HCMV influence of GAD anti level in Diabetic Patients Type I DM in AL-Najaf Governorate

Hawraameer Mubarak¹, Rana Talib Al-Nafakh¹, Saif Jabbar Yasin¹, Mohammed Abd-Zaid Akool²

1. Department of Medical Microbiology, Faculty of Medicine, University of Kufa, Najaf, Iraq
2. Department of surgery, Faculty of Medicine, University of Jabir Ibn Hayyan, Najaf, Iraq

*Corresponding author: hawraameer@gmail.com (Mubarak)

Abstract

This study aims to assess the association of human cytomegalovirus (HCMV) infection with anti-GAD antibody in type one diabetes mellitus patient in AL-Najaf governorate as no such study in this region. The aim has been carried out by the collection of intravenous blood from two hundred patients who were diagnosed as diabetic mellitus type I through detection of Anti Glutamate decarboxylase enzyme antibody in serum, and for measure anti cytomegalovirus IgM and IgG Ab, which has been done by using (ELISA) Enzyme-linked immunosorbent assay and for viral DNA detection which has been done by using (PCR) Polymerase chain reaction technique, patient age was ranged between 20-70 years. ELISA test revealed that 30 patients have IgM and IgG and 60 patients have IgG antibody only against HCMV. Viral nucleic acid detection by molecular technique revealed that in female in 21.9% and most in age group range between 20-29 while in male percent was 47.7%. There is an increase in antibodies to GAD with the increase of HCMV antigens in type 1 diabetic patients with age and are high in older ages.

Keyword: HCMV, GAD anti, ELISA, PCR, Diabetic Mellitus

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1. Introduction

The exact cause of Type 1 diabetes remains unknown, but Longley viruses have been suggested as a possible environmental cause. Although there is a well-recognized genetic origin for type 1 diabetes, its high rate of infection in developed countries must be linked to environmental changes (1). Genetics of population merely do not lead enough alteration between generations to interpreted the dramatic increment in type 1 diabetes prevalence as detected in, it is well known in the medical literature that type 1 diabetes beginning in populations has a seasonal pattern; such outcome which primarily led to a viral etiology creation (2).

The type 1 diabetes mellitus prevalence is increasing all over the world, particularly in developing countries, as well as Iraq due to varying people lifestyles and genetic composition specifically in last few years(3). Diabetes is excessively higher in the adult young population in countries of Asian where it is more common in older people in the West, it will lead to big economic load on health resources in the entire world specifically in developing countries. In Iraq Diabetic patient comprising in younger population i.e. less than 25 years is a worrying condition for social and economic influence on society (4). Type 1 diabetes involves the destruction of specific pancreatic cells, causing a complete need for external insulin. The onset of clinical diabetes begins with the presence of anti-cell antigen antibodies. The Human Hippocampal
Antigen (HLA) region is the single most important genetic factor for type 1 diabetes. However, predictability in the HLA area is expected to show only about 60 percent of the genetic stimulation of the disease. More than 50 recognized polymorphisms from non-HLA genetic provision to the concept that genetics cannot alone clarify type 1 diabetes. Numerous points of sign direct that there is triggers environmental determinants may be vital in promoting of autoimmunity against Beta cell in individuals who liable genetically. The relationship between environmental factors and the onset of symptoms is complicated by the reflection that the rate of symptom progression may be exaggerated by an environmental factor. Therefore, it can be a pathogenic environment as well as etiology. Acceptable independence mechanisms contain viral agents relevant to diet, microbial, psychosocial, and anthropometric factors (5).

Diabetes of type 1 is usually detected in childhood, but about 25% of individuals are identified as adults, sometime even late as the ninth decade of life (6).

The α-decarboxylation of L-glutamic acid catalyzing is by the enzyme glutamate decarboxylase (GAD) to produce gamma-aminobutyric acid (GABA) which is the inhibitory neurotransmitter in the pancreas. GABA controls of β cells function by signaling [4]. There are 2 distinctive isoforms of it which is GAD65 and GAD67, determined by genes in chromosomes 10p11 and 2q31, respectively, chief autoantigen in diabetes type 1 is the GAD65(7).

2. Materials & Methods:

2.1. Study design

The study was designed as an observational study / cross-sectional. Serum sample were collected from 200 hundred patients of 20-70 years of age who were diagnosed as diabetes mellitus type 1 attending the diabetes and endocrine gland center in AL-Seder teaching hospital in AL-Najaf governorate from July to September 2018.

