Investigation of the Etiologic Role of Parvovirus B19 by Immunologic and Molecular Methods in Rheumatoid Arthritis and Systemic Lupus Erythematosus

Romatoid Artrit ve Sistemik Lupus Eritematozusta Parvovirüs B19'un Etiyolojik Rolünün İmmünolojik ve Moleküler Yöntemlerle Araştırılması

Tercan US,[a]
Esin ÇETİN,[b]
Nilgün KAŞİFOĞLU,[a]
Timuçin KAŞİFOĞLU,[c]
Yurdanur AKGÜN,[a]
Cengiz BAL[d]

Departments of
[a]Microbiology,
[c]Internal Medicine,
Division of Rheumatology,
[d]Bioistatistics,
Eskişehir Osmangazi University
Faculty of Medicine,
[b]Microbiology Laboratory,
Eskişehir State Hospital, Eskişehir

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Yazışma Adresi/*Correspondence:*
Nilgün KAŞİFOĞLU
Eskişehir Osmangazi University
Faculty of Medicine,
Department of Microbiology,
Eskişehir,
TÜRKİYE/TURKEY
nkas@mynet.com

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ABSTRACT Objective: Genetic, hormonal, environmental factors and many infectious agents are thought to play role in the etiology of rheumatoid arthritis (RA). In this study, we aimed to investigate the role of Parvovirus B19 in etiology of RA and systemic lupus erythematosus (SLE). **Material and Methods:** Eighty-seven RA and 50 SLE patients diagnosed according to 1987 American College of Rheumatology (ACR) criteria and applied to Eskisehir Osmangazi University Medical Faculty, Internal Medicine Department, Division of Rheumatology between January 2007-January 2008, and a control group of 50 healthy blood donors were included in the study. In serum samples, Parvovirus B19 IgM, IgG (Focus Diagnostics Cypress, USA) were investigated by ELISA method. Genome presence and viral load were investigated by quantitative real-time polymerase chain reaction with Parvovirus B19 RG PCR kit (Qiagen, USA). The results were evaluated statistically by SPSS 20.0 program. **Results:** There was no significant difference between RA, SLE and healthy control groups in terms of Parvovirus B19 IgM (p=1.000). When Parvovirus B19 IgG positivity rate was considered, there was no difference between RA and SLE groups (p=0.277). Although there was no difference between RA and control groups (p=0.133), Parvovirus B19 IgG positivity rate was significantly higher in SLE group compared to the control group (p=0.016). Parvovirus B19-DNA was negative in all groups. **Conclusion:** We could not show any correlation between Parvovirus B19 and RA etiopathogenesis, there could be a relation between Parvovirus B19 and SLE etiopathogenesis. It is concluded that multicenter systematic investigations examining also synovial tissue and fluid samples are needed.

Key Words: Parvovirus B19, human; arthritis, rheumatoid; lupus erythematosus, systemic

