

Parvovirus B19 infection: association with third-trimester intrauterine fetal death

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Objective To identify the presence of parvovirus B19 infection as a possible cause of fetal loss in the third trimester.

Design Prospective study of women experiencing third-trimester intrauterine fetal death (IUFD).

Setting All cases of IUFD at Danderyd Hospital from 1992 to 1998.

Population Ninety-three women with IUFD in 33,759 deliveries (0.3%).

Methods Detection of B19 DNA by polymerase chain reaction (PCR) in placental and fetal tissue. Placental pathology and B19-specific immunohistochemistry. Maternal serology in consecutive samples.

Results Among 93 cases of IUFD, seven (7.5%) had detectable B19 DNA in freshly-frozen placental tissue. The detection of B19 DNA in these tissues was confirmed by detection of B19 DNA in six separately stored paraffin-embedded placental tissues. No other explanations for the fetal deaths were found. None of the women had experienced any clinical signs of infection prior to fetal demise. None of the seven fetuses were hydropic. Histopathologic examination of the placentas revealed only minor abnormalities. Serology on maternal samples at birth revealed delayed or absent B19 IgG responses in five of seven cases. Two women were B19 IgG seropositive at the time of delivery but had unusual infection patterns; persistent viraemia for at least five months before birth in one case and likely persistence or re-infection by B19 in the other.

Conclusion In our study, 7.5% of IUFDs in the third trimester may have been caused by parvovirus B19 infection, without signs of fetal hydrops. This finding indicates that B19 PCR should be included in the routine investigation of IUFD.

INTRODUCTION

Parvovirus B19 infection is a common viral infection with a seroprevalence of 50–70% in the adult population. The virus binds to its cellular receptor, the P-antigen, and has a tropism for immature erythrocytes in the bone marrow or fetal liver¹. Infection leads to inhibition of erythropoiesis, resulting in anaemia. Other tissues, such as the myocardium and endothelial cells, can also be affected². Clinical signs are usually fever and rash (erythema infectiosum, fifth disease), arthralgia, or merely signs like febrile illness with malaise. The infection can also be asymptomatic.

In pregnant women the virus is known to be associated with fetal anaemia, fetal hydrops, spontaneous abortion and intrauterine fetal death (IUFD)^{3,4}. Several

cases of IUFD caused by the combination of infection in the second or third trimester and hydrops are reported^{5–8}. Parvovirus B19 has also been demonstrated to be a significant cause of midtrimester abortions⁹. However, nonhydropic third trimester fetal loss associated with parvovirus B19 has not been a dominant issue^{3,5}.

In a prospective study of the incidence of B19-antibodies in a population of pregnant women, one woman without B19-antibodies delivered a stillborn baby at 37 weeks of pregnancy¹⁰. There were no hydropic changes in the baby, but B19 DNA was found in placental tissue as well as in maternal blood samples collected three weeks before the fetal demise and at delivery. The mother later developed antibodies against B19, but not until six months after delivery. Since no other cause of the IUFD was found, we feel it possible that the B19 infection caused the fetal loss, possibly because of a slow immunological response to the infection by the mother. This case was the incentive for the present study.

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METHODS

Swedish legislation defines spontaneous abortion as fetal loss up to 28 weeks of gestation. At Danderyd Hospital all cases of IUFD are examined according to a standard protocol after consent¹¹. IUFD is defined as fetal death after gestational week 28. The protocol includes maternal blood samples for fetal nucleated red blood cells, IgG and IgM antibodies against toxoplasma, rubella, cytomegalovirus, herpes simplex virus, parvovirus B19, and listeria, thyroid hormones and thyroid hormone antibodies. Bacterial culture from the cervix is collected. Amniocentesis for chromosomal examination and bacterial as well as viral cultures of amniotic fluid are also performed in most cases (but parvovirus B19 cannot be detected by standard viral cultures). After birth, fetal heart blood is sampled for bacterial culture. The placenta undergoes histopathologic examination and, when required, is analysed for microbial pathogens. Autopsy of the fetus is recommended, but not all parents consent.

Since 1992 all women admitted to Danderyd Hospital with third trimester fetal death have been examined for B19 IgG and B19 IgM in maternal serum and for B19 DNA by polymerase chain reaction (PCR) of placental tissues. During the 7-year study period, from 1992 to 1998, ninety-three cases of IUFD occurred. The total number of deliveries during that time was 33,759. The fetal deaths associated with B19 infection in the present study occurred in December 1991 (index case), April 1993, May 1993, January 1994, November 1994, May 1997 and October 1998 (cases 1–7, respectively). IUFD was diagnosed by ultrasound and delivery was initiated within 36 hours.

