Molecular Detection of Human Parvovirus B19 among Blood Donors in Southern Darfur State, Sudan

Eibthal M. Osman¹, Mustafa E Yassin², Abbas B. Mohammed², Nasealdeen M. Bush¹

¹Omdurman Islamic University, Omdurman, Sudan
²Al Neelain University, Khartoum, Sudan.

Abstract

Background: A spectrum of blood infectious agents are transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors. Human parvovirus B19 is an emerging transfusion transmitted infectious agent. Parvovirus B19 infection is connected with severe complications in some recipients. It has been associated with a wide number of diseases and it may cause no clinical symptoms. Mainly it may cause a flu-like illness, an acute infection resulting in anemia, erythema infectiosum (fifth disease), a rash illness of childhood, spontaneous abortion and non-immune hydrops fetalis in pregnant women, and acute symmetrical polyarthropathy in adults.

Objective: To perform molecular detection of human parvovirus B19 among blood donors in Southern Darfur State, Sudan.

Materials and methods: This was a descriptive, cross-sectional study, conducted during the period from September 2016 to April 2017. A total of 110 blood donors were tested for B19 VP1 coding gene using the nested polymerase chain reaction (PCR) technique.

Results: Out of 110 blood donors, 8 samples were positive for parvovirus B19 (7.3%).

Conclusion: The prevalence rate of B19V DNA among Sudanese blood donors in Southern Darfur State was 7.3% and there was no statistically significant difference between the prevalence rate of human parvovirus B19 DNA and other variables in the study group.

Key words: Human parvovirus B19, Blood donors, Nested PCR, Southern Darfur State.

Introduction

Human parvovirus B19 was identified in 1974 by Yvonne Cossart. It is the only member of the Parvoviridae family known to be pathogenic in humans. It belongs to the Parvovirinae.
subfamily, the *Erythroparvovirus* genus, and the *primate erythroparvovirus 1* species. It is a small, non-enveloped, single-stranded DNA virus with a virion diameter of 18-26 nm. The name parvovirus originates from the Latin word parvum, which means small. The virus links to a specific receptor on the surface of host cells called P blood group antigen globoside-4(Gb4). Individuals who lack this antigen are not susceptible to human parvovirus B19 infection. The transmission of human parvovirus B19 occurs mainly via respiratory droplets but it can also spread by contaminated blood, organ transplantation, vertical transmission, contaminated plasma donations, or plasma derived from medicinal products. Transmission of human parvovirus B19 by blood products is favored by two important viral characteristics: a) persistent infection in the bone marrow of asymptomatic individuals; and b) prolonged replication (up to several years) after initial infection/reinfection. Human parvovirus B19 has been associated with a wide spectrum of diseases, generally, human parvovirus B19 infection occurs without any clinical symptoms; or it may cause a flu-like illness. Besides, it may lead to acute infection resulting in anemia, erythema infectiosum (fifth disease), a rash illness of childhood, spontaneous abortion and non-immune hydrops fetalis in pregnant women, and acute symmetrical polyarthritis in adults. Depending on the hematologic state of the host, human parvovirus B19 infection may be associated with hematopoietic disorders like aplastic crisis, thrombocytopenia, and pancytopenia. Hepatitis, myocarditis, myositis, neurological disease, vasculitis and persistent arthropathy may occur occasionally. Persistent human parvovirus B19 infection has been reported in patients both with and without underlying immunodeficiencies. Human parvovirus B19 serology (anti-B19V immunoglobulin IgM and IgG antibodies) can be determined using enzyme-linked immuno-sorbent assay (ELISA), radioimmunoassay, chemiluminescence or immunofluorescence. PCR analysis or alternative nucleic acid amplification technology (NAT) assay (e.g., transcription-mediated amplification) may be a better diagnostic tool as viral titres can reach more than $10^{12}$ genome equivalents (geq) per mL during acute infection. On the other hand, in chronic B19V infection, viral DNA can persist in the host without the presence of B19V IgM or IgG. In the immunocompetent host, viral DNA is detectable for at least one month after infection but can persist even longer at low levels.

