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ORIGINAL ARTICLE

Molecular and Serological Study of Parvovirus B19 Infection among Rheumatoid Arthritis Patients at Menoufia University Hospital

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease. Conflicting reports are available about the association between human parvovirus B19 (B19) infection and RA. Most studies were done in European and Asian countries, but only few studies were performed in Africa. ***Objectives:*** This study aimed to investigate the seroprevalence of parvovirus B19 infection in RA patients compared with healthy controls and to search for possible association of B19 viremia with disease activity and severity. ***Methodology:*** This case-control study was conducted on 50 RA patients who fulfilled the 2010 American College of Rheumatology/European League against Rheumatism classification criteria for rheumatoid arthritis and 30 matched healthy controls. All participants were examined for parvovirus B19 infection by serological detection of anti-B19 IgM and IgG by ELISA and B19 DNA by nested PCR. ***Results:*** Parvovirus B19 DNA was detected in 17 (34%) of patients While controls were (6.7%) with a significant difference (0.005). There was significant difference between patients and controls (P=0.007) regarding IgG anti-B19 antibody but not anti-B19 IgM (P =0.59). There was a significant association between B19 viremia and all activity parameters. B19 positive patients had higher levels of ESR and CRP, higher DAS28 scores and more affected joints than B19 negative patients with statistically significant differences. B19 positive patients had significantly higher levels of RF and anti- CCP. Furthermore, B19 positive patients were more likely to have joint erosion. ***Conclusion:*** This study revealed that parvovirus B19 infection may play a role in the aetiopathogenesis of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease marked by persistent synovitis and progressive joint destruction, limiting daily activities and negatively impacting quality of life. Autoantibodies, like anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF), are commonly detected in Patients' serum[1]. Despite the fact that its pathogenesis is still unknown, it is thought to be a multifactorial complex disorder influenced by both genetic and environmental factors. Nearly half of the risk factors for RA are thought to be due to genetic factors like the HLA class II DR1 and DR4 disease-susceptible haplotypes, whereas the other half of the risks is environmental factors, including smoking and infection[2]. Several infectious agents were identified as risk factors for RA, including *Escherichia coli*, *Proteus mirabilis*, *Mycobacterium tuberculosis*, Mycoplasma, human parvovirus B19 (B19), Epstein–Barr virus, hepatitis B virus, retroviruses and alpha viruses[3].

Parvovirus B19 is a ubiquitous small, single-stranded DNA virus encoding two structural capsid proteins (VP1 and VP2) and a non-structural protein (NS1) that primarily replicates in bone marrow erythroblasts[4]. It is transmitted mainly by respiratory droplets, blood and pooled blood products, organ transplantation and vertical transmission from the pregnant woman to her fetus. Acute infection is marked by a five-day phase of high viremia. Then virus clearance occurs with the appearance of IgM, followed by IgG However, B19 virus can persist lifelong in various body tissues (bone marrow, skin, synovia and the liver)[5].

Parvovirus B19 virus has been linked to erythema infectiosum (fifth disease) in children, persistent pure red cell aplasia in immunocompromised patients and aplastic crisis in hemolytic disorder patients[6]. Moreover, B19 was accused to play a role in several autoimmune disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus, polymyositis and primary biliary cirrhosis[7]. It was observed that B19- induced arthritis is similar to the diagnostic criteria of RA. In addition B19

was suggested to cause joint damage, which may be accompanied by development of RF with identification of B19 DNA in synovial fluid and tissue of affected joints[8].

Although many studies on B19 and its relation with several diseases were conducted worldwide, most studies on this virus in patients with rheumatic diseases are conducted in European and Asian countries. Few studies were done in Africa. So, this study aimed to assess the prevalence of B19 infection among patients with RA in comparison with healthy controls and to determine the association between B19 viremia and disease's activity and severity.

