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Original Article

Erythema nodosum manifestation of *Parvovirus B19*-associated reactive arthritis

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Abstract

Introduction: *Parvovirus B19* virus-mediated viral inflammation and immune-complex deposition generate mainly short-term manifestations in the affected individuals. The objective of this study was to determine *Parvovirus B19* infection in rheumatoid arthritis (RA) patients.
Methodology: The study employed 50 patients diagnosed with RA and 30 healthy individuals. Blood samples were collected from both groups. The blood samples were screened for *Parvovirus B19* infection using polymerase chain reaction to detect *B19* DNA and enzyme-linked immunosorbent assay to detect anti-*B19* IgM and IgG.
Results: 17 (34%) of 50 patients tested positive for parvovirus *B19* DNA. In contrast, the mortality rate in the control group was significantly lower (6.7%; $p = 0.005$). Anti-*B19* IgG antibody levels differed significantly with patients and control ($p = 0.007$), whereas anti-*B19* IgM Ab levels did not ($p = 0.6$). There was a significant correlation between viremia *B19* and all measured parameters. *Parvovirus*-affected patients had significantly higher CRP and ESR, elevated DAS28 scores, and more joint pain compared to *parvovirus* (-) patients.
Conclusions: Anti-CCP and RF values were significantly high in *parvovirus* (+) patients. Joint erosion was also prevalent in patients who tested positive for parvovirus. The findings of this study suggest that infection with *parvovirus* in patients with RA, and a possible role of this viral infection in the pathogenesis of RA may contribute to the pathogenesis of RA.

Key words: Parvovirus; Erythema nodosum; rheumatoid arthritis.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by an aberrant immune response targeting the tissues of the body. It is a pathological condition characterized by aberrant immune response, leading to persistent inflammation and degenerative changes in the joints. This condition significantly impacts everyday functioning and diminishes overall well-being. The serum of arthritis patients frequently contains autoantibodies [1], such as antibodies against citrullinated peptides (ACPA) and rheumatoid factor (RF). Although its exact cause is unknown, researchers proposed a complicated pathophysiology of the disorder is caused by an interaction of genes and the environment. Approximately 50% of the risk factors associated with RA can be related to environmental factors, such as smoking and infection. Meanwhile, the remaining 50% can be linked to genetic factors, specifically the disease-susceptible haplotypes of human leukocyte antigen (HLA) class II alleles DR1 and DR4 [2]. A variety of bacteria, such as *E.coli, P. mirabilis, M. tuberculosis, Mycoplasma, human parvovirus B19 (B19), Epstein-Barr virus (EBV), hepatitis B virus, retroviruses, alphaviruses, and chlamydia* have been identified as potential risk factors associated with the development of RA [3,4].

The *parvovirus B19* is a widely distributed virus with a tiny genome consisting of a single strand of DNA. It encodes two capsid proteins, VP1 and VP2, as well as a non-structural protein known as NS1. This virus typically multiplies in erythroblasts found in the bone marrow [5]. The primary modes of transmission include respiratory droplets, blood and pooled blood products, organ transplantation, and vertical transfer from a pregnant mother to her fetus. A high level of viral particles in the bloodstream characterizes the acute phase of infection, which typically lasts for five days. Virus clearance was observed after the emergence of IgM, succeeded by IgG. Nevertheless, the *parvovirus* can endure throughout an individual's lifetime with many bodily tissues, including bone marrow, skin, synovial fluid, and the liver [5].

The *parvovirus* is associated with the development of erythema infectiosum, often known as the fifth disease in children. Additionally, it was found to cause persistent pure red cell aplasia in individuals with impaired immune systems, as well as plastic crises in patients with hemolytic disorders [5]. Furthermore, *B19* has been implicated in the pathogenesis of other autoimmune illnesses, such as RA, systemic lupus erythematosus, polymyositis, and primary biliary cirrhosis [6]. It was observed that the diagnostic criteria for RA are comparable to those for *B19*-induced arthritis. Joint damage caused by *B19* was hypothesized, and *B19* DNA was found in synovial fluid and tissue from damaged joints, suggesting that the onset of RF may coincide with joint injury. Studies on parvovirus and its association with numerous diseases have been undertaken globally, although most research on this virus in patients with rheumatic disorders is limited to Europe and Asia. Research in Africa is limited [7].