In a study conducted among Korean blood donors, 10,032 plasma specimens were investigated, and the prevalence rate of B19V DNA was 0.1%. Virus titers in B19V DNA positive donors were less than $10^5$ IU/mL (range: 2.7×10^1-3.2×10^4 IU/mL), except for one donor (1.33×10^8 IU/mL). Nine out of 10 B19V DNA positive donors also possessed anti B19V IgG only or IgG and IgM. The prevalence rate of anti-B19V IgG was 60.1%.

In United Kingdom and sub-Saharan Africa (Ghana, Malawi, and South Africa) the prevalence rate of human parvovirus B19 viremia in 2,440 donated bloods were studied. They found that 1:3300 donors were B19 DNA PCR positive, whereas 1:260 was viremic.

Though incidence and prevalence rates of human parvovirus B19 infection in blood donors has been documented in western literature, however, until date there is no reliable data of the prevalence rate among blood donors in Sudan. Thus, there is a need to explore the prevalence rate of human parvovirus B19 among Sudanese blood donors; and thereby, prevent and/or minimize its transmission in various clinical settings.

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Materials and methods

This was a descriptive and cross-sectional study conducted in Southern Darfur State (Sudan) during the period from September 2016 to April 2017. Confidentiality of information obtained from participants investigated was maintained. Verbal consent of blood donors was taken before being enrolled in the study. Laboratory results of specimens collected were handed to all participants included in the study. Permission to collect the specimens was obtained from the blood bank authorities at Nyala Teaching Hospital. Approval to run the study was taken from the Ethical Committee of the Islamic University, Omdurman (Sudan). Complete information regarding risk factors, if any, was explained to all participants under the study without concealment. Data analysis was performed by the statistical package for social sciences (SPSS) version 21. Chi-square test was applied to assess the association between the categorical variants; and p-value < 0.05 was considered significant.

Sampling was a non-probability purposive type, and sample strategy was convenience where participants were chosen on the basis of accessibility. Sample size was 110 blood specimens collected from blood donors. Demographic and clinical data were collected from all participants using a structured questionnaire. 5 ml of blood were collected from each blood donor in plain containers, centrifuged at 3000 rpm for 5 minutes, and plasma was separated and subjected to DNA extraction.

Procedure: DNA was extracted from plasma using the phenol/chloroform/isoamyl alcohol (PCI) protocol. 500 µl of 5% Sodium dodecyl sulfate (SDS) were added to 250 µl of patient's plasma. Then 20 µl of proteinase K were added and incubated at 56°C for one hour. After that, samples were incubated at 95°C for 10 minutes to deactivate proteinase K. Then 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1) solution were added, and centrifuged at 12000 rpm for 10 minutes. The upper layer was transferred into new Eppendorf tube and 1000 µl of absolute ethanol were added and incubated at -20°C overnight. Next day, samples were centrifuged at 16000 rpm for 15 minutes, the supernatant was discharged and the tubes were dried. Then 200 µl of 70% ethanol were added, shaked-up until the pellet disappears, and centrifuged at 16000 rpm for 15 min. After that, the supernatant was discharged, and the tubes were inverted open for 2 hours. Then 250 µl distilled water were added and DNA preserved at -20°C. DNA of control samples (human parvovirus B19 positive plasma) was also extracted as described above.

To detect parvovirus B19 DNA, the virus B19 VP1 coding gene was amplified using nested PCR employing two sets of outer and inner primers pairs:

1. The outer primers pair were:
   (P1) 5’-CAAAAGCATGTTGAGTGAGG-3’(sense)
   (P2) 5’-CTACTAACATGCATAGGCGC-3’(antisense).

2. The inner primers pair were:
   (P3)5’-CCCAGAGCACCATTATAAGG-3’(sense)
   (P4) 5’-GTGCTGTCAATGACCTGTAC-3’(antisense).

The amplification was performed as per the following protocol:

a) First round of amplification: Outer primers (P1 and P2) were used in this round. 30 cycles
were programmed. Denaturation was made for one minute at 94°C, annealing of outer primers was made for two minutes at 55°C, primers extension was made for one minute at 72°C, and then final extension was made for five minutes.

