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Urotensin-II level and its association with oxidative stress in early diabetic nephropathy

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ABSTRACT

Objective: Diabetic nephropathy is the most common cause of end stage renal failure. Early treatment of diabetic nephropathy depends on understanding the underlying mechanisms of the disease. In this study we investigated the role of U-II in early nephropathy and ıts association with oxidative stress, paraoxonase (PON)-1 and arylesterase.

Research design and methods: Twenty-three diabetic patients with microalbuminuria, 23 diabetic patients with normoalbuminuria and 25 healthy individuals were enrolled in the study. Serum total antioxidant status (TAS), total oxidant status (TOS), PON-1, arylesterase, and urotensin-II (U-II) levels were measured. Oxidative stress index (OSI) percent ratio of TOS to TAS level was accepted as OSI.

Results: Serum U-II levels were higher in the microalbuminuric diabetes group compared to the normoalbuminuric diabetic group and the healthy control group ($p = 0.009$ and $p = 0.0001$, respectively). Normoalbuminuric diabetic group's U-II levels were significantly higher compared to those of the healthy control group ($p = 0.0001$). Correlation analysis yielded that plasma U-II levels are negatively correlated to TAS, arylesterase, and PON-1 levels ($r = -0.395$, $p = 0.001$; $r = -0.291$, $p = 0.014$; and $r = -0.279$, $p = 0.018$, respectively) and that they had a positive correlation with OSI levels ($r = 0.312$, $p = 0.008$). These associations were confirmed in the multiple regression analysis. The results of multiple logistic regression analysis showed that oxidative stress is important in the development of microalbuminuria.

Conclusion: The data of this study reveal that increased serum U-II has a role in the development of diabetic nephropathy. This effect of U-II may be related to high levels oxidative stress parameters.

1. Introduction

Diabetic nephropathy is one of the important complications of diabetes mellitus (DM) and is the most common cause of end stage renal failure in clinical practice (Reutens, 2013). Early diagnosis of diabetic nephropathy depends on understanding the underlying mechanisms.

Though microalbuminuria has been identified as the most effective indicator of early diabetic nephropathy, some structural changes might have already occurred by the time microalbuminuria is detected (Araki et al., 2008). It is highly important to prevent development of microalbuminuria.

Urotensin-II (U-II) is recognized as the strongest vasoconstrictor detected among mammals to this day (Ross, McKendy, & Giaid, 2010). U-II and its receptors are found in various tissues such as heart, brain, kidney, smooth muscle, and endothelium (Barrette & Schwertani, 2012; Onan, Hannan, & Thomas, 2004). It is a significant mediator in renal diseases (Adebiyi, 2014; Balat, Karakök, Yilmaz, & Kibar, 2007).

Various studies have shown that diabetic patients have increased serum U-II levels (Ong, Wong, & Cheung, 2008; Totsune et al., 2003). Similarly, in diabetic nephropathy patients, U-II and its receptors' expression has been detected to have increased (Langham et al., 2004). However, there is not sufficient information on U-II's exact role in diabetic nephropathy development.

Oxidative stress increase is an important condition in DM; and it is known to be an important cause of diabetic nephropathy development (King & Loeken, 2004). It is suspected that there is an imbalance between the oxidant and anti-oxidant mediators prior to development of renal lesions, and that the oxidation level increases as the disease progresses (Piarulli et al., 2009). Paraoxonase-1 (PON-1), which has paraoxonase (PON) and arylesterase activities, is a high-

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density lipoprotein cholesterol (HDL-C) bound antioxidant enzyme that protects low-density lipoprotein cholesterol (LDL-C) against oxidative damage. Low levels of PON and arylesterase, antioxidant enzymes, have been reported in situations in which reactive oxygen radicals increase and oxidative stress occurs (Wegner, Piorunska-Stolzmann, Araszkiewicz, Zozulińska-Ziółkiewicz, & Wierusz-Wysocka, 2011).

In this study, we investigated the role of serum U-II in early nephropathy and its association with oxidative stress.

2. Materials and methods

The study was initiated upon obtaining approval from ethics Committee of Harran University Faculty of Medicine numbered (15.06.2009/07-18). All participants were informed and an informed consent was obtained from all of them prior to the study.

2.1. Patient group and study protocol

A total of 71 participants were included in the study: 23 diabetic patients with microalbuminuria (median age = 50; IQR: 9; 12 females, 11 males); 23 diabetic patients with normoalbuminuria (median age = 46; IQR: 9; 13 females, 10 males); and 25 healthy individuals (median age = 47; IQR: 11.5; 15 females, 10 males). People with systemic diseases such as infectious diseases, inflammatory diseases, hypertension, liver failure, cardiovascular diseases, malignancies, neurodegenerative diseases, cerebrovascular diseases; those on antioxidants such as antihypertensive medications, lipid-lowering medications, and vitamin E; and smokers were excluded from the study. Seven patients with microalbuminuria and 2 patients with normoalbuminuria were taking insulin therapy. Other patients were taking at least 2 oral antidiabetic drugs.