For comparison, placental tissues from 50 unselected first-trimester spontaneous abortions were collected during June 1997 to December 1998. These samples were tested in the same way as samples from the IUFDs.

Placental tissue and maternal serum samples were collected following delivery and stored at -20°C within 24 h. Additional placental tissue was fixed in formaldehyde immediately after parturition and subsequently paraffin-embedded. Autopsy was performed after parental consent (cases 1, 2, 5 and 6), and biopsies from fetal tissues were processed according to routine procedures.

Serology was performed immediately after delivery as part of the diagnostic routine. Over the long study period, different assays were used for detection of B19 IgG and IgM. To obtain comparable results from different years, all samples were reanalysed at a later date using one diagnostic kit (Biotrin B19 IgG and B19 IgM immunofluorescence assay, Biotrin Inc, Dublin, Ireland).

Before PCR amplification, frozen tissues were thawed and sections homogenised in a sterile mortar,

followed by heat treatment (95°C for 10 min) and centrifugation (14,000 rpm for 10 min). Serum samples were heat treated and centrifuged in the same way. Supernatant (2 μL) of the respective specimens was used as a template in the PCR.

Paraffin-embedded tissue blocks were heated individually in sterile chambers at 65°C to melt the paraffin before 25 mg sections of tissue were cut using sterile scalpel and forceps. Each section was deparaffinised by washings with xylene and 100% ethanol. After evaporation of the ethanol, DNA was extracted with Qiagen Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions with the exception of final elution with 100 μL of water in order to concentrate DNA. Suspension (2 μL) was used as template in the PCR. Remaining tissue from the blocks was again re-embedded in paraffin for subsequent microtome cutting for immunohistochemistry.

Nested PCR was performed as described earlier¹². Samples analysed before 1997 were amplified by primers corresponding to the B19 VP1 gene¹² and subsequently by primers corresponding to the NS-1 gene¹³. To eliminate sample-to-sample contamination all procedures were performed using sterile material, aerosol-resistant pipette tips and geographically separated facilities for sample preparation, reagent preparation, amplification and detection.

B19 DNA positive and negative controls (tissues or serum samples) were included in each assay.

Representative pieces from different parts of each placenta as well as fetal tissues were formalin-fixed and paraffin-embedded according to standard procedures. Sections were routinely stained with hematoxylin and eosin for detection of viral inclusions in proerythrocytes.

Immunohistochemical analysis for detection of parvovirus B19 was performed in formalin-fixed, paraffin-embedded tissues. Two anti-B19 monoclonal antibodies (No. B0091, DakoPatts, Denmark; No. MAB8292, Chemicon, USA) were used in an automatic staining system (TechMate, DakoPatts) utilising a streptavidin-biotin technique.

RESULTS

IUFD was diagnosed in 93 of 33,759 (0.3%) women during the 7-year study period. Among the 93 cases of IUFD, 7 (7.5%) had detectable B19 DNA in fresh placental tissue. No other explanations for the fetal deaths were found during the routine investigation in these seven cases. Parvovirus B19 infection was therefore considered as a possible aetiology. The number of B19 DNA positive IUFDs was too small for correlations with seasonal or annual variations of B19 epidemics in the community. Such epidemics were noted by the local

clinical virology laboratories in the early summer months of 1994 and 1998, respectively (data not shown). None of the seven women had experienced rash, arthralgia or other clinical signs of parvovirus infection during pregnancy. Neither were there any reported contacts with parvovirus infected individuals.

In addition to B19 PCR performed on freshly frozen placental tissue at birth, paraffin-embedded placental and selected fetal tissues, as indicated, were examined retrospectively (Table 1). B19 DNA in placental tissue was found in six of the seven IUFDs. In addition, B19 DNA was detected in liver and kidney tissue collected from case 6.

B19 DNA by PCR was tested in just a few maternal serum samples. Case 1 was positive one month prior to and at delivery. Case 6 was negative 21 months after delivery and case 7 was positive in three consecutive samples collected five months prior to parturition, at delivery and two months after (data not shown).

As controls, placentas from first-trimester spontaneous abortions were collected. These tissues were handled and analysed in a similar way to placental tissues from the IUFD cases and one of 50 tissue samples was B19 DNA positive.

