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The relationship between urinary and plasma levels of tumor necrosis factor alpha and various stages of chronic kidney disease in patients with type II diabetes mellitus

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ABSTRACT

Introduction: The concept of diabetic nephropathy, as a metabolic disease, is now being replaced by chronic low-grade inflammatory disease. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that plays an important role in the pathogenesis and clinical outcomes of diabetic nephropathy.

Objectives: This study aimed to determine the relationship between plasma and urinary levels of TNF-α and chronic kidney disease (CKD) in patients with type 2 diabetes mellitus.

Patients and Methods: In this descriptive-analytical study, patients with type 2 diabetes mellitus who referred to the endocrine clinic in Kashan (2016) were enrolled in the study and their clinical and laboratory data were recorded. Albumin/creatinine ratio (ACR) and glomerular filtration rate (GFR) were calculated. The patients were divided into three groups based on their GFR. Serum and urinary levels of TNF-α were determined by ELISA and were compared between the studied groups.

Results: A total of 128 patients were evaluated. Of all, 35 patients (27.3%), 39 patients (30.4%), and 54 patients (42.3%), respectively, were suffering from stage 1, stage 2, and stage 3 CKD. The plasma levels of TNF-α in patients with stage 1, 2, and 3 CKD, were 66.20 ± 33.27 pg/mL, 67.47 ± 42.98 pg/mL, and 77.32 ± 47.23 pg/mL respectively, since the difference among them was not significant ($P=0.417$). In addition, the urinary levels of TNF-α in patients with stage 1, 2, and 3 CKD, respectively, were 88.18 ± 26.66 pg/mL, 97.41 ± 57.76 pg/mL, and 101.18 ± 60.47 pg/mL, since no significant difference was observed between the three groups ($P=0.957$).

Conclusion: Based on the results of this study, with changing the stage of CKD, the serum and urinary levels of the TNF-α increases too, although this increase is not significant. Moreover, the plasma and urinary levels of the TNF-α have a direct and significant relationship with each other. It is recommended to conduct further studies in this field.

Implication for health policy/practice/research/medical education:
To determine the relationship between plasma and urinary levels of TNF-α and different stages of CKD in patients with type 2 diabetes mellitus, we conducted a descriptive analytical study on 128 patients. The results showed that plasma and urinary levels of TNF-α had no significant relationship with the stages of CKD. Based on the results of this study, with changing the stage of CKD, the serum and urinary levels of the TNF-α increase as well, although this increase is not significant. Moreover, the plasma and urinary levels of the TNF-α have a direct and significant relationship with each other.

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Introduction

Diabetes mellitus is a disease with multiple causes, which many different environmental and genetic factors are involved in its etiology (1,2). The International Association for Diabetes has predicted that the number of people with diabetes in the world will exceed 439

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million people by 2030 (3). Complications associated with diabetes are one of the most important health problems worldwide. Diabetic nephropathy is one of the most important medical complications of diabetes mellitus. Nearly one-third of patients with diabetes are diagnosed with diabetic nephropathy (4). Diabetes is the most common cause of end-stage renal disease, which causes many social and economic problems for patients (5, 6). Many people with chronic kidney disease (CKD) will also develop renal failure and end-stage renal disease and require alternative renal procedures, such as dialysis or kidney transplantation. Care services provided for patients with end-stage renal disease are costly. For instance, 14.6% of total health care budget in the United States in 2006 was allocated to provide care services for this group of patients. In addition to renal complications, high serum creatinine is recognized as a potential risk factor for cardiovascular diseases and death (7). Hence, in recent years, many studies have been conducted to identify pathophysiological processes leading to diabetic nephropathy, and the knowledge on the disease has dramatically increased. In the classic view, kidney damage is caused by metabolic and hemodynamic changes that increase systemic and glomerular pressure, as well as by changes in molecules that occur in the presence of high blood sugar levels. However, this view has changed into a much more complex scenario in which the pathogenesis of diabetic nephropathy is considered as a multifactorial process that includes genetic and environmental factors; this process triggers a complex set of pathophysiological events (8-10). Moreover, oxygen reactive metabolites have been proposed as a mechanism of injury to renal tubules and interstitial tissue in animal models. In addition, hyperlipidemia is thought to be involved in progressive renal disease through proliferation and mesangial sclerosis. The activation of the renin-angiotensin-aldosterone system (RAAS) and the increase in transforming growth factor beta (TGF-β) also play a critical role in the progression towards kidney fibrosis (11,12). According to recent evidence, subclinical chronic inflammation may play a key role in the onset and progression of diabetic nephropathy, as the infiltration of inflammatory cells in the tubules and interstitial space is evident in the biopsy of the kidneys of patients with diabetic nephropathy (13,14). In previous studies, the relationship between the serum levels of tumor necrosis factor alpha (TNF-α) and diabetic nephropathy have been investigated; however, most of the previous trials have been conducted using anti-TNF-α drugs on animal samples.

