Evaluation of IFN-γ Serum Level in Nephropathic Type 2 Diabetic Patients

1Reza Nosratabadi, 2M.K. Arababadi, 3G. Hassanshahi, 5N. Yaghini, 5V. Pooladvand,
8Ali Shamsizadeh, 8E.R. Zarandi and 8H. Hakimi
1Department of Laboratory Sciences, Faculty of Medicine, Islamic Azad University, Zahedan Branch, Zahedan, Iran
2Department of Microbiology, Hematology and Immunology,
3Department of Biochemistry,
4Department of Physiology, Faculty of Medicine, Rafsanjan University of Medical Science, Rafsanjan, Iran

Abstract: The present study was aimed to examine the serum level of IFN-γ in type 2 diabetic patients with nephropathic complications. In this experimental study, serum samples were obtained from 100 type 2 diabetic patients suffering from nephropathy and 100 healthy controls. Serum level of IFN-γ was analyzed by ELISA. Results of this study showed that the mean serum level of IFN-γ was 16.09±2.04 and 4.03±1.00 pg mL⁻¹ in nephropathic patients and healthy controls, respectively. Statistical analysis of data showed that the difference in the IFN-γ serum level was significant between nephropathic patients and controls. Due to the elevated level of IFN-γ in nephropathic patients, it can be possibly concluded that IFN-γ is involved in nephropathy complication of type 2 diabetes.

Key words: Cytokine, diabetes, nephropathy

INTRODUCTION

Diabetes mellitus is increasing globally and is expected to affect 200 million people by 2010 and 300 million in 2025 (Steyn et al., 2008). Type 2 is the most prevalent form of diabetes (Arababadi et al., 2009). Current studies showed that several genetic and environmental parameters such as nephropathy are involved in pathogenesis and complications of type 2 diabetes (Nathanson and Nystrom, 2008). Immune system related factors, including, cytokines and cytokine-receptors axis are recently under research for their crucial roles in diabetes (Nathanson and Nystrom, 2008). Also, the critical role of cytokine imbalance in type 2 is reported (Tsiavou et al., 2004). It has been suggested that diabetes is an immune dependent disease and is associated with alteration in cytokine expression pattern (Cruz et al., 2008). For example, peripheral blood monocytes of type 2 diabetes tend to produce inflammatory cytokines (Giulietti et al., 2007). Increased serum level of inflammatory cytokines like IL-18 (Skopinski et al., 2005), IL-6 and TNF-α (Pickup et al., 2000) is documented in type 2 diabetes. Several studies referred to the association and key role of IFN-γ in immunological syndromes such as multiple sclerosis (Bever et al., 1991), SLE (Xie et al., 2002), nephrotic syndrome (Seconi et al., 2003), graft rejection (Hoerbelt et al., 2008), asthma (Litonjua et al., 2003) and type 1 diabetes (Ozer et al., 2003). Investigators believe in crucial role of IFN-γ in defense against infectors and autoimmunity (Chen and Liu, 2009). IFN-γ suppresses IL-17 expression by Th1 cells via activation of regulatory T-cells (Chen and Liu, 2009), thus, can inhibit autoimmunity. In other way some investigators proposed that IFN-γ induces Th1 response by enhancement of APC cells activation in order to IL-12 secretion and worsen autoimmune disease (Chen and Liu, 2009). Due to the importance of IFN-γ in autoimmunity, a question can be raised to address the impact of this cytokine on renal disease? Hence, to partially answer to this question, we aimed this study to evaluate the role of IFN-γ as autoimmune associated cytokine in type 2 diabetic patients with nephropathy.

MATERIALS AND METHODS

This study was performed during June 2007 to March 2008 in the Department of Hematology and Immunology, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. The peripheral blood samples were collected from 100 type 2 diabetic patients with nephropathy (end stage
renal complications), who were registered and diagnosed by an internal medicine specialist and 100 healthy controls. Two groups were selected from Rafsanjan population with same sex, roughly age and socio-economic status after ethical approval committee of Islamic Azad University, Zahedan Branch. Fasting blood sugar, urine albumin level, blood pressure and clinical presentations were assessed three times during a period of 6 months to select the patients and healthy controls. The bias factors were eliminated in groups, for instance both groups were free of infections, allergy and smoking. IFN-γ serum level was detected using ELISA (eBioscience, ESP) in patients and control samples immediately after blood collection. Assays were performed as manufacturer's guidance. Nutshell, IFN-γ specific capture antibody (1 μg mL⁻¹) was added to polyester plates and incubated overnight then the plates were washed and standards and samples were added. After 2 h plates were washed, then detection antibody (conjugated with biotin) (400 ng mL⁻¹) was added and incubation followed for 2 h. After addition of enzyme-avidin another washing was performed. Tetramethylbenzidine (TMB) was used as substrate and the reaction was stopped after 15 min and read at 450 nm. The sensitivity of kit was mentioned as 2 pg mL⁻¹ by manufacturer and inter and intra assay study was performed for the kit.

**RESULTS**

The mean age was 48±9 and 48±8 in nephropathic type 2 diabetic patients and controls, respectively. Present results showed that there was not a significant difference between groups regarding age (Table 1) (p<0.05). There were 59 (59%) females and 41 (41%) males in nephropathic type 2 diabetic group whereas 60 (60%) of controls were female and 40 (40%) males. There was not also a significant difference between groups regarding socio-economic status (Table 1) (p< 0.90).