2.2. Evaluation of nephropathy and duration of diabetes mellitus

Mean urine albumin/creatinine index was measured in the spot urine, collected on 3 different days. Urine contaminated with bacteria, red blood cells, and white blood cells were removed. Urinary albumin concentration was measured via latex turbidimetric immunoassay method using commercial kits. When albumin/creatinine = 30–300 mg/g in type 2 DM patients, it was considered as microalbuminuria. The staging criteria recommended by Mogensen et al. were used for early diabetic renal disease diagnosis (Mogensen, Christensen, & Vittinghus, 1983).

For the duration of DM, the time of initial symptoms associated with the disease was considered beginning of the disease. If there were no symptoms, the time of diagnosis was considered as the beginning. The American Diabetes Association's, 2010 criteria were used for DM diagnosis (ADA, 2010).

2.3. Measurements

Systolic blood pressure (SBP), diastolic blood pressure (DBP), height, and weight of each participant were measured. Body mass index (BMI) was calculated as body mass (kg)/height $(m)^2$.

Blood samples were collected in the morning hours after an 8-hour fasting period. Serum samples were stored at −80 °C until total antioxidant status (TAS), total oxidant status (TOS), PON, arylesterase, and U-II levels were measured. Urine microalbumin and creatinine values were measured via turbidimetric method by a Cobas Integra 800 model auto-analyzer (Roche®). Hemoglobin A1c (HbA1c) levels were tested by using the Celldyn 3700 (Abbott, ®USA) auto-analyzer commercial kit. Serum urea and creatinine values were measured spectrophotometrically by routine biochemical methods using Cobas Integra 800 model auto-analyzer (Roche®). Serum triglyceride (TG), total cholesterol, LDL-C, and HDL-C concentrations were measured using an auto-analyzer (Aeroset, Abbott, USA) commercial kit (Abbott, USA).

2.4. Measurement of total oxidant/antioxidant status

Serum TOS was determined using a novel automated measurement method developed by Erel (2005). Oxidants present in the study sample oxidize the ferrous ion-*o*-dianisidine complex to ferric ion. The oxidation is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as mmol H_2O_2 Equiv./l.

Serum TAS was determined using a novel automated measurement method developed by Erel (2004). In the method, hydroxyl radical, the most potent biological radical, is produced first. In the assay, reagent 1 containing ferrous ion solution is mixed with reagent 2, which contains hydrogen peroxide. The sequentially produced radicals, such as brown colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. The anti-oxidative effect of the study sample against the potent-free radical reactions, which are initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values, lower than 3%, and the results are expressed as mmol Trolox Equiv./l.

Oxidative stress index percent ratio of TOS to TAS level was accepted as OSI [OSI (arbitrary unit) = TOS (mmol H_2O_2 Equiv./l)/TAS (mmol Trolox Equiv./l)] (Bolukbas et al., 2005).

2.5. Measurements of PON and arylesterase activities

PON and arylesterase activities were measured with commercially available kits (Relassay, Gaziantep, Turkey). PON measurement was performed either in the presence (salt-stimulated) or in the absence of NaCl. Paraoxon hydrolysis rate (diethyl-*p*-nitrophenyl phosphate) was measured by monitoring increased absorption at 412 nm at 37 °C. The amount of generated *p*-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290/M per cm (Eckerson, Wyte, & La Du, 1983). PON activity was expressed as U/l serum. The coefficient of variation (CV) for individual samples was 1.8%. Arylesterase activity was measured using phenyl acetate as substrate. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310/M per cm. One unit of arylesterase activity was defined as 1 mmol phenol generated per minute under the above conditions and expressed as U/l (Haagen & Brock, 1992). The CV for individual serum samples was 4.1%. The sensitivities of both tests were over 98%.

2.6. Measurement of U-II level

Serum U-II levels were determined by Sandwich ELISA (Phoenix Pharmaceuticals®, USA).

2.7. Statistical analysis

Shapiro–Wilk test was used to test continuous variables for normality. Measurements of normally distributed variables (TAS, OSI, HDL, LDL, urea and arylesterase) are presented as mean ± standard deviation. Those with non-normal distributions are presented as median and interquartile range (IQR). Student's t-test was used in comparison of 2 independent groups of normally distributed variables; one-way analysis of variance (ANOVA) test was used when comparing more than 2 groups; and LSD test was used for paired comparisons to identify which group the difference was caused by. For non-normally distributed variables, Mann–Whitney U test was used to compare 2 independent groups and Kruskall Wallis test was used to compare more than 2 independent groups. DUNN test was used for post-hoc comparisons. Spearman correlation analysis was done to identify