ÖZET Amaç: Genetik, enfeksiyöz ajanlar ve çevresel faktörlerin romatoid artrit (RA) gelişiminde rol oynadığı düşünülmektedir. Çalışmamızda RA etyopatogenezinde etken olabileceği düşünülen enfeksiyöz ajanlardan Parvovirüs B19'un RA etyolojisindeki yerinin araştırılması hedeflenmiştir. **Gereç ve Yöntemler:** Ocak 2007-Ocak 2008 tarihleri arasında Eskişehir Osmangazi Üniversitesi Tıp Fakültesi İç Hastalıkları Anabilim Dalı Romatoloji Bilim Dalı'na başvuran, 1987 Amerikan Romatoloji Derneği (ACR) tanı kriterlerine göre kesin tanısı konmuş, 87 RA hastası, 50 sistemik lupus eritematozus (SLE) hastası ve kan bankasına başvuran sağlıklı donörlerden 50 kişilik kontrol grubu çalışmaya alındı. Gruplardan alınan serum örneklerinde, ELISA yöntemi ile Parvovirüs B19 IgM ve IgG (Focus Diagnostics Cypress, USA) araştırıldı. Genom varlığı ve yükü incelenirken, gerçek zamanlı polimeriz zincir reaksiyonu yöntemi ile Parvovirüs B19 RG PCR kitleri (Qiagen, USA) kullanıldı. Elde edilen sonuçlar SPSS 20.0 programı kullanılarak istatistiksel olarak değerlendirildi. **Bulgular:** RA, SLE ve sağlıklı kontrol grupları arasında Parvovirüs B19 IgM antikor pozitifliğine göre istatistiksel olarak anlamlı fark bulunamadı (p=1,000). Parvovirüs B19 IgG pozitifliği açısından değerlendirildiğinde, RA ile SLE grubu arasında fark tespit edilemedi (p=0.277). RA ile kontrol grubu arasında Parvovirüs B19 IgG pozitifliği açısından anlamlı fark tespit edilemezken (p=0,133), SLE grubunda sağlıklı kontrol grubuna göre anlamlı yükseklik olduğu gösterildi (p=0,016). RA, SLE ve sağlıklı kontrol gruplarının tümünde Parvovirüs B19 DNA negatif bulundu. **Sonuç:** Çalışmamızda Parvovirüs B19, RA etyopatogenezi ile ilişkilendirilemedi. Ancak Parvovirüs B19 ile SLE etyopatogenezi arasında bir ilişki olabileceği gösterildi. Bu konuda daha geniş kapsamlı, sistematik, çok merkezli, sinoviyal sıvı ve doku örneklerinin de incelendiği yeni araştırmaların yapılmasına ihtiyaç olduğu sonucuna varıldı.

Anahtar Kelimeler: Parvovirüs B19, insan; artrit, romatoid; lupus eritematozus, sistemik

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Rheumatoid arthritis (RA) is a systemic, chronic and progressive autoimmune disease. The basic clinical characteristic of the disease is symmetrical, destructive polyarthritis of peripheral small joints. Extra-articular findings may also be seen. The disease causes deformities and loss of function of synovial joints, and long-term morbidity and mortality.[1,2] RA is the most common inflammatory arthritis in the world. Although the prevalence varies in different populations, it is approximately 0.5-1%. It is 2-4 times more frequent in females compared to males. The disease can emerge in all ages from one year-old to advanced ages, but the initial age is usually 40-50 years.[3,4] The etiology and pathogenesis of the disease have not been explained yet. Genetic, hormonal, environmental factors and many infectious agents are considered to play a role.[5] It is suggested that many infectious agents might play a role in pathogenesis of RA. Various theories have been proposed about the roles of these potential agents in the evolution of arthritis. Although numerous studies are performed to explain the role of infectious agents in the pathogenesis of RA, decisive results could not be obtained.

There are some studies demonstrating the relationship between arthropathy and Parvovirus B19. In Parvovirus B19 infections, joint symptoms such as arthritis/arthropathy can be seen in 33% of adults, and these symptoms are more frequent in females. Joint symptoms are acute and usually moderate, with non-erosive peripheral arthritis. These symptoms last for a few weeks and are usually self-limited. However, in some cases they can persist for months or years. Fifty percent of patients with chronic B19 arthropathy fulfill the RA diagnostic criteria. Additionally, B19 arthropathy can resemble early rheumatoid arthritis in the distribution of joint manifestations, symmetry of involvement, and incidence of morning stiffness.[6] Parvovirus B19 is considered to play a role in the etiology of RA by direct synovial infection.[7-9] Rheumatoid arthritis is a disorder with significant social and economic unfavorable effects. Given the burden of the disease, further studies about the etiopathogenesis of RA are needed.

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that can affect many organs such as skin, joints, kidneys, lungs, nervous system, and serous membranes. SLE is an autoimmune disease which presents with the effect of hormonal and environmental (ultraviolet, food, drugs and infectious agents) factors in especially individuals with a genetic predisposition.[10] The purpose of this study is to investigate the role of Parvovirus B19 in etiology of RA and systemic lupus erythematosus (SLE).

