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Association of Toxoplasmosis with Serum TGF-β, IL-17, and IL-6 Levels in Individuals with Diabetes

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ABSTRACT

Cytokines play an essential role in regulating the interaction of immune cells in diabetes and infections such as toxoplasmosis. The purpose of this study was to examine the serum levels of interleukin (IL)-6, IL-17, and transforming growth factor-beta (TGF-β) in patients with type 1 and type 2 diabetes mellitus with toxoplasmosis, and to explore their inter-relationship.

Forty patients with diabetes mellitus, including 20 with type 1 and 20 with type 2, as well as 20 healthy subjects, voluntarily participated in the study. Each subject provided 5 mL of peripheral blood for enzyme-linked immunosorbent assay.

Subjects with type 2 diabetes mellitus who also had toxoplasmosis showed a significant increase in TGF-β levels and a decrease in IL-6 levels. In contrast, patients with type 1 diabetes mellitus displayed a slight increase in IL-6 and IL-17 levels compared to both the patients with type 2 diabetes and the healthy control group.

Our findings show an increase in TGF-β and a decrease in IL-6, which may suggest a reduction in inflammation and beta cell destruction in individuals with type 2 diabetes and toxoplasmosis. The elevated serum levels of IL-6 and IL-17 in individuals with type 1 diabetes further support the exacerbation of inflammation.

Keywords: Cytokines; Diabetes mellitus; Immune system; Inflammation; Toxoplasmosis

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by a deficiency in insulin production or sensitivity to it. Beta (β) cells in the pancreas produce insulin, which facilitates glucose absorption in cells to provide energy and enable the metabolic process. The most common types of DM are type 1 and type 2. DM1 is an autoimmune insulin-resistant disease that is related to the destruction of pancreatic β cells by T lymphocytes. On the other hand, DM2 occurs due to insulin resistance (caused by excess

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fat tissue) and reduced insulin efficacy.[1] In general, DM2 is more prevalent than DM1. The occurrence of DM1 is related to genetic factors, while environmental factors such as inactivity, consumption of sugary drinks, overeating, and a diet high in carbohydrates and fats are considered risk factors for the development of DM2.[2,3]

In the interaction between β cells and immune cells, small molecular proteins such as cytokines play a crucial role. These proteins are essential for regulating the interaction of immune cells with pancreatic β cells and the development of DM1. Immune regulatory cytokines such as interleukin (IL)-10, IL-33, and transforming growth factor-beta (TGF-β) prevent the destruction of β cells by inducing tolerance and modulating immune responses. Conversely, cytokines such as IL-6, IL-17, IL-21, and tumor necrosis factor (TNF) enhance the inflammatory and destructive responses to β cells, leading to the development of DM.[4]

When DM patients are infected with intracellular parasites such as *Toxoplasma gondii*, their cytokine profile may change, potentially impacting their health.[5] The role and importance of cytokines in the pathogenesis of toxoplasmosis have been evaluated in various studies. IL-10 and TGF-β prevent parasites from being destroyed by the immune system's regulatory mechanisms. However, IL-6, when accompanied by TGF-β, enhances antiparasitic immune responses associated with T helper (Th) 17 lymphocytes and inhibits the spread of the infection.[6] This could explain why some studies have considered toxoplasmosis as a risk factor for both DM1 and DM2.[7] The concurrent infection of DM patients with *Toxoplasma*, along with the necessity of TGF-β and IL-6 cytokines to differentiate T_H17 inflammatory cells from $CD4^+$ Th cells, will pose a new challenge for these patients. The aim of this study was to investigate the serum levels of IL-6 and TGF-β cytokines, which are effective in the immune system in DM1 and DM2 patients who were simultaneously infected with *Toxoplasma*.

MATERIALS AND METHODS

This cross-sectional study included 20 patients with DM1 and toxoplasmosis, 20 patients with DM2 and toxoplasmosis, 20 patients with DM1 only, 20 patients with DM2 only, and 20 healthy subjects who volunteered. They were examined according to specific inclusion and exclusion criteria.

The inclusion criteria consisted of subjects with a confirmed diagnosis of DM1 or DM2; Subjects with DM1 had to be between 2 and 20 years old, while those with DM2 had to be between 21 and 75 years old. Participants were not required to follow any specific dietary restrictions and could not be pregnant or breastfeeding. Participants should be free from chronic complications such as retinopathy, nephropathy, and neuropathy, particularly in the week leading up to the study. They should also avoid following any specific diet plans, especially in the month prior to the study. Additionally, participants should refrain from taking any nutritional supplements.

