Detection of coxsackie virus B and measurement level of Tumor necrosis factor alpha in patients suffered in T1DM with coxsackie virus infection

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Abstract---Objective. Infection with Coxsackie virus. This virus that damages pancreatic cells, has long been linked to the onset of insulin-dependent diabetic mellitus (IDDM). Pro-inflammatory cytokines can be produced as a result of this illness. Tumor necrosis factor-a is one of these pro-inflammatory cytokines. Materials and Methods. Blood sample were collected from 180 Iraqi participants. Ninety of them is type 1 diabetic patients and other 90 is healthy control .both groups were tested for the incidence of Coxsackie virus B IgG. So the patients groups is divided to two groups according to sero positivity of CVB-IgG .all 180 patients tested to measure of level of TNF-α. Results. The Results showed increasing in levels of TNF-α in CBV positive Type 1 Diabetes mellitus was (34.85 ± 11.00 pg/ml). The level of this interleukin in Type 1 Diabetes mellitus negative to that virus was (26.16 ± 7.79 pg/ml). While the results of this interleukin in control group was (13.82 ± 3.93 pg/ml) with p-value 0. Conclusion. The concentration of TNF-α, according to results, has been shown to be associated with type 1 diabetes mellitus patients infected with CVB-IgG and diabetic patients without CVB.

Keywords---Viral diseases, T1DM, TNF-α, and Coxsackie virus.
Introduction

Diabetes mellitus (DM) is a severe metabolic illness that has been present for a long time that develop as a result of a complex combination of heredity and environmental variables. It is characterized by hyperglycemia, polyuria and polyphagia (1). T1DM (type 1 diabetes mellitus) is a one type of DM, is the most common chronic autoimmune illness defined by gradual loss of pancreatic beta – cell ,leading in a reeducation of insulin output (2). One and the important etiology of T1DM is exposure to virus such as enter virus like Coxsackie virus implicated in the development of T1DM. (3)

Infection with Coxsackie virus recently been linked with to produce some pro-inflammatory cytokine such as TNF-α. Cytokine are small protein produced by cells that play a role in cellular communication and inflammation. Cytokine are protein that regulate pain ,inflammation, immune response and other processes .they are classified as pro-inflammatory and anti-inflammatory cytokine(4). TNF-α producing DCs and macrophage were found in pancreatic islet infiltrate in early investigation, indicating that these cells were the original and main producers of TNF-α. It was discovered that it up regulated MHC-I molecules ,speeding up B – cell death and then induces islet-infiltrating DCs/ macrophages to cross-present an exogenous islet antigen to CD8+ T cells via a CD40/CD154-independent mechanism(5). The aim of this research is measurement of TNF-α in DM patients with viral infection in correlation with those without it.

Materials and Method

Ninety type 1 diabetes mellitus patients were attended to Imam Hassan center for Endocrinology and Diabetes at the holy Karbala province and 90 healthy controls (non-diabetic patients). All of two groups is tested for detection of Coxsackie virus so this groups is divided according to sero –positivity of CVB-IgG to following groups : First groups : Type 1 diabetes mellitus with Coxsackie virus. Second groups: Type 1 diabetes mellitus without Coxsackie virus. Third groups: healthy control. The following step is measurement of level of TNF-α for the three groups. The laboratory diagnosis was done for detection of Coxsackie virus and determination of level of TNF-α is biotek ELISA system.

Statistical analysis

T tests for (cause and control) and ANOVA tests were performed to investigate the difference between the three groups in the study’s data using the statistical analysis technique employing social sciences (SPSS) version 20.

Results

The 90 sample of T1DM patients and 90 sample of control groups were tested for Coxsackie virus B ,the result show that 18(28%)patients were positive for specific anti-Coxsackie virus B-IgG(anti CVB-IgG) in T1DM patients. In other hand, all the serum samples of non-diabetes patients were negative to anti CVB-IgG. The Results showed increasing in levels of TNF-α in CBV positive Type 1 Diabetes mellitus was (34.85 ± 11.00 pg/ml). The level of this interleukin in Type 1
Diabetes mellitus negative to that virus was (26.16 ± 7.79 pg/ml). While the results of this interleukin in control group was (13.82 ± 3.93 pg/ml) as shown in Table (1) (Figure 4). There was significant differences (P<0.05) of TNF-α level in CBV positive Type 1 Diabetes mellitus than the others in which negative to them.