MATERIAL AND METHODS

Eighty-seven RA and 50 SLE patients diagnosed according to 1987 American College of Rheumatology (ACR) criteria and applied to Eskisehir Osmangazi University Medical Faculty Department of Internal Medicine, Division of Rheumatology between January 2007-January 2008, and a control group consisting of 50 healthy donors applied to blood bank were included in our study. The mean ages of RA and SLE groups were as 53.22±12.68 years (range 24-77) and 35.54±10.93 years (range 17-62), respectively. In RA group, 83.9% were females, and in SLE group 92% were females. In serum samples obtained from different groups, Parvovirus B19 IgM and IgG (Focus Diagnostics Cypress, USA) were studied by ELISA method. This ELISA kit utilizes recombinant VP1 Parvovirus B19 protein. Parvovirus B19 DNA was investigated by quantitative real-time polymerase chain reaction (RT-PCR) method using Parvovirus B19 RG PCR kit (Qiagen, USA). This PCR kit amplifies a 76-bp region of the Parvovirus B19 genome and quantifies by using five quantitation standards. The lowest detection limit of the kit is 0.2 IU/μl. PCR tests were studied duplicate in all groups. The results were evaluated statistically by IBM SPSS for Windows 20.0 program. In the analysis of cross-tables, Chi-square tests (Pearson, Yates, Fisher's exact Chi-square tests) were used. $p<0.05$ was considered as statistically significant.

This study has been approved by our Institutional Ethics Review Board for human studies, and all patients have signed informed consents. The study complies with the principles of the Helsinki

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RESULTS

There was no statistically significant difference in RA, SLE and healthy control groups in terms of Parvovirus B19 IgM antibody positivity (p=1.000) (Table 1). There was a significant difference between groups according to Parvovirus B19 IgG positivity (p=0.033) (Table 1). In SLE group, Parvovirus B19 IgG positivity rate was significantly higher than healthy control group (p=0.016). Although the difference between RA group and the other two groups was not statistically significant, it was remarkable that Parvovirus B19 IgG positivity rate of RA group was higher compared to especially healthy control group (p=0.133). Parvovirus B19 genome presence and viral load was investigated by quantitative real time PCR method in RA, SLE and healthy control groups. The genome results were negative in all groups, therefore could not be evaluated statistically (Table 1).

DISCUSSION

The etiology of RA is not known exactly similar to many other autoimmune diseases. However, it is thought that genetic, hormonal, environmental factors and many infectious agents play role in the development of the disease.[5] In RA etiopathogenesis, it is suggested that many infectious agents play a role in triggering the disease, but exact evidences have not been found yet. Many studies have been done to investigate the relationship of various infectious agents with the etiology of RA.[7-9] Parvovirus B19 is a fairly common cause of infection in the world. There are some studies that indicate the relation of Parvovirus B19 infection with arthropathy. In Parvovirus B19 infections, joint symptoms such as arthritis/arthropathy can be seen in the 33% of adults, and these symptoms are acute and usually moderate. These symptoms last for a few weeks and are usually self-limited. on the other hand, chronic arthropathy can be seen for months or years in some cases. Fifty percent of patients with chronic B19 arthropathy fulfill the RA diagnostic criteria.[11,12] Autoimmune manifestations associated with Parvovirus B19 infections are also defined. It is determined that anti-VP2 antibodies cross-react with some autoantigens. In patients with rheumatologic presentations, the presence of antiphospolipid antibodies that occur by phospholipase A2 activity of VP1 is associated with persistent infections. NS1 protein of the virus stimulates the IL-6 and TNF-α production, and causes inflammatory signs of arthritis and synovial erosion. In addition, Parvovirus B19 causes detectable autoantibody synthesis by polyclonal B cell activation.[13,14] RA-like joint symptoms caused by Parvovirus B19 especially in adults have led to consider it as an etiologic agent. Parvovirus B19 may play an etiologic role in RA pathogenesis via direct synovial infection.[7-9]

Caliskan et al. investigated Parvovirus B19 IgM and IgG antibodies of 31 RA patients, 20 patients with early synovitis, 25 SLE patients, 25 osteoarthritis patients and 50 healthy individuals, and found that antibody positivity rate was significantly higher in RA group than healthy group.[15] However they could not find any difference between RA and other patient control groups in terms of antibody positivity. Nikkari et al. could not find any difference between RA and control

TABLE 1: The distribution of Parvovirus B19 antibodies and Parvovirus B19 DNA among groups.

