Correlation of Pentosidine with Kidney Diseases in Iraqi Patients with Diabetic Nephropathy

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Abstract
Diabetic nephropathy (DN) is a principle cause of microangiopathy and the main reason for kidney disease at the end stage in patients with type 2 diabetes mellitus (T2DM). This work aimed to study the relation of pentosidine with kidney injury in the case of diabetic nephropathy. This study included 75 patients suffering from T2DM and 75 apparently healthy subjects. The patients group was divided into three groups (normoalbumin, microalbuminuria, and macroalbuminuria; 25 patients for each) on the basis of albumin-creatinine ratio (ACR) . The level of serum pentosidine was determined using an ELISA kit. The level of pentosidine was found to be significantly higher in DN patients than in the healthy group. Also, the results revealed a strong positive correlation of pentosidine with each of creatinine and blood urea levels, while a negative correlation was recorded with eGFR. It can be concluded that pentosidine may be associated with disease progression and it may be employed as one of the most efficient markers for the prediction of renal function.

Keywords: Type two Diabetes Mellitus, nephropathy, Pentosidine, microalbuminuria, macroalbuminuria
Introduction

In previous studies, diabetes mellitus was investigated with different complications that include osteoporosis, neuropathy, and nephropathy [1-3]. Diabetic renal disease, termed as diabetic nephropathy (DN), is considered as a popular complication of diabetes and is the major source of renal disease that raises diabetic patient morbidity and mortality. DN morphological anomalies involve early glomerular hypertrophy, thickening of the membrane glomerular basement [GBM], loss of podocytes, extension of the mesangial matrix, and tubular damages [4].

Over 50 percent of T2DM patients and 30 percent of type one diabetes mellitus (T1DM) develop kidney failure which leads to end-stage renal disease (ESRD) in a large number of cases [5]. Furthermore, diabetic patients with ESRD are more likely to undergo adverse macrovascular problems, such as stroke, coronary artery disease, and cerebrovascular diseases, resulting in increased rates of mortality [6]. Diabetic kidney disease is identified by albuminuria and/or an initially raised glomerular filtration rate (GFR), which is reduced in the middle to the end stage [7]. DN is a causative disorder distinguished by association of hemodynamic and metabolic factors that include elevated blood sugar and advanced glycation end products (AGEs) [8]. Reactive derivatives are created by a non-enzymatic Maillard reaction, which occurs among carbohydrates and the free amino groups of proteins, e.g. lysine and arginine residues. These groups are subjected to a sequence of complicated reactions that leads to the production of a complex group of irreversible compounds, namely AGEs, that cause the complex disorder of metabolic syndrome [9]. The covalent coupling between methylglyoxal (MG) and glyoxal, on one side, and the free amino and thiol groups in proteins, on the other side, was found to be responsible for the formation of these compounds [10].

Pentosidine was well described as a biomarker for the production and accumulation of AGEs that are to play an important role in diabetes and vascular disorders [11,12]. Checking diabetic patients with the use of pentosidine would provide a strong long-term glycemic management tool that can have a significant impact on the levels of glycated hemoglobin. Pentosidine is a fluorescent agent that can be tested by using several methods, including spectrofluorimetry, ELISA, HPLC, and mass spectrometry [13]. It is one of the better compounds present in humans in the chemically characterized AGEs. Previous researchers have found that higher serum level of pentosidine is correlated with increased arterial wall thickness and stiffness in diabetic patients [14]. Essentially, the formation of pentosidine can occur as a result to glycosylation and oxidation. The elevated plasma level of pentosidine in diabetes may also be caused by increased oxidative stress. Plasma pentosidine is strongly associated with low GFR, high oxidative stress, and inflammatory conditions in diabetic kidney disorder [15]. Thus, the aim of the current study is to evaluate the relation between pentosidine and kidney diseases in the case of DN.

Materials and methods

Studied groups

Seventy-five T2DM patients aged 35-65 years who attended Baghdad Medical City/Baghdad Teaching Hospital were included in this study. On the basis of albumin-creatinine ratio (ACR), the patients were classified into three main categories: 25 patients suffering from diabetes with ACR < 30mg/g (normoalbuminuria group), 25 patients with ACR range of 30–300mg/g (microalbuminuria group), and 25 patients with ACR > 300 mg/g (macroalbuminuria group). Exclusion criteria involved subjects with T1DM, pregnancy, congestive hard failure, systemic lupus erythematosus, and polycystic kidney disease. In addition, the control group consisted of 75 healthy subjects aged 35-65 years.
Blood Samples
Venous blood samples (10 ml) were collected in vacuum tubes with a clot activator. Samples were centrifuged for 15 minutes at 1500 rpm, then the serum was separated and stored at −20°C until analysis.