METHODOLOGY

This study was performed at the Microbiology and Immunology Department, Faculty of Medicine, Menoufia University in collaboration with Rheumatology & Rehabilitation Department over the period from April 2020 to December 2021. The study included 50 RA patients aged more than 18 years old and fulfilled the 2010 American College of Rheumatology/European League against Rheumatism classification criteria for rheumatoid arthritis[9] and as a control group, 30 healthy subjects were matched for age and sex. The study protocol was approved by Menoufia University's Local Ethics Committee, and all participants gave their written consent. All patients were subjected to:

Full history taking and thorough clinical examination:

Locomotor examinations were performed for all RA patients and included swollen joint count, tender joint count and the existence of extra-articular manifestations (e.g subcutaneous nodules, internal organs involvement and vasculitis manifestations)

Radiological examination:

X-rays films for the hands, wrist and feet (postero-anterior and oblique views) were done for all patients. Joint erosion was estimated using the Sharp/ van der Heijde score (SHS)[10].

Laboratory Investigations:

- Erythrocyte sedimentation rate (ESR) was done by the Wistergren method and recorded in mm/hr.; first hour reading was taken.
- C-reactive protein (CRP) was performed by latex agglutination slide test (Diagnostic Automation/ Cortez Diagnostics, California, USA).
- Rheumatoid factor (RF) was detected in serum using latex agglutination method (Spectrum, Hannover, Germany).

Disease activity:

It was estimated using Disease Activity Score 28 (DAS28). It was measured using a formula composed of the number of tender and swollen joints, patient's global assessment of disease activity using the visual-analog scale (VAS) and ESR or CRP[11] as shown in (Table1).

Table 1: Disease Activity Score 28 (DAS28)

Disease activity stages	DAS28 value
1. Remission	DAS28 ≤ 2.6
2. Low disease activity	2.6 < DAS28 ≤ 3.2
3. Moderate disease activity	3.2 < DAS28 ≤ 5.1
4. High disease activity	5.1 < DAS28

Disease severity:

It was determined according to defined parameters; Health Assessment Questionnaire (HAQ) that measured life quality, anti-CCP, seropositivity for RF and X-ray joint erosion[12].

Viral workup:

Serum samples of patients and controls were stored at -20°C to use for ELISA quantification of anti-B19 IgM and IgG and for nested PCR detection of B19 viral DNA.

- **ELISA RIDASCREEN ParvovirusB19 IgM and IgG (**r-biopharm, Darmstadt, Germany) were used for quantitative measurement of anti-parvovirus B19 IgM and IgG serum levels. Levels > 0.9 were considered positive[13].
- **Nested PCR for parvovirus B19-DNA detection**

As directed by the manufacturer, viral DNA was extracted from serum samples using a DNA-PCR template preparation kit (Thermo Fisher Scientific 81, Wyman Street, Waltham, MA, USA) and an RNA Purification Kit (Lethwania). Then first amplification round was performed by adding 0.4 µl of extracted DNA to the PCR mix for a total volume of 25 µl of DreamTaq Green PCR MasterMix (2′) (Thermoscientific (Fisher Biotec, 198 Cambridge St, Wembley, WA 6014, Australia)), 200 µmol/l deoxynucleotide triphosphate (Stratagen (4040 Lake Washington Blvd NE #201, Kirkland, WA 98033, United States)) and 300 ng of the first round primer. Reaction was performed as following steps: Initial denaturation step at 95°C for 5 minutes, thirty five repeated cycles of: denaturation at 95°C for 1 minute, annealing at 55°C for 1.5 minutes and extension at 72°C for 1 minute followed by final extension step at 72°C for 7 minutes. About 3 µl of the product of this round was added to a second 50 µl PCR mix. The second-round reaction mix contained the same constituents as the first-round, but 300 ng of the second primer was added using the same amplification conditions of the first round. The first round primer was 5′-CTTTAGGTATAGCCAACTGG-3′ (Biosearch Technologies, USA) and 5′-ACACTGAGTTTACTAGTGGC-3′, to yield a product of 1112 bp. The second round primer was 5′-CAAAAGCATGTGGAGTGAGG-3′ and 5′-CC TTATAATGGTGCTCTGGG-3′ to give a product of 104 bp. About 10 µl of second round PCR products

were analyzed by 2% agarose gel electrophoresis then bands were visualized after ethidium bromide staining[14,15].

