Human Parvovirus B19 Infection in Females with Recurrent Spontaneous Abortions

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ABSTRACT

Human parvovirus B19 is a small DNA virus that is transmitted mainly through contact with respiratory secretion or aerosols, but it may be transmitted to the fetus transplacentally from infected mother leading to many complications mainly fetal hydrops, intrauterine fetal death or spontaneous abortion. In this study we aimed at assessment of the relation between maternal human parvovirus B19 infection and recurrent spontaneous abortion (RSA) and comparing the serological diagnostic methods and PCR for diagnosis of B19 infection in females with RSA. Sera were collected from 50 patients with RSA (patients group) and 25 pregnant females without history of abortion (control group) and all sera were tested by nested PCR to detect B19 DNA and by EIA to detect B19 Ig M and Ig G. 19 DNA was detected in 58% in the patients group and in 4 % in control group while B19 Ig M was positive in 6% of the patients group and 4 % in the control group but B19 Ig G was positive in 62% and 8% of the patients and control groups respectively.

Key Words: Human parvovirus B19 - Recurrent spontaneous abortion - Nested PCR- Ig M – Ig G

INTRODUCTION

Human parvovirus B19 (B19) is a member of the genus Erythrovirus within the subfamily Parvovirinae, it is a small nonenveloped with a linear single-stranded DNA genome of ~5.6 kb (1,2).

It was first identified in the sera of normal blood bank donors being screened for the hepatitis B virus (3). Parvovirus B-19 is the only parvovirus that is known to cause disease in humans (4). The virus exclusively infects humans and replication occurs primarily in erythrocytes and erythroblasts, which can lead to anaemia in predisposed individuals (5). The virus has a predilection for rapidly multiplying erythroid progenitor cells. This is explained by the presence of the virus receptor, globoside. Globoside, or blood group P antigen, is mainly found on the erythroid progenitor cells, erythroblasts and megakaryocytes. It is thought to be the cellular receptor for parvovirus B-19 (6).

Transmission occurs most commonly by personal contact via aerosol or respiratory secretions; however, contaminated blood products, such as clotting factor concentrates, are a source of iatrogenic transmission (7). B19 can be transmitted transplacentally from an infected mother to the fetus, which leads to non-immune fetal hydrops (NIHF), spontaneous abortion, or intrauterine fetal death (8). Children who are infected with parvovirus B19 typically develop erythema infectiosum (fifth disease) which is characterized by a “slapped-cheek” rash, low-grade fever, and mild influenza-like symptoms (9).

The maternal infection caused by the virus may be symptomatic or asymptomatic. The fetus seems to be most susceptible to parvovirus B19 infection during the first and second trimester of pregnancy and especially between weeks 10 and 20, which coincide with the major development of the erythroid precursor (10,11). By the third trimester, the fetus is able to mount a more effective immune response to the virus, which may account for the decrease in fetal loss at this stage of pregnancy (12). Destruction of fetal red blood cells can lead to non-immune hydrops fetalis (13).

The infection by parvovirus B19 is diagnosed by the detection of Ig M antibodies in both maternal and fetal blood, but in some cases, maternal anti B19-Ig M can no longer be detected when hydrops fetalis develops (14). Parvovirus B19 Ig M usually appears within 2 to 3 days after acute infection and may persist up to 6 months while Ig G appears a few days later and remains present for life. Presence of Ig G and absence of Ig M suggest immunity while if both are negative suggest lack of immunity and susceptibility to infection. Presence of parvovirus B19 Ig M with no evidence of Ig G suggests either recent infection or a false positive result. Detecting specific Ig M to parvovirus appears to be a reliable screening indicator for women with recurrent abortions (15).
hybridization, and in situ hybridization might be the best indicator of infection, not only in fetal but also in maternal blood, at least in doubtful cases\(^{14}\). Viral DNA detection improves the diagnostic value of the serologic tests.\(^{16, 17}\).

**Aim of the work:**
- Detection of the prevalence of human parvovirus B19 infection in females with recurrent spontaneous abortions.
- Comparing the role of Ig G, Ig M and DNA detection in the diagnosis of the human parvovirus B19 infection in females with recurrent spontaneous abortions.

**SUBJECTS & METHODS**

The study was conducted on 50 patients and 25 control subjects from those attending the gynecology and obstetric outpatient clinics at Kasr Alainy Hospitals, Faculty of Medicine, Cairo University during the period from December, 2012 to August, 2013.