Figure 1. Distribution of positive CVB-IgG according to age groups

Figure 2. Distribution of positive CVB-IgG according to gender groups
Measurement of level of serum interleukin (TNF-α)

The result showed the increasing level of TNF-α in CVB positive type 1 diabetes mellitus was (34.85 ± 11.00 pg/ml). The level of this interleukin in type 1 diabetes mellitus negative to that virus was (26.16 ± 7.79 pg/ml). While the result of this interleukin in control groups was (13.82 ± 3.93 pg/ml).
Table 1. Concentration levels of TNF-α, in the study groups: CBV positive Type 1 DM, CBV Negative Type 1 DM and Control group.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Groups</th>
<th>NO.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>F test</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>T1DM with CBV</td>
<td>18</td>
<td>34.85</td>
<td>11.00</td>
<td>33.522</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T1DM without CBV</td>
<td>72</td>
<td>26.16</td>
<td>7.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>90</td>
<td>13.82</td>
<td>3.93</td>
<td>33.522</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*P-value 0.05

**Discussion**

The result of anti-CVB-IgG of this research, which was 28% sera positivity of anti CVB-IgG results, would refer to previous Coxsackie virus B infection that had been neutralized by adaptive immune response. IgG antibody response are crucial for adaptive immunity because they reflect the immunological memory for previous pathogen exposure (6). The induction of adaptive immunity is associated with clearance of virus. B cell regulate and conduct the production of specific antibodies, which either deactivates the virus opsonisation and neutralization processes or initiates destruction of infected cells (7).

According to figure 1, the result showed that the age group less or 10 years had the highest percentage (66.7%). The high prevalent within 1-10 years age might be due to more predisposing related to poor hygiene habits in that age, which increased transmission of viral infection among them readily (8). According to figure 2, sex showed that the highest percentage of them which constituted 64.28% female groups.

According to figure 3, ABO blood system results of type 1 diabetic patients with Coxsackie virus, the highest percentage O blood group 50%. Human blood is organized into 34 groups, each of which carries hundreds of genes and antigens that are inherited as polymorphic features. Changes in expressed blood group antigens can affect a person’s ability to withstand infectious illnesses in a variety of situations. Blood groups may play a key role in the acquisition of viral illnesses by increasing cellular adhesion and virus engulfment as virus receptors and coreceptors (9).

According to figure 4, and table 1, The Results showed increasing in levels of TNF-α in CBV positive Type 1 Diabetes mellitus was (34.85 ± 11.00 pg/ml). The level of this interleukin in Type 1 Diabetes mellitus negative to that virus was (26.16 ± 7.79 pg/ml). While the results of this interleukin in control group was (13.82 ± 3.93 pg/ml) as shown in Table (4.1) (Figure 4). There was significant differences (P<0.05) of TNF-α level in CBV positive Type 1 Diabetes mellitus than the others in which negative to them. Experimental infection of human peripheral blood mononuclear cells with CBV resulted in increased production of cytokines such as TNF-α and IL-6 (10), that CBV is able to infect β-cells and The similarity of human and viral proteins might provide another reason, and the coxsackie-B
virus does include a protein that is comparable to human glutamic acid decarboxylase-65 (GAD65) (11).

Although, TLR4-dependent mechanism, viral infections cause the production of pro-inflammatory cytokines in human pancreatic cells, including IL-1beta and TNF-alpha. Some research has suggested that these cytokines may have a negative effect on pancreatic function, and TNF-alpha has been linked to cell damage in the early stages of T1DM development by inducing endoplasmic reticulum stress, despite the fact that they appear to play a protective role in the host's defense against infection. (12). The T1DM cells are infected by T1DM, CBV, which causes them to produce CXCL8 and stimulates Th1 and Th2 cells to generate TNF-alpha (13).

Conclusions

This study show, the Coxsackie virus B(CVB) was detected 28% from the serum of type 1 diabetes mellitus. The result of CVB– IgG antibodies revealed that IgG antibody has levels were higher age group less than 10 years than other age groups. The result showed that CVB was predominant in females patients more than male patients. Also the result shows the O blood groups is more susceptible to Coxsackie virus infection. The concentration of the TNF-alpha, according to our results, has been shown to be associated with the type 1 diabetes mellitus patients infected with CVB– IgG and diabetic patient without CVB.

References