	RA		SLE		Healthy control		
	+	-	+	-	+	-	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	p value
Parvovirus B19 IgM	1 (1.1)	86 (98.9)	1 (2.0)	49 (98.0)	1 (2.0)	49 (98.0)	1.000
Parvovirus B19 IgG	46 (52.9)	41 (47.1)	32 (64.0)	18 (36.0)	19 (38.0)	31 (62.0)	0.033
Parvovirus B19 DNA	0	87 (100)	0	50 (100)	0	50 (100)	-

RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus.

group in terms of Parvovirus B19 seropositivity.[16] Akbulut et al. investigated Parvovirus B19 IgM and IgG in 50 rheumatoid factor positive patients and in a rheumatoid factor negative group.[17] Thirty-one of these rheumatoid factor positive patients were RA patients. In that study, significant differences were not found between RA patients and non-RA patients in terms of Parvovirus B19 IgM and IgG. In our study, Parvovirus B19 IgM specific antibody positivity rates were not significantly different in RA, SLE and healthy control groups. Parvovirus B19 IgG positivity rate of RA group was higher than healthy control group, but the difference was not statistically significant. Parvovirus B19 IgG positivity rate of SLE group was significantly higher than the healthy control group. Indeed, many agents including viruses may play role in the etiopathogenesis of SLE which is a chronic autoimmune disease. As previously reported in the literature, Parvovirus B19 may simulate the clinical and laboratory features of SLE, may exacerbate SLE, or SLE may develop following the Parvovirus B19 infection. The true prevalence of B19 infection in SLE patients is difficult to determine as the immunosuppression in SLE can inhibit IgM and IgG seroconversion.[18] Previous Parvovirus B19 infection may be a triggering factor in the pathobiology of inflammatory rheumatologic diseases.

Caliskan et al. detected Parvovirus B19 DNA in 3 of 20 early synovitis and 4 of 31 RA patients.[15] Nikkari et al. could not detect Parvovirus B19 DNA in RA and control groups.[16] Chen et al. found the positivity rate of Parvovirus B19 DNA significantly higher in RA group than control groups.[19] In that study, Parvovirus B19 DNA was positive in 22 (30.6%) plasma samples of 72 RA patients and in 8 (75.0%) synovial fluid of 14 RA patients. It is noteworthy that the ratio in the synovial fluid is high. We could not detect Parvovirus B19 DNA by real-time PCR method in any of the groups. It is suggested that the reason may be that we only studied serum samples and not included synovial fluid and tissue samples. Although it is known that synovial fluid and tissue samples are valuable materials in studies conducted with similar patient groups, we could not include these samples, because clinicians do not prefer these invasive samples unless they are mandatory. We found that the numbers of samples in patient and control groups are lower than ours in most of the studies about Parvovirus B19 in the literature. In addition, molecular methods applied in those studies are usually in-house methods using different primers and prone to contamination, and more subjective methods. In our study, the numbers of samples in patient and control groups are higher than those studies. Moreover, the molecular method we used is a fully automated, CE approved, standardized real-time PCR method with a lowest contamination risk, and with high specificity and sensitivity rates. The lowest detection limit of the kit is 0.2 IU/μl. The quantification range is 10^1-10^8 copies/mL. The PCR kit can detect all relevant genotypes.

In conclusion, when we compare the results of our study and the others, we observe both compatible and different results. These conflicting results can be explained by non-standardized selection of case and control groups, the comparison criteria, follow-up periods, tests performed, statistical analysis, and interregional genetic and socioeconomic variables.

As a result, although we could not show an association between Parvovirus B19 and RA etiopathogenesis, an association with SLE ethiopathogenesis may be suggested. Nevertheless, it is not possible to use our data as a conclusive result for the association between Parvovirus B19 and RA-SLE ethiopathogenesis. Further, prospective, multicenter studies including synovial tissue and fluid samples as well as serum samples are needed to enlighten the role of many infectious agents on RA and SLE etiopathology.

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