**Study population:**
- **Patients group:** fifty patients were selected in this group with history of two or more spontaneous abortions with unknown medical cause. Patients with history of only one abortion or abortions due to known medical cause were excluded from the study.
- **Control group:** twenty five pregnant females were included in the study as a control group and they were 2\(^{nd}\) gravida or more, pregnant in the last trimester (gestational age not less than 28 weeks) and with no previous history of abortion.
- Informed consents were obtained from the patients and control subjects before starting our study.

**Methods**
- All patients and healthy control subjects involved in this study were subjected to full medical history & laboratory investigations.
- All laboratory tests were performed on serum samples.

1- **Sera collection, preparation and storage:**
- A volume of 2 ml of serum was separated aseptically from blood of each patient and healthy control by centrifugation, and divided into 3 aliquots to prevent repeated freezing & thawing of the same aliquot. Only clear, non hemolyzed specimens were used.
- Sera were coded and stored at -20°C till processing.
- Sera were brought to room temperature prior to testing. Frozen specimens were completely thawed and mixed well prior to testing.

2- **Detection of human parvovirus B19 antibody Ig M in the serum samples:**
- It was done using a third generation qualitative capture enzyme immunoassay (Diasorin- Biotrin; catalog no. V619IM, Diasorin-ltd/Biotrin-international-ltd, Dublin, Ireland) that detect Ig M antibody against human parvovirus B19 structural proteins VP1 and VP2.
- The kit contained 3 standard controls (S1, S2 & S3) that were used in interpretation of the results as recommended by manufacturer briefly; samples with B19V-IgM index value of \(\leq S1\) (negative standard control) were considered negative, samples with B19V-IgM index value of \(\geq S3\) (low positive standard control) were considered positive and samples with B19V-IgM index value of equal S2 (cut-off standard control) were considered equivocal samples and were retested and if the results were equivocal again they were considered as negative samples as recommended by the manufacturer.

3- **Detection of human parvovirus B19 antibody Ig G in the serum samples:**
- It was done using a third generation qualitative indirect enzyme immunoassay (Diasorin- Biotrin catalog no. V619IG, Diasorin-ltd/Biotrin-international-ltd, Dublin, Ireland) that detect Ig G antibody against human parvovirus B19 structural proteins VP1 and VP2.
- The interpretation of the results was done according to manufacturer recommendations and it was on the same rules as that of Ig M.

4- **Detection of the human parvovirus B19 DNA by Nested PCR:**

**A) DNA Extraction:**
- The sera were allowed to thaw at room temperature.
- A volume of 200 µl of each sample was used to extract DNA using QIAamp DNA mini kit (QIAGEN, Germany).
- The extracted DNA was placed at -20°C until PCR testing was performed.

**B) DNA amplification by Nested PCR:**
- It was done using Biometra T-Personal thermal cycler (48 wells, Ver 09/10, Germany).
- Two sets of lyophilized specific oligonucleotide primers (table 1), that amplify well-conserved region in human parvovirus B19 genome, were used.
The first PCR amplification reaction was done by adding 10 µl of the extracted DNA to 13 µl of a PCR master mix 2X solution Promega (catalog no. M7502) (Promega, BioSciences, CA, USA) (containing; 2.5 U of Taq DNA polymerase, 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3mM MgCl2.) and one µl of each primer of the outer primer set to reach a final amplification reaction volume of 25µl. The amplification conditions were; 5 minutes at 95 ºC and 35 cycles of 91 ºC for 1 minute, 50 ºC for 1 minute, and 67 ºC for 3 minutes.

The second PCR amplification (nested) reaction was done by adding 10 µl of the first PCR product to 13 µl of a PCR master 2X solution Promega and one µl of each primer of the inner (nested) primer set to reach a final amplification reaction volume of 25µl. The amplification conditions were; 35 cycles of 91 ºC for 1 minute, 50 ºC for 1 minute, and 67 ºC for 3 minutes (17).

C) Detection of the amplified nested PCR product:

Ten µl of the amplified PCR products were analyzed by 1.5% agarose gel electrophoresis with a molecular size marker gave different bands ranging from 100-1000 base pair and were visualized using UV transilluminator (Biometra TI 1, Prod.No.1706301) to see the amplification band of 218 bp specific to human parvovirus B19.

