Original Article

Parvovirus B19 in Children with Juvenile Idiopathic Arthritis

Reza Shiari[1], Fariba Shirvani[2*], Abdollah Karimi[2], Shahnaz Armin[2], Alireza Fahimzad[2], Roxana Mansour-Ghanaei[2], Sedigeh Rafiei Tabatabaei[2] and Fatemeh Fallah[2]

1. Department of Pediatric Rheumatology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Pediatric Infectious Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*** Corresponding author**

Faribs Shirvani, MD
Pediatric Infections Research Center, Research Institute for Children Health, Mofid Children Hospital, Tehran, Iran
Tel: +98 21 2285 9142
Email: shirvanifariba@rocketmail.com

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Abstract

Background: The causal role of Parvovirus B19 (B19V) in Juvenile Idiopathic Arthritis (JIA) is still a matter of debate. In this study, an attempt was made to investigate the frequency of B19V infection and the association between patients' characteristics and B19V infection in children with JIA.

Methods: Synovial fluid samples were obtained from 27 children (13 boys, 14 girls, aged 3-16 years) with JIA and were analyzed by polymerase chain reaction to detect B19V DNA. Age, sex, number of involved joints, time elapsed between beginning of symptoms and arthrocentesis, serum Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) were compared between JIA patients with and without B19V.

Results: Six patients (22.2%) were B19V+. There was no significant association between presence of B19V DNA in synovial fluid and number of joints involved, duration of disease, treatment with Disease-Modifying Anti rheumatic Drugs (DMARD) or glucocorticoid therapy and mean ESR and CRP levels. However, there was a slightly significant relationship between sex and age and detection of B19V DNA in the synovial fluid of JIA patients.

Conclusion: Our study demonstrated a 22% prevalence of B19V infection in JIA patients, and also that there was a significant relationship between sex and age and detection of B19V DNA in the synovial fluid of JIA patients.

Keywords: Juvenile idiopathic arthritis, Parvovirus B19, Polymerase chain reaction

Introduction

Parvovirus B19 (B19V) is a DNA virus of the family Parvoviridae which is classified as a member of the genus Erythrovirus due to its tropism for erythroid precursors (1). In immunocompetent individuals, B19V is primarily known as the causative agent of erythema infectiosum, a self-limiting exanthematous disease mainly affecting children aged 5-15 years; however, B19V infection can lead to serious complications such as chronic anemia and lethal cytopenias in immunocompromised hosts (2,3).

B19V infection can also induce arthropathy in about 8% of infected children and up to 80% of infected adults, manifesting as arthralgia and/or arthritis (4). In adults, B19V-related arthritis often resembles Rheumatoid Arthritis (RA), *i.e.* an acute, symmetric polyarthritis mainly involving proximal interphalangeal and metacarpophalangeal joints, knees, wrists and ankles, accompanied by prominent morning stiffness and even a positive Rheumatoid Factor (RF) test (2-4). On the other hand, the arthritis in children is often asymmetric and pauciarticular, and often involves knee joints and mimics oligoarticular Juvenile Idiopathic Arthritis (JIA) (4).

The association between B19V infection and chronic arthritis has been previously investigated; however, the nature of this association is still a matter of debate. Some studies have demonstrated the role of B19V in the etiopathogenesis of RA and JIA. A higher prevalence of B19V DNA and/or anti B19V-specific antibodies in JIA patients compared to controls has been previously reported (5,6). Moreover, there is evidence of a correlation between the clinical activity of RA and B19V infection (7,8). On the other hand, it has been shown that the percentage of anti B19V-IgG positive JIA children doesn't significantly differ from age-matched control groups (9).

B19V infection may also be associated with disease activity, level of cytokines and clinical outcomes in chronic arthritis. It has been reported that B19V infection is significantly associated with higher disease activity and aggressiveness (9), increased plasma levels of IL-6 (8) and higher rate of clinical complications (10) in RA patients. However, in both studies, the study populations were limited to adult patients with RA (8,10).

The aim of the present study was to investigate the prevalence of B19V infection in patients with a confirmed diagnosis of JIA. Also, the possible association between B19V infection and clinical manifestations was investigated in children diagnosed with JIA.

