Parvovirus B19 Infection Frequency in Placenta of Fetal Loss Cases in Children Medical Center, Tehran, Iran

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ABSTRACT

Background and Objectives: Infection with parvovirus may induce spontaneous abortion, non-immune hydrops fetalis, and intrauterine death in full term infants. The aim of this study was to determine the frequency of parvovirus B19 in paraffin-embedded formalin fixed placental tissues in lost fetuses by PCR method and comparison with its frequency in healthy full term neonates as controls.

Materials and Methods: In a case control study, thirty-one formalin fixed paraffin embedded placental tissue from autopsies related to fetal losses and also the same number of control cases were collected and the PCR for internal control and target sequence were performed.

Results: Thirty-one subjects and 31 controls were enrolled and three out of 31 cases were positive for parvovirus (9.7%) using PCR. Hydropic changes were noted in two of the positive cases for parvovirus.

Conclusion: Parvovirus B19 infection during pregnancy may cause red precursor cells damage resulting in anemia and congestive heart failure. We suggest that studies with greater sample sizes are carried out to determine the frequency and management of parvovirus B19 infection because of timely diagnosis and transfusion of severe hydropic fetuses can reduce the risk of fetal death.

Keywords: Human Parvovirus B19, Placentas, Fetal Deaths, Autopsy
Introduction

Discovered in 1974 by Cossart et al., *parvovirus B19* is an encapsulated, single stranded DNA virus that causes fifth disease in children. Although fifth disease is benign and self-limiting, parvovirus infection during pregnancy can cause severe damage to fetus. The rate of transplacental transmission of infection is 25–33% and the risk of adverse fetal outcome is 3% to 12% (1, 2).

Studies have shown that parvovirus infection can cause fetal hydrops, anemia and even fetal demise, yet this virus has not reported to cause congenital malformations (3,4). Anemia and hydrops develop because of the predilection of parvovirus to erythroid cells. Parvovirus receptor is P blood group antigen which has high expression on erythroid and megakaryocytic precursors (4,5).

Infection may induce spontaneous abortion, non-immune hydrops fetalis, intrauterine fetal death, or stillbirth (6). Therefore, the infection is serious at any time during pregnancy. In addition to red blood cells, *parvovirus B19* can affect liver and myocardial cells of fetus without permanent sequels (4).

Other parvovirus-associated diseases include arthropathy, persistent anemia in immunodeficiency states and aplastic crisis in patients with hemolytic diseases.

Infection can be suspected based on clinical findings, serology and PCR studies, but testing abortus or stillbirth infant for parvovirus at prenatal care units is not routinely performed. Management of newly infected women depends on gestational age. If infection occurs before 20 weeks of gestation, when the risk of fetal mortality is high (around 10%), serial ultrasound for detection of fetal hydrops is advocated. If middle cerebral artery doppler sonography supports presence of anemia or the fetus shows evidence of hydrops, the main treatment will be intrauterine transfusion (7,8). Nevertheless, there is controversy in the management of these patients because some cases spontaneously resolve (9).

It is recommended that tissue samples obtained after fetal death such as placenta or fetal organs be tested for this virus routinely (10,11).

Few studies have been conducted so far for determining the frequency of parvovirus infection in fetal loss cases. There is no study in Iran for determining the frequency of Parvovirus; the aim of this study was to determine the frequency of *parvovirus B19* in paraffin embedded formalin fixed placental tissues in lost fetuses by PCR method to understand any possible role of *parvovirus B19* in fetal death and comparison with its frequency in healthy full term neonates as controls.

Material and Methods

In this case control study, thirty-one formalin fixed paraffin embedded placental tissue from autopsies related to fetal loss cases (range 12 weeks to full term) who were autopsied between 2004-2008 collected at children's medical center hospital, affiliated to Tehran University of medical sciences. In addition to being a referral tertiary care center, this hospital is the major teaching children Hospital of Tehran University of Medical Sciences, Tehran, Iran and patients are admitted from all regions of Iran, representing a wide spectrum of socioeconomic levels.

