Viruses Induce Type 1 Diabetes Mellitus in the Presence of HLA-DR3, DR4 Genes

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Abstract: Recently, the increasing of type 1 diabetes (T1DM) was revealed among children in Kurdistan - Iraq that passed through several wars and economic sanctions. It has been demonstrated that virus infection induced T1DM by destruction of beta cells through direct invasion or autoimmune reactions. Genetic factors also lead to immune disorders which induce T1DM. Despite the reports on effects of viral infection and autoimmune disturbance in the presence of HLA genes, there are no enough studies to show the relationship between them in Kurdish race. Therefore, Serum glucose, three viral (CMV, Rubella and Coxsackie virus), two genes (HLA DR3 and HLA DR4) and anti-glutamic acid decarboxylase (GAD) Ab. were studied to evaluate their relationship with T1DM. Study was performed by interviewing 230 samples (122 females and 108 males) of T1DM patients and another 130 non-diabetic individuals as a control group for collecting blood samples and personal and family information related to this study. Indirect Enzyme-Linked Immuno Sorbent Assay (ELISA) used for detection of anti-viruses antibodies and anti-GAD antibody. While, Polymerase Chain Reaction (PCR) - SSP technique were used for detection of HLA-DR3 and DR4. The statistical results revealed that the mean±SD concentration of anti-CMV IgG, rubella, CSV-B antibodies in the sera of patients was (1.22±0.36 IU/ml), (0.80±0.08 IU/ml), (0.81±0.14 IU/ml) respectively when compared with (0.79±0.32 IU/ml), (0.38±0.17 IU/ml), (0.26±0.21 IU/ml) respectively in sera of control group (P-value < 0.05). This means that there is a significant increase in mean concentration of anti-CMV, rubella, CSV-B antibodies in the sera of patients in comparison with control group. The statistical results of anti-GAD antibody revealed (89.54±0.43 IU/ml) with control group (P-value < 0.05). While, results of the HLA-DR3 and DR4 genes revealed that there is a relationship between these genes and viral infections that induce T1DM and these genes also related with the elevation of anti-GAD Ab in the blood circulation of T1DM patients. This study supports the claims that viruses are responsible for T1DM especially in the presence of HLA-DR3 or/and DR4.

Key words: HLA-DR3 • DR4 • Viral induced type 1 diabetes • GAD

INTRODUCTION

Diabetes mellitus is chronic metabolic disorder characterized by disturbance in glucose metabolism, insulin defect in secretion and prolonged exposure of the cells and tissues to hyperglycemia [1]. It leads to several complications including macrovascular complications (myocardial infarction and stroke) and microvascular complications (neuropathy and nephropathy) [2]. Type 1 diabetes mellitus (T1DM) results from a complete lack or
a severe deficiency of insulin [3]. It has been attributed to a number of factors including environmental, genetic, immunological factors [4]. Moreover, others factors have been proposed to be the cause of diabetes such as: stress and lack of breast-feeding [5], chemical agents [6] and other unknown factors [7]. T1DM is the most common among children and young adults [8]. There are many studies on the relationship between T1DM and viral infections which are still a matter of critical debate [9]. Presently, there are 14 viruses that are believed to induce or trigger T1DM [10, 11]. The common viruses that induce T1DM include; rubella, CSV-B, CMV, adenovirus and mumps [12]. Genetic [13], immune disturbances [4] and environmental factors form a dread triangle which their interaction can induce T1DM [14].

Viruses can damage β-cells in two ways; by, direct infection of β-cells [15], or by molecular mimicry. When immune disorder occurs during molecular mimicry in which viral antigen with homology to self-epitopes cross react resulted in the activation of self-reactive lymphocytes that in turn mediate β-cell destruction [16]. The viral infection could make auto antigens in the pancreatic β-cells appear for cytotoxic (CD8) cells and this provokes CD8 cells to attack these auto antigens [17]. It has been revealed that CMV, CSV-B and rubella virus is related to T1DM. CMV, as a common virus, causes immune disturbance in the human worldwide [18]. According to the report it has estimated that approximately 55% of adults in developed countries and 90% in developing countries are suffering from CMV [19]. Al-Saeed reported that 79.5% of pregnant women in Iraq had CMV IgG infection [20]; while in another study 94.4% of the T1DM patients had CMV IgG positive while the value among non-diabetic people was 77% [10]. CSV-B causes damage to β-cells. Therefore the finding of CSV-induced T1DM is also related to the presence of anti-GAD antibody [21]. Rubella virus is known as a common viruses among patients with T1DM and induces T1DM [22].