B19 IgG was negative in five of seven maternal blood samples collected at birth (cases 1–5, Table 1). Case 1 seroconverted between samples collected at two and six months after birth. Case 5 was still B19 IgG negative eight months after birth. Case 6 was already B19 IgG positive in a blood sample collected 25 months prior to IUFD and at the time of a previous spontaneous abortion. Analysis of avidity and epitope-type specific reactivity, kindly performed by Dr. Hedman (University of Helsinki, Finland), excluded a recent (< 6 months)

primary infection at that time (data not shown). Case 7 was B19 IgG and B19 IgM positive five months prior to IUFD. B19 IgG titres increased during these five months and then decreased during the following two months. B19 IgM was highest in the first sample and later decreased but was still detectable two months after parturition. This pattern is usually seen following a recent primary infection.

None of the seven IUFD cases were hydropic. Macroscopic examination of the placentas revealed only minor, occasional findings such as small intervillous thromboses. No extensive infarction or bleeding was noted. Histopathologic examination of the placentas revealed minor abnormalities, i.e. focal villous oedema, small intervillous thromboses or increased erythropoiesis (Table 1).

B19-specific immunohistochemistry for viral capsid proteins was negative and no viral inclusions were found in the placental or fetal tissues (data not shown). It must be noted that some of the tissues were severely autolysed and therefore difficult to evaluate.

DISCUSSION

This is to our knowledge the first long term, prospective study describing the frequency of third-trimester IUFD associated with B19. Only 60–70% of IUFD can be explained in terms of the standard investigative protocol. This frequency is comparable to that of other centres¹⁴. Since 1992 we have found seven B19 PCR positive placental tissues among 93 (7.5%) cases of third-trimester IUFD. B19 DNA was analysed by PCR on freshly frozen placental tissue shortly after delivery. At the end of the study period we tested archival material

Table 1. Clinical and laboratory parameters in 7 cases of intrauterine fetal death (IUFD).

Case no.	Maternal age (years)	Para	Gest. week	Weight at birth (g)	Cord complication	B19 IgG (months after partus)	B19 IgM (months after partus)	Placental pathology	B19 DNA in paraffin-embedded tissue*
1	31	1	37	2880	No	Neg (-5, -1, 0, 2) Pos (6)	Neg (-5, -1, 0, 2, 6)	Normal	Pos (placenta) Neg (kidney, spleen, lung, liver, heart)
2	38	0	32	1870	Yes	Neg (0)	Neg (0)	Normal	Neg (placenta)
3	29	1	41	4010	Yes	Neg (0)	Neg (0)	Normal	Pos (placenta)
4	38	3	41	3375	Yes	Neg (0, 1)	Neg (0, 1)	Focal villous oedema, increased erythropoiesis	Pos (placenta)
5	38	2	27	970	No	Neg (-4, 0, 3, 8)	Neg (-4, 0, 3, 8)	Intervillous thromboses, focal villous oedema	Pos (placenta) Neg (liver, lung)
6	37	0	40	2444	Yes	Pos (-25, -4, 0, 1, 21)	Neg (-25, -4, 0, 1, 21)	Chorionitis	Pos (liver, kidney) Neg (heart, lung)
7	31	1	34	1815	No	Pos (-5, 0, 2)	Pos (-5, 0, 2)	Normal	Pos (placenta)

*All cases were B19 DNA positive in placenta as examined on freshly frozen tissue (as defined in the standard programme following all cases of IUFD in our clinic). The results here show the presence (pos) or absence (neg) of B19 DNA in placenta and fetus which were extracted from paraffin-embedded tissue samples.

(paraffin-embedded placental tissues) from these cases and could confirm B19 DNA positivity in six of seven tissue samples. The case with divergent results had been stored for five years and it is possible that the DNA was not stable, although a false positive result in the original sample cannot be ruled out. An indication of the specificity of the PCR assay was obtained by collecting placentas from first-trimester spontaneous abortions since B19 infection seems to be uncommon in these cases¹⁵ and only a few case reports have been published^{16,17}. The tissues were handled and analysed in a similar way to placental tissues from the IUFD cases and one of 50 tissues was B19 DNA positive.

According to different studies^{18,19}, the incidence of fetal death after maternal B19 infection, irrespective of gestational age, is 6.5–14%. However, fetal death due to B19 infection after 20 weeks of gestation is reported to be very rare¹⁹. Fetal death, as examined in the second trimester, usually occurs within three to six weeks after maternal B19 infection, although longer periods have been described¹⁸. At this time B19 IgG is present in maternal blood, whereas B19 DNA has been cleared^{20–22}. Our third-trimester IUFD cases had a different serologic pattern. Only two out of seven mothers were B19 IgG positive at delivery. Follow up samples were available from three of the B19 IgG-negative mothers, showing seroconversion between two and six months postpartum in one case, while the other two cases were consistently B19 IgG-negative after one and eight months, respectively. It is interesting to speculate whether the fetuses were especially vulnerable because of lack of maternal antibody protection. For comparison, a primary varicellae-zoster virus infection in late pregnancy can lead to a fatal situation if the baby is born before the mother has developed antibodies.