Table 1: Sequences of the Oligonucleotide primers

<table>
<thead>
<tr>
<th>Primer set</th>
<th>sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer set</td>
<td></td>
</tr>
<tr>
<td>- Forward (P1)</td>
<td>5-TGT GGT AAG AAA AAT AC-3</td>
</tr>
<tr>
<td>- Reverse (P6)</td>
<td>5-TCA TTA AAT GGA TTT-3 (17).</td>
</tr>
<tr>
<td>Inner set</td>
<td></td>
</tr>
<tr>
<td>- Forward (P2)</td>
<td>5-GGA ACA GACTTA GAG CTT ATTC-3</td>
</tr>
<tr>
<td>- Reverse (P5)</td>
<td>5-ACC CAT CCT CTC TGT TTG ACT TAG TTG CTC GTAT-3 (17).</td>
</tr>
</tbody>
</table>

RESULTS

Our study included 50 female patients with recurrent spontaneous abortions; their age ranged from 19 to 39 years with mean age of 28.68 ± 5.53. Also the study included 25 control pregnant females in the last trimester with no history of abortion and their age ranged from 21 to 38 years with the mean age of 28.84 ± 5.07.

Out of 50 patients with recurrent spontaneous abortions, 29 patients (58.0%) had human parvovirus B19 DNA in their serum samples detected by PCR while only one female subject (4.0%) out of the 25 females of the control group had human parvovirus B19 DNA in her serum detected by PCR and this deference was statistically highly significant (P = < 0.001) (table 2).

Three patients out of 50 (6%) in patients group had human parvovirus B19 Ig M antibodies in their sera and all of them were human parvovirus B19 DNA positive and also had human parvovirus B19 Ig G antibodies in their sera. While human parvovirus B19 Ig M was not detected in 47 patients (94%) in the patients group; 25 patients of them (50%) were human parvovirus B19 DNA – PCR positive and had human parvovirus B19 Ig G in their serum and one patient of them (2%) was positive for only parvovirus B19 DNA – PCR. On the other hand human parvovirus B19 Ig M antibodies was detected in the serum of only one subject of the control group who was positive for human parvovirus B19 DNA but negative for human parvovirus B19 Ig G. These results showed that; there was no statistically significant difference between results of Ig M detection in patients and control groups (P = 0.720), the correlation between the results of Ig M detection and DNA detection in the patients group was statistically insignificant (P = 0.245).
but this correlation was statistically significant in the control group (p = 0.040) (table 3).

Human parvovirus B19 Ig G antibodies was detected in 31 patients out of 50 (62%) in the patients group; 28 patients of them (56%) were human parvovirus B19 DNA positive by PCR, while 3 patients of them (6%) were PCR negative. While human parvovirus B19 Ig G was negative in 19 out of 50 (38%) in the patients group and one of these Ig G negative cases was PCR positive. Regarding the control group; human parvovirus B19 Ig G was detected in 2 females out of 25 (4%) but these 2 females were PCR negative. Twenty three females of the control group (92%) had no human parvovirus B19 Ig G in their sera and in one of them (4%) human parvovirus B19 DNA was detected by PCR (this female was human parvovirus B19 Ig M positive) (table 4). There was a statistically significant difference between the results of human parvovirus B19 Ig G in both patients and control groups (P = < 0.001) and there were statistically significant correlation between the results of detection of human parvovirus B19 Ig G and PCR in both patients and control groups (P= < 0.001 for both of them) (table 4).

On evaluation of the human parvovirus B19 Ig M & Ig G detection tests statistically; our study showed that; there was a statistically highly significant differences between the results of the human parvovirus B19 Ig M & Ig G detection tests in both patients and control groups (P = < 0.001 for both of them). Although the overall sensitivity of the Ig M detection test was low, its specificity and positive predictive value were 100%, while its negative predictive value and test accuracy were of moderate values. On the other hand the human parvovirus B19 Ig G detection test had high values regarding its sensitivity, specificity, positive predictive value, negative predictive value and accuracy (table 5).

