Is IL-6 Increased in Type 2 Diabetes Mellitus Patients Independent of Nephropathic Complication?

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Abstract

Introduction: Interleukin IL-6 is a pleiotropic pro-inflammatory cytokine with a key impact on both non-immune events and immune regulation in a wide range of cells and tissues outside the immune system. The aim of this study was to investigate the roles of IL-6 in the pathogenesis of type 2 diabetes (T2D) patients with and without nephropathy.

Methods: We enrolled 50 T2D patients with nephropathy and 52 T2D patients without nephropathy who referred to the diabetes clinic (Ali Ebn- Abitaleb Hospital, Rafsanjan, Iran) from April 2010 to November 2012 were included in the study. Serum levels of IL-6 were measured by ELISA. Data were analyzed using ANOVA (and Tukey’s post hoc test), and Chi-square test through SPSS version 18 software. Significant level was set at 0.05.

Results: Our findings indicated that serum levels of IL-6 were significantly elevated in all of patients in comparison to healthy subjects (P < 0.01) whereas, there was no significant difference between T2D patients with and without nephropathy.

Conclusion: It appears that IL-6 is a potential biomarker in the diagnosis of diabetic patients. Based on our results it may be concluded that IL-6 levels may not be related to nephropathic complication in T2D patients. In addition to IL-6, other inflammatory cytokines may also possibly are involved in the pathogenesis of nephropathy.

INTRODUCTION

Interleukin-6 is a well-known pro-inflammatory cytokine which is mainly produced by T cells and macrophages, renal cells, muscle cells, adipocytes, and osteoblasts [1]. IL-6 has been reported to serve as a regulator for the production of cell adhesion and chemotactic molecules, as well as secretion of cytokines involved in amplification of the inflammatory responses [2]. Furthermore, over 90% of people who are suffering from diabetes have T2D and this pathologic state involves severe complications in different body organs, including eye, nervous system and the kidneys [3]. Attractions have been drawn to word the fact that renal involvement is a paramount cause of morbidity and mortality in the diabetic patients. Inflammatory responses play an important role(s) in development and further progression of diabetic nephropathy (DN) with recruitment and activation of innate immune cells and expansion of pro-inflammatory cytokines [4]. Pro-inflammatory and fibrogenic cytokines which are generated and released by mentioned cells in the local microenvironment can directly damage kidney construction and consequently activate the epithelial-to-mesenchymal transition process [5], subsequent in extracellular matrix accumulation. In addition to production of pro-inflammatory cytokines, expression of both chemo attractant cytokines and adhesion molecules are up-regulated in kidney cells from patients and animal models of diabetes. Furthermore, IL-6 has a direct impact on glomerular and infiltrating cells and this in turn influences extracellular matrix dynamics, disturbing membrane thickening in renal glomeruli [6,7]. Different immune cells and molecules such as T-lymphocytes, macrophages, chemokines [8], adhesion molecules [9], growth factors, nuclear factors, and cytokines [8] which proved to be prominent in diabetic glomeruli have been involved in various pathogenic pathways associated with DN [5,10-12].

According to the before mentioned introductory comment, present study aimed to delineate and examine role(s) of IL-6 and its related inflammatory processes, in order to determine its pivotal parts in the pathogenesis of T2D with and without nephropathy in a sample of south-Eastern, Iranian patients.

MATERIAL AND METHODS

In this experimental case-control study, peripheral blood samples were collected from 158 subjects who were fitted in three groups as 50 DN, 52 T2D without nephropathy and 56 healthy subjects and sample size is about 50 in each group and totally is 150 subjects [13]. The patient and control groups were selected within the Rafsanjan population with similar demographics (Table 1) who were referred to the diabetic clinic (Ali Ebn-Abitaleb Hospital, Rafsanjan, Iran) from April 2010 to November 2012. The human ethical approval for this study was granted by the Ethical Committee of the Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Clinical characteristics, including one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, or pruritus) fasting blood sugar, urine albumin level, blood pressure, being overweight or obese (BMI > 25 kg/m2), hypertension, cardiovascular disease symptoms, and increased levels of triglycerides, low concentrations of high-density lipoprotein cholesterol, or both were assessed three times during a period of 6 months for each individual patient as well as the control group subjects. Patients with co-existing acute sicknesses including malignancy, infectious diseases within the past one week, and active immune-based disease; medical history of clinical cardiovascular disease; renal insufficiency (serum creatinine > 1.5 mg/dL); or confounding factors for proteinuria such as severe uncontrolled hypertension (> 160/100 mm Hg) and smoking history were excluded from the study. Our inclusion criteria for T2D patients without nephropathy was having fasting plasma glucose 140 mg/dl or random plasma glucose 200 mg/dl attending the diabetic clinic of Ali Ebn-Abitaleb hospital with normal albuminuria (urinary albumin excretion [UAE] persistently lower than 30 mg/d). Also subjects with T2D symptoms plus macroalbuminuria (persistent UAE greater than 300 mg/d) were considered as DN patients. Additionally, normal control group were randomly selected from volunteer donors of Rafsanjan Blood Transfusion Center.

Cytokine assay

IL-6 serum levels were detected using ELISA (eBioscience, Esp) in different groups, immediately after blood collection. Assays were performed according to the manufacturer’s guidelines. The sensitivity of the kit was 2 pg/ml and inter- and intra-assay assessments of reliability of the kits were conducted (CV < 14% and CV < 0.03%, respectively).