In addition to delayed or absent seroconversion, another unexpected finding revealed that two women in the study group had persistent B19 infection prior to IUFD. One was positive for B19 DNA, B19 IgG and B19 IgM in all three consecutive samples (collected five months prior to delivery, at delivery, and one month postpartum, respectively). Changes in levels of IgG (increasing titres) and IgM (decreasing titres) in the three samples indicated a recent primary infection prior to the first sample. The other woman had B19 IgG antibodies more than two years prior to IUFD. That sample was collected at the time of a spontaneous abortion. Unfortunately, no archival material is available from that event, which rules out further confirmation of whether or not B19 caused the fetal demise.

The latter case is, to our knowledge, the longest duration of B19 IgG in a prenatal sample before the time of B19-associated fetal death. The case may represent either a re-infection of the mother or viral persistence. Cassinotti *et al.*²³ reported a B19 IgG positive woman

four years prior to B19-induced fetal hydrops. However, they questioned the specificity of the IgG since two other assays gave negative results. B19 viraemia, as detected by PCR in serum, is usually cleared within a few days after symptomatic infection¹⁸. This is in contrast to some of the women in the present study who had a persistent infection for several months. Persistence of B19 infection is seen in immunosuppressed patients²⁴ and only in rare cases of immunocompetent patients^{25,26}. Cases of re-infection have also been found²⁷. Viral persistence has been associated with lack of functional (neutralising) activity in spite of high titre IgG²⁸ whereas the role of cellular immunity has not been evaluated. The fact that none of the women had experienced clinical signs of infection or had been exposed to other infected persons, as recalled at the time of IUFD, is another difference from second-trimester B19-associated fetal hydrops or spontaneous abortion, where 27% of the women could recall signs of infection²⁹.

The most prominent feature of our cases was the lack of fetal hydrops. Hydrops is a frequent finding in B19-infected second-trimester fetuses⁶. The possibility of B19 infection should therefore be considered in any case of IUFD and not just in those with hydrops. No signs of malformation were noted in any of the seven fetuses upon external examination. Three of the fetuses underwent autopsy. Our inability to detect immunochemically demonstrable antigenic viral material or viral inclusions in nucleated erythrocytes in any of the examined placentas and fetal tissues is striking. This is in contrast to the ease of detecting viral antigens in cases of second trimester hydropic spontaneous abortions (own unpublished results). One explanation may be the prolonged storage of samples over many years or the advanced autolytic changes in many tissues. However, we (unpublished data) and others³⁰ have been able to detect viral antigens in severely macerated fetal tissues using a similar technique. Thus, this phenomenon might reflect a genuine biologic paucity of viral antigenic expression, feasibly associated with the lack of fetal hydrops and the other maternal serologic abnormalities, as compared with B19 infection during the second trimester. The differences may partly be due to greater haematological reserve and a more mature immune response in third-trimester fetuses.

The virus can affect other tissues as well, with particular affinity to liver and myocardial cells^{6,31}. Direct myocardial damage may lead to intrauterine congestive heart failure. Cord complications, as seen in some of our cases, might not be the primary cause of fetal demise. In theory, in severely sick fetuses the tension of the cord might be minimised, and a lack of elasticity might make a secondary contribution to the entanglement.

Treatment of B19 infection is still a problem and a point of discussion. All women in the present study had followed the recommended prenatal care programme. There was no indication of fetal or maternal illness at the last check-up prior to IUD. The question arises whether the IgG negative, or persistently infected, woman should be treated with immunoglobulin prior to or during a possible new pregnancy. In cases of fetal hydrops, intrauterine cord transfusion and immunoglobulin treatment of the mother have been tried^{7,32}. At prenatal counselling after a suspected B19-exposure or previous IUD, B19 IgG positive women are told that they are immune. We feel that further studies are needed before such information should be reconsidered. B19 IgG positive, immunocompetent individuals who are persistently infected, or susceptible to re-infection, are presumably rare.

The death of a child is always a traumatic event. When an IUD occurs, it will certainly help the parents if a rational explanation can be given, preferably with the assurance that it will either not happen again (for example an infection that leads to immunity), or that it can be prevented by some kind of intervention. All investigations that can elucidate fetal demise are of the utmost interest. As seen in the present study, serology is an insensitive method for diagnostic purposes in B19-associated IUDs. Only one of the cases was B19 IgM positive at delivery. Examination for B19 DNA by PCR clearly increased the number of cases that received a probable aetiological explanation of the fetal death. This indicates that B19 PCR should be included in the routine investigation of IUD.

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