Statistical analysis

All data were tabulated and analyzed using statistical package for the social sciences (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) software, on an IBM compatible computer. The results were presented as ranges and mean ± SD. Chi-square test was used for the analysis. P values of less than 0.05 were regarded as statistically significant.

RESULTS

The demographic, clinical and laboratory and radiological characters of RA patients were summarized in table 2.

Table 2: Demographic and clinical data of 50 patients with rheumatoid arthritis:

Characters	RA patients (No = 50)
Demographic characters	
Age (years)	
Range in years	**29.0 – 59.0**
Mean ± SD	**42.10±10.35**
Gender [female: No (%)]	**38 (76.0%)**
Positive Family history of autoimmune diseases: No (%)	**11 (22.0%)**
Clinical characters	
Disease duration	
Range in years	**1.0 – 19.0**
Mean ± SD	**7.98±5.43**
Morning stiffness	
Range in minutes	**20.0 – 150.0**
Mean ± SD	**52.22±29.60**
Number of tender joints	
Range	**5.0 – 30.0**
Mean±SD	**16.16±7.65**
Number of swollen joints	
Range	**1.0 – 19.0**
Mean±SD	**12.68±4.20**
Lab characters	
ESR (mm/1st hour)	
Range	**20.0 – 110.0**
Mean±SD	**51.60±28.05**
CRP (mg/dl)	
Range	**1.0 – 36.0**
Mean±SD	**15.12±9.40**
Patients with **RF+**: No (%)	**39 (78.0)**
Patients with **anti-CCP+**: No (%)	**35 (70.0)**
Radiological characters:	
Patients with joint erosions: No (%)	**15 (30.0)**
Extra-articular manifestations:	
Patients with extra-articular manifestations: No (%)	**20 (40.0)**

Among the 50 RA patients, anti-B19 IgM was positive in 3 (6%) with non-significant difference (P =0.59) between patients and controls. On the other hand, anti- B19 IgG was detected in 21 (42%) of RA patients and in 4 (13.13%) of the controls with a significant difference between them (P= 0.007). Regarding Nested PCR results, a higher rate of viral B19 DNA was detected among RA patients 17 (34%) when compared with controls (6.7%) with a statistically significant difference (0.005). Thirteen out of 17 B19 positive RA patients were IgG-positive (2 of these patients also had IgM antibodies), but the other 4 patients had no anti-B19 antibodies as shown in table 3& Fig 1.

Table 3: Viral markers of B19 in studied groups:

Marker	Group I (RA Patients No=50)		Group II (Control No=30)		X_2	*P value*
	No.	%	No.	%		
IgM positivity	**3**	**6**	**1**	**3.3**	**0.28**	**0.59 NS**
IgG positivity	**21**	**42**	**4**	**13.3**	**7.17**	**0.007 S**
B19 DNA detection by nested PCR	**17**	**34**	**2**	**6.7**	**7.73**	**0.005 S**

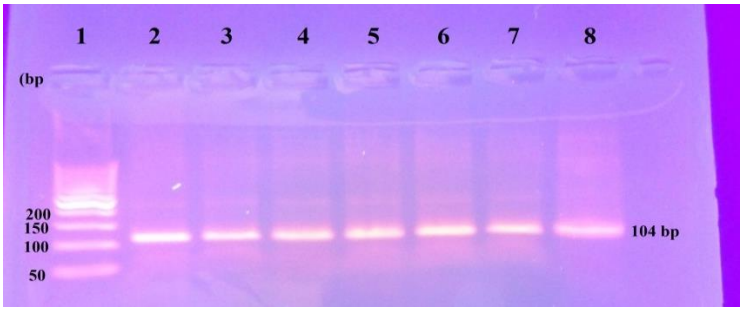

Fig. 1: Agarose gel electrophoresis of amplification product of of B19 at 104 bp. Lane (1) shows 50 bp ladder. Lane (2) positive control, Lanes (3, 4, 5, 6, 7 and 8) show the amplification product of B19 at 104 bp