This investigation aimed to examine the relationships between *B19* viremia, disease activity, and severity by comparing the prevalence of *B19* infection in RA patients to that in healthy controls.

Materials and Methods

Ethical Statement

Ethical approval for conducting this study was requested from the College of Medicine Baghdad, (June 2023).

Consent procedure

The procedure was already carried out according to the method approved by College of Medicine, University of Baghdad.

Study design and sampling

A prospective cross-sectional study was conducted at the rheumatology outpatient clinic at Baghdad Teaching Hospital in Baghdad. Iraq. The data was collected from the patients regularly attending the outpatient clinics from 1st October 2021 to 31st May 2022.

Inclusion and exclusion criteria

All patients aged ≥ 40 years, of both genders, who signed the consent form and were willing to participate are included. Young individuals, or individuals with a history of disability, psychological and mental diseases, malignant tumors, or incomplete data, who refused to sign the consent, and were unwilling to participate were excluded from the study

Table 1. Dissemination of information to all RA patients

Characteristics	RA patients
Age (years) Range in years	29.0 – 59.0
Range of disease duration in years	1.0 – 19.0
Range of morning discomfort in minutes	20.0 – 150.0
Number of tender joints, Range	5.0 – 30.0
ESR (mm/st hour) Range	20.0 – 110.0
CRP (mg/dL) Range	1.0 – 36.0

Sample collection

A total of 50 individuals were recently diagnosed with RA and their disease duration was less than one year. The age range of participants was 17-58 years. The individuals were categorized into two groups: patients diagnosed with RF+ and RF-, in addition to a control group consisting of 30 healthy individuals with no RA disease as approved by specialists.

Blood samples of 5 milliliters were obtained from both patients and healthy individuals, specifically to separate the serum.

Laboratory analysis

Each patient who has self-reported "Doctor-Diagnosed Arthritis" undergone further laboratory tests including the rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), and C-reactive protein (CRP). The normal RF < 5 by nephelometry method, and normal CRP levels 0–5 were detected using turbidimetric inhibition immune assay, and normal ACPA < 20 by ELISA method was determined using chemiluminescent immunoassay for detection of anti-parvovirus IgG & IgM Ab. These assessments encompassed the evaluation of various factors, such as the count of swollen joints, the count of sensitive joints, and the presence of extra-articular symptoms. Examples of such manifestations include subcutaneous nodules, involvement of internal organs, and signs of vacuities. Radiographic imaging was performed on the hands, wrists, and feet of all the patients, utilizing posteroanterior and oblique perspectives [8].

Disease activity

Disease activity was estimated using the disease activity score (DAS28). Joint pain and swelling were evaluated, and the affected patients' perception of their illness severity was determined using a visual analog scale (VAS) and either the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) [9], as depicted in Table 1.

The magnitude of the sickness

The study assessed anti-CCP seropositivity, rheumatoid factor (RF) seropositivity, and joint

degeneration by X-ray imaging. The given coordinates are presented [10].

Disease activity stages, DAS28 values

Recovery DAS28 ≤ 2.6, minimal illness activity was 2.6 < DAS28 ≤ 3.2, moderate disease activity was 3.2 < DAS28 ≤ 5.1, and severe disease activity was 5.1 < DAS28.

Quantification and detection of viral particles

The serum samples obtained from both patients and controls were appropriately preserved at a temperature of -20 °C. These samples were subsequently utilized to quantify anti-*B19* IgM and IgG through the employment of ELISA techniques, as well as for the detection of *B19* viral DNA using nested polymerase chain reaction (PCR) methodology. The ELISA assay is a widely used diagnostic tool in the field of immunology. The quantitative analysis of serum levels of anti-parvovirus IgM and IgG Ab was performed using parvovirus IgM and IgG Ab assays (Germany). Positive levels were defined as values greater than 0.9 [11].

Procedure for the nested polymerase chain reaction

The utilization of nested *PCR* is a molecular methodology commonly employed in the identification and analysis of *parvovirus B19*-DNA. The extraction of viral DNA from serum samples was conducted following the guidelines provided by the manufacturer. The experimental procedure entailed the utilization of a DNA-PCR template preparation kit (Thermo Fisher Scientific, USA), in conjunction with an RNA purification kit. The initial amplification step involved the addition of 0.4 µL of extracted DNA to the PCR mix, resulting in a final volume of 25 µL. The PCR mixture utilized in this research comprised Taq Green PCR Master Mix (2′) procured from Thermo Scientific (Fisher Biotec, Australia). The solution comprised a concentration of 200 µmol/L of deoxy nucleotide triphosphate (Stratagen) along with 300 ng of the primer used in the initial round.

