

Serum Cytokine and Vitamin Levels in Experimental Diabetic Rats

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Abstract: It is considered several cytokines are effective in pathogenesis and therapy of diabetes mellitus. The aim of this study was to investigate serum concentrations of interleukin-1 β , interleukin-2, interleukin-6 and interleukin-10 cytokines thought to have relationship with diabetes mellitus and serum retinol, α -tocopherol and vitamin D₃ levels notified they have constructive effects on diabetes in streptozotocine induced diabetic rats. Male Wistar-Albino rats between 7-8 weeks old and weighing 180-210 g were used. Streptozotocin induced and blood glucose levels were found between 230-390 mg dL⁻¹ diabetic 25 rats formed the study group and 15 healthy rats constituted the control group. Serum cytokine levels in both groups were evaluated by solid phase sandwich ELISA method and serum vitamin levels were established by HPLC. Serum interleukin-1 β , interleukin-6 and interleukin-10 levels were not sufficient to constitute a statistical importance between groups. However, in diabetic group decreased interleukin-2 levels ($p \leq 0.05$) were established. Besides, decreased retinol and vitamin D₃ levels ($p \leq 0.05$) were detected in diabetic group but reduction in levels of α -tocopherol did not reach statistical significance. In conclusion, decreased serum interleukin-2 levels established in diseased group may indicate this cytokine has a part in the pathogenesis of diabetes mellitus and reduced vitamin levels may exhibit they used for disposal of free oxygen radicals. Besides, increased vitamin D₃ consumption may be due to its disposal in T cell regulation.

Key words: Diabetes mellitus, cytokine, vitamin, rat, oxygen radicals, Turkey

INTRODUCTION

Diabetes mellitus is defined as a metabolic disease characterized by hyperglycemia (Broedl and Goke, 2006; Lencioni *et al.*, 2008). The two main forms of diabetes are type1 and type2 diabetes. Both types are characterized by progressive β -cell failure. In type 1 diabetes, this is typically caused by an autoimmune assault against the β -cells inducing progressive cell death. The pathogenesis of type 2 diabetes is more variable, comprising different degrees of β -cell failure relative to varying degrees of insulin resistance (Cnop *et al.*, 2005).

Cytokines are a diverse group of regulatory proteins or glukoproteins that are secreted by specific cells of the immune system and glial cells which act as chemical communicators between various cells, inducing their effect by binding to specific cell surface receptors thereby triggering various intracellular signal transduction events. They play a central role both immune and inflammatory function and related processes such as haematopoiesis and wound healing (Walsh, 2004). In type 1 diabetes, autoimmune processes destroy the pancreatic β -cells that synthesize insulin causing a nearly complete absence of this hormone (Lernmark, 1999). In several studies, it is

informed interleukin-1 β (IL-1 β) and interleukin-2 (IL-2) cytokines may contribute the development of diabetes by inducing β -cell destruction (Rabinovitch, 1994; Grunnet *et al.*, 2009) whereas interleukin-6 (IL-6) and interleukin-10 (IL-10) cytokines may have protective effects against the occurrence of diabetes because of their roles in stimulating humoral immune responses (Pennline *et al.*, 1994; Tisch and McDevitt, 1996). Besides it is suggested in several studies that retinol, α -tocopherol and vitamin D are effective in prevention of β -cell dysfunction by acting as free radical scavenging antioxidants or by suppressing cytokines which are responsible in diabetes mellitus progression (Mathieu *et al.*, 1994; Tajiri and Grill, 1999; Kang *et al.*, 2004). As only limited data are available on the circulating levels of cytokines and vitamins in diabetes in the present study we have measured serum levels of IL-1 β , IL-2, IL-6 and IL-10 cytokines and serum retinol, α -tocopherol and vitamin D₃ levels in Streptozotocine (STZ) induced diabetic rats.

MATERIALS AND METHODS

Male Wistar-Albino rats between 7-8 weeks old and weighing 180-210 g were used in this study. The rats were

fed on standard laboratory diet and water *ad libitum* and kept in cages at an ambient average temperature of 21±1°C. All the implied processes in the animals were approved by the institutional committee. Forty rats were randomly allocated into two groups.