In addition, 31 paraffin blocks of placental tissue belonging to healthy alive neonates provided by Valiasr Teaching Hospital were used as controls. All controls were full term placentas from normal deliveries and for none of cases medical termination were performed. Detailed clinical, autopsy and placental examination reports were reviewed on all
intrauterine death cases. The time from the last menstruation was used for calculation of gestational age.

In each case, stained sections from liver, spleen, heart and placenta were evaluated histologically for the presence of parvovirus nuclear inclusion bodies defined histologically (1,2). One 5-micrometer section of each block was prepared for PCR reaction. For each paraffin block new disposable knife was used and sections were collected in clean PCR microtubes. After deparaffinization, DNA extraction was performed by means of High pure template nucleic acid extraction kit (Roche, Germany).

Statistical analysis was performed using SPSS version 16.0.1 (SPSS Inc., Chicago, IL, U.S.A.) and $P$ value < 0.05 was considered as significant. The study was approved by the Ethical Committee at the Tehran University of Medical Sciences as well as nowhere in the study, the patient name was mentioned and they were not charged for tests.

**PCR**

The PCR for internal control and target sequences were performed in 20μL reaction containing 0.5μM of each forward and reverse primers, 0.25 μM of probe and 10μL Premix Ex Taq (Takara Bio, Ostu, Shiga, Japan)

Internal control amplification was performed for Homo sapiens hydroxymethylbilane synthase (HMBS). Primers and probe sequences are shown in Table 1.

**Table 1-** Primers and probe sequences

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
</tr>
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<tbody>
<tr>
<td>ParvoF2</td>
<td>AGTTATCTGACCACCTCCATGC</td>
</tr>
<tr>
<td>ParvoR2</td>
<td>CTAACCTGCCAGGCTTGTG</td>
</tr>
<tr>
<td>ParvoP</td>
<td>CCAGTAGAGTCATGCAGAACCTAGAGGAGA</td>
</tr>
<tr>
<td>HMBSF</td>
<td>GCCTGCAGTTTGAATCAGTG</td>
</tr>
<tr>
<td>HMBSR</td>
<td>CGGGACGGGCTTTAGCTA</td>
</tr>
<tr>
<td>HMBSP</td>
<td>TGGAAGCTAATGGAAGCCAGTACC</td>
</tr>
</tbody>
</table>

The real-time PCR method for detection of parvovirus was performed as described by Aberham *et al.* with modifications (12). The reactions were performed in a Rotor-Gene 3000 real-time machine (Corbett Research, Mortlake, Australia) as follows: initial denaturation at 95 °C for 30 seconds; subsequently, 40 cycles of 95°C for 5 seconds and 61°C for 20 seconds. Acquisition was performed in FAM channel. Primers and probe sequences are shown in Table 1.

**Result**

Obtained during 2003 to 2009, 31 cases and 31 controls were enrolled in this study. All cases of fetal loss autopsied in children hospital center in Tehran, Iran. Twenty-two cases were male and the rest were female. The mean gestational age of fetuses was 22.25±7.9 weeks. Reviewing their H&E slides none of cases or controls showed characteristic nuclear changes related to viral cytopathic effect. Three out of 31 cases were positive for parvovirus (9.7%) using PCR, all of which were male with mean gestational age of 17.3±3weeks. Hydropic changes were noted in two of parvovirus-positive cases. From eight other fetuses that showed hydropic changes but were negative for parvovirus DNA, one showed generalized cytomegalovirus cytopathic effect; the cause of death of the other seven cases was not found by routine light microscopy. No sign of infection was found in the mothers’ pregnancy records around or before abortion.
Sex and gestational age differences between virus negative and positive cases were not statistically significant ($P=0.53$ and $P=0.26$, respectively).