The amplification procedure consisted of an initial denaturation step conducted at 95 °C for 5 minutes. Subsequently, a sequence of thirty-five repeated cycles was performed, wherein each process encompassed denaturation at 95 °C for 1 minute, followed by annealing at 55 °C for 1.5 minutes, and ultimately extension at 72 °C for 1 minute. Subsequently, a conclusive extension step was executed at 72 °C for 7 minutes. An aliquot of 3 µL. of the resulting PCR product from the first round was added to a second PCR mixture comprising a reaction volume of 50 µL. The composition of the reaction mixture used in the second round was the same as that of the first round. Then, 300 ng of the second primer was added to the experimental setup, employing identical amplification conditions as those used in the initial round. The nucleotide sequences of the primers utilized in the first round include 5′-CTTTAGGTATAGCCAACTGG-3′ (Biosearch Technologies, USA) and 5′-ACACTGAGTTTACTAGTGGC-3′. In the second round of the PCR, the nucleotide sequences of the primers used include 5′-CAAAAGCATGTGGAGTGAGG-3′ and 5′-CCTTATAATGGTGCTCTGGG-3′. At the end of the second round of PCR, 10 µL of the product was separated by electrophoresis using a 2% agarose gel. Following ethidium bromide staining, the resultant bands were subsequently detected [12].

Statistical analysis

The data obtained were analyzed with the Statistical Package for the Social Sciences (SPSS; version 20), a statistical programming software. The difference in percentages (qualitative data) was tested using the Pearson Chi-square test and the *p* value was considered statistically significant when it was equal to or less than 0.05.

Table 2. Distribution of data to parvovirus B19 DNA (+) and (-) patients.

Characteristics	RA patients with B19 (+)	RA patients with B19 (-)	*p*
Variation in Age (years)	29.0 – 59.0	41.15±10.	0.37 NS
Average Standard Deviation	42.10 ± 10.35	29.0 – 58.0	
Range of disease duration in years (Mean ± SD)	8.94 ± 5.12	7.48 ± 5.59	0.37 NS
Variation in minutes of morning stiffness (Mean ± SD)	1.0 – 18.0	1.0 – 19.0	
Count of aching joints; Range, (Mean ± SD)	21.11 ± 7.51, 5.0 – 30.0	13.60 ± 6.45, 5.0 – 30.0	0.002 S
ESR (mm/ 1st hour), Range, (Mean ± SD)	64.64 ± 34.0, 25.0 – 110.0	44.87 ± 22.13, 20.0 – 90.0	0.03 S
CRP (mg/dL), Range, (Mean ± SD)	19.05 ± 10.65, 12.0 – 36.0	13.09 ± 8.14, 6.0 - 30.0	0.03 S
DAS28 > 5.2, Mean ± SD, Range	3.32 ± 0.91, 1.7 – 4.5	2.76 ± 0.67, 1.9 – 4.20	0.01 S
Patients with extra-articular manifestations (nodules of rheumatoid arthritis, myositis, vasculitis, lung, heart, or eye disorders)	9 (52.9)	11 (33.3)	0.18 NS

Figure 1. Relationship between RA with several markers.