The experimental group (n = 25) was made diabetic by a single intraperitoneal injection of freshly dissolved streptozotocin (STZ, Sigma) (45 mg kg⁻¹ body weight in citrate buffer 0.1 M, pH 4.5) into overnight fasted rats. The control group (n = 15) was injected with buffer only. About 48 h after injection of STZ, development of diabetes in rats was confirmed by measuring blood glucose levels in blood samples taken from a tail vein. Rats with blood glucose levels ≥250 mg dL⁻¹ were considered to be diabetic. Diabetes mellitus was confirmed by the use of PlusMED Accuro glucometer (Plusmed Trad., Estonia). Blood samples were taken by cardiac puncture under ether anesthesia. Then serum samples were obtained after centrifugation at 3000 rpm for 5 min and were stored at -70°C until used. Serum IL-1β (BioSource Rat IL-1β kit, California, USA), IL-2 (BioSource Rat IL-2 kit), IL-6 (BioSource Rat IL-6 kit) and IL-10 (BioSource Rat IL-10 kit) levels were determined by solid phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using an ELISA reader (Triturus, Grifols Diagnostico, Barcelona, Spain).

Quantitative analysis of serum retinol, tocopherol and vit D₃ levels was performed by High Performance Liquid Chromatography (HPLC, Agilent-1100, Gemany) method (Zaspel and Csallany, 1983; Reynolds and Judd, 1984; Miller and Yang, 1985). Results are expressed as mean±SD Independent t-test was used for statistical analysis and statistical significance was set at p≤0.05.

RESULTS AND DISCUSSION

In this study, we measured serum levels of IL-1β, IL-2, IL-6 and IL-10 cytokines thought to have relationship with diabetes. As shown in Table 1 there were no significant differences (p>0.05) in serum IL-1β, IL-6 and IL-10 cytokine levels among the groups. However, IL-2 levels were significantly decreased in diabetic group compared to controls. Recent studies suggest that aberrant cytokine expression plays an important role in the pathogenesis of diabetes by triggering β-cell apoptosis (Rabinovitch *et al.*, 2007; Gysemans *et al.*, 2008; Haller and Shatz, 2008; Tilg and Moschen, 2008).

However, only limited data is available about if there is a correlation between circulating levels of cytokines and diabetes. Fidan *et al.* (2005) notified diabetic group did not differ in serum concentrations of TNF-α, IL-1, IL-2, IL-6 and IL-10 from controls (p>0.05) in STZ induced

Table 1: Serum cytokine levels in control and diabetic rat groups

Parameters (pg mL ⁻¹)	Control group (n = 15)	Diabetic group (n = 25)	p-value
IL-1 β	106.08±1.47	108.85±1.12	p>0.05
IL-2	135.71±0.99	131.27±1.33	p≤0.05
IL-6	19.69±1.14	20.45±0.64	p>0.05
IL-10	63.23±2.80	63.35±0.53	p>0.05

± values are expressed as Mean±SD

Table 2: Serum vitamin levels in control and diabetic rat groups

Parameters (µg mL ⁻¹)	Control group (n = 15)	Diabetic group (n = 25)	p-value
Rational	0.8230±0.0356	0.6800±0.0430	p≤0.05
α-Tocopherol	0.9930±0.2279	0.5170±0.0584	p>0.05
Vitamin D ₃	0.0878±0.0152	0.0393±0.0146	p≤0.05

± values are expressed as Mean±SD

diabetic female rats. Also Schloot *et al.* (2002) did not determine an increase in the serum levels of IL-10 in NOD mice compared to controls. Besides, Cavallo *et al.* (1991) measured IL-1, IL-2 and IL-6 in sera from patients affected by type 1 diabetes. In this research, detectable levels of IL-1, IL-2 and IL-6 were found in the serum of a small percentage of subjects and were not significantly different between patients and controls.

In several studies, deficient production of IL-2 has been reported in type 1 diabetes but its cause has not been elucidated (Kaye *et al.*, 1986). Zier *et al.* (1984) suggest that decreased IL-2 synthesis is specific for type 1 diabetes not explainable solely as a consequence of poor metabolic control and thus might be involved in the pathogenesis of the disease.

Besides Wedrychowicz *et al.* (2004) informed the low levels of IL-2 might be explained by an abnormal consumption or by the presence of increased IL-2 receptor levels or by a serum factor which interferes with IL-2 production. In the research, we established decreased IL-2 levels (p≤0.05) in diabetic group too. This data supports the suggestion that IL-2 cytokine has a part in the pathogenesis of type 1 diabetes. In the present study, we also evaluated serum concentrations of retinol, α-tocopherol and vitamin D₃ in STZ induced diabetic rats. As shown in Table 2 decreased retinol and vitamin D₃ levels (p≤0.05) were detected in diabetic group. However, reduction in levels of α-tocopherol in diabetic group did not reach statistical significance.

