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Investigation of Parvovirus B19 Infection Among Iranian Patients with Behcet's Disease

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ABSTRACT

Background and Aim: Behcet's disease is rare and can cause inflammation in blood vessels throughout the body. Although various studies have been conducted on the possible association between BD and various pathogens such as viruses, the major cause of this disease is still unknown. Our study aimed to evaluate the presence of B19 in Behcet patients and healthy carriers.

Materials and Methods: For the current case-control study, we examined 103 samples including 54 males and 49 females, and 40 healthy control samples. At first, all samples were checked by ELISA technique and then, the level of B19 DNA was confirmed by Realtime PCR. Finally, the results of patients were compared to healthy control samples.

Results: A wide range of clinical manifestations was observed in the BD patient group. We found statistical differences in the prevalence of B19 IgG between patients and healthy populations (84.46% vs. 55%, respectively). However, the prevalence of B19 IgM was similar between patients and healthy control groups (4.58% vs. 2.5%, respectively). We couldn't observe any detectable levels of B19 DNA in the patient and healthy carrier groups.

Conclusion: Our results failed to establish a relationship between B19 infection and BD development, but such a correlation has been reported. However, there may be an indirect association between genetically susceptible people after a viral infection.

Keywords: Behcet Disease, Parvovirus B19, Iran, ELISA, Real-time PCR

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1. Introduction

Behcet's disease is a rare syndrome with inflammation of several organs characterized by severe and systemic vacuities, but the major cause of this disease is still unknown **(1)**. The main clinical manifestations of this disease are recurrent oral aphthous, genital aphthous, skin lesions, and uveitis. Clinical manifestations, organ involvement, severity and duration of the disease, recurrence of aphthous, response to treatment, and prognosis can vary from person to person **(2-4)**.

BD is an endemic disease from the Middle East to the Far East. With such a wide geographic spread, the disease's claimed prevalence could vary greatly based on the population's ethnicity, geographical area, and methodology. Generally, BD affects males and females of all ages equally, while the disease is most prevalent in the third decade of life **(2, 4)**. Turkey and Iran with a prevalence of 420 cases **(5)** and 68 cases **(6)** per 100,000 people are the first and second most common countries for BD. In the United States, the prevalence of BD was 5.2 people per 100,000 population **(7)**.

Despite a long time since the introduction of BD, the exact cause of this disease has not yet been determined. Studies have demonstrated that genetics and environment can influence the prevalence of Behcet's disease. Among environmental factors, infectious agents such as viruses are considered effective factors in causing Behcet's disease. When a skin lesion is medically cleansed, it has been found that positive pathergy reduces **(8)**. For example, some studies demonstrated the effectiveness of penicillin on the mucosal lesions of BD patients. The virus was isolated from various fluids of BD patients including ocular fluid, according to Sezer *et al.* When the virus was inoculated into animal models and showed symptoms similar to BD disease, he judged it to be the BD causal agent. However, these findings were not thereafter corroborated by others **(9, 10)**. With the advent of modern tools, more researchers have been looking for indications of the possible relationship between various pathogens such as viruses, and BD development **(10-12)**.

Eglin R *et al.* claimed that the RNA and DNA strands of herpes simplex virus 1(HSV-1) are more in the peripheral blood cells or saliva samples of BD patients than in healthy carriers **(13, 14)**.

Hepatitis viruses, which have been linked to a variety of rheumatologic illnesses, can also be considered the cause of BD **(15)**.

Parvovirus B19 is a non-enveloped DNA virus that can cause a variety of clinical manifestations in humans. This virus can bind erythrocyte P antigen and targets a variety of cells such as erythroid precursor cells, placental cells, and megakaryocytes **(16-18)**. B19 can cause a variety of clinical manifestations according to age and immunological status. In immunocompetent children, B19 can induce the fifth disease and mild manifestations, but in immunocompromised patients, it can cause lethal cytopenias **(19, 20)**.

