Serological parvovirus B19 screening of patients with rheumatoid arthritis and ankylosing spondylitis

ABSTRACT
Parvovirus B19 is single-stranded DNA virus and can cause general flu-like symptoms or asymptomatic infection with rash and, more commonly in adults, arthritis, especially in the fingers and hands but also in the wrists and ankles. The present study aimed to investigate the involvement of parvovirus B19 in the development of rheumatoid arthritis and ankylosing spondylitis by demonstrating the presence of specific antiviral antibodies in patient serum samples. Serological methods (indirect ELISA test and Immunoblot test) to demonstrate the specific parvovirus B19 IgM/IgG antibodies were used. A total 36 serum samples, collected in 2015 from patients with rheumatoid arthritis (n=24) and ankylosing spondylitis (n=12) were tested. The average age of patients studied was 50 ± 13.2 years. Positive results for B19-IgM antibodies which are marker for acute B19 infection in 2/36 (5.55%) serum samples were detected. These two sera were from patients with diagnosis rheumatoid arthritis. Protective B19-IgG antibodies evidence of past infection in 17/36 (47.22%) patients were found. Thirteen of them were diagnosed with rheumatoid arthritis and four with ankylosing spondylitis. Two of the tested serum samples from patients with rheumatoid arthritis were positive for both investigated B19 markers. Rheumatoid diseases are one of the oldest problems of mankind, which is widespread today. This study is the basis for future more detailed tests aimed enrichment of diagnostic range on these diseases and inclusion of parvovirus B19 assays.

Key words: parvovirus B19, ELISA IgM/IgG, rheumatoid arthritis, ankylosing spondylitis

Introduction
Parvovirus B19 (B19V) is single-stranded DNA virus and can cause general flu-like symptoms or asymptomatic infection with rash and, more commonly in adults, arthritis, especially in the fingers and hands but also in the wrists and ankles. (Cossart et al., 1975, Young et al., 2004). B19V infection is widespread, especially in the developed countries of Europe, Asia and America. Specific protective B19V-IgG antibodies in 2-10% among children under 5 years, in 15-40% among children of 10 – 19 years, in 45-60% among adults over 20 years and in more than 75 % among adults over 55 - 60 years are found (Heegaard & Brown, 2002, Mossong et al., 2008, Yermorloich et al., 2012). According Colmegna and Alberts-Grill (2009), 80% of adults experience joint pain as a result of B19V infection, such as arthritic symptoms were observed in 60% of women compared with 30% for men (Simo et al., 1994). Chronic arthritis occurring...
after spending B19 infection is quite similar clinical picture in rheumatoid arthritis (RA) and in 50% of patients met the criteria for diagnosis of RA (Kerr et al., 2005). About 8% of children infected with B19V has joint pain. However, arthralgia is more common in adolescents and adults spent B19 infection and affects up to 60% of these people (Nesher & Moore, 1997).

The diagnostic of parvovirus B19V infection is performed using ELISA or Immunoblot, which selectively detect anti-B19 IgG or anti-B19 IgM antibodies using a viral structural protein as antigen (Lehmann et al., 2008). They are the standard method for laboratory testing and are of particular significance.

The present study aimed to investigate the involvement of parvovirus B19 in the development of rheumatoid arthritis and ankylosing spondylitis by demonstrating the presence of specific antiviral antibodies in patient’s serum samples.

Materials and Methods

Clinical samples

The study started in January 2015 and included 36 patients, who were diagnosed at Clinic of rheumatology of University Hospital for Active Treatment “St. Ivan Rilski”, Sofia with ankylosing spondylitis (AS) (n=12) - according to the modified New York criteria (Van der Linden et al., 1984), and with RA (n=24) according to American College Rheumatology criteria 1987 (Arnett et al., 1988). Variables, as patient’s age and disease duration, are labeled as mean±standard deviation (SD).

The ethics committee of Medical University, Sofia approved the protocol for this study, and informed consent was obtained from all patients.

