Is Parvovirus B19 Infection Incriminated in Chronic Fatigue Syndrome?

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ABSTRACT

Background: Parvovirus B19 is a small DNA virus that belongs to the genus Erythrovirus. It is the cause of erythema infectiosum in children and incriminated in the pathogenesis of chronic fatigue syndrome (CFS), which is a heterogeneous disorder of unknown pathogenesis and etiology. Objective: We aimed to determine the possible role of parvovirus B19 in the etiology of CFS. Methods: 50 patients with CFS and 45 people with no symptoms comparable with CFS as a control were investigated for specific anti B19 IgG and IgM, also B19 DNA in their sera. Results: No specific anti B19 IgM was detected in both CFS patients and control, anti B19 IgG was detected in 26 of 50 CFS patients (52%) and in 23 of 45 control (51.1%). Also B19 DNA was found in 4 of 50 CFS cases (8%), and in 3 of 45 control (6.7%). There was no statistical difference between patients with CFS and control for the presence of parvovirus B19. Conclusion: B19 infection bears no apparent relationship to CFS.

INTRODUCTION

Human Parvovirus B19 (PVB19) (genus, Erythrovirus) was discovered fortuitously in 1975 by Cossart et al., who unexpectedly found viral particles in the sera of asymptomatic patients being screened for hepatitis B infection(1).

Biochemical and molecular characteristics subsequently demonstrated that these particles were paroviruses and, because specimen 19 of panel B contained the unexpected virus, PVB19 was so designated . The B19 virus is a small (20-25 nm) in diameter, non enveloped, single stranded DNA icosahedral virus that encodes 3 major proteins, 2 structural proteins (96% VP1 and 4% VP2), and a nonstructural protein (NS1)(1,2).

Human PB19 belongs to the family Parvoviridae (from parvum, the Latin word for small) and to the genus Erythrovirus. It is a heat-stable virus and can survive at 60°C for up to 12h. For the purpose of replication, PB19 is dependent on erythroid precursor cells in the bone marrow, which are destroyed in the process(3).

Human PB19 is usually transmitted via the respiratory route, but can also be acquired by blood transfusions or can be passed from mother to the fetus (vertical route). It is estimated that, in the western world, by 15 years of age 50% of all individuals have been infected, and these figures could rise to 80% or 100% in the elderly. The virus causes a variety of human diseases, including erythema infectiosum (fifth disease) in children, spontaneous abortion in early pregnancy, pure red blood cell aplasia in immunocompromised persons and aplastic crisis in patients with hemolytic disorders(4).

Chronic fatigue syndrome (CFS) was first described in the 1980s, is a heterogeneous disorder affecting more than 267 per 100,000 people. The reported prevalence of CFS is 0.2–2.6%, with women being affected almost twice as often as men.

A similar prevalence was found in different geographic locations and in diverse ethnic groups(5).

The pathophysiology and etiology of CFS are unknown, because there are no characteristic physical signs or diagnostic laboratory abnormalities. It is defined by self-reported symptoms and disability, but only about 1% of the patients who are given the diagnosis in primary care settings meet the criteria for CFS(6).

CFS patients suffer from disabling fatigue, headaches, concentration difficulties and memory deficits (90%). Additional symptoms are often observed, such as sore throat (85%), tender lymph nodes (80%), skeletal muscle pain and feverishness (75%), sleep disruption (70%), psychiatric problems (65%), and rapid pulse (10%). Due to these complaints patients often face social problems, the loss of jobs and the break-up of marriages(7).