Exclusion criteria included individuals outside the age range of 2 to 20 years for DM1 and 21 to 75 years for DM2, as well as those with dietary restrictions. Additionally, pregnant or lactating women, DM patients with chronic complications (such as retinopathy, nephropathy, and neuropathy), especially in the week prior to the study, and those who had recently followed a specific diet plan or used a specific nutritional supplement were not eligible to participate.

Diagnosis of DM was done by measuring serum HbA1c levels. The subjects with HbA1c > 6.5% were considered to have DM. In addition, the fasting blood sugar of DM patients was also above 200 mg/dl. The healthy control group was selected from individuals referred to the central laboratory of Zabol City. They had fasting blood sugar levels below 110 mg/dL and serum HbA1c levels < 6.5%.

Demographic Data

The study population consisted of five groups: DM1 with toxoplasmosis (n=20; 8 men, 12 women; mean age 15.00 ± 3.16 years), DM2 with toxoplasmosis (n=20; 11 men, 9 women; mean age 58.00 ± 7.35 years), DM1 only (n=20; 7 men, 13 women; mean age 16.00 ± 4.47 years), DM2 only (n=20; 15 men, 5 women; mean age 62.00 ± 6.93 years), and a healthy control group (n=20; 10 men, 10 women; mean age 44.00 ± 12.58 years).

Sampling and ELISA

Five milliliters of peripheral blood were collected from each subject, and the sera were separated for enzyme-linked immunosorbent assay (ELISA). The ELISA kit was purchased from Pishtaz Teb Co., Iran.

Analysis of specific immunoglobulins (Ig) to *Toxoplasma* (IgM and IgG) was conducted following the manufacturer's instructions of the kit (Pishtaz Teb Co., Iran). The analysis was qualitative, determining whether

the antibodies were positive or negative. Additionally, samples from each group were used for cytokine analysis using an ELISA kit from Karmania Pars Gene Co., Iran (data were not shown).

The ELISA kit obtained from Karmania Pars Gene Co. was specifically used to measure serum levels of IL-17, TGF-β, and IL-6. The minimum detectable concentrations for IL-17, IL-6, and TGF-β were 3 pg/mL, 2 pg/mL, and 6 pg/mL respectively.

Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics version 26. The data is presented as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the means across all groups. A p value < 0.05 was considered significant.

RESULTS

Serum Levels of TGF-β

The serum level of TGF-β was significantly higher in patients with DM2 and toxoplasmosis (223.8 ± 44.7 pg/mL) compared to the healthy control group (46.3 ± 19.1 pg/mL) ($p < 0.001$), as well as compared to patients with DM1 and toxoplasmosis (27.4 ± 6.9 pg/mL) (p=0.000). However, there was a significant difference when comparing it to the TGF-β levels in the DM2-only group (90.9 ± 5.09) (p=0.029) (Figure 1A).

However, the TGF-β serum level in DM1 patients with toxoplasmosis (27.4 ± 6.9 pg/mL) did not show a significant difference compared to the healthy control group or the DM1-only group (85.59 ± 2.06) ($p > 0.05$). The TGF-β serum level in DM2 patients with toxoplasmosis (223.85 ± 44.79) showed a significant difference compared to the DM1 patients with toxoplasmosis and the healthy control group ($p < 0.01$). Additionally, it showed a significantly higher level compared to the DM2-only group (p=0.029) (Figure 1A).

Serum Level of IL-17

There was no significant difference in the serum levels of IL-17 between patients with DM1 and DM2 who had toxoplasmosis (10.2 ± 0.93 pg/mL and 9.85 ± 0.6 pg/mL, respectively) compared to the healthy control group (9.12 ± 0.9 pg/mL). Furthermore, there was no significant difference between DM2 patients with toxoplasmosis compared to the DM2-only group (21.81 ± 1.58). Similarly, there was no significant difference between DM1 patients with toxoplasmosis compared to the DM1-only group (32.46 ± 1.85). Moreover, there was no significant difference between the DM2-only group (21.81 ± 1.58) and the DM1-only group (32.46 ± 1.85) ($p > 0.05$) (Figure 1B).