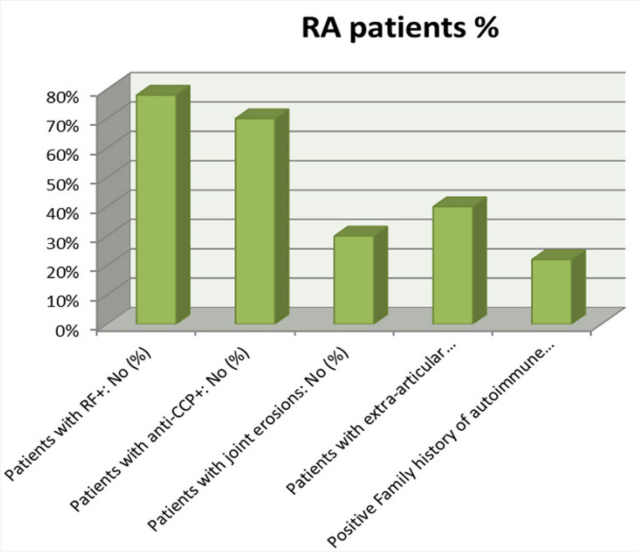
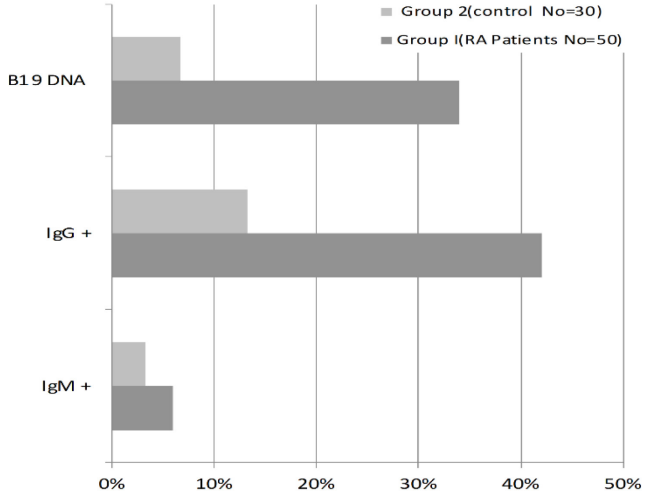


Figure 2. Virological indicators of parvovirus B19 in the groups that were examined.



Results

Descriptive and general characteristics of related factors

Table 1 provides a complete overview of the demographic, clinical, laboratory, and radiographic attributes of patients diagnosed with RA. Among the cohort of 50 patients who were diagnosed with rheumatoid arthritis RA, a subset of 3 patients (6%) were found to exhibit positive results for anti-*B19* IgM. Nevertheless, there was no statistical significance (p = 0.59) in the observed disparity between the patients and the control group. On the contrary, a significant disparity was observed in the prevalence of anti-*B19* IgG between the RA patients and the control group. Specifically, 21 individuals (42%) within the RA cohort revealed the presence of this antibody, whereas a significantly lower proportion of the control group, consisting of four individuals (13.13%), displayed the same antibody. The observed difference between the two groups was found to be statistically significant.

Molecular study of parvovirus DNA with rheumatoid arthritis

In the nested PCR, the first set of primers utilized in the first round of the PCR produced 1,112 bp DNA fragments, while the primers employed in the second round resulted in a product of 104 bp in length. Based on the results obtained from the nested PCR analysis, a significant disparity in the prevalence of *parvovirus* DNA was identified between patients diagnosed with RA and the control group. Specifically, the prevalence of *parvovirus* DNA among RA patients accounted for 34% of the cases, but in the control group, the prevalence was only 6.7%. The observed deviation demonstrated statistical significance, as indicated by a p-value of 0.005. The present investigation involved the examination of a cohort of 17 patients diagnosed with RA who exhibited positive test results for *B19*. Out of the patient population under consideration, thirteen individuals were identified as having IgG-positive antibodies, with two of these individuals additionally presenting IgM antibodies. However, it is worth noting that the four remaining patients did not exhibit any detectable levels of anti-*B19* antibodies.

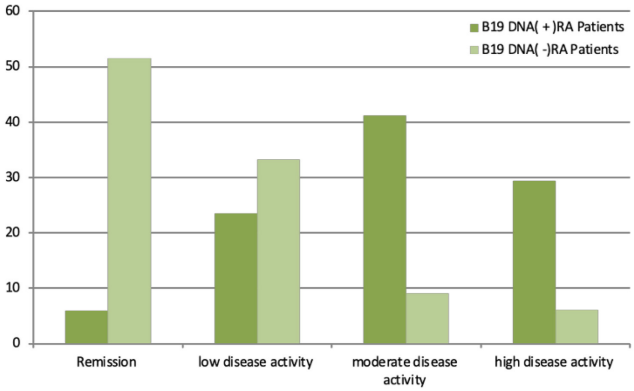
Disease activity stages, DAS28 values

Observed in the results presented in Table 2 and Figures 1 and 2. A statistically significant correlation was found between the 28-joint Disease Activity Score (DAS28) and *B19*-DNA detection in RA patients as determined by nested PCR. According to the study, 41.2 and 29.4% of RA patients who tested positive for *B19*-DNA were found to be in the phases of moderate and high disease activity, respectively. This study revealed a significant disparity between stages of remission and low disease activity in patients with *B19*-DNA-negative RA. Specifically, 51.5 and 33.3% of patients were classified into the remission and low disease activity stages, respectively. The observed distinction was determined to have statistical significance (p), as illustrated in Figures 3 and 4.