GAD 65 Kilodalton (kDa) in the β-cells in pancreas is considered as a target (autoantigens) for T cells which causing autoimmunity T1DM [23]. Type 1 A, resulting from an autoimmune reaction (immune-mediated) leading to β-cell destruction. In type 1 A, the elevated titer of anti-GAD Ab can be found in the blood circulation. The immune system produces Ab against antigenic part of CSV-B virus, which is the most common childhood viral infection. Unfortunately, part of GAD in the human pancreas is similar to this part of CSV-B virus and this similarity confuses the immune system [24]. In vivo and vitro investigations revealed that T lymphocytes can distinguish GAD auto antigens as a foreign body, following B-lymphocyte cells which captures and present it to T cytotoxic [25]. Moreover, the auto reaction of T cells against pancreatic GAD is a critical point in the development of T1DM as a result of imbalance of T-lymphocyte cells [26]. Whole genome linkage scan have shown that the major histocompatibility complex (MHC)/HLA region on chromosome 6p21 contains the major genetic component of the T1DM [27]. The results of the two studies concluded among Iraqi Arab population that the presence of HLA- DR3, DR4 and DQ genes can induce T1DM [28, 29]. The presence of HLA-DR or/and DR4 alleles strongly associated with T1DM, i.e. chance of acquiring diseased is more than those of the same population who lack this type of alleles [30-32].

In Erbil city, in best of knowledge there is no record of studies that focus on the relationship of viral infection and anti-GAD Ab in the presence of HLA genes that triggers T1DM. This study is aimed to compare the sero-positive T1DM patients and healthy (non-diabetic) individuals’ response towards rubella virus, CMV, CSV-B and anti-GAD Ab in the presence of HLA-DR3, DR4 in a sample of Kurdish patients in Erbil city – Kurdistan region of Iraq with the sole target of reducing the T1DM virus among infected patients.

**MATERIALS AND METHODS**

This study is concerned with T1DM patients at the Layla Qassim Centre for Diabetic Patients, Erbil, Iraq. The ethical approval and permission to perform the work was granted by the local scientific committee of Hawler Medical Institute. Furthermore, a consent form was obtained from each participant prior to the study. Kurdish T1DM patients were selected randomly. Non-Kurdish, such as Arabs and Turkmanis were excluded from the study. The diagnosis of T1DM followed the 1997 criteria of the American Diabetes Association (ADA). The molecular tests were carried out at the Forensic Department, Ministry of Health and Baghdad. The blood glucose tests were carried out at Nanakali Hospital for Blood Diseases, Erbil. Testing for cytokines by ELISA was conducted at Zanko Private Laboratory for Advanced Immunological Investigations, Erbil. The analyses and the interpretations of results were obtained at the Department of Biosprocess, Faculty of Chemical Engineering and University Technology Malaysia (UTM).
Size of the Sample: Two hundred and thirty T1DM patients comprising 108 males and 122 females, aged between 3 and 40 years, were selected randomly for this study in addition to 130 healthy subjects (non-diabetic patients) made up 68 females and 62 males were also included in this study as the control group. The control group consisted of Kurdish volunteers with age ranges similar to those of the selected patients.

Blood Collection: Peripheral blood samples were collected by intra-venous puncture and aspiration from the cubital vein. From each patient, 10 ml of blood was drawn and divided into two parts. The first part was left to clot and after centrifugation, sera were aspirated and preserved in the liquid till they were used for measuring the glucose levels, detection CMV IgGAb, rubella IgGAb, CSV-B IgGAb and anti-GAD IgG Ab. The second sample was used for the HLA study where genomic DNA (1-2 µg) was extracted from peripheral blood leucocytes using DNAzol kit from (BAG HEALTH CARE COMPANY). And stored at 4°C for further study. Confidentiality of patients’ information was assured by coding all the test samples and the master list available only to the investigators.