The diagnosis of CFS is complex due to its similarity with other ill-defined disorders, such
as fibromyalgia, Gulf War syndrome and Sjögren syndrome. In 1994 Fukuda et al.\(^8\) reported the significant overlap between CFS and fibromyalgia, and considered CFS as a subclass of prolonged fatigue. They proposed a method for obtaining the correct diagnosis: a patient must present four or more symptoms concurrently for at least 6 months. These criteria are: a) a coexisting medical or neuropsychiatric condition that does not explain the chronic fatigue; b) the level of fatigue, including subjective and performance aspects; c) the total duration of fatigue; and d) the level of overall functional performance. Characteristics excluding patients from CFS include active medications, past or current major depressive disorders, alcohol abuse, and severe obesity\(^8\).

CFS was thought to be the consequence of a viral or bacterial infection, because of the patients' immunological findings. One of the first suspected pathogens was Epstein-Barr virus, because patients often have higher titers of IgM to the EBV viral capsid antigen. Also, antibodies against cytomegalovirus and human herpes virus-6 were detected more often in CFS patients, although other reports failed to repeat these results\(^9\). Another virus family studied as a possible cause of CFS is the enterovirus, since RNA copies were detected in muscle biopsies of CFS patients but not in a healthy control group. In other studies no association between enteroviruses and serological tests was recorded\(^10\).

Human Parvovirus B19 is considered one of the most probable causes of CFS, based on several case reports of patients with a chronic course of fatigue after infection, fulfilling the criteria for CFS diagnosis. In addition, a higher prevalence of Mycoplasma infections has been reported in CFS patients than in healthy subjects\(^9\).

One characteristic of B19 infection (erythema infectiosum) is the presence of viremia, as shown by detection of B19-DNA in blood, sometimes for several years after infection. While the significance of this is uncertain, it provides a mechanism whereby B19 infection could be related to CFS. Previous studies testing this relationship have been inconclusive because of the small number of samples tested for prevalence\(^11\). Therefore, we examined the prevalence of B19 infection in a larger number of samples

**SUBJECTS, MATERIAL & METHOD**

Sera from fifty patients with CFS attending Benha University Out Patient Medical Clinic were collected in the period between March 2008 to January 2009. They were 18 males and 32 females, their ages ranged between 28 to 47 years (34.7± 8.6).

Also sera from forty five people between 19 and 51 years old (37.2 ±9.5) who did not show any symptoms comparable to CFS were used as controls. Sera were stored at −70°C in 1.5-mL Eppendorf tubes until processing.

1. **Enzyme immuno assay (EIA)**

   Detection of specific B19 IgG and IgM antibodies in serum was achieved using commercial ELISA kits that use recombinant capsid protein VP2 as antigen. Assays were carried out following the instructions of the manufacturer, Immunobiological Laboratories, Inc. (IBL-USA) for Human Parvovirus B19 IgM ELISA and Human Parvovirus B19 IgG ELISA). Sera that were positive in the IgM ELISA were treated with adsorbent solution to avoid false-positive results caused by the presence of rheumatoid factors and re-tested with the (IBL- USA IgM ELISA kit).

2. **Real-time PCR**

   B19 DNA was recovered from serum according to the method described by Cassinotti et al. (1993)\(^12\). A volume of 100 ul of sample was mixed with 10ul of 10 K buffer (0.5% Tween 20 in 500mMKCl, 15mMMgCl2, 100mMTris-HCl, pH8.3), then 3ul of proteinase K was added at a final concentration of 600ug/ml. The sample was incubated at 50 °C for 60 min, then at 95 °C for 10 min to inactivate proteinase K. Finally, the sample was centrifuged and 3 UL of cleared supernatant was used in the amplification reaction.

   PCR amplifications were carried out with two successive rounds of 35 cycles performed in a 2400 thermocycler (Perkin-Elmer, Norwalk, CT). In the first round, each cycle consisted of a denaturation step at 94 °C for 1 min, followed by an annealing step at 50 °C for 2 min. Since satisfactory primer extension occurred during the heating from annealing to denaturation temperature we omitted a specific elongation step. For the second round the annealing temperature was changed from 50 to 60 °C for 2 min. Final extension steps at 72 °C for 7 min followed both rounds.