Objectives

Since diabetic nephropathy is one of the debilitating complications in diabetic patients, its prevention helps to reduce the illness and mortality of patients and decrease the costs of treatment as well. The aim of this study was to investigate the relationship between plasma and urinary levels of TNF-α and the stages of CKD in patients with type 2 diabetes mellitus. Proving a significant relationship between these two will provide evidence to verify whether anti-TNF-α drugs play an important role in preventing diabetic nephropathy or not.

Patients and Methods

Study population

In this descriptive analytical study, patients with type 2 diabetes mellitus referring to the endocrine clinic of Shaheed Beheshti hospital in Kashan were enrolled in the study (2016).

To calculate the sample size, we used the sample size formula to compare the two mean values in two domains, with a reliability of 95%, strength of 90%, and the mean (standard deviation) TNF-α obtained from a study by Lampropoulou et al (15). The minimum number of samples required for each group was 22 patients, however considering the possibility of sample loss, a minimum of 35 patients were enrolled in each group. The minimum sample size was 105 patients. The inclusion criteria were as follows: primary diagnosis of type 2 diabetes mellitus over the age of 30 years, passing at least one year after the primary diagnosis of type 2 diabetes mellitus, and giving consent to participate in the study. On the other hand, patients with infectious diseases, liver disease, autoimmune diseases, stages 4 or 5 CKD, heart failure, tumors, patients with a history of taking antibiotic, non-steroidal anti-inflammatory drugs, corticosteroid, and cytotoxic drugs, and patients who were reluctant to continue the study were excluded from the research. After selecting the samples, the patients were divided into three groups based on the level of glomerular filtration rate (GFR) and the stages of CKD. The first group included patients with stage one CKD with GFR ≥ 90 ml/min/1.73m^2. The second group included patients with stage 2 CKD with 60 ≤ GFR <90 mL/min/1.73 m^2. The third group included patients with stage 2 CKD with 30 ≤ GFR <60 mL/min/1.73 m^2. Blood samples were taken from the patients to measure the plasma levels of the TNF-α, BUN, LDLC, HDL-C, and triglyceride. In addition, urine samples were also taken and used to determine albumin/creatinine ratio (ACR) and the urinary level of the TNF-α.

To conduct the tests, 10 mL of blood was centrifuged at 1300 rpm for 10 minutes and the supernatant was stored in a freezer at 20°C until performing the ELISA test. Moreover, 10 mL of urine was centrifuged at 1500 rpm for 10 minutes and the supernatant solution was stored in

the freezer at 20°C until performing the ELISA test. After the completion of the sampling process, the samples were stored out of the freezer to reach the ambient temperature and then used for the ELISA test. To perform the ELISA tests for measuring the TNF-α levels we used Eastbiopharm kit (Nano Zist Kala Fanavaran Diana Company, China) with a kit sensitivity of 5 pg/mL (with enough sensitivity to determine TNF-α in urine) and urine micro-alumina kits prepared by the Pishtaz Teb Company.

The data on patients including sex, age, and duration of type 2 diabetes mellitus were recorded through using a questionnaire.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. All patients signed a written informed consent form to participate in the study. This study was approved by the Ethics Committee of Kashan University of Medical Sciences (ethical code #Ir.kaums.res.1395.134). This study was extracted from the internal medicine residency thesis of Forouz Farzadnejad at this university.

Statistical analysis

The data were entered into and analyzed by SPSS-16 software. Kolmogorov-Smirnov test was used to determine the normality of the data. First, the variables of the three groups were described. Central and dispersion indices were presented for quantitative variables. Absolute and relative frequency and frequency distribution tables were presented for qualitative variables. To analyze the data, the mean values of quantitative variables of the three groups were determined using one-way ANOVA after checking parametric conditions; otherwise they were calculated using the Kruskal–Wallis test. The significance level was set at P <0.05.

Results

In this study, 128 patients with type 2 diabetes mellitus were investigated to determine the relationship between plasma and urinary levels of TNF-α and CKD. Of all, 35 patients (27.3%), 39 patients (30.4%), and 54 patients (42.3%), respectively, were suffering from stage 1, stage 2, and stage 3 CKD. The mean age of the patients was 47.02 ± 10.94 years in the first group, 55.82 ± 8.42 years in the second group, and 65.59 ± 8.46 years in the third group. There was a statistically significant difference between the three groups in terms of age (P<0.001). In addition, 88.58%, 56.42%, and 57.41% of the patients in the first, second, and third group were female, and a statistically significant difference between the three groups in terms of gender was seen (P=0.004). Table 1 presents the frequency distribution of gender and mean age of the patients in the three groups.

Table 2 shows the mean plasma and urinary levels of TNF-α and renal function of patients in different stages of CKD. Plasma level of TNF-α in the first, second, and third stages of CKD was 66.20 ± 33.27 pg/mL, 67.47 ± 42.98 pg/mL, and 77.32 ± 47.23 pg/mL, respectively, while the difference was not significant (P=0.417). Moreover, the urinary level of TNF-α in the first, second, and third stages of CKD was 88.18 ± 26.66 pg/mL, 97.41 ± 57.76 pg/mL, and 101.18 ± 60.47 pg/mL, respectively and there was no significant difference between the three groups (P=0.957).

