The Role of IL-18 and Human Cytomegalovirus in Type 1 Diabetes Mellitus in Thi-Qar Province

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ABSTRACT

Previous studies indicated that elevated serum levels of Interleukin-18 (IL-18) and human cytomegalovirus (HCMV) antibodies have a fundamental role in the pathogenesis of Type I diabetic mellitus (T1DM). Therefore, the aim of the current study was to investigate the possible overlap between human cytomegalovirus (HCMV) and Interleukin-18 (IL-18) with the pathogenesis of T1DM. The serum of (45) T1DM patients and (45) healthy controls were used to detect IL-18 levels with an ELISA Kit and detect HCMV with cobas c411 apparatus for detect IgM and IgG HCMV.

The results showed that the levels of IL-18 and human CMV IgM, IgG were significantly higher in patients with type 1 diabetes compared to the control group (45.8773 ± 12.196 and 0.52 ± 0.25, 63.52 ± 13.0 pg/ml for patients respectively) versus (12.611 ± 3.9 and 0.11 ± 0.01, 0.261 ± 0.68 pg/ml for healthy subjects, respectively); The values were at P value ≤ 0.05.

The results of the data concluded that there was a significant increase of IL-18 and human CMV Abs, compared to the lower levels in the control group, indicating that these markers may be involved in the pathogenesis and development of T1DM.

Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common chronic diseases that occur during childhood and accounts for about 10% of all cases of diabetes. In this disease, the insulin-producing from beta-cells located in the pancreas are gradually destroyed until the amount of insulin produced is not sufficient to keep up a normal level of blood glucose [1]. T1DM is an autoimmune disease, which reveals the role of immune response effectors in the pathogenic processes and failure of immune tolerance towards β-cell antigen [2]. The pathogenesis of T1DM is complex and results from a combination of genetic and environmental factors. Among environmental factors a series of viruses including cytomegalovirus (CMV) infections have been associated with the development of beta cell autoimmunity [3]. CMV infection is already relatively common among small children. In the Finnish population, 27% of infants at the age of 7 months tested positive for CMV IgG antibodies [4]. CMV is a common herpes group virus that causes a lifelong latent infection. CMV seroprevalence in adults varies geographically from 40% to 100% [5]. In Finland, similar to other Western countries, approximately 50% of pregnant women have seropositive for CMV-specific IgG antibodies [6]. More than 75% of childhood CMV infections are acquired in early childhood, transmitted typically perinatally or during the first year of life through saliva, maternal genital secretions or breast milk after reactivation of the latent virus in the mother. Thereafter, the incidence rate of CMV infection, stays rather stable until it peaks again at the age of 4 to 5 years [4].
(HCMV) belonging to the Betaherpesvirinae subfamily, is the largest member of the the human herpesvirus family [7]. In the fetus, newborns, and immunocompromised patients, HCMV infection can cause a range of severe clinical effects [8-10]. HCMV is one of the important viral factors that are believed to be linked with the occurrence of T1D because of its ability to induce the immunological damage of β-cells [11]. (CMV) is an important factor believed to be associated with type 1 diabetes due to its role in stimulating damage to immune beta cells (beta cells) [12]. Evidence supporting the role of inflammation in type 1 and type 2 diabetes had increased, the mechanism of inflammation process involved the release of inflammatory cells, and cytokines profibrotic growth factors recording interleukin-1 (IL-1), interleukin-18 (IL-18), interleukin-6 (IL-6) [13]. Interleukin-18 (IL-18) considers as a proinflammatory cytokine (member of the IL-1 cytokine super family) that secreted from mononuclear and plays an essential role in many inflammatory and autoimmune diseases [14].

A previous study by [15] indicated that high levels of IL-18 in type 1 DM patients are associated with short- and long-term glycemic control. In Iraq, Study for [16] emphasized that IL-18 has a critical role in the initiation, development, and promotion of T1DM and T2DM in Iraqi patients. Also, Study for [17] indicated that infectious diseases are more common and / or serious in people with diabetes, which raises their mortality rate. Interleukin (IL) -18 is a pro-inflammatory cytokine that has been involved in causing a number of inflammatory diseases like type 1 diabetics [18]. IL-18 has also been recorded to be included in the pathogenesis and promotion of diabetes [19].

An elevation IL-18 levels have been indicated in the sera of patients at high risk for developing T1D [20]. Yaribeygi et al. (2019) study reported that interleukin 18 and other pro-inflammatory cytokines are increased by hyperglycemia in subjects with impaired glucose tolerance, suggesting a causal role for hyperglycemia in the immune activation of diabetes [13].

This study was designed to compare the circulation serum levels of IL-18 and HCMV Abs between type 1 diabetes and healthy to detect the crucial role of these markers in pathogenicity of T1DM.