STATISTICAL ANALYSIS

Data were expressed as mean ± SEM. Statistical analysis was conducted using SPSS version 18 (SPSS, Inc, Chicago, IL, USA). Differences were evaluated with the ANOVA, Tukey’s post hoc test and, Chi-square test. Normality of data was checked using One-Sample Kolmogrov-Smirnow Test. Significant level of differences was set at 0.05.

RESULTS

Results of the present study showed that there was not a significant difference between groups regarding the mean age, gender status, duration of diabetes, and weight of the participants (Table 1).

Statistical analysis of our findings showed that triglyceride, cholesterol, LDL, fasting blood sugar (FBS) and proteinuria were significantly increased in patient groups thereby compared with control (P < 0.05). Conversely, glomerular filtration rate (GFR) and HDL were significantly decreased in patient groups when compared with control (P < 0.01) (Table1).

Serum levels of IL-6

The mean IL-6 serum level were 1.92 ± 0.08 pg/mL, 5.13 ± 0.20 pg/mL and 4.94 ± 0.19 pg/mL in control, T2D patients with nephropathy and T2D patients without nephropathy groups, respectively (Figure 1). Statistical analysis of the data showed that IL-6 serum levels were significantly increased in both groups of patients in comparison with healthy subjects (p < 0.001) whereas, there was no significant difference between T2D with and without nephropathy patients (P = 0.054).

DISCUSSION

There exist some reports, proposing association between different inflammatory mediators, the occurrence and severity of DN, although with conflicting results. Moreover, inflammatory cytokines such as IL-6 play important variety of activities involved in DN, from progression to the initial stages of diabetes to development and end stages of renal failure. Several are in favor of the fact that inflammatory cytokines are strong predictors for the development of diabetes [14,15]. Findings of our study demonstrated that serum levels of IL-6 were elevated in T2D patients either with or without nephropathy complications. In consistent with our findings, previous studies have reported that higher levels of IL-6 are associated with the elevated risk of diabetes, supporting an association between chronic inflammation and development of diabetes in populations with different ethnical variations [16]. Conversely, several studies showed a significant association between IL-6 and glomerular basement membrane thickening, a crucial lesion of diabetic nephropathy and a strong predictor for development of renal failure [17,18]. These data are supportive for the notion that IL-6 may play a role in the pathogenesis of DN. Hasegawa and colleagues showed that in diabetic rats, glomerular basement membranes had significantly generated greater amounts of IL-1 and Tumor necrosis factor-alpha (TNF-alpha) in cultured peritoneal macrophages than whenever cells were incubated with basement membranes from non-diabetic rats [19]. These reports were among the first studies that addressed a role for inflammatory cytokines network which contributing in the pathogenesis of DN [19,20]. Nowadays, it is well-delineated that inflammatory cytokines such as, IL-1, IL-6, IL-18 and TNF-α are contributing to the process of DN expansion [9,18,21]. Recently, Perlman and colleagues reported that in peripheral blood of DN patients the transcript level of seven mediators including Interleukin 1α (IL1α), TNF-alpha, transforming growth factor beta (TGFβ), Interleukin 8 (IL8 or chemokine (C-X-C motif) ligand 8, CXCL8), Interleukin 17 receptor A (IL17RA), Interferon gamma (IFNg), and Myeloid differentiation primary response gene 88 (MYD88) were significantly increased at all disease stages as compared to...
control but IL-6 elevated with disease progression until stage 4-5 [22]. These findings indicated that IL-6-expression patterns could variously be altered in different types of nephropathy, according to the stage of the disease. Krystallenia et al., reported that the differences of inflammatory state in the two types of diabetes may indicate their various clinical history, but the similarities of inflammatory mediator impairment may reflect a common susceptibility to the insulin dependence and the possible reduction of the inflammation [23]. The grade of inflammation in patients suffering from DN is perhaps one of the reasons for the altered levels of IL-6. Vestra and co-workers showed that IL-6 affects extracellular matrix dynamics at mesangial cell and podocytes and low-grade inflammation is related to glomerular basement membrane (GBM) thickening and nephropathy status in T2D [24]. Their findings suggested that grade of inflammation can affects the level of inflammatory cytokines in DN patients. Thus, the serum levels of IL-6 may possibly be changed in accordance with different grades of inflammation and nephropathy. Our study was limited to several limitations. The patients, who participated in the present study were at different stages of the disease and under different therapeutic agents for diabetes or its related complications. Also, self-reported data and lack of prior research studies with same results in DN patients was another important limitation of the study.

CONCLUSION

Overall, concerning results discussed here, it could be concluded that the IL-6 is a potential biomarker for the diagnosis of T2D. Also this cytokine may probably affects nephropathy in T2D, but nephropathic complications of T2D are very complex and depend on several environmental and genetic factors. Therefore, stage of nephropathy and inflammation should be considered in the measurement of IL-6 in DN patients. IL-6 and other inflammatory cytokines such as IL-1 and TNF-α as a network perhaps have a significant impact in the pathogenesis of nephropathy in T2D patients. These data may suggest that further studies requisite to be performed for determination of the roles of inflammatory cytokines in different stages of nephropathy, using an array of inflammatory cytokines in array-based techniques.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study, prior sample collection.

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REFERENCES