ELISA technique: A. Detection of autoantibodies: For anti-GAD Ab used the kit from Euroimmun Company Germany) 2011, catalog (EA 1022-9601 G). Briefly, the procedure is as follows 25 µl of the calibrators, negative and positive controls or patient samples were transferred into the individual microplate wells according to the pipetting protocol and the frame is covered and incubated for 1 hour at room temperature. After incubation, the microtite ELISA plate is washed three times with wash buffer and after blotting on dry paper towels, 100 µl of GAD (biotin-labelled GAD) added into each of the microplate wells and mixed well. The plate was covered the frame and incubated for 1 hour at room temperature. After incubation the plate washed and 100 µl of enzyme conjugate (peroxidase-labelledavidin) was added into each of the microplate wells and covered. This is incubated for 20 minutes at room temperature (+18°C to +25°C) on a microplate shaker set at 500 rpm. The plate washed again and 100 µl of chromogen/substrate solution was added into each of the microplate wells. The plate was incubated for 20 minutes at room temperature and it was protected from direct sunlight. The reaction was stopped by the stop solution and the color read spectrophotometrically at 450 nm.

Viral Studies: The quantitative test of CMV and rubella virus was detected and evaluated as follows. The procedure is implemented according to the catalog of Biotech, Inc. USA. Company and the steps involved are: One hundred ml of diluted sera, calibrator, positive and negative and controls were added into the appropriate wells. For the reagent blank, 100ml of sample dispensed diluents in 1A well position. The air bubbles were removed from the liquid and mixed well. The plate was incubated at 37c for 30 minutes. After the incubation, the liquid removed from all wells. Then the wells rinsed carefully according to the kit protocol. One hundred ml of enzyme conjugate was dispensed into each well. Then the added substance mixed gently for 10 seconds. The plate was incubated at 37c for 30 minutes. The enzyme conjugate was removed from all wells. The wells rinsed and flicked 5 times with diluted wash Buffer. One hundred ml of TMB reagent was added into each well. Then it is mixed gently for 10 seconds. The plate was incubated at 37c for 15 minutes. Hundred ml. of the stopping solution (1N.HCl) added to each well to stop reaction. The liquid was mixed well for 30 seconds. All air bubbles were removed from wells. The optical density (O.D.) of the well contents was read at 450 nm using micro-titer plate reader (ELISA) within 15 minutes. While for CSV-B, the procedure steps was performed according to the catalog of ELISA Kit/. DIACHECK. Switzerland Company. All reagents and serum samples were brought to room temperature before used. One hundred µl of diluted sera, positive, negative and differential control reagent were added to the appropriate micro-titer wells. The plate was covered and incubated at 37°C for 30 minutes. At the end of the incubation, the wells were washed four times with diluted wash solution according to the kit protocol by adding 300 µl of washing solution. After incubation, the liquid was removed from all the wells. Then the wells rinsed carefully according to the kit protocol. One hundred µl of anti -human IgG-PO conjugate solution was added to each well and mixed gently. The plate was covered and incubated at 37°C for 30 minutes. One hundred µl of TMB-Chromogen/substrate solution were added to each well and mixed well. The plate was incubated at 37°C for 15 minutes at room temperature and shielded from light. One hundred µl of stop solution was added to each well to stop reaction. The optical density (O.D.) of the well contents was read at 450 nm using micro-tier plate reader (ELISA) within 15 minutes. ELISA-based determination of serum IgG Ab (I.U./mL)
against CMV, rubella and CSV-B were used. The concentration of Ab at the cut-off absorbance was: 15 I.U. /mL (absorbance 2 at λ 450nm), 1.2 I.U. /mL (absorbance 1.2 at λ 450nm) and 100 I.U./mL (absorbance 1.5 at λ 405nm) against CMV, rubella and CSV-B respectively. The serum Ab concentration was calculated according to the following equation.

\[
\text{Serum Ab (IU/mL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of cut – off} \times \text{Concentration of cut-off}}
\]