Discussion

There is evidence to suggest a correlation between some viral infections and the development of autoimmune illnesses in genetically predisposed persons. The correlation between *B19* infection and RA is a subject of great interest [13]. The current study

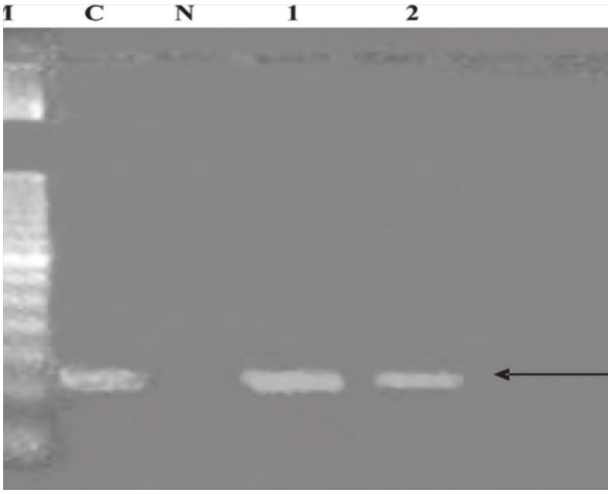
Figure 3. The observed variations in Disease Activity Score (DAS28) among patients, comparing those with and without the presence of Parvovirus B19 DNA



aimed to compare the effects of *B19* infection and RA on many aspects of human health. The infection caused by parvovirus in arthritis may potentially serve as an infectious etiology for RA. The presence of anti-*B19* antibodies (specifically IgM and IgG) was observed in 6 and 42% of individuals diagnosed with RA, respectively, as determined in the present study. A statistically significant disparity ($p \leq 0.07$) was observed in the occurrence of anti-B19 IgG among individuals with RA, compared to the occurrence of anti-*B19* IgM in the same patient population.

In a study conducted in Taiwan, Chen *et al*. (2006), [14] observed that occurrence of plasma anti-*B19* antibodies (particularly IgG and/or IgM) was identified in 93.6% of patients who were diagnosed with the illness. The results of this study demonstrated a statistically significant difference ($p < 0.001$) compared to the control group. Regaya *et al*. (2007), [15] reported similar results. The study observed that among individuals in the Tunisian population, the prevalence of anti-*B19* IgG antibodies in the blood was observed to be 80.7% in patients diagnosed with RA, but only 43% of the control group exhibited the presence of these antibodies. A rationale for the heightened occurrence of anti-*B19* antibodies in individuals diagnosed with RA was reported by Da Silva *et al*. (2014) [16]. They proposed that this phenomenon can be linked to the augmented vulnerability of RA patients to viral infections due to immunosuppression or other immunological traits evident within the patient cohort. It is important to consider the possibility that *B19* may serve as a potential catalyst for the onset of RA. The study utilized nested PCR to detect the presence of *B19*-DNA in the sera of individuals diagnosed with RA, as well as in a group of control people. The findings indicated that *B19*-DNA was identified in the sera of 34% of patients diagnosed with RA, whereas only 6.7% of

Figure 4. The agarose gel electrophoresis technique was employed to analyze the amplification product of parvovirus B19, which had a length of 104 base pairs. Lane 1 displays a ladder consisting of 50 base pairs. Lane 2 is designated as the positive control in this experimental setup, while Lanes 3, 4, 5, 6, 7, and 8 demonstrate the presence of the amplification product of parvovirus B19 (104 base pairs).



individuals in the control group demonstrated the presence of *B19*-DNA. The observed discrepancy was determined to have statistical significance ($p = 0.05$). The findings of this study indicated a possible involvement of *B19* in the development of RA, which also demonstrated a possible involvement of *B19* in the etiology of RA. The *B19* viral infection can initiate RA in individuals with a genetic predisposition to the development of this condition. Multiple investigations have yielded comparable results. For instance, Chen *et al*. (2006) [14] observed a synergistic impact of DR4 and plasma *B19*-DNA on the susceptibility to RA. Moreover, a Japanese study showed that infection with the *B19* virus led to an increase in the production of both tumor necrosis factors (TNF) and interleukin-6 (IL-6).

