Egypt J Pediatr Allergy Immunol 2011;9(2):71-76.

Original article

Parvovirus B19 viremia in children with systemic lupus erythematosus

Background: *Parvovirus B19 infection may present with fever, rash, non-erosive arthritis, hepatitis, anemia, thrombocytopenia, leucopenia and positive ANA, B19 infection may be misdiagnosed as new onset systemic lupus erythematosus. At the same time, B19 infection and systemic lupus erythematosus may occur simultaneously in some patients. A casual relationship between B19 infection and classic idiopathic systemic lupus erythematosus has not been demonstrated yet.* ***Objectives:*** *This study was undertaken to investigate the seroprevalence of parvovirus B19 in SLE patients and to search for the different correlates of this viremia with positive results.* ***Methods:*** *This case-control study was conducted on 30 patients with SLE and 30 normal controls. All the children were subjected to detailed medical history, Clinical examination, laboratory estimation as sera from them were examined for parvovirus B19 infection by serological assays using nested polymerase chain reaction and IgG and IgM antiB19 antibodies by ELISA.* ***Results:*** *Parvovirus B19 DNA was detected in 11 of the 30 patients with SLE (33.3 percent) while it was not detected in any of our normal controls. Of the 11 patients with B19 DNA, only two had IgG anti-B19 antibody and one had IgM anti-B19 antibodies, whereas IgG and IgM anti-B19 antibodies were detected in 11(57.8%)and 9 (47.3%)of 19 SLE patients without B19 DNA respectively. B19 DNA was found more commonly in sera from SLE patients without anti-B19 antibodies than in those with anti-B19 antibodies (P<0.05).* ***Conclusions:*** *parvovirus B19 might induce either idiopathic SLE in a person who is genetically susceptible or it might induce a SLE-like picture. Parvovirus B19 infection in patients with SLE may be due to lack of anti-B19 antibodies because of either the immunocompromised nature of the host or the use of immunosuppressive drugs. There was a higher prevalence of hypocomplementemia in patients with parvovirus B19 viremia than in those without parvovirus.*

Keywords: Human parvovirus B19, Systemic lupus erythematosus, Nested PCR

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INTRODUCTION

Human parvovirus B19 (PB19) is a single-stranded DNA virus that was first discovered in 1975 by Yvonne Cossart and her colleagues[1]. It has been associated with a variety of clinical manifestations, including rash, thrombocytopenia, leukopenia, fetal wastage, hypocomplementemia, autoimmune hemolytic anemia, arthritis and vasculitis[2-6]. It is the causative agent of erythema infectiosum (EI). In addition, B19 infection can be associated with elevated levels of antinuclear antibody, anti-double stranded DNA antibody, antineutrophil cytoplasmic antigens and anti-cardiolipin[7]. Also, antiphospholipid antibodies, when seen in acute parvovirus B19 infection, may have the same specificity and cofactor dependence as antiphospholipid antibodies associated with systemic lupus erythematosus[8].

B19 is a small, non-enveloped virus containing a single-stranded DNA of 5600 nucleotides and composed of two capsid proteins, VP1 (781 amino acids) and VP2 (554 amino acids), and a non-structural protein, NS1[9,10]. It targets early erythroid progenitor cells. It requires rapidly dividing cells in order to replicate. The cellular receptor is the P antigen (globoside, a glycosphingolipid), expressed in most individuals on mature erythrocytes and other cells[11]. B19 infection is found worldwide in persons of all ages. Most people become infected at some time during their life, up to 15% of individuals developing infection between 1 and 5years of age, 15–60% between the ages of 5 and 19yeas, and 30–60% in adulthood[12].

The detection of anti-VP1 and anti-VP2 antibodies is the basis for the diagnosis of acute or past B19 virus infections. The dominant humoral

immune response is to VP2 during early convalescence and to VP1 during late convalescence. Anti-VP1 and anti-VP2 antibodies play a major role in limiting B19 infection in man[13]. Antibodies against NS1may have utility as an indicator of chronic or persistent forms of parvovirus B19 virus infection[14].

The association of B19 infection with autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), primary biliary cirrhosis (PBC) and polymyositis (PM), has been suggested[5,6,15-18], although the exact relationship between the infection and these disorders is not understood. Recently, it has been suggested that B19 exacerbates or even induces SLE[19,20]. There are striking analogies between the clinical features and hematological findings of SLE and those of B19 infection.

