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The possible Association between Epstein-Barr Virus and Type 1 Diabetes Mellitus

Ahmed H. Mohammed[1] *PhD*, **Alzahraa Albatool I. Sabr**[2] *MSc*

[1]Dept. of Pathological Analysis, College of Science, University of Thi-Qar, Thi-Qar, Iraq

Abstract

Background Type-1-diabetes (T1D) also known as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes results from the progressive destruction of pancreatic beta cells resulting in insulin deficiency. Genetic factors are thought to be a major component for the development of T1D. The studies on the risk of developing T1D suggesting that the environmental factors, particularly viruses may be implicated in the initiation of beta cell destruction leading to T1D.

Objective To investigate the possible relationship between Epstein-Barr virus and T1D.

Methods The sera were collected from 56 T1D patients and 30 controls of age range 3-22 years old and from both sexes. The sera divided into two parts, one part for serology for detection anti-EBV EBNA-1 IgM and IgG antibodies by enzyme linked immunosorbent assay (ELISA) technique and the other for viral genomic extraction and conventional polymerase chain reaction (PCR) to detect the viral target gene.

Results The results by ELISA technique indicated that only 7 (12.5%) of T1D patients were positive for anti-EBV IgM antibody and 24 (42.9%) of T1D patients showed positive results for anti-EBV IgG antibody. In contrast, the control group showed negative results for both anti-EBV IgM and IgG antibodies. The results of PCR technique revealed that 15 (26.79%) of T1D patients have EBV DNA compared with none of the controls have EBV DNA (P<0.001).

Conclusion EBV infection may contribute to the pathogenesis or progression of T1D.

Keywords EBV, Type 1 Diabetes Mellitus, ELISA, PCR

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List of abbreviations: APC = Antigen presenting cell, CMV = Cytomegalovirus, EBV = Epstein-Barr virus, MHC = Major histocompatibility complex, T1D = Type 1 diabetes mellitus, T2D = Type 2 diabetes mellitus, TCR = T cell receptor

Introduction

Type 1 Diabetes Mellitus (T1D) is a rising worldwide health problem and the most common form of diabetes in childhood characterized by the body's inability to produce insulin due to selective loss of insulin-producing β-cells in the pancreatic islets [1,2]. Although the etiological factors for T1D are still obscure, epidemiological and genomic studies have been associated T1D with both environmental factors and genetic factors, i.e. polymorphisms in human leukocyte antigen (HLA) haplotypes [3,4].

It seems that viruses play a significant role among many environmental factors in the pathogenesis of T1D [5]. Most of the available data indicated that the viral infections are implicated in the development of T1D. A potential relationship between viruses and T1D is suggested by the evidence that some viruses can stimulate the disease in experimental animals and can be isolated from the pancreas of patients with newly diagnosed T1D [6-8].

Various viruses have been reported to be related with human T1D: Enteroviruses especially, Coxsackie B virus [9], Cytomegalovirus [10], Epstein-Barr virus, Rubella virus, Mumps virus, Rotavirus [11], and human Parvovirus [12].

Epstein–Barr Virus (EBV), also known as human herpes virus 4, is a γ-lymphotropic herpes virus and the causative agent of infectious mononucleosis (IM). The EBV genome is composed of a linear, double-stranded DNA with a relatively large genome size of approximately 180 kilobase pairs (kbp) that is encoded for many of the genes [13]. It was first identified in cells isolated from African Burkitt's lymphoma, later, it has been detected that it is highly prevalent around the world [14,15].

EBV has been associated with development of auto-immune diseases, this virus possesses a number of immune evasion mechanisms and immune-modulating proteins that make it a good candidate for initiation and progression of autoimmune diseases [16]. Therefore, it has been suggested that it is related to the development of T1D [17]. The mechanism by which EBV can contribute to the pathogenesis of T1D include the molecular mimicry. EBV infection may lead to cross-reactive autoimmune response through molecular mimicry between viral antigens and host proteins. A five amino acids-long sequence (GPPAA) in the Aspartic-57 region of the HLA-DQ8 β chain, which suggested to be important in defining the risk of T1D, is repeated 6 times in the BRF4-encoded EBNA3C protein of the EBV. Therefore, individuals who carry this sequence (GPPAA) in their HLA-DQ molecule present cross- reactivity to this epitope in EBV and may affect the pathogenesis of T1D [18]. The researchers found when EBV infects human immune cells, a protein produced by the virus -EBNA2- recruits human proteins called transcription factors to bind to areas of both the EBV genome and the cell's own genome. EBNA2 and its related transcription factors activate some of the human genes related to the risk for several autoimmune diseases, including T1D [19].

