Effect of Parvovirus B19 in Diabetes Mellitus Type 1

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Abstract. Type 1 diabetes mellitus (T1D) is one of the complicated diseases and many studies indicated the contribution of viruses in the consequences of T1D. The present study aimed to detect of Parvovirus B19 *NS1* gene in the sera of T1D patients by using conventional PCR. Forty-five patients with T1D have been conducted in this study based on clinical examination and laboratory evaluation of random blood sugar, fasting blood sugar, and glycated hemoglobin. Viral genome extraction was made according to the kit manufacture's manual then conventional PCR and gel electrophoresis was performed to detect the target genome. The results showed the presence of *NS 1* gene in 7 (14%) of 45 patients included in this study, so conclude that the detection of viral genome in the blood samples of T1D patients is not sufficient to prove that human Parvovirus B19 has a role in the pathogenesis and consequences of T1D.

Keywords: Parvovirus B19, type 1 diabetes mellitus, PCR, fasting blood sugar.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disorder that usually strikes individuals at almost any age. It is caused by autoreactive T cells [1]. The 2019 International Diabetes Federation (IDF) states that T1D incidence, prevalence, and mortality estimates for children < 15 years for all 211 countries. The estimated cases were about 600,900 and incident cases around 98,200. So many countries were continuing to be affected and remain to have the highest rate and others have seen lowest rate as in East and South-East Asia. Generally speaking 3-4% per year there is an increase of incidents for such type of illness. On top of that T1D mortality has drastically decreased, , especially in people with diabetic nephropathy [2].
It is known that genetic and environmental factors could affects the major contribution for the development of the disease. Viruses may consider the main onset of T1D [3]. It is found in some cases there is a link between T1D and viruses including DNA viruses from families Herpesviridae and Parvoviridae and RNA viruses of families Togaviridae, Paramyxoviridae, Retroviridae, and Picornaviridae. There is a strong relationship between T1D and enteroviruses [4]; wherever, there is the uncertainty of the relation of diabetes to enteroviruses [3]. Multi models applied in some cases to highlight the triggering to beta-cell pancreatic destruction however, there is no indication of introducing other viruses [5]. Parvovirus B19 (B19V), is a member of enteroviruses that belongs to the family Parvoviridae, genus Erythroparvovirus may have a role in the development of T1D. Several cases of autoimmune disease that occur after

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acute infection with B19V have been reported. Kasuga *et al*. reported an association between T1D and infection with parvovirus B19in young adults who showed serum levels of B19 IgM and antibodies to the diabetic autoantigen IA-2 were significantly elevated [6,7]. Whereas rat virus parvovirus Kilham showed autoimmune diabetes in rats [8] for which T1D had not clarified [6]. This work targeted the presence of B19V in T1D patients by detection of the B19V *NS1* gene in the blood sample of T1D patients using conventional PCR.

2. Patients and Method

2.1. Study Design
A study was covered with 45 T1D patients attending the Special Center of the Endocrine Glands and Diabetes in Al-Nasiriyah city, south of Iraq. Known cases of T1D patients (males and females) of age range 3-16 years with their demographic or clinical information regarding age, sex, weight, the duration of the disease, insulin therapy (Number of doses during the day and type of insulin), family members with T1D and T2D and clinical history of the patients were mandatory for inclusion criteria.

2.2. Sample Collection
Five milliliters of venous whole blood were withdrawn by 5.0 ml syringe sterile disposable. About 1.0 ml was separated in the EDTA tube for the HbA1c test and the remaining (4.0 ml) of the blood samples was collected in a sterile gel tube, subsequently allowed to clot, and centrifuged at 3000 rpm for 10 min for serum separation to perform the blood sugar tests and extraction of the viral genome.

2.3. Blood Sugar Tests
The blood sugar test was done by using the RANDOX kit/ UK. Glucose oxidase and hydrogen peroxide were considered and formed the basis for tests requirements.

2.4. HbA1c Test
It is a test to count 2 to 3 months' average blood sugar level. It is based on the principle of fluorescence immunoassay technology by using the Finecare HbA1C Rapid Quantitative test kit/China. The test uses a sandwich immunodetection method.

2.5. Viral genome extraction and PCR
The viral genomic DNA was considered from serum samples using the viral nucleic acid extraction kit from Qiagen/USA, according to the manufacturer's protocol. The conventional PCR technique has been used in this study for amplification of the NS1 gene of B19

V and the primers designed by (Alpha DNA/ Canada) include: (Forward primer 5'-GCC GCC AAG TAG AGG AA-3' and reverse primer 5'-CCA CGA TGC AGC TAC AA-3'). The PCR reaction mix consisted of 5μl of extracted DNA, 1μl of forward and reverse primers, and 13μl distilled water. All these components were placed in the PCR tubes that contents all other components and the final volume in the reaction tube was 20μL. The extension for the PCR product was at 72°C for 7 min. The PCR was validated over 2% gel electrophoresis with Green Star™ Nucleic Acid Staining solution I upon preparation.

3. Results

The present study included 45 patients having T1D with age range 3 – 16 years old. Demographic data for those patients showed that the percentage of males among T1D patients was (35.5%) and the percentage of females was (64.5%). While the age and the bodyweight of all subjects representing by mean ± SD are as follows, (10.02 ± 3.14) and (31.85 ± 11.73), respectively.