   The two nested primer pairs map to a fragment of the major capsid protein VP2 gene.
and were designed to yield highly specific and sensitive amplifications Cassinotti et al.\textsuperscript{(12)}, B19: P1 (nucleotides 1417–1434), 5'’-GGG CCG CCA AGT ACA GGA-3’’ and, P2 (nucleotides 2160–2141), 5’’-AGG TGT GTA GAA GGC TTC TT-3’’ for outer PCR; and P3 (nucleotides 1498–1525), 5’’-AAT GAA AAC TTT CCTTT AAT GA-3’’ and P4 (nucleotides 2088–1965), 5’’-TCC TGA ACT GGTCCC GGG GAT GGG-3’’ for internal (nested) PCR.

For a single amplification reaction we used 3ul of DNA solution, 2.5 ul dNTPs, 1ul of each primer, 0.5ul of AB taq 2.5ul phosphate buffer and bidistilled water to final volume of 25ul. Finally a drop of mineral oil was added. Amplified DNA fragments were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide. Fetal calf serum (FCS), manipulated along with the specimens was used as negative controls in order to check for possible contamination (Figure 1). The different procedures of DNA extraction, PCR assay and amplicon analysis were carried out in physically separated rooms.

The statistical analysis of quantitative data of positive serum samples was carried out by analysis of variance using the Log Transformation of data and Scheffe’ test for Post Hoc comparison. Analysis was performed using the statistical software StatView 5.0.

**RESULTS**

This study was conducted on 95 subjects, that were classified into two groups: group A (50 patients), diagnosed as CFS, and group B (45 persons), as a control. Patients’ details were summarized in Table I.

<table>
<thead>
<tr>
<th>Table I: Patients’ data</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>34.7 ± 8.6</td>
<td>37 ± 9.5</td>
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<tr>
<th>Table II: Results of anti B19 IgM , IgG , and B19 DNA by PCR</th>
<th>Group A</th>
<th>Group B</th>
<th>P</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti B19 IgM antibodies %</td>
<td>0%</td>
<td>0%</td>
<td>&gt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Anti B19 IgG titre ( IU/ml)</td>
<td>170 ± 80</td>
<td>98 ±100</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>Anti B19 IgG antibodies</td>
<td>No</td>
<td>%</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Anti B19 IgG antibodies</td>
<td>26</td>
<td>52</td>
<td>23</td>
<td>51.1</td>
</tr>
<tr>
<td>Anti B19 IgG antibodies</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>B19DNA</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Sig.: significance   S : significant   NS : non significant

<table>
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<tr>
<th>Table III. Positive B19samples versus age.</th>
<th>Anti-B19-IgG antibodies</th>
<th>B19-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td>29 ± 8</td>
<td>37 ± 4</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>24 ± 5</td>
<td>30 ± 8</td>
</tr>
</tbody>
</table>

Fig. 1. PCR products of PVB19. Lane 1, 50-bp marker; lane 2 : negative control,lanes3-4-5&6 Group I cases , lanes 7-8&9 control group.
DISCUSSION

CFS is a disease characterized by severe and debilitating fatigue, myalgia, sore throat, sleep abnormalities, impaired memory and concentration, musculoskeletal pain, stress, and secondary depression\(^{(9)}\). It has been widely reported that the onset of disease coincides with both clinical and laboratory evidence of infection with a variety of viruses and bacteria\(^{(10)}\).

Parvovirus B19 is a one-strand small (5,600 base-long) DNA virus, classified as an erythrovirus from the family of parvoviridae\(^{(4)}\).

Some studies found that CFS was preceded by a parvovirus B19 (B19) infection. However, other studies, using small numbers of samples, could not support any relationship between B19 and CFS.

In the present study, the B19 specific IgM in both groups was very similar to the profiles observed by Kato et al.\(^{(11)}\).