**PCR-SSP Technique:** The HLA-gene study started with the isolation of the DNA with the EXTRA-GENE kit [33]. Briefly, the procedure included adding 500 µl of blood to 900 µl of lysis-buffers in a nuclease – free, 1.5 ml micro-centrifuge tube. After centrifugation for 1 minute at 8000 rpm, the sediment is washed twice then the leukocyte sediment is re-suspended in 240 µl aqua dest and 120 µl of the extraction-buffer is added, mixture vortexes. To the clear solution is added 120 µl of the protein-precipitation-reagent. The preparation was washed and centrifuged twice for 5 minutes at 13000 rpm. The supernatant is then transferred into a new nuclease-free 1.5 ml centrifuge tube. The sediment is then washed twice in 96% and then in 70% ethanol the tube is placed upside down on a filter paper for about 5 minutes. The DNA pellet is dissolved in 100 µl of aqua dest, for complete DNA dissolution followed by incubation of the tube at 56°C for about 10 minutes and the DNA obtained is stored at -20°C. For determining the DNA concentration, five µl of the DNA solution was diluted with 495 µl distilled water and the optical density was determined at 260 nm in UV spectrophotometer (Schimadzu-Japan) using distilled water (D.W) as a reference. The contaminating proteins were measured at 280 nm and the quotient A260/A280 was calculated. Pure DNA will give an A260/A280 ratio of 1.8 or higher. The Polymerase Chain Reaction-sequence specific primers (PCR-SSP): procedure. Amplification was performed by the prealiquoted and dried reaction mixture which already contained allele-specific primer sets, internal control primers and nucleotides. The HLA –SSP plates were removed from -20 C and the 10x per –buffer was thawed at room temperature. In an appendorf reaction tube, a master mix was prepared for each typing test. A master mix contained Aqua dest, 10 xPCR- buffer, taq-polymerase (5u/ µl) and the DNA solution (50-100 ng/ µl). The contamination control should be performed; the master mix without the DNA solution was prepared first and pipettes 10 µl of this mix in the contamination control. Following that, 10 µl of the mix was pipette into each of the dried primer mixes in the plate. The PCR plate semi tubes were tightly closed with the reactive caps. The plate was turned down-wards to ensure dissolution of the dried reaction mixes at the bottom of the plate. The plate was transferred to the thermo cycler and PCR program was started.

**Gel Electrophoresis:** After the amplification has been finished, the samples were taken out of the thermo cycler and the reaction mixtures carefully loaded each on a slot of the gel. The electrophoresis separation of the horizontal agarose gel was followed by UV-detection of the DNA bands in a UV transilluminator [34].

**Statistical Analysis:** SPSS version 20 software was used for all data analysis. The results are presented as number, percent and mean±SE. The data had normal distribution and were analyzed using two tailed unpaired “t” test and 95% confidence intervals (95% C. I.) test taking P= 0.05 as the lowest limit of significance.

**RESULTS**

The demographical analysis of this study shows in (Table1). It is clear that the number of females was higher than males. The number of females was 122 (53%) while; the number of males was 108 (47%). The number of patients having family history of diabetes was higher 169 (73.5 %) in comparison with the number of patients who have no family history of disease 61 (26.5 %) as illustrated in table in (Table 2.1). Table number 1 also shows the mean value of serum glucose between T1DM patients and control group. The (Mean±SE) for T1DM patients was (237.79±6.13 mg/dl) compared with (118.42±0.83 mg/ dl) for control group.

The result of this study revealed that there is elevation in the amount of anti-viral Ab in the blood circulation of T1DM patients and control group (Table2). The statistical results revealed that the mean±SD concentration of anti-CMV IgG, rubella, CSV-B antibodies in the sera of patients was (1.22±0.36 IU/ml), (0.80±0.08 IU/ml), (0.81±0.14 IU/ml) respectively when compared with (0.79±0.32 IU/ml), (0.38±0.17 IU/ml), (0.26±0.21 IU/ml) respectively in sera of control group and (P-value < 0.05). This means that there is a significant increase in mean concentration of anti-CMV, rubella, CSV-B antibodies in
Table 1: Demographical picture of the studied groups

<table>
<thead>
<tr>
<th>No.</th>
<th>Demographical Parameters</th>
<th>Diabetic Patients (n=230)</th>
<th>Healthy Control (n=130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age(years) [Mean±SE]</td>
<td>15.57±0.69</td>
<td>11.95±0.61</td>
</tr>
<tr>
<td>2</td>
<td>Number of male and female</td>
<td>Male: 108</td>
<td>Male: 62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female:122</td>
<td>Female: 68</td>
</tr>
<tr>
<td>3</td>
<td>Positivity of Family History</td>
<td>169 (73.5 %)</td>
<td>61 (26.5 %)</td>
</tr>
<tr>
<td>4</td>
<td>Random Plasma Glucose (mg/ dL) [Mean±SE]</td>
<td>237.79±6.13</td>
<td>118.42±0.83</td>
</tr>
</tbody>
</table>

Table 2: The presence of anti viral and GAD IgG Ab among T1DM and control group.