Laboratory analysis

All serum samples were tested for anti-parvovirus B19 IgM and IgG antibodies with a commercial indirect enzyme-linked immunosorbent assay (Euroimmun, Anti-Parvovirus B19 ELISA (IgM/IgG). This assay was found to have a high sensitivity of 100% and a specificity of 98%. The assay was performed as recommended by the manufacturer and the results were interpreted qualitatively as positive, negative or equivocal.

With the purpose of detection of structural (VP1, VLP and VP2) and non-structural (NS1) B19 viral proteins and to avoid false positive results Immunoblot test (Euroimmun, Anti-Parvovirus B19 EUROLINE (IgM/IgG), with a sensitivity of 98% and a specificity of 100%) were used.

Results

A total 36 serum samples were tested and the average age of patients was 50 ± 13.2 years. Positive results for B19V-IgM antibodies which are a marker for acute B19 infection in 2/36 (5.55%) serum samples were detected. These two sera were from patients with diagnosis RA (2/24, 8.33%). Protective B19V-IgG antibodies evidence of past infection in 17/36 (47.22%) patients were found. Thirteen of them were diagnosed with RA (13/24, 54.16%) and four with AS (4/12, 33.33%). Two of the tested serum samples from patients with RA were positive for both investigated B19 markers (B19V-IgM and B19V-IgG antibodies) (Table 1).

All samples with positive B19V-ELISA results were investigated with the B19V-Immunoblot test. This test is based on recombinant highly specific antigens purified by affinity chromatography, which contain linear and conformational epitopes (Table 2). We detected positive results with stronger signal intensity on antigen bands for structural viral proteins VP1 and VLP (Immunoblot B19V IgG) in 17 of 17 positive ELISA IgG samples. In two ELISA IgG positive samples antigen bands at the three structural proteins VP1, VLP and VP2 were found. One of 17 positive ELISA IgG samples from patient with RA had positive antigen bands for the three structural proteins and also for non-structural (NS1) protein.

About ELISA IgM positive samples (n=2), in all of them structural proteins VP1, VLP and VP2 (Immunoblot B19V IgM) were found (Figure 1). In one sample four positive bands (VP1, VLP, VP2 and NS1) were shown.

Discussion

Rheumatoid arthritis and ankylosing spondylitis are musculoskeletal conditions that cause severe long term pain and disability which prevalence increased with ageing. They are observed both in adults and in the people of working age, even children (Heegaard & Brown, 2002).
There are many different reasons for the development of these diseases, but most often they are infectious, toxic, metabolic and others. The present study aimed to determine the involvement of parvovirus B19 in the pathogenesis of these diseases and was based on samples collected during a 2015. B19V markers by ELISA and Immunoblot were detected. Overall, 47.22% of the investigated patients were B19V IgG positive which is similar to observations from previous study in Finland (Han et al., 2007) and is close to the average frequency of the spread of the B19V in the general population (Heegaard & Brown, 2002). In all samples positive for B19V IgG and IgM, positive signals for structural viral proteins VP1 and VPL were found. In two samples from patients with diagnosis RA both B19V IgG and IgM antibodies were found. These results are associated with the study of Hannu et al. (2007) who demonstrated acute B19V infection in 3.3% (2 of 60 tested samples) of testing patients with RA. One of the B19V IgG and IgM positive samples had positive antigenic bands for all tested viral proteins including non-structural protein NS1. Antibodies against NS1 protein have no relevance in diagnosis, but they can be detected, for example, in patients with forms of RA caused by B19V (Heegaard & Brown, 2002, Lehmann et al., 2008). Regarding to clinical diagnosis B19 infection was demonstrated more frequently in patients with RA (13/24, 54.16%) compared to those with AS (4/12, 33.33%).
Rheumatoid diseases are one of the oldest problems of mankind, which is widespread today. This study is the basis for future more detailed tests involving serological and molecular methods and aimed enrichment of diagnostic range on these diseases and inclusion of parvovirus B19 assays. On the other hand, understanding the pathogenesis of B19V arthropathy may provide insight into the mechanisms by which rheumatoid arthritis develops.

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References