Furthermore, the B19 virus can persist in patients for a long time and induce autoimmune inflammatory disease **(21, 22)**. B19 is identified worldwide, and its seroprevalence is enhanced with aging. B19 virus can be transmitted from various routes including respiratory route, vertical transmission, and transmission via blood transfusion **(23-26)**. B19 infection has been associated with some autoimmune diseases including collagen vascular diseases that are similar to lupus erythematous **(2, 27)**. Furthermore, patients with the latest B19 infection have been shown to develop leukocytoclastic vacuities **(27, 28)**. Therefore, in this study, we investigated the presence of parvovirus B19 DNA and antibodies in the serum of BD patients and compared them with healthy individuals.

2. Materials and Methods

2.1 Study Population

The current study is a case-control study performed on the Behcet patients referred to the Iran University of Medical Sciences affiliated hospital from January 2016 to February 2019. A total of 103 subjects (49 female and 54 male) Behcet cases and 40 controls matched by age (± 2 years), were analyzed in the present study. All patients who were referred to the hospital were eligible. After obtaining written consent, about 6 ml of blood was obtained from each participant. Serological assays, DNA extraction, and Real-time PCR for B19V were measured according to the standard methods and were recorded.

2.2 Sampling and Preparation of Samples

We collected 6 mL of whole blood from each person who entered the study. Serum was isolated for B19V DNA and serological tests, and stored at –80°C and –20°C, respectively.

2.3 Serological Assays

For the detection of serological markers encompassing IgM and IgG of B19, the Vircell ELISA kit (Vircell, Granada, Spain) was used, according to the manufacturer's instructions. The sensitivity and specificity of the ELISA kit were 98.19% and 95.38%, respectively.

2.4 DNA Extraction

The B19V DNA was extracted by a High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the adapted manufacturer's instructions. After genome extraction, DNA elution was performed by using RNAase / DNAase-free buffer. Then, the purity and concentration of genomic extracts were analyzed using the NanoDrop™ spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All extracted DNAs with a 260/280 ratio of less than 1.8 and a 260/230 ratio of less than 2 were

excluded from further analyses. Elusions were preserved at -70°C until use.

2.5 Real-time PCR for B19V

The B19V genome was analyzed by TaqMan Real-time PCR, which amplified a 154-base pair fragment of the previously mentioned highly conserved region (NS1 gene). To make a total reaction volume of 16 µL, 8µL of 2X qPCR master mix, 0.5µM of each forward and reverse primer, 0.2 µM probe, 3 µL of extracted DNA, and DNase RNase-free distilled water were mixed. A Rotor-Gene Q instrument was used to perform real-time PCR (Qiagen, Germany). The following amplification cycles were programmed: a 10-minute initial denaturation at 95˚C, 35 cycles of 95˚C for 20 seconds, and 30 seconds at 62˚C. B19 Forward Primer (5'-CCACTATGAAAACTGGGCAATA-3'), B19 Reverse Primer (5'-GCTGCTTTCACTGAGTTCTTCA-3'), and B19 PROBE (5'-FAM-AATGCAGATGCCCTCCACCCAG-TAMRA-3') **(29)**. We used the Beta-actin gene as an internal control. The positive control used in the real-time PCR assay was obtained from a patient who was positive for human Parvovirus B19 by conventional PCR assay.

2.6 Statistical Analysis

All data was analyzed by using the Social Science Statistics Package (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). All comparisons were made using the Chi-square test and t-test. The Chi-square test was used for comparing categorical variables between the two groups. The odds ratio (4) and confidence interval (CI 95%) for BD were analyzed. A P-value of <0.05 was considered an indication of a significant difference.

3. Results

3.1 Demographic Information

For the present study, we analyzed 103 patients and 40 healthy carrier samples. In the Behcet group, 54 (53%) of patients were male, with a mean ±SD age of 39.71±7.86 years, and the mean disease duration was 13.22±9.12 years, while 22 (55%) of healthy controls were male, with a mean ±SD age of 37.25±9.73 years. The most frequent clinical symptoms of patients were Oral ulcer (100%), Genital ulcer (67.96%), joint involvement (56.31%), ocular involvement (50.48%), and Skin lesion (47.57%), while, neurological involvement (10.67%), vascular involvement (6.79%), and intestinal involvement (5.82%) were the less common clinical findings.