<table>
<thead>
<tr>
<th>Type of virus Ab.</th>
<th>Most frequency age group 0 - 10 years</th>
<th>Mean±SD (I.U/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti CMV IgG</td>
<td>Patient</td>
<td>45.65 %</td>
<td>1.22±0.36</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>35.38 %</td>
<td>0.79±0.32</td>
</tr>
<tr>
<td>Anti RubellaIgG</td>
<td>Patient</td>
<td>26.95 %</td>
<td>0.80±0.08</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>49.23 %</td>
<td>0.38±0.17</td>
</tr>
<tr>
<td>Anti CSV-B IgG</td>
<td>Patient</td>
<td>6.15 %</td>
<td>0.81±0.14</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.15 %</td>
<td>0.26±0.21</td>
</tr>
<tr>
<td>Anti GAD Ab. IgG</td>
<td>Patient</td>
<td>76.1%</td>
<td>89.54±0.43</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19.12%</td>
<td>9.73±0.38</td>
</tr>
</tbody>
</table>

Table 3: Correlation between the presence of HLA-DR3, DR4 genes and anti GAD Ab

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HLA-DR3 Positive</th>
<th>N (%)</th>
<th>HLA-DR4 Positive</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti GAD Ab (IgG) among patients</td>
<td>Positive</td>
<td>135 (58.69 %)</td>
<td>125 (54.34 %)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>36  (15.65 %)</td>
<td>44  (19.13 %)</td>
<td></td>
</tr>
</tbody>
</table>

The result of statistical analysis showed that there is a relationship between the presence of HLA-DR3 and DR4 with anti-GAD antibody. Table 3 showed that there are 135 (58.69 %) of patients having positive result for anti-GAD Ab (IgG) with presence of HLA-DR3. The results showed that there are 125 (54.34 %) of patients having positive result for anti-GAD Ab (IgG) with presence of HLA-DR4.

**DISCUSSION**

The demographic picture of the studied groups showed the following: The T1DM is more common among young ages. In this study, majority of patients were young age (under 19 years). This findings were supported by other study which reported that majority of T1DM patients were under 20 years [35]. While, other study recorded that 11.5 years are a mean age for T1DM [36]. Third study recorded that the mean age is 8.2 years [37]. The data is in agrees also with similar results were obtained from many studies from different countries by different researchers which showed that three-quarters of cases of T1DM which diagnosed were in the age less than 18 years of age and it showed that the frequent average population in every of the age groups (0–4), (5–9) and (10–14) years [36]. The findings of current study supported by similar result of study which reported that the number of female patients was slightly elevates in populations of African or Asian origin [38]. The results supported by second study which performed in Najaf city of Iraq by other author who concluded that the T1DM is more common among females than males [39]. In opposite, While, other Brazilian study reported that the incidence of diabetes is higher in males than females [40]. This result agrees with result of other study which concluded that the diabetes mellitus is higher among individual with family history of disease especially those who have 1st degree relatives than others who have other degrees [41]. The results of the current study supported by result of similar study which reported that the disease is commonest among families who have parents suffer from diabetes [35]. The result of this study also supported by results of other authors who reported that 80% of infants who have T1DM in Finland have parents suffering from diabetes, 20% of patients have 2nd and 3rd relatives
degree of disease [42]. Furthermore, the UK diabetic medicine reported that family history relates to T1DM is stronger than T2DM [35]. The elevated level of glucose in the blood stream is the main difference between diabetic and non-diabetic individuals. This difference is due to the defect in the insulin secretion or its function [43].

The results of current study confirmed by result of other study that mentioned the potent effects of acute CMV infection which leads to the weakness and disturbances of the immune system including diabetes, osteoporosis, cognitive dysfunction and cardiovascular disease [44]. The findings of current study supported by result of one study which mentioned that infection with CMV leads to T1DM [45]. The result of current study supported by many others studies mentioned the relationship between CMV and T1DM. First, the study supported by result of study which reported that the infection with CMV is one of the causes that leads to T1DM [46]. The results of second study supported our result in that CMV leads to T1DM through an autoimmune reaction against pancreatic islet cells and cause severe insulin deficiency in the body [47]. Moreover, the result of current study signaled the size of the problem of CMV which affects the growing of T1DM. The result of a study which performed by one author also supported the results of current study and mentioned that CMV is one of the risk factors that leads to increase the disease in Iraq [48]. While, other studies reported that infection with CMV not related to T1DM even in the presence of HLA genes [49] and second study is also stated that CMV is not responsible for T1DM [50].