The objectives of this study were to detect the anti-EBV antibodies (IgG and IgM) in the sera of patients with T1D by using enzyme linked immunosorbent assay (ELISA), and to confirm the presence of EBV genome in the sera of patients with T1D using conventional polymerase chain reaction (PCR).

Methods

Patients and controls

This case-control study included 56 patients with T1D were collected from The Special Center of The Endocrine Glands and Diabetes in Al-Nassyrieh City during the duration from September to December 2018. The subjects were included 24 males and 32 females' patients with diagnostic features of T1D and age range was 3-22 years old. The diagnosis was based on the clinical and laboratory examinations. The second group is the control group, which included 30 apparently non-diabetic healthy people of males and females within the same age range of patient group. This study was approved by the Committee of Ethical Standards in the College of Science, Thi-Qar University and informed consent was obtained from all patients and controls before taking samples.

Five ml of fasting venous blood was taken from patients and controls. The blood was collected in coagulate gel tubes and centrifuged at 3000 rpm for 15 minutes to separate the serum. Each serum sample was separated in several 1.5 ml tubes stored at -20 ˚C for the serological and molecular tests.

Serological and Molecular study of EBV

Serological study of EBV

Detection of the serum level of the anti-EBV EBNA-1 IgM and IgG antibodies is based on the same principle of indirect ELISA assay using the EBV EBNA-1 IgM and EBNA-1 IgG antibody test kits from Demeditec/Germany, these kits are designed for the qualitative and the quantitative determination of specific IgM or IgG antibodies against EBV EBNA-1 in the

serum. The Microtiter strips are coated with EBV EBNA-1 antigen. Standards and diluted serum samples are pipetted into the wells of the Microtiter plate. The intensity of the color is directly proportional to the concentration of the IgM antibodies or IgG antibodies and measured at a wavelength of 450 nm.

Molecular study of EBV

The viral genomic DNA was extracted from serum samples by using the viral nucleic acid extraction kit from Geneaid/Taiwan, according to manufacturer's protocol.

The conventional PCR technique has been used in the current study for amplification the EBNA-1 gene of EBV DNA and the primers, which were used in this study were obtained by previous study. The PCR reaction mix consisted of 10 µl of extracted DNA, 2 µl of forward and reverse primers and 6µl distilled water. All these components were placed in the PCR tubes that contents all other components which needed to PCR reaction such as (Top DNA polymerase (1U), dNTPs, Reaction buffer (1x) with 1.5 mM $MgCl_2$, and stabilizer and tracking dye). The final volume per reaction tube was 20 µL. The PCR thermocycling conditions were done as shown in table (1). Then the PCR products were analyzed by 2% agarose gel electrophoresis and at molecular position 270 bp [20].

Table 1. Primer's sequence and PCR conditions for EBVNA-1 gene

Primer's sequence (5'- 3') of EBNA-1 gene	PCR conditions (Temperature (c) / Time)				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
		40 cycles			
F:GTCATCATCATCCGGGTCTC R:TTCGGGTTGGAACCTCCTTG	95/5min	95/30 sec	58/30 sec	72/30 sec	72/8min

Statistical analysis

The statistical analysis of this case-control study 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. Chi-square test or fisher exact test used to estimate the association between variables.

In order to measure the potential risk of pathogen in disease group, relative risk is a ratio of the probability of an event occurring in the exposed group versus the probability of the event occurring in the non-exposed group.