This study was undertaken to investigate the seroprevalence of parvovirus B19 in SLE patients and to search for the different correlates of this viremia with positive results.

METHODS

Study population

This case-control study was conducted on 30 children and adolescents fulfilling the American College of Rheumatology Classification Criteria for SLE younger than age of 16 years. They were 13males and 17 females. Their ages ranged between 9 and16 years with a mean age±SD: 12.7±1.8 years (median=13 years).They were recruited on one of their routine visits to the dermatology clinic, pediatric nephrology clinic, children's Hospital, Zagazig University, Egypt. In addition, 20 age and sex matched healthy children were studied as controls. They were 14 males and 16 females. Their ages ranged between 11and 16 years with a mean age±SD: 13.3±1.2 years (median=13.7 years).They were recruited from the out patient clinic of the same hospital.

All the children included in this study were subjected to detailed medical history with particular stress on age of the onset of SLE, the duration of the disease, any family history of autoimmune diseases and history of hospital admission. Cases and controls were asked about drugs which might affect anti-B19 antibodies level (e.g. steroids, cyclosporine, azathioprine and methotrexate) and had no associated illnesses (e.g. other inflammatory, immune infection or malignant diseases) which might affect B19 antibodies level before enrollment into the study. Also, antibodies against Epstein-Barr virus and cytomegalovirus were performed to be excluded from the study.

Clinical examination was performed on patients and controls. For each patient, the clinical parameters of disease were also studied. These included the affection of various systems by the disease (skin, kidney, CNS, joints, serous membranes), and to exclude blood diseases and malignancies by chest, cardiac and abdominal examination, features of chronic anemia, hepatosplenomegaly. Assessment of growth by measuring weight and height and putting on their percentile was done.

All individuals were subjected to the following investigations:

a. Autoantibody profile (ANA and anti-dsDNA) by ELISA.

b. complements(C 3, C4) levels by nephelometery.

c. erythrocyte sedimentation rate (ESR) by Westergen method.

d. anti-cardiolipin antibody (aCL) by ELISA.

e. Detection of Parvovirus B19-DNA by nested PCR:

Viral DNA was extracted using AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit (Axygen; CA; USA) as directed by the manufacturer. Nested PCR was done according to Soliman et al.[22].In the first round of amplification, 0.2µM of nucleotide primers corresponding to nucleotides 2381-2400(B19S1) and 2781-2800 (B19AS1) (5' CCTTTTCTGTGCTAACCTGC-3' and 5'-CCCAGGCTTGTGTAAGTC TT-3', respectively), were used. Two microlitres of each sample was used in a 50 µl reaction containing 5µl of 10x buffer (500mM Tris HCl,PH 8.7, 50 mM NH_4Cl, 20mM $MgCl_2$, 400 mM KCl, 1% TritonX-100), 4µl of 25 mM dNTP, 1U of Tth DNA polymerase and 36 µl water. The reaction was overlaid with mineral oil. After an initial denaturation for 5 min at 94^oC, 38 cycles of denaturation (94^oC for 45 sec), annealing (54^oC for 45sec) and extension (72^oC for 1min). Two µl of the first PCR product was then added to the second round PCR mixture containing 2µM of each oligonucleotide primers corresponding to nucleotides 2429-3448 (B19SII) and 2730-2751 (B19ASII) (5'-AAAGCTTTGTAGATTATGAG-3' and 5'-GGTTCTGCATGACTGCTATGG-3', respectively). Then 25 cycles of amplification were performed using the same parameters as the first round. Subsequently, the nested PCR products of size 322 base pairs (bp) were visualized by electrophoresis on 1% agarose gel together with a molecular weight marker.

f. Quantitative measurement of Parvovirus B19 IgM and IgG antibodies by ELISA [RIDASCREEN

ParvovirusB19 IgM and IgG (r-biopharm, Darmstadt, Germany)] were used to determine the presence of specific IgM and IgG against parvovirus B19 VP1 and VP2. Levels>0.9 are considered positive.

Statistical methods

The data of the study was statistically analysed by SPSS (Statistical package for social sciences) version 15. Chi square test was used for comparison between studied groups. Spearman correlation coefficient rank test was used to rank different variables against each other in linear correlation.

RESULTS

Table 1. Clinical manifestations of SLE patients with and without B19 DNA.