b) Second round of amplification: This round was performed in the presence of the inner primers (P3 and P4). The cycling parameters of the second amplification round were same as the first round expect that the primers annealing step is carried out at a temperature of 57°C instead of 55°C. Positive and negative controls were processed using the same protocol. Later, the PCR products were analyzed using gel electrophoresis on 2% (w/v) agarose gel in TAE buffer and stained with ethidium bromide. The amplicon of 288 base pairs (bp) were visualized by using the gel documentation system of Syngene, Bioimaging, UK (Fig.1).

Fig. (1): Nested PCR product on 2 % agarose gel electrophoresis


Results

A total of 110 blood donors samples were enrolled in this study. Out of these 8 (7.3%) blood donors were found positive for parvovirus B19 DNA. The prevalence rate of parvovirus B19 DNA among different age groups was 5.3%, 8.3% and 8.3% in the age groups 18-26 years, 27-35 years and 36-44 years respectively (Table 1).

Regarding the education level, the frequency rate of parvovirus B19 DNA was 16.7% among uneducated blood donors, 8.3% among primary educated blood donors, and 9% among university educated blood donors (Table 1).

The prevalence rate of parvovirus B19 DNA was 9.5% among donors with blood group

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O-positive, 6.7% among donors with blood group A-positive, and no detectable parvovirus B1 DNA among donors with other blood groups (Table 1). There was no statistical significant difference between the prevalence rates of parvovirus B19 DNA in all study variables (p > 0.05).

Table (1): Frequency rate of parvovirus B19 DNA according to age incidence, education, and blood groups of blood donors investigated

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive parovirus B19 DNA</th>
<th>Negative parovirus B19DNA</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age incidence:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-26 years</td>
<td>2 (5.3%)</td>
<td>36 (94.7%)</td>
<td>38</td>
<td>0.840</td>
</tr>
<tr>
<td>27-35 years</td>
<td>4 (8.3%)</td>
<td>44 (91.7%)</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>36-44 years</td>
<td>2 (8.3%)</td>
<td>22 (91.7%)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td><strong>Education:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uneducated</td>
<td>3 (16.7%)</td>
<td>15 (83.3%)</td>
<td>18</td>
<td>0.096</td>
</tr>
<tr>
<td>Primary School</td>
<td>1 (8.3%)</td>
<td>11 (91.7%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>High School</td>
<td>0 (0%)</td>
<td>36 (100%)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>4 (9 %)</td>
<td>40 (91%)</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td><strong>Blood group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-positive</td>
<td>7(9.5%)</td>
<td>67 (90.5%)</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>B-positive</td>
<td>0 (0%)</td>
<td>11 (100%)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>A-positive</td>
<td>1(6.7%)</td>
<td>14 (93.3%)</td>
<td>15</td>
<td>0.824</td>
</tr>
<tr>
<td>O-negative</td>
<td>0 (0%)</td>
<td>4 (100%)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>AB-positive</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B-negative</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Blood transfusion is a branch of medicine in the healthcare section. An incorporated strategy for blood safety is required for elimination of transfusion-transmitted infections and for provision of safe and adequate blood. The prevalence rate of parvovirus B19 DNA among blood donors in Southern Darfur State (Sudan) was found to be 7.3%. This result was higher than that reported by Ke and his colleagues (0.58%) in Chengdu, China7 and higher than the findings of two recent studies (8, 9).

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investigated plasma donations and serum samples of asymptomatic blood donors and found a prevalence rate of parvovirus B19 DNA as approximately 1:800 and 1:3915 respectively. There was no statistically significant difference between the prevalence rate of parvovirus B19 DNA and other variables in the study group.

Conclusion: The prevalence rate of B19V DNA among Sudanese blood donors in Southern Darfur State was 7.3% and there was no statistically significant difference between the prevalence rate of parvovirus B19 DNA and other variables in the study group.

References


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