The result of this study shows a part of the incidence of Rubella in Erbil city and this supports the report of the ministry of health, Kurdistan region of Iraq as published 2011, in which it was reported that 85% of children under age 1 were vaccinated against rubella in Erbil city. This implies that non-vaccinated children recorded higher values before 2011 as the quality of health services in Kurdistan increased most especially after the war and economic sanctions of 1991 to 2003 which destroyed the infrastructure of the city. The results of this study agrees with results of other study which reported that the rubella virus infection spreading among patients with T1DM [22]. The findings of current study are consistent with results of other study which concluded the a relationship between T1DM and rubella virus infection [51]. The result of this study supported by the results of other study performed by Dezayee which reported that the infection with the rubella is one of the causes of T1DM [52]. The results also supported by the results of third study which reported that the rubella can induce T1DM through the antigen mimicry leads formation of antibodies against pancreatic β-cells [11]. The result of this study supported by the results of fourth study which recorded that the rubella infection is one of viral infections more common among T1DM [53]. On the other hand, many researchers believed that there is no evidence that rubella virus leads to immune disturbance that induce T1DM [54].

The result of this study about CSV-B agrees with results of other studies which revealed that there are a relationship between CSV- B and T1DM [9]. The result of this study supported by results of second study which reported that CSV- B is one of the causes of T1DM [55]. The result of current study supported also by the results of third study which refer that the incidence of CSV-B leads to T1DM is due to high similarity between glutamic acid decarboxylase (GAD) in the beta cells and antigenic part of the virus which infects these cells [51]. While, a team of researchers believed that there is no convince evidence for the relationship between Coxsackie B virus infection and T1DM [56]. Other study mentioned that there was no evidence fully prove that an enterovirus infection including CSV-B can induce T1DM among children or from mothers infected by this virus, but may play a role in a number of susceptible subjects [57].

The results of current study is agrees with the result of one study which mentioned the presence of high level of anti-GAD antibody in the sera of T1DM patients and he reported that the exact triggering actions causing a release of GAD antibody into the blood stream are still unclear; however, damage to β-cells for various reason causes release of GAD into extracellular and in hereditarily susceptible individuals, activates the immune system [42]. The findings of this study supported by the result of second study which mentioned a significant increase in the mean concentration of anti-GAD antibody in the sera of patients when compared with the mean concentration of anti-GAD antibody in the sera of control group. It is mentioned also that the significant increases in mean concentration of anti-GAD antibody in the sera of patients is indicator for damage of β-cells in the pancreas [58]. The results in current study also supported by third study which concluded a presence of high level of anti-GAD antibody in the sera of T1DM patients [59]. It is also supported by fourth study which performed by Indian author who reported that there is a presence of anti-GAD antibody in the sera of T1DM patients [60]. Furthermore, the presence of anti-GAD antibodies increase among T1DM in all ages including those who are in 30 years age and above which known as: Latent
autoimmune diabetes in adults (LADA) [35] and this result also supported by the result of other study mentioned the presence of anti-GAD antibody in the blood circulation of T1DM patients in adult ages [41]. While a study linked the presences of anti-GAD antibodies with the presence of HLA-DR3, DR4 and family history with diabetes [61].

The statistical analysis showed that there are a most of patients who have HLA-DR3, or/and DR4 also have high level of anti-GAD antibody. The results of current study supported by other study which reported that the elevation of anti-GAD antibody in the sera of T1DM patients is related with HLA-DR genes [62]. The finding supported also by result of other author who mentioned that the presence of one or more autoantibodies against islet cells are related to the presence of HLA gene. Moreover, result of this study agrees with findings of third studies which mentioned that the formation of autoantibodies against β-cells related to the presence of genetic risk factors especially HLA genes [63]. Result of fourth study performed by other author who reported that the formation of autoantibodies against islet cells observed among patients who have a 1st degree relatives of family history with diabetes [37]. Finally, most of the patients have the HLA genes and immune markers characteristic of T1DM. In addition, CSV-B, CMV, adenovirus and mumps have been the cause for inducing certain cases of T1DM. It is generally believed that environmental agents can induce T1DM among individuals that are genetically susceptible [64, 65].

**CONCLUSION**

The significant difference between T1DM and healthy individuals was observed for the results of viral infections in the presence of HLA-DR3, or/and DR4 genes and anti-GAD Ab. Further study is recommended to assess these viral parameters related with other factors such as the immune responses (cellular and genetic-molecular). It also recommends that the link between T1DM and viral infection needs further study.

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