Table 2: Results of PCR for detection of human parvovirus B19 DNA in patients and control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCR Patients</th>
<th>PCR Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative count</td>
<td>21 (42.0%)</td>
<td>24 (96%)</td>
<td>45 (60.0%)</td>
</tr>
<tr>
<td>% within group</td>
<td>29 (58.0%)</td>
<td>1 (4.0%)</td>
<td>30 (40%)</td>
</tr>
<tr>
<td>Positive Count</td>
<td>50 (100%)</td>
<td>25 (100%)</td>
<td>75 (100%)</td>
</tr>
<tr>
<td>% within group</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Correlation between human parvovirus B19 Ig M and PCR results.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ig M Positive n = 4</th>
<th>Ig M Negative n = 71</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients Group n = 50</td>
<td>PCR +ve (100%)</td>
<td>PCR -ve (0%)</td>
<td>PCR +ve (55.32%)</td>
</tr>
<tr>
<td>Control Group n =25</td>
<td>PCR +ve (100%)</td>
<td>PCR -ve (0%)</td>
<td>PCR +ve (100%)</td>
</tr>
<tr>
<td>Total n = 75</td>
<td>PCR +ve (100%)</td>
<td>PCR -ve (0%)</td>
<td>PCR +ve (100%)</td>
</tr>
</tbody>
</table>

Table 4: Correlation between human parvovirus B19 Ig G and PCR results.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ig G Positive n = 33</th>
<th>Ig G Negative n = 42</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients Group n = 50</td>
<td>PCR +ve (90.32%)</td>
<td>PCR -ve (9.68%)</td>
<td>PCR +ve (5.26%)</td>
</tr>
<tr>
<td>Control Group n =25</td>
<td>PCR +ve (0%)</td>
<td>PCR -ve (100%)</td>
<td>PCR +ve (4.35%)</td>
</tr>
<tr>
<td>Total n = 75</td>
<td>PCR +ve (84.85%)</td>
<td>PCR -ve (15.15%)</td>
<td>PCR +ve (4.76%)</td>
</tr>
</tbody>
</table>
Table 5: Statistical values of human parvovirus B19 Ig M & Ig G detection tests.

<table>
<thead>
<tr>
<th>Statistical value</th>
<th>Parvovirus B19 Ig M detection test</th>
<th>Parvovirus B19 Ig G detection test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>13.33%</td>
<td>93.33%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>88.89%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100%</td>
<td>84.85%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>63.38%</td>
<td>95.24%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>65.33%</td>
<td>90.67%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Infection with human parvovirus B19 primarily takes place during childhood. The infection is contagious, and transmission occurs mainly through respiratory secretions\(^{18,19}\). Human parvovirus B19 infection may occur in pregnant women and is often asymptomatic or not evocative but transplacental transmission and fetal infection may occur 4 to 5 weeks later\(^{20}\) leading to a range of complications, including abortion, severe fetal anemia, non-immune hydrops fetalis (NIHF), and even fetal demise\(^{21,22}\).

Many studies suggested that fetal loss occurred through infection of fetal erythroid progenitor cells leading to profound anemia and cardiac failure\(^{18,19}\). Cardiac failure may also be associated with myocarditis, which can cause arrhythmias or even cardiac arrest without presence of anemia\(^{23,24}\).

In the presented study we tried to find if there is a correlation between recurrent spontaneous abortion (RSA) and the presence of human parvovirus B19 by investigating a group of 50 female patients with history of two or more spontaneous abortions with unknown medical cause in comparison to a group of 25 pregnant females 2 prior gravida or more with gestational age ≥ 28 weeks and without previous history of abortion as a control group.

The diagnosis of human parvovirus B19 infection can't be relied on viral cultivation but be made most reliably by the detecting virus specific Ig M and / or Ig G antibodies or by the isolation of parvovirus DNA detected by molecular techniques\(^{25}\). We used nested PCR to detect the presence of human parvovirus B19 DNA as a confirmed definitive diagnosis of the presence of the virus and also we used EIA as serological technique to detect presence of Ig M and Ig G against human parvovirus B19.

In our study 58.0% of the patients with RSA had a confirmed human parvovirus B19 infection as evidenced by the presence of its DNA detected by nested PCR while only 4.0% of the control group had human parvovirus B19 DNA with a highly statistically significant deference between the two groups (\(P = 0.001\)).

In accordance with our results; Xu et al.\(^{26}\) stated that there may be a relationship between HPV B19 infection and spontaneous abortion as they found HPV B19-DNA in serum samples of 25.8% (47/182) of spontaneous abortion patients, and in no one of the control group. Also El-Sayed & Goda\(^{17}\) reported that by nested PCR they detected the viral DNA in serum of 48% of RSA patients and in 0% of the control subjects and this was a highly statistically significant deference (\(P = 0.002\)).