2.2. Patients:

All patients were and diagnosed as symptomatic diabetes militias type one by physician and the patient that included in the study are diabetic type one and has anti-GAD antibody. The cases such as renal frailer, malignancy, organ transplant, liver dysfunction, congenital and inherited disease as thalassemia and sickle cell anemia were excluded.

2.3. Serological technique:

The patient was diagnosed as type 1 diabetes mellitus by measuring glutamic acid decarboxylase (GAD) antibodies. Anti-GAD antibody and specific anti-cytomegalovirus antibody (IgM, IgG) were done by ELISA (kit from BioCheck. Inc). (Bio-Rad) Isoform GAD65 from human recombinant glutamic acid decarboxylase was used. The values of GADA-ELISA cut-off test are 5.0 U/mL. The limitation of GADA-RIA and GADA-ELISA tests lower detection were 0.11 U/mL and 0.57 U/mL.

2.4. PCR technique

Whole blood sample with EDTA were obtained from study sample for detection of cytomegalovirus DNA. DNA extraction kit and PCR amplification kit are (DNA –Sorb-B) where supplied by Sacaca Biotechnology, Italy. DNA isolation and PCR amplification and thermocycling condition and procedure were performed according to the instruction of kit. The PCR product was amplified and detected by ethidium bromide staining visualization in agarose gel electrophoresis.

2.5. Statistical Analysis
For determining the statistical importance of data, Chi-square test was applied. The significant consideration was value of $P < 0.05$.

3. Result

3.1. Human cytomegalovirus infection detected by IgM and IgG antibody related with age groups

Patients totally were 200 as 104 men and 96 women. The mean age was 32±2 and BMI of the patients was 20 (± 4) kg/m$^2$. The result revealed that 76(38%) patients was positive for IgM and 142 (71%) for IgG. IgM antibody detected more commonly in age group that range between 60-69 and less commonly in age group between 20-29 years and IgG antibody found commonly at age range from 50-59 and in less commonly in age group range from 20-29 as in below table and the $p$ value of IgM positive patient among age group was non-significant (0.63) while $p$ value of IgG antibody with age groups was significant (0.0001).

Table (1) Human cytomegalovirus infection detected by IgM and IgG antibody related with age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>No. of cases</th>
<th>IgM +</th>
<th>IgG +</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20 – 29)</td>
<td>47</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>(30 – 39)</td>
<td>28</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>(40 – 49)</td>
<td>34</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>(50 – 59)</td>
<td>48</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>(60 – 69)</td>
<td>43</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>76 (0.63)</td>
<td>142</td>
</tr>
</tbody>
</table>

3.2. Human cytomegalovirus infection detected by PCR according to age groups and gender

The below table show that HCMV antigen that detected by PCR technique has been found mostly in age group range between 60-69 years and less commonly in age group range between 20-29 years and there was no significant difference between patient in related to age group as the $p$ value was 0.51 and HCMV antigen was detected in female in 21.9% and most in age group range between 20-29 while in male percent was 47.7% and mostly in age groups that range between 50-59 and 60-69 years and the is no significant difference between age group and gender as the $p$ value are 0.47 and 0.71 in female and male respectively.

Table (2): Human cytomegalovirus infection detected by PCR according to age groups and gender

<table>
<thead>
<tr>
<th>Age groups</th>
<th>No. of cases</th>
<th>PCR +</th>
<th>PCR+/Female</th>
<th>PCR+/Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20 – 29)</td>
<td>47</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>(30 – 39)</td>
<td>28</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>(40 – 49)</td>
<td>34</td>
<td>13</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>(50 – 59)</td>
<td>48</td>
<td>17</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>(60 – 69)</td>
<td>43</td>
<td>18</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>70</td>
<td>21</td>
<td>49</td>
</tr>
</tbody>
</table>
3.3. Human cytomegalovirus detection by ELISA test according to IgM and IgG antibodies and gender in different age group

The below table show comparison between IgM antibody detection in both gender and revealed that IgM antibody found in 34.5% in female and mostly in age group range 60-69 while in male the percent was 41.3% and more in both age group 50-56 and 60-69 years and there is no significant difference between age group as the p value is 0.5 and IgG antibody found in 72.9% in female and mostly in age group range 50-59 while in male the percent was 69.2% and more in both age group 50-69 years there are no significant difference between age group and IgM in female and male as p value are 0.83 and 0.873 respectively

Table (3): Human cytomegalovirus detection by ELISA test according to IgM and IgG antibodies and gender in different age group