Results

Serological results

Serological results of the current study revealed that only 7 (12.5%) out of 56 T1D patients were positive for anti-EBV IgM antibody and the controls showed negative results for that type of antibody. Statistically, there was significant differences between the patient group and the control group (P = 0.043) in the presence of anti-EBV IgM. On the other hand, the results detected that (42.9%) of T1D patients were positive for anti-EBV IgG antibody compared with (0.0%) of the control group, indicating a highly significant difference (P < 0.001) as shown in tables (2) and (3).

Results of PCR

EBV genome was positive in 15/56 of T1D patients (26.76%) in the present study while the result of EBV genome detection was negative in controls. The results showed high significant difference (P <0.001) between T1D patients and controls in the EBV infection and the relative risk indicates to the positive relation between EBV and T1D as in table (4).

The positive results appeared in the molecular position about 270pb in gel electrophoresis and this position is a specific product size for EBNA-1 gene that used in this study to detect EBV infection (Fig. 1).

Table 2. Serological detection of anti-Epstein Barr virus IgM antibody in T1D patients and controls

			Study groups		Total
			T1D patients	Control	
Anti-EBV IgM	Positive	Count	7	0	7
		%	12.5%	0.00%	8.13%
	Negative	Count	49	30	79
		%	87.5%	100%	91.86%
Total		Count	56	30	86
		%	100%	100%	100%
P value				0.043	
Relative Risk with (CI)				1.612 (1.357-1.916)	

Table 3. Serological detection of anti-Epstein Barr virus IgG antibody in T1D patients and controls

			Study groups		Total
			T1D patients	Control	
Anti-EBV IgG	Positive	Count	24	0	24
		%	42.9%	0.00%	27.9%
	Negative	Count	32	30	62
		%	57.1%	100%	72.1%
Total		Count	56	30	86
		%	100%	100%	100%
P value				< 0.001	
Relative Risk with (CI)				1.938 (1.523-2.466)	

Table 4. Detection of the presence of EBV DNA by conventional PCR in T1D patients and control groups

			Study groups		Total
			T1D patients	Control	
EBV DNA	Positive	Count	15	0	15
		%	26.79%	0.0%	17.44%
	Negative	Count	41	30	71
		%	73.21%	100%	19.76%
Total		Count	56	30	86
		%	100%	100%	100%
P value			<0.001		
Relative Risk with (CI)			1.73 (1.42 - 2.11)		

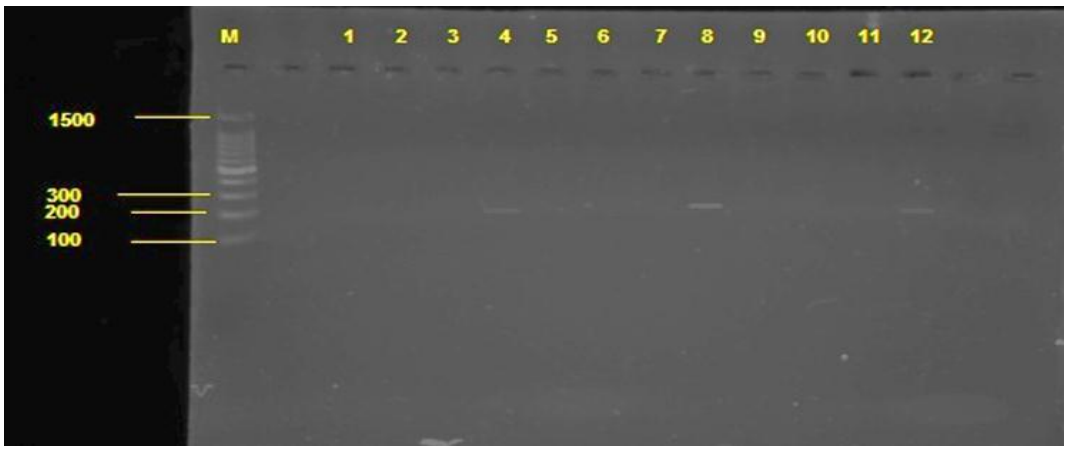

Figure 1. Detection of EBNA-1 gene for EBV by conventional PCR at molecular position 270bp, the positive result appeared in the samples (4, 8 and 12) in this image and other samples were negative