**Material and Methods**

A total of 45 T1D patients, who attended The Special Center for Endocrine Glands and Diabetes in Al-Nassyrieh city in Thi-Qar province, Iraq in addition to (45) apparently non-diabetic healthy people representing the control group. This case-control study was conducted from September 2019- May 2020. The patients are diagnosed as by the consultant medical staff, according to checked clinical examination and biochemical analysis. The patients divided into (23) males and (22) females with age range from 1-40 years. The control included (22) males and (23) females within the same age range of patients group. Five ml of venous blood was drawn from each subject (diabetic patients and controls). The blood samples were collected in coagulates gel tubes and left to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum samples were separated in several 1.0 ml tubes that were stored frozen at -20°C until used to avoid thawing and repeated freezing.

IL-18 levels measurement was quantitatively determined in sera of patients and healthy control subjects by means of ELISA (Enzyme Linked Immunosorbert Assay) using ready kits manufactured by Bioassay company(China). While CMV IgM & IgG levels measurement by Cobas c411 automated instrument.

To conduct the research ethical permission was obtained from the hospital and from all participants in this work patients and healthy. The patients Selection were accomplished with the assistance of Physicians in the hospitals.

**Statistical analysis**

The statistical analysis of this case-control study was performed with the statistical package for social sciences (SPSS) 20.0 and Microsoft Excel 2013. Numerical data with normal distribution were described as mean and standard deviation, independent sample T-test used for comparison between two groups. Categorical data were described as count and percentage. The lower level of accepted statistically significant difference is below or equal to 0.05 and the high level of accepted statistically significant difference is below or equal to 0.01.

**Results and Discussion**

**1. Distribution of type 1 diabetes patients by gender:**

Regarding to gender distribution, the results showed that the percentage of males in the T1D patient group was 23(51.11%) and the percentage of females was 22(48.89%), where the ratio of male to females was 1.05: 1. The persons in the control group were chosen similar to the gender percentage of the patient group to avoid having this factor effect on the quality of the study. The percentage of the males and females in the control group was 22(48.89%) and 23(51.11%), respectively. As explained in the below Table (1).

<table>
<thead>
<tr>
<th>Table 1: Distribution of the two Study Groups by Gender:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>

The present data was compatible with Hussein et al. (2016) in Baghdad province who showed that Male to female ratio was 1.058:1 [21].
Also, the study was relatively agreed with two Iraqi studies by Majeed (2008) in Thi-Qar province and Saleh et al. (2012) where both studies showed that Male to female ratio was 1:2:1 [22,23]. The finding by Matteo Apicella et al. (2020) indicated that Male percentage was 51%, and female was 49% [24]. But the current results incompatible with El-sehmany and his colleagues (2019) who found that Male and female percentage was male 47.5%, female 52.5% [25]. Also, Thomas et al. (2003) demonstrated that male to female ratio was 0.89:1 [26]. In our current study, we made sure that both groups of the study were identical in terms of the number of samples and gender to avoid the influence of the sex factor on the study criteria. So according to the current results that support previous data we conclude there is no significant effect to the gender on diabetics type I.

### 2. Distribution of type 1 diabetes patients according to age groups

The results indicated that there was a significant decrease in TID 1 patient percentage in third age of patients with a percentage 11.11% for the age group (21 -30) years, while the highest incidents of TID 1 were in second age group with a percentage 33.33 % for the age group (11-20) years. Compared to the control group, which showed decreases in the second group with a percentage 17.77 % and increase in the first and third groups with a percentage (28.89%). As listed below in the Table (2) below.

**Table 2: Distribution of the two study groups by age groups.**

<table>
<thead>
<tr>
<th>Age groups (Years)</th>
<th>Study group</th>
<th>T1DM Patients</th>
<th>Control Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count %</td>
<td>Count %</td>
<td></td>
</tr>
<tr>
<td>(1-10)</td>
<td>14</td>
<td>31.11</td>
<td>13 28.89</td>
</tr>
<tr>
<td>(11-20)</td>
<td>15</td>
<td>33.33</td>
<td>8 17.77</td>
</tr>
<tr>
<td>(21-30)</td>
<td>5</td>
<td>11.11</td>
<td>13 28.89</td>
</tr>
<tr>
<td>(31-40)</td>
<td>11</td>
<td>24.45</td>
<td>11 24.45</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>100%</td>
<td>45 100%</td>
</tr>
</tbody>
</table>

The current finding was comparable with Nuha et al. (2016) who found that the high frequency of onset of TID was recorded at the childhood before the age of puberty [27]. Also, the study was compatible with the Majeed. (2008) in Thi-Qar province who noted that the majority of patients (79.3%) were between 5-12 years of age [22]. Derraik et al. (2012) found that increasing incidents of Type 1 were recorded in age group (10-14) years [28]. This age range was in agreement with the study of Wherrett et al. (2000) who found that TID recorded at this childhood stage [29].

### 3. The levels of IL-18 in patients and Healthy:

According to the levels of IL-8, the study results recorded a significant increase in the values of IL-18, where the values were (45.8773 ± 12.196), as a comparison with a control group with values (12.611 ± 3.9). As the study showed a significant difference between IL-18 in patients with type 1 diabetes. These results also showed that there were statistical significant differences at p < 0.01 in the levels of IL-18 in patients and healthy. As shown in the Table (4-5) below.