All disease activity parameters had a significant association with B19 infection. B19 positive RA patients had higher levels of ESR (P= 0.03) and CRP (P=0.03), higher DAS28 scores (P= 0.01) and more affected joints than B19 negative RA patients with a statistically significant difference. RF (P= 0.04) and anti-CCP (P= 0.04) levels were significantly higher in B19 positive RA patients. Furthermore, B19 positive RA patients were significantly more susceptible to have joint erosion (P= 0.01) indicating higher disease aggressiveness among B19 positive RA patients. However, there was non-significant difference (P=0.18) between B19 positive RA patients and B19 negative RA patients regarding extra-articular manifestations (rheumatoid nodules, myositis, vasculitis, lung, cardiac or eye diseases) as shown in table 4.

Table 4: Demographic and clinical data of B19 DNA positive and negative patients:

	B19 positive RA patients (17)	**B19 negative RA patients (33)**	X_2	P value
Demographic data				
Age				
Mean ± SD	43.94±11.08	41.15±10.0	t=	0.37
Range	29.0 – 59.0	29.0 – 58.0	0.90	NS
Gender**:** female No (%)	11 (64.7)	27 (81.8)	1.80	0.29 NS
Disease duration (years)				
Mean ± SD	8.94±5.12	7.48±5.59	t=	0.37
Range	1.0 – 18.0	1.0 – 19.0	0.89	NS
Disease severity				
HAQ				
Mean ± SD	2.17±0.72	2.69±0.52	t=	0.006
Range	1.0 – 3.0	1.0 – 3.0	2.89	S
Patients with joint erosion, No (%)	9 (52.9)	6 (18.2)	6.45	0.01 S
Patients with RF+, No (%)	16 (94.1)	23 (69.7)	3.89	0.04 S
Patients with anti-CCP+ No (%)	15 (88.2)	20 (60.6)	4.07	0.04 S
Disease activity				
Tender joints number				
Mean ± SD	21.11±7.51	13.60±6.45	U=	0.002
Range	5.0 – 30.0	5.0 – 30.0	3.09	S
swollen joints number				
Mean ± SD	15.05±2.68	11.45±4.34	U=	0.006
Range	10.0 – 19.0	1.0 – 19.0	2.75	S
ESR (mm/h)				
Mean ± SD	64.64±34.0	44.87±22.13	U=	0.03
Range	25.0 – 110.0	20.0 – 90.0	2.10	S
CRP (mg/dl)				
Mean ± SD	19.05±10.65	13.09±8.14	U=	0.03
Range	12.0 – 36.0	6.0 - 30.0	2.15	S
DAS28 > 5.2				
Mean ± SD	3.32±0.91	2.76±0.67	t=	0.01
Range	1.7 – 4.5	1.9 – 4.20	2.43	S
Patients with extra-articular manifestations (rheumatoid nodules, myositis, vasculitis, lung , cardiac or eye diseases)	9 (52.9)	11 (33.3)	1.79	0.18 NS

There was a significant association between detection of B19 DNA in RA patients by nested PCR and 28-joint Disease Activity Score (DAS28) as 41.2% and 29.4% of B19 DNA positive RA patients were in moderate and high disease activity stages respectively. However, 51.5% and 33.3% of B19 DNA negative RA patients were in remission and low disease activity stages respectively with a statistically significant difference (P= 0.01) as shown in Fig 2.