<table>
<thead>
<tr>
<th>Age groups</th>
<th>HCMV IgM +/female</th>
<th>HCMV IgM +/male</th>
<th>IgG +/female</th>
<th>IgG +/male</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 29</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>30 – 39</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>40 – 49</td>
<td>7</td>
<td>9</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>50 – 59</td>
<td>7</td>
<td>10</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>60 – 69</td>
<td>8</td>
<td>10</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>33</strong></td>
<td><strong>43</strong></td>
<td><strong>70</strong></td>
<td><strong>72</strong></td>
</tr>
</tbody>
</table>

4. Discussion

The theory that viral infection may aggravate autoimmune illness in predisposed genetically persons is accepted generally (8). Together immune-arbitrated destructive of beta cell and direct way have been suggested as pathogenic theory. Cytomegalovirus was associated with diabetes Type 1(9)

There is a strong correlation between the presence of HCMV genome in islet cells, lymphocytes and antibodies to serum in newly diagnosed Type I diabetes, Additionally, it has been reasoned that HCMV can lead to cross reactivity of T cell with the autoantigen GAD65 of beta cell and has been noticed in islet cells from diabetic cases of Type 1(10).

The study reveals that there is high prevalence of HCMV in Iraqi patient and this agree with result of (11). The study revealed that seroprevalence increase gradually with age this agree with (12,13).

Autosomal disease is known to be common in young adults but can also occur in the middle or older age group. Viral presence or infection may be a longitudinal factor during the induction of a single antibody to the island, a simulation of single-island antibodies into bodies Counter-multiple islet, or progression of autoimmune cell-to-clinical onset to T1D (14).

Several studies reported that both the initial development of the progression to multiple and autoantibodies ensued at an early age. Consequently, individuals progress to clinical T1D at different paces during which viral infections may act as an accelerator.
Although HCMV has been predicted to increase the risk of diabetes, literature is rare, possibly due to the low rate of active viral infection and type 1 diabetes in the general population. On the other hand, both HCMV infection and new diabetes were frequently observed in recipients of kidney transplantation (15,16). Therefore, this group of patients is particularly important to study the potential relationship between HIV and diabetes. In addition, the results of a recent study suggest that weak defense of beta-cell anti-virus may increase the risk of diabetes after HCMV infection.

It is clear that the current study does not establish a causal relationship between HCMV infection and diabetes, but adds some support to the hypothesis that the HCMV virus may induct the development of diabetes in individuals who are ready. HCMV infection was detected prior to the diagnosis of diabetes (17).

Although many HCMV products have anti-apoptotic effects, HCMV has also been revealed to promote apoptosis in various cells. Hence, viral triggering of apoptosis may be a potential mechanism that leads to cell destruction. (18).

HCMV-specific cytotoxic T cells or Natural killer cells may kill infected cells infected with the virus. Furthermore, automated T cells may kill β cells, for example by identifying the self-antigen GAD 65, which is similar to sequencing of molecular mimicry through HMLV DNA binding protein PUL57 (18). Interestingly, a biopsy from a patient with recurrent diabetes after receiving a HLA-matched pancreas showed that T cells assigned to HCMV with cytotoxicity and destruction of autoimmune cells, monocytes, activated phagocytes, and granule cells that attract cells of the pancreas infected with HCMV may be toxic of cells by reactive intermediate nitrogen, oxygen secretion or other toxic compounds. Present study revealed that no significant difference between male and female patients and this disagree with (19,20) and this disagreement may be due to the effect of glucose level in immune status and increase susceptibility to cmv infection as all the patient was diabetes (21).

Present study shows no significant difference in age group with seroprevalence in male and female and this disagree with (22) and this difference can be due to seroprevalence differences between countries as this study in middle of Iraq where as Lechman study in Germany and this Can be explained by differences in the prevalence of major exposures related to transmission of HIV (HCMV): rate and duration of breastfeeding, congestion, childcare arrangements and sexual behavior. Limitations of our study include: 1-cross-sectional study design, which devoid of a longitudinal component and 2-implied assumption that time and course of HCMV pathogen exposure were equal among subjects.

5. Conclusion:

This study show there is increase detection of HCMV antigen with the ageing and this agree to a well-known that there is decline in naive CD8 cells with age progression and this decrease in cellular immunity can associated with reactivation of latent cytomegalovirus infection rather than primary infection thus type one diabetes mellitus can be develop even in older age rather than in young age and this explain that anti GAD antibody find in this patient even IgM antibody against cytomegalovirus are detected in same time

Research not involve human participants and animal

Informed consent was obtained from all individual participants included in the study.

Acknowledgements

To anyone who help us to complete the research
6. References