Table 3 shows the relationship between the studied variables. As shown, there was a significant relationship between the plasma and urinary levels of TNF-α.

Table 1. Frequency distribution of sex and mean age of the patients in the three groups

Variable	First group (n = 35)	Second group (n = 39)	Third group (n = 54)	*P* value
Age (year)	47.02 ± 10.94	55.82 ± 8.42	65.59 ± 8.46	<0.001
Gender				
Male	4 (11.42%)	17 (43.58%)	23 (42.59%)	0.004
Female	31 (88.58%)	22 (56.42%)	31 (57.41%)	

Table 2. GFR, ACR, mean plasma and urinary levels of TNF-α, and renal function of patients in different stages of CKD

Variable	First group (n = 91)	Second group (n = 93)	Third group (n = 14)	*P* value
GFR (mL/min/1.73 m^2)	108.33 ± 16.35	74.13 ± 9.97	44.33 ± 8.31	<0.001
ACR (mg/mmol)	0.36 ± 1.59	0.17 ± 0.80	0.17 ± 0.44	0.620
Plasma level of TNF-α (pg/mL)	66.20 ± 33.27	67.47 ± 42.98	77.32 ± 47.23	0.417
Urinary level of TNF-α (pg/mL)	88.18 ± 26.66	97.41 ± 57.76	101.18 ± 60.47	0.957

Abbreviations: GFR, glomerular filtration rate; ACR, albumin/creatinine ratio; TNF-α, tumor necrosis factor alpha.

Table 3. Relationship between age, ACR, GFR, plasma level of TNF-α, and urinary level of TNF-α

Variable	GFR	Age	ACR	Plasma level of TNF-α	Urinary level of TNF-α
GFR	-	r = - 0.696 *P* = 0.001	r = 0.031 *P* = 0.719	r = 0.112 *P* = 0.183	r = 0.115 *P* = 0.173
Age	r = - 0.696 *P* = 0.001	-	r = - 0.012 *P* = 0.887	r = - 0.051 *P* = 0.543	r = 0.067 *P* = 0.428
ACR	r = 0.031 *P* = 0.719	r = - 0.012 *P* = 0.887	-	r = 0.002 *P* = 0.977	r = - 0.016 *P* = 0.846
Plasma level of TNF-α	r = 0.112 *P* = 0.183	r = - 0.051 *P* = 0.543	r = 0.002 *P* = 0.977	-	r = 0.798 *P* <0.001
Urinary level of TNF-α	r = 0.115 *P* = 0.173	r = 0.067 *P* = 0.428	r = - 0.016 *P* = 0.846	r = 0.798 *P* <0.001	-

Abbreviations: GFR, glomerular filtration rate; ACR, albumin/creatinine ratio; TNF-α, tumor necrosis factor alpha.

Discussion

The aim of this study was to determine the relationship between plasma and urinary levels of TNF-α and different stages of CKD in patients with type 2 diabetes mellitus. The results showed that plasma and urinary levels of TNF-α had no significant relationship with the stages of CKD.

Hasegawa et al showed that in diabetic rats, as compared with non-diabetics, stimulated the production of more amounts of TNF-α and IL-1 by macrophages cultured in peritoneal fluid (16). Experimental studies have always shown an increase in mRNA encoding TNF-α gene and protein levels in proximal and glomerular tubule cells in diabetic rats (17-23). In normal mode, the plasma level of TNF-α is 3-30 pg/mL; in addition, with the progress of nephropathy, the plasma levels of inflammatory molecules that contain pro-inflammatory cytokines increased in diabetic patients (24-26). The factors that inhibit TNF-α have been successfully used in induced diabetes in animals. For instance, DiPetrillo et al showed that the treatment of diabetic rats by an anti-TNF-α agent reduced urinary excretion of TNF-α and prevented urinary retention and renal hypertrophy (27). Similarly, inhibiting TNF-α by infliximab significantly reduced albuminuria and urinary excretion of TNF-α in streptozotocin-induced diabetic rats (28). Previous studies also demonstrated the effect of pentoxifylline in the treatment of diabetic nephropathy through inhibiting TNF-α. However, the mechanisms that are used to treat and reduce albuminuria by inhibiting TNF-α remain unclear (29-32).

Conclusion

Based on the results of this study, with changing the stage of CKD, the serum and urinary levels of the TNF-α increase as well, although this increase is not significant. Moreover, the plasma and urinary levels of the TNF-α have a direct and significant relationship with each other.

Limitations of the study

The limitations of this study were the small sample size, the lack of an examination on the effect of anti-TNF-α drugs, and the lack of evaluation of other effective factors. Therefore, it is suggested to conduct further studies to determine the effect of anti-TNF-α agents in different stages of CKD in patients with type 2 diabetes mellitus. It is also necessary to simultaneously consider other factors affecting the serum and urinary levels of TNF-α and follow up the patients and re-measure serum and urinary levels of TNF-α to determine its effect on control therapy of diabetes.

Authors' contribution

FF and AS conducted the research. FF prepared the primary draft. MS and MRT revised and prepared the final manuscript. All authors read and approved the final paper.

Conflicts of interest

The authors declare no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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