Experimental section
The enzyme-linked immune sorbent assay (ELISA) kit (Al-Shkairate establishment for Medical Supply, Jordan) was used to determine the level of pentosidine. The enzymatic procedure was used to determine the levels of fasting blood glucose [FBG], urea, and creatinine. The particle enhanced turbidimetric inhibition immunoassay (PETINIA) [13] was used to measure microalbumin level. The collected urine samples were used to estimate albumin and creatinine. A modified kinetic Jaffe technique was used to evaluate creatinine in urine. Albumin/creatinine ratio (ACR) was calculated by dividing microalbumin level by creatinine level in the urine. To calculate eGFR the formula of the “modification of diet in renal disease” (MDRD) was applied [16].

Statistical analysis
The data was analysed by IBM SPSS for Windows, Version 22.0. One-way analysis of variance (ANOVA) was used for determining whether the mean variations among the four different analyzed groups are statistically significant. Pearson correlation analysis was applied to obtain the value of the correlation coefficient (r). ROC curve analysis was used also in this study to estimate the strength of each marker to be useful in diagnosis of the disease [17-18].

Results
Table 1 shows mean ± SD values of age, BMI, FBG, duration of diabetes, ACR, GFR, urea and creatinine. High significant increases (p=0.00) in the duration of disease values were observed among the three groups of patients. In the patients group, there was also a significant increase in the level of FBG (p=0.00) relative to normal subjects. In addition, significant increases (p=0.00) were found in urea and creatinine levels in the microalbuminuria and macroalbuminuria groups in comparison to patients with normoalbuminuria and the control groups. Microalbuminuria and macroalbuminuria patient groups showed a substantial decline in eGFR value compared to patients with normoalbuminuria and control group. The results of ACR showed a significant difference (p=0.00) among all patient groups.

Table 1-Demographic and Clinical Characteristics of the Studied Groups

| Parameters          | Control group (n=75) | DM with normoalbuminuria group (n=25) | DM with microalbuminuria group (n=25) | DM with Macroalbuminuria group (n=25) | P value |
|---------------------|----------------------|---------------------------------------|--------------------------------------|---------------------------------------|---------|
| Age (years)         | 52.70±2.63a          | 53.68±9.68a                          | 54.56±5.14a                         | 54.40±6.34a                          | 0.36    |
| Duration (Years)    |                      | 5.52±2.04a                           | 9.84±1.67b                          | 12.88±2.24c                          | 0.00    |
| BMI (Kg/m²)         | 28.67±5.76a          | 28.82±5.30a                          | 27.28±6.09a                         | 27.93±5.52a                          | 0.70    |
| FBG (mg/dl)         | 87.87±11.48a         | 213.85±95.12a                        | 196.86±82.16a                       | 214.65±73.71a                        | 0.00    |
| B.urea (mg/dl)      | 32.34±8.23a          | 36.77±13.12a                         | 64.10±27.30b                        | 87.10±23.36b                         | 0.00    |
| S.creatinine (mg/dl)| 0.73±0.20a           | 0.90±0.34a                           | 1.41±0.61b                          | 2.35±0.60f                           | 0.00    |
| eGFR(ml/min/1.7 3m²) | 106.88±29.26a        | 89.60±32.56a                         | 47.44±20.89b                        | 23.16±9.98c                          | 0.00    |
| ACR(mg/g)           | -                    | 17.94±8.12a                          | 116.90±51.35b                       | 674.31±199.87                        | 0.00    |

FBG, fasting blood glucose; eGFR; estimated glomerular filtration rate ,ACR; albumin creatinine ratio, S.Cr; serum creatinine, B.U; blood urea, BMI; body mass index.
- Different small letters represent the comparison between 2 groups.
- Similar small letters denote non-significant differences.
there is no significant differences among patients groups. The results of the correlation between pentosidine and the studied parameters in this work are recorded in Table 3. The results revealed a positive correlation between pentosidine and duration of disease in the macroalbuminuria group \((r=0.4, p=0.04)\), while a negative correlation was recorded with FBG in the same group \((r = -0.5, p =0.01)\).