Discussion

T1D has a worldwide distribution with global variation in the incidence [21]. In Iraq, most of the e epidemiological studies do not distinguish between T1D and T2D in reporting the prevalence or incidence of the disease. However, there is a study reporting that the incidence of T1D in Basrah Province, southern part of Iraq was 7.4 per 100,000 during 2012-2016 [22]. EBV is one of the most common latent viruses inside the humans' B-lymphocytes and it has been documented as a causative agent of many cancers, where it has been shown that EBV infection strictly related to the malignant lymphomas in Iraq [23,24]. In addition, EBV may be considered a significant cause of renal impairment and kidney rejection in renal transplant patients [25].

The result of the current study showed presence of significant differences between T1D patients and control (P<0.001) in the detection of anti EBV IgG antibody. While regarding anti-EBV IgM antibody, the results of the current study also showed a significant difference between T1D patients and control (P= 0.043). Therefore, these findings might explain a positive correlation between the presence of anti-EBV IgM and IgG with T1D and

this probably suggested the role of the EBV infection in the pathogenesis of T1D.

Detection of antiviral antibodies, IgM or IgG refer to a viral infection then inflammation occurs. This inflammation has a role in the development and pathogenesis of T1D in different mechanisms. Many studies suggest that chronic infection result in increased processing and presentation of viral antigen which may have mimicry with host proteins. This mechanism involves a continuous acquisition of autoreactive events, leading to a chronic inflammatory state. This activation of multiple autoreactive T cells, as a result of tissue damage, is commonly referred to as epitope spreading and could be related to viral infections, by activating virus-specific T-cells or by direct virus-mediated self-tissue destruction. Virus-specific T cells become activated and migrate to the target tissues where they recognize the viral epitopes. The tissue destruction and release of self-antigens results in activation of autoreactive T cells and as a consequence an autoimmune response [26-28].

The inflammation that is induced by viral infection consists in the nonspecific activation of autoreactive T cells that have escaped thymic selection in a chronic inflammatory environment induced by viruses. Only a small fraction of activated T cells in viral infections are actually virus-specific. The others proliferate in the absence of the first signal (T cell receptor (TCR) + major histocompatibility complex (MHC) + Antigen). Cytokines, chemokines and other inflammatory mediators are secreted to promote a Th1 response, and increase the expression of MHC molecules, adhesion molecules and costimulatory molecules in the antigen presenting cells (APCs). The altered pattern of expression also affects the target cells, i.e. hyperexpression of MHC, adhesion molecules and antigen processing molecules among others. The release of self-antigens in the tissue and its presentation by macrophages or dendritic cells may prime virus-specific and autoreactive T cells. This effect has been observed by the transgenic expression of IFN-γ in insulin producing cells or oligodendrocytes resulting in the spontaneous development of diabetes or CNS demyelination respectively [29-32].

The results of PCR came to strengthen the possible association between EBV and T1D, especially our results showed significant differences ($P<0.001$) in the presence of EBV DNA between T1D patients and controls.

Detection of EBV by serology or by PCR gave evidence of the validity of the hypothesis that there is a relationship between EBV and T1D but the mechanism through which EBV might contribute to the pathogenesis of T1D remain uncertain. However, several possible scenarios can be included. First, EBV infection enhances immune cell cytotoxicity and tissue destruction through inducing the release of inflammatory cytokines. Second, EBV is resulting in local antiviral immune response that damage beta cells when the virus spread from B lymphocyte to pancreatic tissue [33,34]. Third, EBV infection may trigger a cross reactive autoimmune response through molecular mimicry of viral antigens and host proteins [18]. Other evidence showed that viral infections may play a role in accelerating the progression from beta cell autoimmunity to clinical T1D [35].

In conclusion, the findings of this study suggested EBV infection may have a role in the pathogenesis, development and progression of T1D.

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Author contribution

Dr. Mohammed: Idea, methods, and reviewing. Sabr: Materials, writing and publishing.

Conflict of interest

There is no conflict of interest.

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Correspondence Dr. Ahmed H. Mohammed
E-mail: ahmedhasan5@sci.utq.edu.iq
ahmedlab79@gmail.com
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