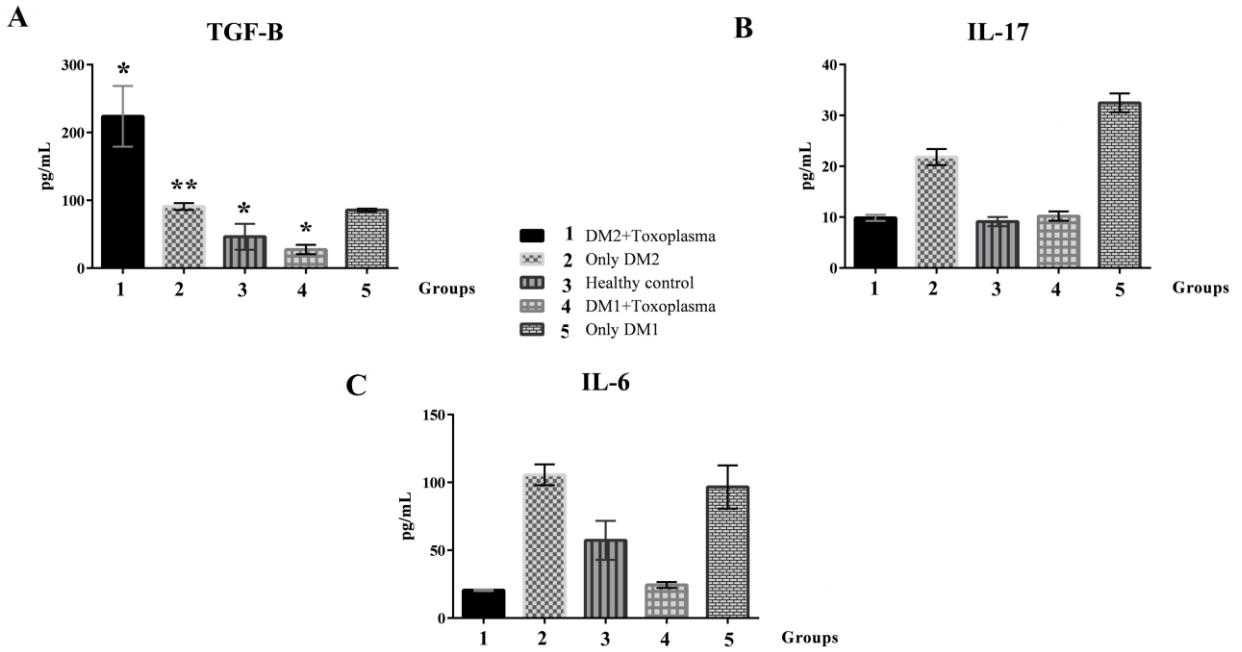

Figure 1. TGF- β, IL-17 and IL-6 serum levels in DM2+Toxo, Only DM2, Healthy control, and DM1+Toxo and Only DM1 are shown. There were significant differences in TGF-β groups (* $p < 0.001$, **: p=0.029)

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Serum Level of IL-6

Patients with DM2 who had toxoplasmosis exhibited lower serum levels of IL-6 (20.5 ± 0.51 pg/mL) compared to the healthy control group (57.3 ± 14.4 pg/mL), and DM2-only group (105.6 ± 17.72) ($p > 0.05$). Additionally, patients with DM1 who had toxoplasmosis had IL-6 levels of 24.4 ± 2.16 pg/mL, while the DM2-only group had levels of 105.6 ± 17.72 pg/mL, and the DM1-only group had levels of 96.64 ± 15.97 pg/mL ($p > 0.05$) (Figure 1C).

DISCUSSION

In this study, we evaluated the concentration of inflammatory and regulatory cytokines IL-6, IL-17, and TGF-β in DM patients with and without *Toxoplasma* infection, as well as in healthy individuals.

An increase in blood sugar is expected to weaken the cellular immune responses associated with T_H1, T_H2, and T_H17 cells. [5] This can be especially concerning for DM patients who need efficient cellular immunity to fight parasitic diseases such as toxoplasmosis,[8] making them more susceptible to infection. Additionally, toxoplasma can present additional challenges for these patients.

Abdel-Moneim et al acknowledged the role of IL-17 and its secreting cells (T_H17) in the pathogenesis of inflammatory diseases, as well as DM1 and DM2. They demonstrated that an increase in the number and activity of T_H17 cells has detrimental effects on the β cells of the Langerhans islets. However, neutralizing IL-17 antibodies can reduce the number and activity of T_H17 cells.[9] Our subjects likely have antibodies that bind to the cytokines and target cells, and then some of the innate immune components such as opsonization and the reticuloendothelial system eliminate them. Certainly, antibodies for *Toxoplasma* were present in their peripheral blood, and IL-17-secreting cells that are involved in the pathogenesis of DM1 may also be present in toxoplasmosis. Therefore, a decrease in cell count and cytokine levels would be expected. However, further studies utilizing flow cytometric analyses to estimate T_H17 cell counts are necessary. Mohsen et al (2018) confirmed the presence of $CD4^+CD8^+$ (double positive) cells secreting IL-17 as a characteristic of individuals with DM1.[10]