**Table 2-Levels of Pentosidine in all the Studied Groups**

| Parameter      | Control group (n=75) | DM with normoalbuminuria group (n=25) | DM with microalbuminuria group (n=25) | DM with Macroalbuminuria group (n=25) | P value |
|----------------|----------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------|
| Pentosidine (ng/ml) | 100.03±35.98\(^a\) | 263.55±69.33\(^b\) | 282.65±72.46\(^b\) | 272.86±71.06\(^b\) | 0.00    |

Small letters represent the comparison between 2 groups.

Also, in this study, a negative correlation was found between pentosidine and urea levels in the normoalbumin group \((r =-0.61, p =0.001)\). In parallel, a positive correlation was observed between pentosidine and urea levels in the micro and macroalbuminuria patient groups \((r=0.54, p=0.005 \text{ and } r=0.47, p=0.01, \text{ respectively})\). Furthermore, a positive correlation between pentosidine and creatinine levels was noticed in the micro and macroalbuminuria groups \((r=0.71, p=0.00 \text{ and } r=0.6, p=0.001, \text{ respectively})\). Whereas, the results revealed a strong negative correlation between pentosidine and GFR levels in micro and macroalbuminuria groups \((r =-0.71, p=0.00 \text{ and } r =-0.76, p=0.00, \text{ respectively})\). Additionally, a positive correlation was observed between pentosidine level and ACR in normoalbumin patient group \((r =0.39, p =0.04)\).

**Table 3-Correlation of Pentosidine levels(ng/ml) with Studied Parameters**

| Parameters | DM with normoalbuminuria group (n=25) | DM with microalbuminuria group (n=25) | DM with Macroalbuminuria group (n=25) |
|------------|-------------------------------------|--------------------------------------|-------------------------------------|
| Age        | \(-0.33\) | \(0.10\) | \(-0.17\) | \(0.40\) | \(0.19\) | \(0.36\) |
| BMI        | \(-0.11\) | \(0.61\) | \(0.02\) | \(0.91\) | \(-0.10\) | \(0.62\) |
| Duration Years | \(0.39\) | \(0.05\) | \(-0.22\) | \(0.28\) | \(0.40^*\) | \(0.04^*\) |
| FBS        | \(-0.17\) | \(0.40\) | \(-0.06\) | \(0.75\) | \(-0.50^{**}\) | \(0.01^*\) |
| B. urea    | \(-0.61^{**}\) | \(0.001^*\) | \(0.54^{**}\) | \(0.005^*\) | \(0.47^{**}\) | \(0.01^*\) |
| S. creatinine | \(-0.12\) | \(0.54\) | \(0.71^{**}\) | \(0.00^*\) | \(0.60^{**}\) | \(0.001^*\) |
| GFR        | \(0.17\) | \(0.39\) | \(-0.71^{**}\) | \(0.00^*\) | \(-0.76^{**}\) | \(0.00^*\) |
| ACR        | \(0.39^*\) | \(0.04^*\) | \(0.10\) | \(0.63\) | \(0.06\) | \(0.77\) |

Linear regression analysis
\(r=\) correlation coefficient
\(*=\) significant difference
\(**=\) strong correlation

**Receiver Operator Characteristics (ROC) Analysis Curve**

The ROC analysis data demonstrate that pentosidine possesses an excellent ability to predict nephropathy in the diabetic group, which included patients with normo, micro, and macroalbuminuria, in comparison with healthy subjects. This result was achieved based on investigations that included the parameters of sensitivity and specificity of the test, as well as area under the curve and some other relevant characteristics, as shown in Table3.
Table 3-ROC analysis data of Pentosidine level of Patient Groups Related to Healthy Group

| Parameters          | DM with normoalbuminuria | DM with microalbuminuria | DM with macroalbuminuria |
|---------------------|--------------------------|--------------------------|--------------------------|
| AUC                 | 0.97                     | 0.99                     | 0.99                     |
| P value             | 0.00                     | 0.00                     | 0.00                     |
| CV                  | 160.00 (ng/ml)           | 183.50 (ng/ml)           | 179.00 (ng/ml)           |
| Specificity         | 88.00%                   | 96.00%                   | 94.70%                   |
| Sensitivity         | 88.00%                   | 96.00%                   | 92.00%                   |
| PPV                 | 71.00%                   | 88.90%                   | 85.20%                   |
| NPV                 | 95.70%                   | 98.60%                   | 97.30%                   |
| PLR                 | 7.33                     | 24.00                    | 17.36                    |
| NLR                 | 0.14                     | 0.04                     | 0.08                     |

AUC: area under the curve, CV: cut off value, NLR: negative likelihood ratio, NPV: negative predictive value, PLR: positive likelihood ratio, PPV: positive predictive value.

