMA levels in patients with type 2 diabetes mellitus and those in controls (19, 20). Dash et al. and Borderie et al. suggested that IMA as an ischemia marker might indicate the presence of underlying subclinical disease or vascular dysfunction in DN (21, 22). Hyperglycemia is directly related to the overproduction of ROS, leading to oxidative protein damage (23). Chronic hyperglycemia triggers increased generation of ROS, which is responsible for chemical and molecular damage of carbohydrates, lipids, proteins, DNA, and nucleotides. These changes profoundly influence cellular function and lead to abnormal vascular remodeling in the kidneys leading to the development of DN (24). Hyperglycemia-induced ischemia, oxidative stress, and inflammation increase serum IMA levels, resulting in podocyte malfunction in the kidneys. Because of the decreased degradation of structurally

modified albumin in the kidneys, oxidatively modified forms of albumin in the serum are possibly increased (25). However, in our study, all study participants had normal serum albumin levels. This excluded the possible influences of higher albumin concentration on increased IMA levels in patients with DN in this study.

Furthermore, to asses, if IMA levels were related to the degree of renal dysfunction, Pearson's correlation analysis was performed between the kidney dysfunction markers creatinine and MA and the altered albumin molecule. Statistically significant positive correlations were observed between IMA and creatinine and between IMA and MA. Similar results were reported by Dahiya et al., whereas contrasting results were reported by Bilgi et al. who reported no correlation between IMA levels and albuminuria and creatinine (26, 27). Based on the association between IMA and the parameters used in the routine diagnosis of renal dysfunction, our results suggest that IMA is a useful marker for detecting early renal dysfunction in DN.

Conclusion

IMA can be considered as a useful marker for the early detection of DN along with the established markers such as serum creatinine and urine MA.

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Authorship Contributions

Idea/Concept: K. Udaya Bhaskhar, Harini Devi, Aparna. R. Bitla, Srinivasa Rao, Siva Kumar, Alok Sachan; Design: K.Udaya Bhaskhar, Harini Devi, Aparna.R.Bitla, Srinivasa Rao, Siva Kumar, Alok Sachan; Control/Supervision: K. Udaya Bhaskhar, Harini Devi, Aparna. R. Bitla, Srinivasa Rao; Data Collection and/or Processing: K. Udaya Bhaskhar, Harini Devi; Analysis and/or Interpretation: K. Udaya Bhaskhar, Harini Devi; Literature Review: K. Udaya Bhaskhar, Harini Devi; Writing the Article: K. Udaya Bhaskhar, Harini Devi; Critical Review: K. Udaya Bhaskhar, Harini Devi, Aparna. R. Bitla; References and Fundings: K. Udaya Bhaskhar, Harini Devi, Aparna.R.Bitla; Source(s) of support: SBAVP faculty scheme for financial assistance.

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