Also, findings of this study indicated that the mean IFN-γ serum level was 16.09±2.04 and 4.03±1.00 pg mL⁻¹ in type 2 diabetic patients with nephropathy and healthy controls, respectively (Fig. 1). Therefore, present results demonstrated that the level of IFN-γ is higher in type 2 diabetic patients with nephropathy than controls and the difference is statistically significant (p<0.02).

While, female patients showed a markedly higher level of IFN-γ than males (p<0.001) (Fig. 2), age, sugar levels and duration of diabetes had no significant effects on this parameter.

Present results also showed that inter and intra assays were CV<14 and 0.03%, respectively.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group</th>
<th>Nephropathic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean±SD)</td>
<td>48±9</td>
<td>48±9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>60 (60)</td>
<td>59 (59)</td>
</tr>
<tr>
<td>Male</td>
<td>40 (40)</td>
<td>41 (41)</td>
</tr>
<tr>
<td>Socio-economic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>22 (22)</td>
<td>24 (24)</td>
</tr>
<tr>
<td>Medium</td>
<td>47 (47)</td>
<td>42 (42)</td>
</tr>
<tr>
<td>High</td>
<td>31 (31)</td>
<td>34 (34)</td>
</tr>
</tbody>
</table>

Values in brackets are percentages

![Fig. 1](image1.png) Fig. 1: Presentation of IFN-γ serum level in type 2 diabetic patients with nephropathy and healthy controls. Figure demonstrates that serum level of IFN-γ is higher in patients than controls and it is significant. *Significant difference in IFN-γ serum level in nephropathic type 2 diabetic patients and controls (p<0.001, t-test, case vs. control). Data are shown as Mean±SE

![Fig. 2](image2.png) Fig. 2: IFN-γ serum level in male and female suffer from type 2 diabetes with nephropathy. Figure demonstrates that IFN-γ serum level in male is lower than female. *Significant difference in IFN-γ serum level in males and females diabetic patients that suffer from nephropathy (p<0.001, t-test, case vs. control). Data are shown as Mean±SE

747
DISCUSSION

It is believed that type 2 diabetes is an immune related disease (Steyn et al., 2008). Cytokines network play key roles in orientation of immune responses (Arababadi et al., 2009). Expression and secretion of cytokines depend on several different factors including infectious agents, hormonal conditions, cytokine gene polymorphisms etc (Arababadi et al., 2009). The main causes of type 2 diabetes and its inflammatory complications is still unclear but some investigators suggested autoimmune mechanisms like type 1 diabetes. The situation of IFN-$\gamma$ as a pro-inflammatory cytokine in the scenario of autoimmunity and its complications is obscure (Chen and Liu, 2009). In this study, we evaluated the serum level of IFN-$\gamma$ in type 2 diabetic patients who suffer from nephropathy complications. We matched this study groups (case and control) for variables such as sex, age, monthly income and level of education. We found a significant difference in IFN-$\gamma$ serum level in nephropatic diabetic patients and controls. Because nephropathy is known as an inflammatory disorder and the elevated level of IFN-$\gamma$ was shown in several types of inflammatory diseases, hence, we hypothesized that the level of IFN-$\gamma$ could be applied as an prognostic factor in diagnosis and severity of nephropathy in type 2 diabetic patients suffer from nephropathy. To this knowledge this is the first study on the level of IFN-$\gamma$ in these patients. Wong et al. (2007) demonstrated elevated elevated level of other inflammatory cytokines like IL-6, IL-18 and TNF-$\alpha$ in type 2 diabetic patients with nephropathy. The increased serum level of IFN-$\gamma$ related chemokine, IP-10 was also showed in these patients (Xu et al., 2005). These data confirm effective roles of pro-inflammatory cytokines in renal complication of type 2 diabetes. Present data also showed that the level of IFN-$\gamma$ is higher in nephropatic type 2 diabetes, hence, confirm with the results of Wong et al. (2007) and Xu et al. (2005). Because present unpublished data showed an increased serum level of IFN-$\gamma$ in type 2 diabetic patients without nephropathies; thus, it seems that the enhancement of IFN-$\gamma$ level is probably related to the diabetes more than nephropathies. On the other hand, interestingly some investigators reported a decreased level of IFN-$\gamma$ in type 1 diabetes and referred to inability of some cells to produce and secret IFN-$\gamma$ and accordingly influence the IL-17A production and finally leading to the autoimmune type 1 diabetes (Halminen et al., 2001). Kukreja et al. (2002) demonstrated that type 1 diabetic immune cells are unable to produce adequate level of IFN-$\gamma$ in response to experimental stimulators. Finding of this study confirms that the elevated of IFN-$\gamma$ is related with the severity of the nephropathy in type 2 diabetic patients. Studies which focused on the etiology of type 1 and 2 diabetes showed a different pattern of IFN-$\gamma$ production. It seemed that the role of cytokines in type 2 diabetes etiology is obscure, because other environmental parameters (e.g., stress), which affect cytokine production, are involved in this type of diabetes. It is notable that present research team is performing a set of experiments to examine the level of other cytokines and also IFN-$\gamma$ related chemokines in these patients. Finally, nephropathic complications of type 2 diabetes are very complex and are associated with several genetic-environmental factors that these aspects of the disease should be investigated in further studies.

ACKNOWLEDGMENTS

Authors of this study take this chance to appreciate Islamic Azad University, Zahedan Branche as well as all diabetic patients and healthy controls, who attended in this research and also all staff working at the diabetes clinic of Ali Ebn-Abi Taleb Hospital for their warm co-operation and technical aids.

REFERENCES