**Table 3: Sera levels of IL-18 in patients and healthy.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL 18 Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>45.8773±12.196</td>
</tr>
<tr>
<td>Control</td>
<td>12.611± 3.9</td>
</tr>
<tr>
<td>T value</td>
<td>17.427</td>
</tr>
<tr>
<td>Sig.</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Regarding to the IL-18 results, the findings were high compatible with Dong et al. (2007) also compatible with Harms et al. (2015) who found that IL-18 is significantly increased in the sera of juvenile T1Ds compared to controls [30,18]. Esposito et al. (2002) demonstrated that IL-18 and other proinflammatory cytokines are increased by hyperglycemia in subjects with impaired glucose tolerance, suggesting a causal role for hyperglycemia in the immune activation of diabetes [31].

### 4. Level HCMV IgM and HCMV IgG in study groups:

The study showed a significant increase in the values of HCMV IgM and HCMV IgG, where the values were (0.52 ±0.25) and (63.52 ± 13.0) respectively, as a comparison with a control group, the values were (0.11 ± 0.01) and (0.261 ± 0.68) respectively. These results also showed that there were statistical significant differences at p ≤ 0.01 in the levels of HCMV IgM and HCMV IgG in patients and healthy, as shown in the Table (4) below.

**Table 4: Levels of HCMV IgM and HCMV IgG in study groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>HCMV IgM Mean± SD</th>
<th>HCMV IgG Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0.52 ± 0.25</td>
<td>63.52 ± 13.0</td>
</tr>
<tr>
<td>Control</td>
<td>0.11 ± 0.01</td>
<td>0.261 ± 0.68</td>
</tr>
<tr>
<td>T value</td>
<td>10.77</td>
<td>32.465</td>
</tr>
<tr>
<td>Sig.</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

The present finding appeared compatibility with Yasir et al. (2013) for results of the levels of HCMV IgM in Najaf [32]. The study also agreed with Ahmad et al. (2014) in Khartoum and Abdel-Moneim et al. (2017) in Egypt for results of the levels of HCMV IgG [33,34]. On the other hand, Where the study showed incompatible with Ibrahim Saber & Mohammed (2019) for results of the levels of HCMV IgM and HCMV IgG [12]. The current result was inconsistent with Al-Hakami et al. (2016) the rise in CMV (IgM) in type 1 diabetic patients [35], and it was non-compatible with Aarnisalo et al. (2008) for results of the levels of HCMV IgG [36]. A study by Dedinská et al. (2016) revealed the existence of anti-HCMV IgM antibody in these patients in conjunction with the onset of the disease and presence of hyperglycemia and elevation of HbA1c might suggest the role of the asymptomatic HCMV infection with the development of TID [37]. A study reported cytomegalovirus (CMV) do appear to be somehow associated with autoimmune type 1 diabetes since
there is a strong correlation between the presence of islet cell autoantibodies and persistent infections [38]. These findings indicate that the immunorespose caused by HCMV infection was associated with b cell injury [39].

References

Conclusion

According to these results, the current study concluded that IL-18 and (HCMV) infections may have a role in the pathogenesis, development and progression of Type 1 diabetes mellitus in Iraqi patients of Thi-Qar province.

دور الانترلوكين-18 والفيروس المضخم للخلايا البشرية في داء السكري من النوع الأول

في محافظة ذي قار

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الملخص

أشارت الدراسات السابقة إلى أن مستويات المصل المرتفعة من إنترلوكين-18 والاجسام المضادة أم وكذلك جي للفيروس المضخم للخلايا البشرية التي كان لها دور أساسي في امراضية السكري من النوع الأول. لذا كان الهدف من الدراسة الحالية هو التحقيق في التداخل المحتمل بين الفيروس المضخم للخلايا البشرية وأنترلوكين-18 المشتبه في امراضية السكري من النوع الأول. تم استخدام مصل (45) مريضًا بداء السكري من النوع الأول و (45) شخص سليم كعينات سيطرة للكشف عن مستويات الإنترلوكين-18 باستخدام عدة الاليزا ELISA والكشف عن الأجسام المضادة أم وكذلك جي للفيروس المضخم للخلايا البشرية باستخدام جهاز cobas c411.

اظهرت النتائج أن ارتفاع مستويات الإنترلوكين-18 والاجسام المضادة أم وكذلك جي للفيروس المضخم للخلايا البشرية حيث كان أعلى بشكل ملحوظ في مرضى السكري النوع الأول مقارنة بتقاطع المجموعة السيطرة (45.8773 ± 12.196 و 0.52 ± 0.25 بيکوغرام / مل للمرضى على التوالي) مقابل (12.611 ± 3.9 و 0.11 ± 0.01 بيکوغرام / مل للأصحاء على التوالي) وكذالك القيم عند خصصت نتائج البيانات إلى أن هناك زيادة كبيرة للإنترلوكين-18 والاجسام المضادة أم وكذلك جي للفيروس المضخم للخلايا البشرية في مصل مجموعة المرضي مقارنة بالمستويات الأقل في المجموعة الضابطة، مما يشير إلى أن هذه العلامات قد تكون مترابطة في التسبب بأمراضية وتطور داء السكري من النوع الأول.

P value ≤ 0.05