In our study, all of the subjects were adults, and flow cytometry analysis was not carried out. Additionally, we did not conduct any investigation on subjects with only DM1 or DM2 without toxoplasmosis. However, this difference was only observed when compared to the healthy control group. Analysis of TGF-β in all groups showed that its level in DM1 had decreased non-significantly compared to the healthy control group. This finding could explain the increase in IL-17 levels in DM1 compared to DM2 and the healthy control group, which was consistent with our expectations.

Roohi et al analyzed the levels of IL-17, IL-23, and TGF-β in DM1 and DM2. They found no differences in IL-17 and IL-23 levels compared to the healthy control group. However, they did observe a decrease in TGF-β serum levels in DM1 compared to the other groups.[11] Since forkhead box P3 $(FoxP3)^+$ regulatory T cells (Tregs) are the main producers of TGF-β and have an immune regulatory role, it is logical to expect that their serum levels would decrease in autoimmune DM, especially DM1, compared to the healthy control group. However, our findings revealed a significant decrease in the serum levels of TGF-β in individuals with DM1 compared to those with DM2 and the healthy control group. There is likely a strong synergistic effect in the increase of T_H1 cells in individuals with DM1 who are suffering from toxoplasmosis compared to other groups. It is suggested that the cell populations of Treg $FoxP3^+$, T_H17, and other $CD4^+$ Th cells in all groups be sorted by immunohistochemistry analysis.

Li et al discovered a new type of IL-17-secreting cell called iNKT-17 in subjects with DM1 and NOD mice. These cells are responsible for the persistent high serum levels of IL-17 and the intense inflammatory response seen in DM1. Additionally, they can also secrete interferon-gamma (IFN-γ), which further promotes the destruction of β-cells.[12] In our study, we found that the serum level of IL-17 in individuals with DM2 was slightly higher compared to the healthy control group. However, it was slightly lower compared to individuals with DM1. Despite the differences in the diseases, this result contradicts the findings of Li et al. This finding may be related to the function of iNKT cells. However, an increased serum level of IL-17 in patients with DM1 compared to DM2 and the healthy control group perfectly matched the findings of Li et al Toxoplasmosis as an intracellular infection related to T_H1 cells and macrophages, can increase IL-1β and IFN-γ, intensifying inflammatory responses in patients with DM1 compared to the other groups.[13] Kikodze et al also examined the cellular immune responses and specific

cytokines in the pathogenesis of DM1. They reported that T_H1 cells and their cytokines (IL-2, IFN-γ) contribute to the deterioration of insulin-secreting β cells. Conversely, TGF-β-secreting Th3 cells, as well as IL-10 and TGF-β secreting Tregs contribute to the protection of DM1.[14] In our study, we only evaluated serum levels of IL-17, IL-6, and TGF-β. The level of TGF-β, an inhibitory cytokine was higher than the level of IL-17, an inflammatory cytokine. According to various sources, we are unable to definitively identify a specific cell that secretes these cytokines. However, some studies have shown that Tregs and T_H2 cells secrete TGF-β, while T_H17 and iNKT cells secrete IL-17. [14] It should be considered that toxoplasmosis may be present in DM patients in this study. If immunohistochemistry analysis, such as flow cytometry analysis, were performed, these findings would be more likely to be authenticated.

In this study, we observed a significant increase in TGF-β levels and lower levels of IL-6 in patients with DM2 and toxoplasmosis. This finding may indicate a reduction in type 1 (monocyte) inflammation in these patients compared to the other groups, which could be advantageous in preventing further destruction of β cells. Furthermore, the slightly elevated levels of IL-6 and IL-17 in DM1 compared to DM2 and the healthy control group confirm the presence of increased inflammation and suggest that conditions may be conducive to an increase in T_H17 cells. When considering the concurrent toxoplasma infection in subjects with DM, it is important to take into account the inhibitory role of Tregs in DM2 compared to DM1 subjects. These cells are likely activated to prevent the development of a synergistic relationship between toxoplasmic inflammation and DM. Further studies such as flow cytometry analysis, are necessary to confirm this assertion.

STATEMENT OF ETHICS

This study was approved by the Research Ethics Committee of Zabol University of Medical Sciences on 17 May 2022, and received the code IR.ZBMU.REC.1400.074.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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