We found that the B19 specific IgG in CFS was 52% and that in control group was 51.1% (Table II & III). Our findings are consistent with the results reported by Kato et al.\(^{(11)}\) who reported that the B19 specific IgG was 51.7% in patients with CFS and 57.1% in control group. Schmid et al.\(^{(12)}\) found that B19 specific IgG was 50% of young adults from 10-20 years old, 46.8% in the age group of 21–30 years and 80% in the age of 70- 80 years\(^{(13)}\). Also Ronaldo et al.\(^{(14)}\) reported 28% positivity for B19 specific IgG in 889 patients from the city of Sa˜o Paulo (Brazil). The present study showed that there were a significant difference in the mean anti-B19-IgG titer between CFS patients and control (table II). A possible explanation for the elevated anti-B19-IgG titer in CFS patients is excessive, nonspecific activation of cytokines. CFS is associated with changes in the immune system, particularly with cytokine activation. It has been reported that cytokine activation may lead to the elevation of the antibody titers.

Our study showed that there were no significant difference in detection of B19 DNA by PCR between patients with CFS and control group (Table II).

Similar results was reported by Kato et al.\(^{(11)}\), who studied the prevalence of parvovirus B19 by PCR in 58 patients with CFS and 49 age matched controls and found that parvovirus DNA was found in 5.2 % of CFS patients and 4.1% in controls, with no significant difference between the two groups.

Also Kolle et al.\(^{(15)}\), who investigated twenty two monozygotic twin pairs of which one twin had criteria of CFS and the other twin was healthy, he studied different viral infections included parvovirus B19 and found that there were no difference in the presence of parvovirus B19 between the group of twins with CFS and the healthy ones.

Ilaria et al.\(^{(16)}\), investigated seven patients with CFS for parvovirus B19 by PCR in bone marrow aspirate and concomitant serum samples , their sera were also investigated for the presence of IgM and IgG antibodies to the virus. They concluded that their were no evidence of marrow involvement with the virus in any patient.

Our results are not similar to that obtained with Kerr and Mattey\(^{(17)}\), who investigated thirty–nine patients with laboratory–documented acute parvovirus B19 infection by serology and PCR and concluded a highly significant association between psychological stress and development of acute and chronic fatigue and arthritis several years following laboratory documented acute parvovirus B19 infection.

Seishima et al.\(^{(18)}\), determined how often CFS appears after B19 infection and whether prolonged B19 DNA presence, antibody production and persistently reduced complement levels occur in CFS patients after B19 infection. Clinical findings were examined in 210 patients after B19 infection, and CH50 (the total hemolytic complement), C3 and C4 levels were determined. B19 DNA and antibodies to B19 were also tested in 38 patients' sera including 3 with CFS.

They found that, serum B19 DNA disappeared after 4-5 months in all patients tested. There were no differences in B19 DNA-positive period between patients with and without persistent symptoms. IgM antibody titers to B19 became reduced after 2 months in all patients. Complement levels persistently decreased in a greater proportion of patients with persistent symptoms. They concluded that the possibility of CFS after B19 infection should be considered and that CFS may be derived from several aspects other than prolonged B19 DNA presence in sera\(^{(16)}\).

Similarly McGhee et al.\(^{(19)}\), reported a 16 year old boy, who had two years history of CFS after parvovirus B19 viremia.

Also Matano et al.\(^{(20)}\), suggested that parvovirus B19 caused clinical features similar
to those of chronic fatigue in cases who had prior life stressors. Kerr and Tyrell (21), stated that, although CFS may be caused by varius microbial and other triggers, that triggered by B19 virus were clinically indistinguishable from idiopathic CFS and exhibited similar cytokines abnormalities. Kerr et al (22), successfully treated three cases of CFS that followed acute parvovirus B19 viremia, with improvement in physical and functional ability in all patients.

Conclusion
The present study support that parvovirus B19 infection bears no apparent relationship to CFS.

REFERENCES