Wang et al.\(^{27}\) did their study on the aborted embryonic tissues and they found that 24.5% were B19 DNA positive in the cases and 2.5% in the control group and the difference was significant (\(P < 0.05\)). Also Xu et al.\(^{28}\) did their study on the abortive tissue and can detect the B19 DNA in 34 out of 116 cases (27.3%) and in only 4% in the control group with statistical significant difference (\(P < 0.05\)).

Unlike our results; Sifakis et al.\(^{29}\) mentioned that B19 DNA was positive in 2 out of 102 (1.96%) abortive materials of RSA cases. Also Wermelinger et al.\(^{30}\) observed positive B19 DNA in 11% of the cases of RSA.

The use of different PCR primers or different samples or performing studies on different ethnic groups may be the causes of these discrepancies in results of different studies.

Human parvovirus B19 Ig M was detected in 6% of our patients and in 4% in our control subjects; all of them were B19 DNA positive resulting in 100% specificity and positive predictive value of the test. But B 19 Ig M was not detected in 71 cases (47 in patients group and 24 in control group); 26 cases of them were B19 DNA positive resulting in low sensitivity, moderate negative predictive value and moderate accuracy of the test (13.33%, 63.38% & 65.33% respectively). These false negative cases may be due to persistence of viral DNA...
after complete disappearance of the Ig M antibody. 

Kishore and Gupta\textsuperscript{31} found that the frequency of anti-B19 IgM antibodies in women with RSA was 19.8\% and in pregnant females it was 11\%. Another study mentioned that human parvovirus Ig M had been detected in 84\% of RSA cases\textsuperscript{47}. In Kuwait, sero prevalence of parvovirus Ig M was 2.2\% in pregnant women without recurrent abortions\textsuperscript{32} and in Libya it was 5\%\textsuperscript{33}.

In a recent study made by Abiodun et al\textsuperscript{34} to detect seroprevalence of parvovirus B19 among pregnant women in Nigeria Ig M was detected in 4\% of the pregnant females without RSA and the sensitivity of the test when compared with PCR was 100\%, specificity was 47.4\%, accuracy was 67.7\%, positive predictive value was 54.5\%, and negative predictive value was 100\%.

Embissegen et al\textsuperscript{35} stated that immunoglobulin M is the unique diagnostic marker in 20.8\% of documented infections and in the diagnosis of recent symptomatic infection when B19 DNA was no longer detectable. The value of serologic determination of parvovirus Ig M was discussed by Gallinella et al\textsuperscript{36} who reported that Ig M determination detected only 60\% of parvovirus B19 documented infection.

We found that human parvovirus B19 Ig G antibody was detected in 31 patients (62\%) of the patient group and 2 subjects (8\%) in the control group; five cases of these positive Ig G cases were B19 DNA negative while only 2 Ig G negative cases were B19 DNA positive. These finding resulting in high sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the test (ranged from 84.85\% to 95.24\%).

Maksheed et al\textsuperscript{32} mentioned that in Kuwait sero-prevalence of human parvovirus B19 Ig G was 53.3 \% in pregnant women without recurrent abortions. Also Jensen et al\textsuperscript{37} in their study on 3596 pregnant females found that the prevalence of B19 Ig G sero-positive at the first 24 weeks of gestation was 66\%.

In pregnant Swedish women, the prevalence of parvovirus Ig G antibody was 81\% in\textsuperscript{38} Russia, it was 66.9\%\textsuperscript{39}.

Many studies had been done in African countries to detected the sero-prevalence of human parvovirus B19 Ig G in pregnant women without RSA; it was 58.4\% in Malawi, 65\% in Tunisia and 61\% in Libya 27.5\% in Nigeria\textsuperscript{33,40,41,38}.

The differences between results of serologic tests in various studies for human parvovirus B19 might be the result of the differences of the studied populations, the difference in maternal gravidity and the use of different serological kits targeting detection of antibodies against different B19 virus.

In conclusion in our study the high detection rate of parvovirus among patients with recurrent spontaneous abortions supports the hypothesis that parvovirus could be one of the main causes of early recurrent abortions. Also the contradictory results between serologic tests and PCR indicated that the maximum sensitivity of parvovirus testing would require both techniques to be performed.

REFERENCES