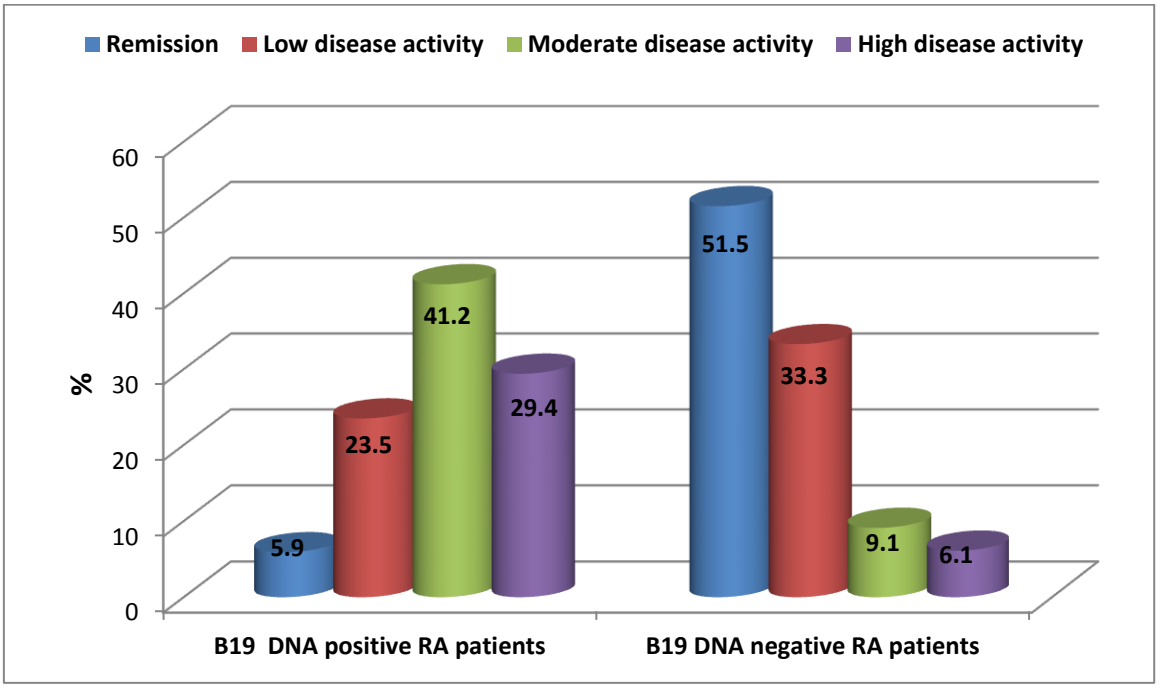

Fig. 2: Comparison between B19 DNA positive and negative patients with regard 28-joint Disease Activity Score (DAS28).

DISCUSSION

Viruses have been accused of triggering several autoimmune diseases in genetically susceptible individuals. The relation of B19 infection with RA is considered an issue of interest. Similarities between B19-induced arthritis and RA suggested that parvovirus infection can be considered as an infectious etiology for RA[16].

In our study, the prevalence of anti-B19 (IgM and IgG) detected in RA patients was 6% and 42%, respectively. Anti-B19 IgG prevalence was higher among RA patients compared with controls (13.3%) with a statistically significant difference (*P* =0.007) but IgM showed non-significant difference between patients and controls. Similar results were reported by Regaya et al.[17] in Tunisian; anti-B19 IgG was detected in the sera of 80.7% and 43% of RA patients and controls respectively but none of them had B19 IgM antibodies. ELsedig et al.[18] reported a significant incidence of anti B19 in Sudanese patients suffering from RA as IgM and IgG were detected in 34.4% and 54.4% of RA patients respectively. Similar results were obtained in Brazil, Silva et al.[19] reported positive anti-B19 IgG in 86. 0% of RA patients. In Taiwan, Chen et al.[20] found that plasma anti- B19 (IgG and/or IgM) was positive in 93.6% of patients with highly significant differences when compared with controls (*p* <0.001). Silva et al.[19] explained the higher prevalence anti-B19 among RA patients for the existence of a higher susceptibility of RA patients to acquire viral infections due to immunosuppression or by other immunological characteristics in these patients. Also, the probability of B19 acting as a trigger for development of RA cannot be rolled out.

By nested PCR, B19-DNA was detected in 34% and 6.7 % of our RA patients' sera and controls respectively with a statistically significant difference (*P* =0.05). The same result was published in Latvia in two different studies that were conducted by Kozireva et al.[21] and Naciute et al.[22] Also, Chen et al.[20] mentioned that viral DNA was positive in 30.6% and 9.1% in plasma samples from patients and controls respectively (p = 0.005) and Silva et al.[19] detected B19-DNA in 30.6% of plasma samples obtained from RA patients.