A large percentage of the patients (N=94, 91.26%) were using systemic Immunomodulatory medicine. 80.58% of them (N=83) had cytotoxic treatment and 98.05% (N=101) had corticosteroid drugs and Disease-modifying antirheumatic drugs (DMARD) Table 1. Lists the related clinical data of the study participants.

3.2 Virus Detection

The prevalence of B19 infection was evaluated by the ELISA technique and detection of antiB19 IgM and IgG antibodies. The B19 viral DNA load was identified by Real-time PCR technique.

Out of 103 Behcet patients, 5 (4.58%) were positive for B19V-IgM antibody, 87 (84.46%) for B19V-IgG antibody, and 0 (0%) for B19V viral DNA, respectively. Out of 40 healthy individuals, 1 (2.5%) and 22 (55%) were positive for B19V-IgM and IgG antibodies, respectively, with no detection of B19V viral DNA. Among Behcet patients, four (3.88%) cases were positive for B19V-IgM and IgG-antibodies Simultaneously (Table 2).

Table1. Comparison of Disease Characteristics between Behcet's Disease and control group

characteristics	Behcet patients (n=103)	Control (n=40)	p-value#	OR (95% CI)
Age (year)*	39.71±7.86	37.25±9.73	0.16##	2.46 (-0.98,5.90)$
Male**	54 (53%)	22 (55%)	0.78###	0.9 (0.43,1.88)
Disease duration (years)*	13.22±9.12	-	-	-
Oral ulcer**	103/103 (100)	-	-	-
Genital ulcer**	70(67.96)	-	-	-
Skin lesion**	49(47.57)	-	-	-
Ocular involvement**	52(50.48)	-	-	-
Neurological involvement**	11(10.67)	-	-	-
Vascular involvement**	7(6.79)	-	-	-

characteristics	Behcet patients (n=103)	Control (n=40)	p-value#	OR (95% CI)
Intestinal involvement**	6(5.82)	-	-	-
Joint involvement**	58(56.31)	-	-	-
Positive pathergy**	43(41.74)	-	-	-
Immunomodulatory drugs**	94(91.26)	-	-	-
Cytotoxic drugs**	83(80.58)	-	-	-
Corticosteroid drugs**	101(98.05)	-	-	-

* Mean ± SD
**Number (percent)
P-value less than 0.05 was statistically significant

Table2. Comparison of B19 infection between Behcet's Disease and control group

characteristics	Behcet patients (n=103)	Control (n=40)	p-value#	OR (95% CI)
B19-IgM positive**	5 (4.58%)	1 (2.5%)	0.53####	1.99 (0.22,17.77)
B19-IgG positive	87 (84.46%)	22 (55%)	0.0002###	4.45 (1.87,10.57)
B19-DNA positive	0(0%)	0(0%)	----	-----

**Number (percent)
P-value less than 0.05 was statistically significant

4. Discussion

Viruses are considered the major causes of human diseases such as cancers, infertility, and autoimmune disease **(30-33)**. Though the main cause of BD development is still unknown, environmental and genetic factors that induce immunological changes can be considered stimulation factors. The potential connection between BD development and some viruses has been studied. Various studies indicated that some viruses such as hepatitis C virus (HCV) **(32)**, hepatitis b virus (HBV) **(33)**, Occult hepatitis B **(19)**, Epstein-Barr virus (EBV), cytomegalovirus (CMV) **(32)**, herpes simplex virus-1 (HSV-1), and varicella-zoster virus (VZV) are among the most important viruses which related to BD development. The BD is a multi-organ syndrome that can cause inflammation in blood vessels throughout the body. The B19 virus was recommended as the causative agent of BD because both diseases caused vasculitis and arthritis **(32, 34)**.

In this study, we selected 103 patients who met the best criteria according to the ISG guidelines and described their signs and symptoms as thoroughly as possible. Plasma samples were analyzed for the presence of B19 viral DNA and IgM and IgG antibodies against the virus. Then the results were compared with control samples with the same conditions from the same geographical region. The analysis was carried out using commercial high-sensitivity Realtime PCR and ELISA kits.