Characteristics of pentosidine in diabetic patients with normoalbuminuria group
Pentosidine showed an excellent capability (AUC= 0.97) to predict nephropathy in diabetic patients from those without any disease. In term of prior probability, the p value was found to be 0.00 with very high sensitivity and specificity values (88%). In terms of posterior probability, the PPV value was 71% and the NPV value was very high (95.7%), which indicates that this marker has equal roles as for confirming and excluding disease. A value of pentosidine >160 ng/ml indicates that the patients in probability have nephropathy compared to the normal persons, in term of likelihood probability (positive PLR ratio indicate the cumulative probability of confirming the disease, while negative NLR ratio indicate cumulative probability of excluding the disease). In term of positive PLR since its value 7.33, while in term of negative NLR since its value is 0.14, as shown in Table 3.

Characteristics of pentosidine in diabetic patients with microalbuminuria group
Pentosidine showed an excellent ability (AUC= 0.99 and p value= 0.00) to distinguish diabetic patients with microalbuminuria from normal control. Pentosidine level can be considered as a strong parameter to diagnose DM patients with microalbuminuria, since the AUC value was found to be 0.99. The best cut-off point derived from the ROC curve, with a sensitivity of 96% and specificity of 96%, was found to be 183.5ng/ml. Accordingly, values above 183.5ng/ml are considered abnormal (diabetes with microalbuminuria). in term of posterior probability of the PPV is (88.9%) and NPV is very high 98.6%), this indicate that this marker have equal roles as for confirming and excluding disease. The likelihood probability (positive PLR ratio indicate the cumulative probability of confirming the disease, while negative NLR ratio indicates cumulative probability of excluding the disease). In term of positive PLR since its value 24, while in term of negative NLR since its value is 0.04, as shown in Table 3. The significance level was obtained at (P=0.00).

Characteristics of pentosidine in diabetic patients with macroalbuminuria group
Pentosidine showed an excellent ability (AUC= 0.99) to identify diabetic patients with macroalbuminuria from normal persons, with a p value of 0.00. The best cut-off point (>179ng/ml) derived from the ROC curve shows a sensitivity of 92 % and specificity of 94.7%. Accordingly, a test value above 179ng/ml is considered abnormal (diabetes with macroalbuminuria), as shown in Table 3. The significance level was P=0.00.

Discussion
The level of AGEs plays an essential role in the progress of DN pathophysiology. It represents one of different symptoms of hyperglycemia. AGEs are deposited in the kidneys and may also be one of the causes for modifying the renal architecture. A study in humans and mice have shown, unsurprisingly, that AGEs are cross-linked with matrix proteins in the glomerular basement membrane [19]. Diabetic patients with renal dysfunction were reported to have double the volume of AGE deposition compared with diabetic patients without kidney
disease [20]. The role of AGEs in DN was further explained by another study which indicated that increased level of AGEs in serum is an indicator of reduced renal activity [21]. Pentosidine is the well-characterized AGE intermolecular cross-link and is utilized as an indirect indicator of AGEs [22]. When the kidney is the central pentosidine removal site[23]. In patients with diabetes with overt nephropathy, several experiments, recorded elevations in serum pentosidine levels [24]. Renal dysfunction may also contribute to pentosidine accumulation in the blood, since the main determinant of serum pentosidine is renal [25]. Our results are in agreement with those of Gohda and Kerkeni, who found that the AGEs (including pentosidine) may play an important role in the development of microvascular complications and are associated with the severity of renal dysfunction in type 2 diabetic patients [26-27]. In patients with diabetes, the measurement of pentosidine will provide a beneficial long-term indicator of glycemic regulation that could significantly affect the amount of glycated hemoglobin. A previous study reported major variations in pentosidine formation between patients with diabetes mellitus and controls. Elevated pentosidine concentrations were reported to be a beneficial biomarker for clinical outcome surveillance in end-stage renal disease [28].

Conclusions
Pentosidine level is significantly increased in patients groups as compared to control group. Furthermore, it has a positive correlation with urea and creatinine, but negatively correlated with eGFR. Accordingly, it may play a significant role that leads to diabetic nephropathy progression and its association with renal function. Thus, it may be employed as a good marker for the prediction of this disease.

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