It has been suggested that oxidative stress may play an important role in the pathogenesis of diabetic complications because hyperglycemia may cause increased production of free radicals (King and Loeken, 2004). Zunino *et al.* (2007) informed increasing vitamin A levels in the diet may have profound effects on suppressing inflammatory immune cells and reducing the oxidative damage in the islets that contributes to loss of β cells.

Also, Kang *et al.* (2004) examined the effects of retinoic acid on cytokine-induced β-cell dysfunction in

rats and established retinoic acid significantly protects IL-1 β and IFN- γ -mediated cytotoxicity of rat insulinoma cells. Besides, Tajiri and Grill (1999) informed that vitamin E is one of the most important exogenous free radical scavengers exerts moderate beneficial effects on β -cell functions during short to intermediate length of high glucose exposure.

Baena *et al.* (2002) and Espe *et al.* (2007) declared serum and plasma levels of retinol concentrations were significantly lower with type 1 diabetes compared with healthy subjects (Baena *et al.*, 2002; Espe *et al.*, 2007). However, Basu *et al.* (1989) notified although, lower serum vitamin A levels were detected in the type 1 diabetic patients compared with nondiabetic subjects, serum concentrations of vitamin E were not significantly different between the two groups and they suggest that the reduced serum vitamin A levels in the diabetic patients reflect reduced mobilization of vitamin A from the liver. Besides, Hozumi *et al.* (1998) measured plasma α -tocopherol and retinol levels in children with type 1 diabetes and established the plasma α -tocopherol levels of the children with type 1 diabetes were significantly higher than those of the control children but there were no differences in plasma retinol levels. Nevertheless, Vessby *et al.* (2002) detected higher tocopherol levels in patients with type 1 diabetes and suggested no vitamin E supplementation is necessary for subjects with type 1 diabetes. In the present study, we found a significant decrease in retinol levels but no significant changes in α -tocopherol levels between diabetic and control groups similar to that reported by Basu *et al.* (1989). Recent evidence suggests a role for vitamin D in pathogenesis and prevention of diabetes mellitus (Takiishi *et al.*, 2010). The Nonobese Diabetic (NOD) mouse experiences disease pathogenesis similar to the human including autoimmune destruction of β cells. Studies on NOD mouse showed that pharmacologic doses of 1,25-dihydroxyvitamin D prevent insulinitis and type 1 diabetes, possibly by immune modulation as well as by direct effects on β -cell function (Mathieu *et al.*, 1994). Moreover, several epidemiological studies provide evidence that vitamin D intake can prevent type 1 diabetes in humans (Hypponen *et al.*, 2001; Stene and Joner, 2003).

In view of the immune-mediated nature of type 1 diabetes mellitus Baumgartl *et al.* (1991) investigated serum 1,25-dihydroxyvitamin D levels in recently diagnosed diabetic patients and compared with healthy controls. A marked decrease of 1,25-dihydroxyvitamin D levels was found at onset of type 1 diabetes compared to normal controls. Accordingly, Frazer *et al.* (1981) and Imura *et al.* (1985) declared the reduction of serum 1,25-dihydroxyvitamin D levels in type 1 diabetic patients compared with healthy controls in their researches. We determined decreased serum vitamin D levels ($p \leq 0.05$) in

diabetic group as those studies mentioned above. However, Munoz-Torres *et al.* (1990) did not find overall significant differences in 1,25-dihydroxyvitamin D levels between diabetic patients and control individuals and suggested exogenous insulin administration may have relevance for the interpretation of these results.

CONCLUSION

In this study, we established no significant differences in serum IL-1 β , IL-6 and IL-10 cytokine levels among the groups. However, IL-2 levels were significantly decreased in diabetic group compared to controls. The alterations of serum IL-2 levels detected in diabetic group may indicate this cytokine has a part in the pathogenesis of diabetes. Furthermore, future investigations including other cytokines may help clarify the role of interleukins in the etiopathogenesis of diabetes. We also evaluated serum levels of retinol, α -tocopherol and vitamin D₃ in this research. Although, significantly decreased retinol and vitamin D₃ levels were detected in diabetic group compared to controls, we did not find a statistically significant depletion in serum levels of α -tocopherol between groups. So, reduced vitamin levels may exhibit they used for disposal of free oxygen radicals and increased vitamin D₃ consumption may be due to its disposal in T cell regulation.

ACKNOWLEDGEMENT

This research was support by a grant from the Yuzuncu Yil University Research Fund (2006-SBE-B51).

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