Clinical feature	**With B19 DNA (*n*=11)** Count (%)	**Without B19 DNA (*n*=19)** Count (%)	***p***
Fever	4 (36.3)	5 (26.3)	>0.05
Rash	8 (72.7)	12 (63.1)	>0.05
Arthritis	8 (72.7)	11 (57.8)	>0.05
Serositis	3 (27.2)	5 (26.3)	>0.05
CNS	1 (9)	2 (10.5)	>0.05
RP	4 (36.3)	4 (21)	>0.05
High ESR	7 (63.3)	9 (47.3)	>0.05
Cytopenia	6 (54.5)	10 (52.6)	>0.05
ANA	7 (63.3)	10 (52.6)	>0.05
Anti-dsDNA	4 (36.3)	7 (36.8)	>0.05
Low C3/C4	8 (72.7)	5 (26.3)	**<0.01**
Proteinuria	4 (36.3)	8 (42.1)	>0.05
aCL	1 (9)	3 (15.7)	>0.05
B19 IgM +ve	1(9)	9 (47.3)	**<0.05**
B19 IgG +ve	2(18)	11(57.8)	**<0.05**

*Numbers in parentheses are percentages.
*CNS=central nervous system; ESR=erythrocyte sedimentation rate; ANA=antinuclear antibodies; anti-dsDNA=anti-double-stranded DNA; aCL=anti-cardiolipin antibody

Table 2. Parvovirus B19 IgM and IgG antibodies levels in children with SLE.

	With B19 DNA (*n*=11)	**Without B19 DNA (*n*=19)**	***p***
B19 IgM (pg/dL)			
Mean (SD)	0.45 (0.26)	1.3 (1.1)	**0.01**
Median	0.4	0.7	
Minimum	0.2	0.1	
Maximum	1.1	3.4	
B19 IgG (pg/dL)			
Mean (SD)	0.65 (0.33)	4 (3.8)	**0.03**
Median	0.5	1.2	
Minimum	0.3	0.45	
Maximum	1.3	10	

Table 2 shows that there is significant difference between IgG and IgM anti-B19 antibodies levels in SLE patients.

Table 3. Correlation between anti-B19 antibodies IgG and IgM levels in SLE patients.

	B19 IgG (pg/dL)	
	r	p
B19 IgM (pg/dL)	0.942	<0.05 **S**

Table 3 shows that there is positive correlation between the level of IgG and the level of IgM Ianti-B19 antibodies in SLE patients.

DISCUSSION

The relationship of parvovirus B19 infection with SLE is an issue of interest. There are striking similarities between B19 infection and SLE. It is difficult to differentiate B19 infection from SLE clinically[6]. Parvovirus B19 may be accompanied by a transient sub clinical state of autoimmunity[21] and may mimic or exacerbate SLE[6,20]. It may be implicated in the development of SLE as well as other chronic arthropathies[5,21].

Kurtzman et al.[23] demonstrated that the production of antibody to the B19 capsid protein plays a major role in limiting parvovirus infection in man. It has also been reported that, in the immunocompetent host, the production of B19 specific antibodies results in clearance of the viremia within a few days, whereas in immunocompromised patients the virus persists[14,24].

The appearance of B19 specific neutralizing antibodies might alter the course of viral infection. The persistence of infection with parvovirus B19 in our patients with SLE may have been due to lack of antibodies against parvovirus B19 because the host was immunocompromised or because of the use of immunosuppressive agents. However in El-Eishi et al.[25] study, this is not feasible because both groups, the parvovirus positive and negative groups were on similar regimens of immunosuppression.

Parvovirus B19 infection may have exacerbated the clinical course of SLE. However, there was no apparent association between the presence of B19 DNA and other clinical manifestations, such as skin rash, arthritis and proteinuria, in patients with SLE.