Materials and Methods

This cross-sectional study was performed on JIA patients aged 16 years or younger recruited from the rheumatology ward of Mofid Children's Hospital, Tehran, Iran and private offices of the researchers, who had to undergo arthrocentesis for diagnostic or therapeutic purposes during a two-year period (March 2016-March 2018). Only patients whose diagnosis was confirmed by the pediatric rheumatologists were enrolled. Diagnosis of JIA was made based on the criteria proposed by the International League of Associations for Rheumatology (ILAR) (10). The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Ethics committee code: 1391-87-10033-12964).

A 5 cc synovial fluid sample was obtained from each patient and stored at -70 °C until processing. Polymerase Chain Reaction (PCR) was performed to detect B19V in the synovial fluids of JIA patients as described by Petty *et al* (11). DNA was extracted after digestion by proteinase K, using DNA extraction kit (iNtRON, South Korea) according to the manufacturer's instructions. A 10 *µl* sample of DNA extract was added to 40 *µl* of master mix fluid, containing 10 *mM* Tris-HCl, pH 8.3, 50 *mM* KCl, 1.5 *mM* $MgCl_2$, 041 *mg/ml* gelatin, 200 *uM* each dNTP, 2-5 units Taq DNA polymerase, 50 *pmol* each primer and 5 *µl* template (iNtRON, South Korea). The PCR amplification steps (45 s at 94°*C*, 60 s at 63°*C* and 120 s at 72°*C*) were repeated 40 times. The following sequences of the primers were used: P1(*5-GTACGCCCATCCCCGGGACCAGTTCAGG-3*), P5(*5-CCCACATGGCAGCTACATCGCACCAAAT-3*), B19 I (*5-ATGGGATACTCAACCCCATGG-3*), B19 II (*5-CCTGTAGTGCTGTCAGTAACC-3*). Data regarding patients' age, sex, number of involved joints, time elapsed between beginning of symptoms and arthrocentesis, history of using corticosteroids and Disease-Modifying Anti rheumatic Drugs (DMARDs), serum Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) and results of

synovial fluid analysis were recorded in a checklist. The data were analyzed with SPSS for Windows 21.0 (SPSS Inc., Chicago, IL, USA).

Descriptive data were reported as mean±SD or medians [Interquartile ranges (IQRs)] for continuous and frequencies (percentages) for categorical variables. Based on detection of B19V DNA in their synovial fluid by PCR, the patients were categorized as either B19V+ or B19V-. The two groups were compared using Chi-square/Fisher's exact test for categorical variables and independent t-test/Mann–Whitney U test for continuous variables. p-value of <0.05 was considered statistically significant.

Results

A total of 27 patients (13 boys, 14 girls, aged 3-16 years) with a confirmed diagnosis of JIA were investigated. Of these, 10 patients were recruited from Mofid Children's Hospital and the rest were recruited from private offices. Disease duration previous to study (the time between beginning of symptoms and arthrocentesis) ranged from 3 months to 5 years. Patients recruited from the hospital were hospitalized for 4.1±1.66 days before arthrocentesis. The majority of patients (74.1%) had received DMARDs during the course of their disease and one third were treated with intra-articular or systemic corticosteroids. None of our cases showed typical signs and symptoms of B19V infection (*i.e.* classic bright red macular exanthem on the cheeks or maculopapular rash on extremities). Table 1 shows the clinical and paraclinical characteristics of the study population.

According to analysis of synovial fluid samples by PCR, 6 patients (22.2%) were B19V+. There was no significant association between presence of B19V DNA in synovial fluid and number of joints involved, duration of disease, treatment with DMARD or glucocorticoid therapy and mean ESR and CRP levels. However, there was a slightly significant relationship between sex and age and B19V infection (Table 2).

Discussion

Despite the well-established association between B19V and acute reactive arthritis, the relationship between B19V infection and chronic arthritis, including JIA and RA, in adults and children is still not determined. The present study was performed to investigate the frequency of B19V infection in JIA patients. Also, the patients' characteristics were compared across B19V+ and B19V- groups.