The biochemical test results of fasting blood sugar, random blood sugar, and HbA1c test for patients group represented by mean ± SD were (217.02 ± 107.61), (328.87 ± 117.92) and (10.74 ± 3.43) respectively table (1).

The results of the PCR technique detected the presence of the B19V *NS1* gene in only 7 (14%) of T1D patients, whereas the others (86%) gave negative results (figure 1). This study evaluates the effect of the presence of B19V on clinical data which was measured for T1D patients, and the results showed there was no effect for the B19V on each of fasting blood sugar, random blood sugar, and glycated hemoglobin. On the other hand, the results also showed, and weight in the presence or absence of the B19V table (2).

Table 1. List of elements of diabetes mellitus type 1.

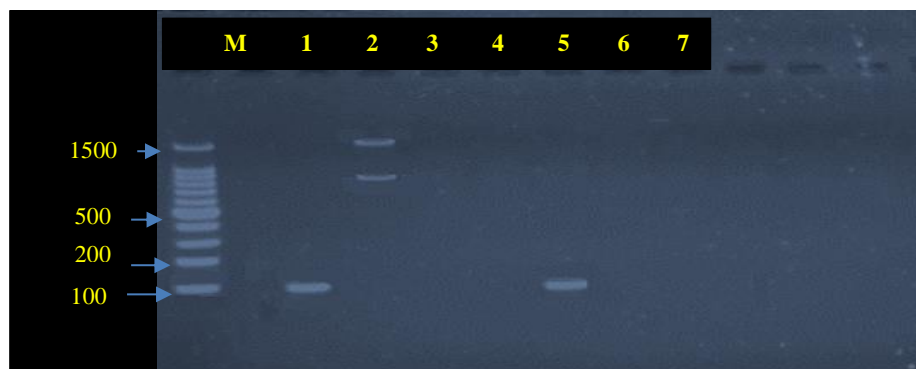| Demographic or Clinical data	Percentage or Mean (± SD)
Sex	Male: 35.5% Female: 64.5%
Age	10.02 ± 3.14
Weight	31.85 ± 11.73
FBS	217.02 ± 107.61
RBS	328.87 ± 117.92
HbA1c	10.74 ± 3.43

Fig 1. Detection of Parvovirus B9 (NS1) Gene by Conventional PCR. NS1Gene molecular position 120bp, lines (2, and 6) in this image and other samples are negative.

Table 2. Type 1 diabetes mellitus patients according to PCR results.

Item	PCR positive Mean ± SD	PCR Negative Mean ± SD	P-value
Age	10 ± 2.1	10 ± 3.3	P = 0.978
Weight	35 ± 11.5	31 ± 11.8	P = 0.452
FBS	194.5 ± 100	221 ± 109.7	P = 0.540
RBS	298 ± 41.7	334.5 ± 126.6	P = 0.168
HbA1c	10.5 ± 2.4	11 ± 2.3	P = 0.587

4. Discussion

For a long time, there are many studies have been suggested that viruses such as Coxsackie B, Rubella, Mumps, Cytomegalo viruses, Epstein Barr virus, Parvovirus B 19, Hepatitis C virus, Retrovirus, Rotavirus, and HIV appear to play a vital role among the numerous environmental factors, which together with the genetic susceptibility, may be implicated in the pathogenesis of T1D [9, 10, 11, 12, 13].

The present study included some of the demographic parameters (such as sex, age, and body weight) and biochemical parameters (such as F.B.S, R.B.S, and HbA1c) to give a clear picture of the epidemiological and clinical situation of T1D patients, who included in this study. A large proportion of patients with T1D was females (64.5%) compared with males (35.5%). This result corresponds with the results of studies conducted in Australia, Oman, Saudi Arabia, Libya, and Egypt [14] but, it differs from other studies in Kuwait, Tunisia, and Basra city which showed that T1D was higher in males than females [15, 16].

Patients in this study were selected from the age of children and adolescents with a mean of (10.02 ± 3.14) for early detection of the virus would be easier and more possible than the patients if they were older. In addition, T1D is frequently diagnosed during childhood and adolescence [17]. The epidemiological trends reported the number of children and adolescents with T1D at school are continued to increase [18, 19]. where the average global incidence rate increasing by 3% to 4% per annul [20]. Many countries have also reported that children are much younger at the time of diagnosis [21,22]. While another study showed an increase [23].

This study also included biochemical tests such as (F.B.S, R.B.S, and HbA1c). They are one of the basic tests approved by the World Health Organization (WHO) since they have an essential role in determining the management of diabetes mellitus. Also, a very young child does not always show clear symptoms [24,25]. The results of these tests in the present study showed significant elevation than the normal value because the patients with T1D have a disturbance in the pancreas, so a little or no insulin is released, resulting in glucose accumulation in the bloodstream and, as a result, an increase in glucose concentrations in diabetic patients [26].

The molecular diagnosis represented by the PCR assay of the current study showed the presence of the B19V *NS1* gene in 7 (14%) of T1D patients. Although this is a small percentage, it may show a potential relationship between B19V infection and T1D consequences [27-28]. However, we cannot assert an association between current or past B19V infection and the development of T1D in these patients and required further investigations.

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