Our finding indicated that the prevalence of B19-specific IgG was 84.46% and 55% in patients and control groups respectively, which was statistically significant. Furthermore, B19-specific IgM antibody against B19-V was 4.58% and 2.5% in patients and control individuals respectively. No virus genome was detected in the patient and healthy carriers groups. However, the lack of a viral genome in IgM-positive individuals may be due to short-term DNAemia.

One of the possible factors of pathogenesis in Behcet's patients could be parvovirus B19 infection. For this reason, Behcet's patients are likely more exposed to the B19 virus compared to the healthy group, and therefore the amount of IgG antibody production will also be higher compared to the healthy group **(35, 36)**.

One of the reasons for the difference between the results of ELISA and real-time PCR techniques can be attributed to the type of marker identified. So that in the ELISA technique, antibodies are investigated, but in the PCR assay, the genome of the virus is examined. Since the duration of the presence of the virus genome in the blood (viremia) is short, and on the other hand, the time of appearance of antibodies in the blood is at least three weeks after infection, it is reasonable that the identification of these two markers is not made at the same time. Hence, the chance of investigation of the virus genome in blood with PCR technique is low, and instead, the antibodies will be present in the blood for a longer time, therefore the detection rate of antibodies will be much higher than the virus genome **(37, 38)**.

Other researchers have also made such observations in this field. Kozireva *et al.* analyzed rheumatoid arthritis patients (83.3%) and healthy carriers indicating that they had detectable levels of both IgG and IgM anti-B19 antibodies in their sera **(39)**. Studies indicated B19 viremia starts 1 week after exposure to the virus and lasts up to 5 days. Anti-B19 antibody is produced about 10-12 days after exposure to the virus and can persist in the body **(40)**. Also, the viral genome can be identified in the plasma for a long time. In this regard, Lindblom *et al.* could find the B19 genome in the infected person 128 weeks after the anti-B19 IgG antibodies development **(41)**. DNA is not detectable in plasma specimens from all persons with anti-B19 IgM or IgG antibodies. The real-time probe-based PCR technique was performed to identify the B19 genome. This indicated relatively low background and linear results in the detection range in all investigations. Although various factors including sequence variations and regional genotype differences can be effective in test results and cause false-negative results. Like our results, some other studies also failed to investigate B19 DNA in their studied patients, although these BD patients were seropositive for the B19 virus **(42, 43)**.

In the study conducted by Irschick, and Philipp **(44)**, they couldn't find B19 viral DNA in BD patients **(44)**.

All available data indicated that the association between B19 infection and BD development is controversial. Furthermore, the findings demonstrated that other infectious agents such as HSV-1, HBV, Chlamydophila pneumonia, or hepatitis viruses can also be related to BD development, although their role as the main cause of BD is under consideration **(28-31, 45, 46)**. Similar to some previous studies, our study couldn't confirm the role of the B19 virus as an etiologic agent for BD development.

The current study has some limitations that may affect the results. First the small number of patients because of rigid clinical criteria in the selection process, second diseases that may overlap with Behcet's disease. Further studies with larger sample sizes, and sampling from various hospitals and regions are recommended.

5. Conclusion

The etiological role of infection in BD and the controversy over the symptoms of various diseases may reveal a more complex cause of the disease, rather than simply blaming the infectious microorganisms for the cause. In patients with a genetic predisposition, such as HLAB51, infection with a particular virus or bacterium can cause a cascade of unwanted or pathological immune responses.

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Ethical Considerations

The study was approved by the Medical Ethics Committee of the Iran University of Medical Sciences (Ethical code: IR.IUMS.FMD.REC.1398.134). Informed consent was obtained from all participants, which was carried out according to the Declaration of Helsinki.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

Authors' Contribution

S.Gh. and S.H.R.M. Conceived and designed the study. Z.S., S.Gh., A.T., S.J.K, and K.Gh. performed laboratory tests and extracted the data. H.S. and S.G. analyzed the data, and S.Gh., Z.S., S.H.R.M, and S.J.K. wrote the paper. All authors read and approved the final manuscript.

Data Availability Statement:

All data used and analyzed are available from the corresponding author.

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