It seems that PB19 might induce either idiopathic SLE in a person who is genetically susceptible or it might induce a SLE-like picture[26]. In our study, Parvovirus B19 DNA was detected in 11 of the 30 patients with SLE (33.3%) while it was not detected in any of our normal controls. Of the 11 patients with B19 DNA, only two had IgG anti-B19 antibody and one had IgM anti-B19 antibodies, whereas IgG and IgM anti-B19 antibodies were detected in 11 (57.8%) and 9 (47.3%) of 19 SLE patients without B19 DNA respectively. B19 DNA was found more commonly in sera from SLE patients without anti-B19 antibodies than in those with anti-B19 antibodies ($p<0.05$). In harmony with our results, El-Eishi et al.[25] parvovirus could be detected in 30% of the SLE population studied and in none of the normal controls ($p<0.05$). Also, Hsu et al.[27] Parvovirus B19 DNA was detected in 17 of 72 (24%) patients with SLE by PCR and was confirmed by Southern blotting and the prevalence of IgG and IgM anti-B19 antibodies in sera from SLE patients with B19 DNA was much lower than in patients without B19 DNA ($p<0.05$).

In contrast to our study, El-Eishi et al.[25] studying the characteristics of the two groups of SLE patients, those with PCR positive and those with PCR negative test results, they could not detect any statistically significant differences between the two groups to help delineate a cause-effect relationship. Moreover, the two groups of patients were similarly immunosuppressed by steroids and cytotoxic agents in various combinations.

The presence of B19 DNA in patients with SLE may not be causative. It may rather reflect a superimposed B19 infection in patients with SLE due to lack of antibodies against B19.

As B19 DNA was detected only in patients with SLE, its clinical significance was studied further. Hypocomplementemia was significantly more common in patients with B19 viremia than in those without B19 DNA ($p<0.05$). Hsu et al.[27] demonstrated that hypocomplementemia and RP were significantly more common in patients with B19 viremia than in those without B19 DNA. We had questions that needed answers, firstly, if parvovirus B19 infection is a cause of SLE, secondly did it cause idiopathic SLE or it just induced a SLE-like picture that was not actually idiopathic SLE? Most of the case reports implicating parvovirus B19 as a cause of a lupus-like illness described, in addition to the lupus-like clinical features, positive serological tests for SLE, including anti-Smith antibody and also the anti-double stranded DNA which is considered by many investigators to be highly specific and committing for the diagnosis of idiopathic SLE. For this reason, we could not rely on any specific serological tests for differentiation between SLE and a parvovirus-induced lupus-like syndrome; rather we relied on disease duration and the absence of renal affection. The disease duration of the self-limiting lupus-like disease induced by B19 infection tended to be two years or less in most of the reported cases. Concerning the absence of renal affection; El-Eishi et al.[25] noted that the spectrum of clinical and serological features of such patients included common features and less common features. The common features included rash, fever, malaise, fatigue, arthritis, leucopenia, thrombocytopenia, and hypocomplementemia with a broad spectrum of autoantibodies. Less common ones were oral ulcers and Raynaud's phenomenon (a single report of each). As far as our knowledge, renal affection was never reported in the setting of the self-limiting lupus-like illness induced by parvovirus. Interestingly, this observation was never commented upon in the literature. However Hsu et al.[27] noted that proteinuria was present in (35.3%) of SLE patients with B19 DNA and in (32.7%) of SLE patients without B19 DNA.

The lupus illness is characterized by the classical features of SLE, which are the probability of renal disease and disease duration of not less than two years. On the other hand, the lupus-like disease does not have usually comprised renal affection and it runs a much more benign course than that of the idiopathic type.

Nevertheless, in order to decide that disease duration less than two years duration and the absence of renal affection should suggest a parvovirus-induced lupus-like illness, further studies are needed. From this study, we suggested that B19 may exacerbate or even induces SLE by autoimmune mechanism (molecular mimicry), in favor to this theory, affinity purified anti-VP1 IgG

from patient with skin rash and chronic B19 arthritis has been found to cross react with human keratin, collagen type II, denatured DNA and cardiolipin[28].

Antibodies against Epstein-Barr virus and cytomegalovirus were performed to be excluded from the study as it was known that false positive ParvovirusB19 IgM and IgG results may be seen with other acute viral infection. Previous viral studies have shown antigenic relationship between peptides from the Epstein-Barr nuclear antigen and Sm, the latter associated mainly with SLE[29].

In conclusion parvovirus B19 might induce either idiopathic SLE in a person who is genetically susceptible or it might induce a SLE-like picture. B19 infection in patients with SLE may be due to lack of anti-B19 antibodies because of either the immunocompromised nature of the host or the use of immunosuppressive drugs. There was a higher prevalence of hypocomplementemia in patients with parvovirus B19 viremia than in those without parvovirus.

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