Generally, two methods are used to diagnose viral infections: serological tests for antibody detection

Table 1. Clinical and para-clinical characteristics of JIA patients

Sex, n(%) **Male** **Female**	 **13 (48.1%)** **14 (51.9%)**
Age, years, mean±SD	9.64±3.87
Number of joints involved, mean±SD	5.25±2.29
Corticosteroid, n (%)	9 (33.3%)
DMARDs, n (%)	20 (74.1%)
Duration of the disease, months, mean±SD	55.51±35.6
Synovial fluid WBC, mean±SD	10240±8520
Synovial fluid PMN, percent, mean±SD	70±21
Synovial fluid glucose, mean±SD	53±18.3
Synovial fluid protein, mean±SD	2684±1911
Mean ESR, *mm/h*	34.07±16.56
Mean CRP, *mg/l*	15.64±6.87

DMARDS, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

Table 2. Comparison of B19V+ and B19V- patients

	B19V+ (n=6)	B19V- (n=21)	p-value
Sex, n(%) **Male** **Female**	 **1 (16.7%)** **5 (83.3%)**	 **12 (57.2%)** **9 (42.8%)**	**0.08**
Age, years, mean±SD	6.914.24±	10.42±3.48	0.04
Number of joints involved, mean±SD	5.33±2.16	5.23±2.38	0.928
Corticosteroid, n (%)	5 (83.3%)	17 (81%)	0.697
DMARDs, n (%)	5 (83.3%)	15 (71.4%)	0.498
Duration of the disease, months, mean±SD	23.5±20.2	12.8±0.96	0.083
Mean CRP, *mg/l*	19.83±8.81	18.97±3.41	0.14
Mean ESR, *mm/h*	47±16.37	37.38±15.43	0.13

DMARDS, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

and direct viral detection methods (particularly PCR). In our study, the frequency of B19V DNA in the synovial fluid of JIA patients was 22% and PCR was used as the detection method. Kakurina *et al* reported a 53.9-72.2% prevalence of B19V specific IgG in serum samples from different subgroups of JIA (7). According to Gonzalez *et al*, B19V specific IgM was detected in 20% and B19 DNA genome by PCR in 48% of the cases (5). In a study by Weissbrich B *et al*, B19V DNA was found in 34% of samples taken from serum, synovial fluid and peripheral blood leukocytes of RA patients (9). The detection of B19V DNA in JIA and RA patients indicates that the patients have not been able to eliminate the virus. This may result from immunosuppression caused by corticosteroids and DMARDS used in these patients. Different frequencies of viral DNA can be reported by differences between study populations and detection techniques.

There is no consensus on the association between B19V infection and JIA. According to some studies, B19V DNA is also found in control samples with varying frequency, so it is still unclear whether the presence of B19V DNA in synovial fluid is of pathogenic relevance. According to Weissbrich *et al*, the frequency of B19V IgG in JIA children was not significantly elevated compared with age-matched control groups (9). On the other hand, Gonzalez *et al* and Nikkari *et al* reported a significantly higher prevalence of viral DNA in JIA patients compared to controls (5,12). It is not still determined whether B19V is a causative agent associated with pathogenesis of JIA or only triggers the manifestations. The causative role of B19V in pathogenesis of RA or JIA has been described more accurately by some studies. According to a study by Nocton *et al*, 6 of 22 children with joint complaints related to a recent B19V infection developed chronic arthritis fulfilling the diagnostic criteria for JIA during the following 2 to 13 months (13). Since the prevalence of B19V DNA between JIA and a healthy control group was not compared in this study, further comparative studies (preferably longitudinal) with larger sample sizes are recommended to determine the possible causative role of B19V in pathogenesis of JIA.

Our results showed that there was a significant relationship between sex and age and detection of B19V DNA in the synovial fluid of JIA patients; however, no significant difference was observed between other patients' characteristics and detection of B19V DNA in the synovial fluid (Table 2). To our knowledge, previous studies have not investigated the relationship between JIA patients' characteristics and B19V infection. Weissbrich B *et al* reported no significant differences between RA patient groups with respect to age, disease duration, number of affected joints, and presence of erosive arthritis as well as ESR and CRP values (9). One of the main reasons for this difference is that they categorized their RA patients based on presence of specific anti-B19V antibodies as well as PCR findings. Moreover, our sample size was relatively smaller than Kozireva *et al*'s (10).

Due to financial limitations, only patients who had to undergo arthrocentesis for diagnostic or therapeutic purposes were included in our study, leading to a small sample size which posed a limitation to our study. For similar reasons, controls could not be included. Longitudinal studies with larger sample size and a control group are recommended.

Conclusion

Our study demonstrated a 22% prevalence of B19V infection in JIA patients, and also that there was a significant relationship between sex and age and detection of B19V DNA in the synovial fluid of JIA patients.

Conflict of Interest

The authors declare that there is no conflict of interest.

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