These findings suggested that B19 may be involved in the development of RA. It seems that B19 virus might induce RA in genetically susceptible individuals. Several previous researches supported this suggestion; a study conducted by Chen et al.[20] suggested a synergistic effect between DR4 and plasma B19 DNA on susceptibility to RA. Also, two studies done in Japan, one showed that infection with B19 induced the expression of both tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, that represent the key cytokines in RA pathogenesis[23]. The other study was a six years follow up study in which the patients developed RA with destructive changes in joints after acute infection with B19. In addition, a clinical improvement was observed after intravenous immunoglobulin therapy against B19 and this also supported the presence of a relation between RA and B19 infection[24].

The mechanism for B19 induction of RA is exactly still unknown. However, some authors used the mechanism of molecular mimicry to demonstrate the role of B19 in RA pathogenesis. Cross reaction may occur between Anti-VP1 IgG and type II collagen, which represents a target antigen for autoantibodies in the RA[19]. Others suggested another scenario in which non-structure protein (NS1) play a serious role as it is a transcriptional activator on the promoters of IL-6 gene leading to up regulation of IL-6 together with persistence of B19 in B cells, T cells, dendritic cells and macrophages, this may alter host cellular immunity. The synovial cells and lymphocytes secrete inflammatory cytokines continuously with persistent B19 infection in the joints leading to polyclonal B cell activation together with synovial cell proliferation in synovium of RA patients[25].

However, other studies denied any role for B19 infection in RA pathogenesis. A long-term follow up research of 54 patients having recent B19 infection was performed by Speyer et al.[26] They found that no one of the patients having acute arthralgia developed RA. Also, Peterlana et al.[27] found that amplification of both NS1 and VP genes of B19 DNA in synovial membranes via nested PCR had revealed that B19 DNA levels were the same in synovial membranes of both RA patients and controls. Thus, the role of B19 virus in RA pathogenesis is still under discussion and needs further researches

Since B19-DNA was detected in 34% of our patients' sera with a statistically significant difference between patients and controls, we analyzed how B19 viremia affects the disease's activity and severity. Our data showed that there was a significant association of B19 infection with all disease activity parameters. B19 positive RA patients had higher levels of ESR and CRP, higher DAS28 scores and more affected joints than B19 negative RA patients with a statistically significant difference. B19 positive RA patients had significantly higher levels of RF and anti- CCP. Furthermore, B19 positive RA patients were significantly more likely to have joint erosion indicating higher disease aggressiveness among B19 positive RA patients. Similar results were obtained by Kakurina et al.[28] who studied the correlation between the disease activity in RA patients and B19 infection. The highest level of disease activity was detected in patients having active B19 viral infection. Also, Naciute et al.[22] detected that RA patients with B19V DNA positive had higher anti-CCP levels and higher DAS28 scores suggesting higher disease aggressiveness and activity, respectively. This was in harmony with Ray et al.[29] Who observed that B19 serum-treated human synovial fibroblasts displayed greater invasiveness than fibroblasts cultured in medium alone or medium with B19-negative serum.

On the contrary, Kozireva et al.[21] denied any significant association between RA activity and B19 infection. Also, Silva and colleagues[19] reported that there was no correlation of B19 infection neither with DAS28 nor with quality of life (HAQ). They also reported that there was no relation between B19 infection with clinical and laboratory aspects. However, they did not deny that B19 may play a role in rheumatoid arthritis pathogenesis. Different results may be related to several factors: RA disease entity heterogeneity, differences in sampling timing with regard to the onset of arthritis in different studies, different races with different responses to B19 virus, unknown viral pathogenesis, additional infectious triggering agents or deficient prospective researches in this subject.[30]

CONCLUSIONS

Not only, parvovirus B19 infection has been linked to the development of rheumatoid arthritis but also, it has been linked to the disease's activity and severity. To confirm the serious role of B19 in the clinical course of rheumatoid arthritis, larger studies are required.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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