The precise mechanism through which *B19* induces the development of RA remains uncertain. Nevertheless, some scientists have utilized the molecular mimicry technique to demonstrate the involvement of *B19* in the pathophysiology of RA. The presence of anti-VP1 IgG antibodies was observed to exhibit cross-reactivity with type II collagen and was recognized as a specific antigen for autoantibodies associated with RA [13].

An alternative hypothesis was proposed indicating that the non-structural protein (NS1) may have a substantial impact by functioning as a transcriptional activator on the promoters of the IL-6 gene. This activation could result in an increase in IL-6 levels and the continued presence of *B19* in various immune cells, such as B cells, T cells, dendritic cells, and macrophages. Consequently, this process can modify

the immune response of the host at the cellular level. The prolonged presence of *B19* infection within the joints leads to the activation of polyclonal B cells and facilitates the development of synovial cells in the synovium of individuals with RA [17].

Ray *et al* (2001), [18] observed that the amounts of *B19*-DNA in the synovial membranes of patients with RA were comparable to those of the control group. This finding was obtained through nested PCR to amplify the NS1 and VP genes of *B19*-DNA. Hence, further investigation is important to ascertain the exact role of the *B19* virus in the pathogenesis of RA. Given the observed statistically significant disparity in *B19*-DNA detection levels (34%) between patient and control sera, the present study aimed to examine the influence of *B19* viremia on the progression and severity of the disease [18]. The findings of the current study revealed a statistically significant association between *B19* infection and various indicators of illness severity.

B19-positive RA patients exhibited elevated levels of ESR, CRP, and DAS. Patients who tested positive for *B19* infection recorded higher scores in the joint assessment [19], as well as a statistically significant increase in the number of affected joints, compared to patients with RA who tested negative for *B19.* The elevated levels of RF and anti-cyclic citrullinated peptide (anti-CCP) were observed in RA patients who tested positive for (*parvovirus B19*) [20]. The prevalence of joint erosion was found to be significantly higher in individuals with RA who tested positive for the *B19* virus, suggesting a more aggressive disease course in this subgroup.

Similarly, Kakurina *et al*. (2015), [21] discovered a correlation between *B19* infection and heightened disease activity among individuals with RA. The presence of an active *B19* viral infection was found to be significantly correlated with the highest level of disease activity. In the study conducted by Naciute *et al*. (2016), [22], it was shown that individuals diagnosed with RA and found to have detectable *B19V*-DNA exhibited elevated levels of anti-CCP antibodies and DAS28 scores. These findings suggest a correlation between the presence of *B19V*-DNA with a more severe and active form of the disease. This observation is in agreement with the results reported by Ray *et al*. (2001) [18], wherein it was demonstrated that human synovial fibroblasts treated with *B19* serum exhibited a higher degree of invasiveness compared to fibroblasts cultured in medium alone or in *B19*-negative serum.

Nevertheless, Kozireva *et al*. (2008), [23] have provided evidence that contradicts the existence of a causal relationship between RA activity and *B19* infection. There was no significant correlation observed by Da Silva *et al*. (2014), [16] between *B19* infection and the health assessment questionnaire (HAQ) or DAS28. Furthermore, the researchers discovered that there was no significant association between *B19* infection and quantifiable laboratory and clinical results. Nevertheless, the potential role of *B19* in the pathogenesis of RA was not disregarded.

Conclusions

The patient showed both erythema nodosum and joint symptoms attributed to reactive arthritis which is a very rare finding in itself. Because of the asymptomatic cases, parvovirus *B19*-associated ReA may remain under-diagnosed. When the physicians suspect REA or joint effusions with exanthema *parvovirus B19* tests should be conducted together with conventional laboratory investigations.

Recommendations

RA is a common problem, and we advise that there should be more studies about this disease.

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Author contributions

Maryam Kareem Ali and Jaafar Sataar Shia collaborated equally during the experimental work and discuss the results.

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