Ten out of 31 cases had gross malformations (34.2%) other than hydrops, none of them was positive for parvovirus B19.

Of the 31 placental tissues from normal pregnancies, none was positive for parvovirus B19 DNA. In addition, viral cytopathic effect was not seen in their H&E stained slides.

**Discussion**

Parvovirus B19 infection during pregnancy may cause damage to red cell precursors resulting in anemia and congestive heart failure. Meanwhile, infection of placenta may be associated with a local release of inflammatory cytokines (e.g. IFN-$\gamma$, TNF-$\alpha$) which causes fetal demise with no clear sign of fetal infection in first weeks of pregnancy (3,13). When there is no hydropic sign in stillborn fetus in late gestation, the rate of parvovirus B19 is much higher compared to uncomplicated normal pregnancies (3,14).

When the fetus shows signs of hydrops and anemia such as pleural effusion, ascites, ventriculomegaly and placentomegaly, determination of etiology of hydrops is important to choose the best management. Cordocentesis and intrauterine blood transfusion are indicated in cases caused by parvovirus B19 infection because the chance of fetal survival may increase 60% to 80%, in contrast to 15% to 30% in untreated cases (8,10-12).

It has been shown that intrauterine transmission of parvovirus B19 may occur throughout pregnancy; the earliest maternal infection causing congenital infection can occur around the 7-8th week of gestation (10). For selecting the best battery of screening tests, the frequency of etiologic factors in the target population must be determined. Because, to the best of our knowledge, the frequency of this infection in cases of fetal death are not known, this case control study was conducted to determine the frequency of fetal loss related to parvovirus B19 infection. Around 10% of dead fetuses in our study were infected with parvovirus. Other studies conducted in Tunisia (Landolsi et al., 17.24%), Brazil (quemelo et al., 5.9%) and Greece showed similar results (15-17). There is 34 to 65% susceptibility to parvovirus B19 in different parts of the world (18).

No control subject showed positivity for parvovirus B19 in our study which is in accordance with the results of previous studies (10,19,20).

In the study conducted by Enders at al. on 1018 parvovirus infected pregnant women, all of fetal demise occurred less than 20 week gestational ages (4); our positive cases also occurred before 20th week.

Yet, no study has determined the susceptibility of women in Iran. Therefore, we suggest that high-risk women, for example teachers and daycare workers, are monitored for parvovirus. If hydrops fetalis is suspected in these groups, by reviewing prenatal findings the etiology would be easier and management could be started sooner.

In our study, all parvovirus-infected cases were male which might be owing to sampling error because other studies showed no sex predilection.

One of the limitations of our study was that although virus was detected in the placenta of dead fetuses, an etiologic role could not be proven for this virus. As mentioned by other authors, the outcome of intrauterine parvovirus B19 infection is not completely known and depends on factors such as variations of viral spread or gestational age of subjects and other causes also may be responsible for fetal
death such as chromosomal abnormalities (21). Women with confirmed infection show a significantly higher rate of second-trimester fetal loss (11.8%) than control group (10, 22). Another limitation of the current study was that the some of the prenatal records regarding to the occupation of mother were not available. Therefore, environmental predisposing factors could not be sought. Because serologic markers become positive too late, studies conducted based on antibody are unreliable to detect recent B19 infections, on the other hand, clinical signs of infection such as rash are seldom seen in adults. One should also consider alive mothers and fetuses with available serums to study parvovirus. PCR employment to clarify the presence of parvovirus B19 could save time for further therapeutic modulation (10, 11).

Finally we suggest that studies with larger sample size are carried out to determine the frequency parvovirus B19 infection and for suspicious cases during pregnancy.

Conclusion

Our study shows the presence of DNA virus in some of the cases, which there was no other obvious cause. Using PCR for detection of this infection in fetal death and in alive hydropic fetuses can aid to improve outcome of pregnancy.

Acknowledgement

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References


