

ORIGINAL ARTICLE

Long term follow up of serostatus after maternofetal parvovirus B19 infection

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Background: Maternofetal parvovirus B19 infection may result in fetal hydrops or abortion. Chronic infection has been associated with long term complications (polyarthritis, persistent aplastic anaemia, hepatitis). In pregnancy maternal immunosuppression caused by a TH2 dominant response to viral antigens has been observed. There is little information on long term reactivity to intrauterine infection.

Aims: To assess the serological status in children and their mothers after maternofetal parvovirus B19 infection and development of fetal hydrops.

Methods: A total of 18 children and their mothers, and 54 age matched control infants were studied. Main outcome measures were parvovirus B19 DNA, specific IgM and IgG against the virus proteins VP1/VP2, and NS-1 in venous blood.

Results: Parvovirus B19 DNA and antiparvovirus B19 (IgM) were undetectable in all sera. A significant larger proportion of maternal sera compared to study children's sera contained IgG against the non-structural protein NS-1. Mean levels of VP1/VP2 IgG antibodies were significantly lower in the children than in their mothers (48 (36) v 197 (95) IU/ml). There was no history of chronic arthritis in mothers and children. Five women had subsequent acute but transient arthritis postpartum, which was not correlated with antibodies against NS-1.

Conclusions: Serological evidence of persistent infection after maternofetal parvovirus B19 disease could not be detected. Increased maternal prevalence of anti NS-1 (IgG) and increased levels of antiparvovirus B19 (IgG) may reflect prolonged viraemia compared to fetal disease.

Parvovirus B19 is a species in the genus *Erythrovirus*, family Parvoviridae. The incidence of parvovirus infection in pregnancy is approximately 1–2%, but may rise during epidemics to >10%.¹ The vertical transmission rate may be up to 50%.² Infection in the first trimester may result in spontaneous abortion, whereas during mid and late gestation fetal anaemia and hydrops may develop. Intrauterine fetal death in late pregnancy has been observed without signs of fetal hydrops.^{1,3} Control of acute parvovirus B19 infection in pregnancy is achieved at three levels: maternal host defence, the placental barrier, and the fetus itself. Maternal host defence against viral antigens is shifted towards a TH2 dominant response in pregnancy. This may result in prolonged virus elimination and enhanced synthesis of specific NS-1 antibodies.^{4–7} IgG antibodies become detectable approx. 12 days after the infection and persist as a postinfection marker. Placental immunocompetence to parvovirus B19 is shown by increased numbers of CD3 T cells and interleukin 2 production.⁸ Long term control of parvovirus B19 infection depends on T and B cell memory. Specific antibody levels in infancy following fetal disease have not been reported in the literature.

Fetal anaemia is the direct result of high affinity of virus particles towards erythroid precursor cells. This process depends on the receptor function of blood group p antigen. Cells other than myeloid cell lines have been shown to express p antigen receptors, and extramyeloid organ manifestations have been described. Myocarditis may be clinically relevant and postmortem examinations have shown inflammatory tissue lesions in parvovirus B19 disease.^{9,10} Mortality of parvovirus B19 associated fetal hydrops may be considerable without therapy. With introduction of red cell intrauterine transfusion regimes (IUT) guided by appropriate anaemia z score,¹¹ established in the 1980s, survival has improved significantly.¹¹ Respective survival rates of transfused fetuses and conservatively managed pregnancies of 82% versus 55% have been

reported.⁹ As a result, long term outcome data of these severely affected infants and their mothers has become available. However, to date there has been no information on persistence of fetal infection in childhood and adolescence. On the other hand, parvovirus B19 has been associated with various diseases such as polyarthritis, persistent aplastic anaemia, pancytopenia, thrombocytopenia, hepatitis, myocarditis, and encephalitis. Therefore the question whether any of these disease states may be related to persistent parvovirus B19 infection is of major interest. An increased seroprevalence of NS-1 antibodies has been observed in pregnancy complicated by parvovirus B19 infection.⁵ There are few data on long term follow up and incidence of arthritic or haematopoietic symptoms in affected women and their offspring. This study presents serological and clinical information of long term follow up in infants and their mothers after proven acute intrauterine parvovirus B19 infection.

METHODS

Serum samples from peripheral venous blood of 18 infants after proven, clinically relevant fetal parvovirus B19 infection with formation of fetal hydrops and their mothers were analysed after informed consent had been obtained. From 1989 to 1998, 37 fetuses were treated by IUT at a single tertiary care centre for parvovirus B19 associated hydrops. Fetal hydrops was considered if effusions of at least two compartments were visualised on fetal ultrasound. Fetal haemoglobin levels ranged from 22 to 85 g/l (median 34.5 g/l). Diagnosis was established and the first transfusion was initiated at a median gestational age of 23 weeks (range 17–29 weeks). Transfusion regimen was guided by appropriate anaemia z scores. Acute fetal and maternal parvovirus B19 infection was verified by the presence of specific IgM and/or parvovirus B19 DNA in fetal and maternal blood. There were 31 live births, of which 20 children (12 girls, eight boys) and their mothers were

Table 1 Levels of IgG against parvovirus B19 VP1/VP2 and anti NS-1 positive samples in 18 mothers and their children after fetomaternal infection

	Children n=18	Mothers n=18	p value
Antiparvovirus B19 VP1/VP2 (IgG) (IU/ml)*	48 (36)	197 (95)	<0.0001
Antiparvovirus B19 NS-1 (IgG) (IU/ml); n (%)	5 (28)	13 (72)	0.022

*Results expressed as mean (SD)

Table 2 Levels of IgG against parvovirus B19 VP1/VP2 in children after fetomaternal infection and 54 controls

	Study group n=18	Controls n=54	p value
Antiparvovirus B19 VP1/VP2 (IgG) (IU/ml)*	48 (36)	68 (50)	NS
Age at testing (months)*	57 (27)	51 (26)	NS

*Results expressed as mean (SD). NS, not significant

available for follow up. Eleven children were lost to follow up because of lack of contact address or consent. Of the 20 mothers (parents) of the study group, two declined consent. Serological tests could be performed in 18 children (age 57 (27) months) and mothers as well as in a control group of 54 age matched children (age 51 (26) months).

Sera were tested for specific IgG and IgM by indirect immunofluorescence assay (Biotrin, Sinsheim, Germany). Quantitative analysis of specific IgG was performed by ELISA (Biotrin, Sinsheim, Germany) with virus-like particles consisting of VP2 and the internal standard for antiparvovirus B19 (IgG) (NIBSC 93-724). IgG and IgM against NS-1 and three different structural protein fragments was detected by Western blot (Recomblot, Mikrogen GmbH, Munich, Germany). Presence of viral DNA was shown by nested polymerase chain reaction (PCR). Serological tests were also performed in a control group of 54 age matched children. For statistical analysis data were analysed with SPSS software, version 10.07, using the Mann-Whitney U test and multivariate analysis of variance; a p value less than 0.05 was considered to indicate statistical significance.

RESULTS

Serological testing

Specific IgM and parvovirus B19 DNA indicating acute or recent infection were undetectable in all sera of the study group and the control group.

IgG against VP1 and VP2 capsid proteins was detectable in all children and their mothers. Mean levels of IgG antibodies were 48 IU/l in children and 197 IU/l in their mothers ($p < 0.001$; table 1). Levels were not correlated to the interval between infection and testing ($p > 0.05$). Antibodies of the IgG class against NS-1 were detected in children and their mothers in the study group. A significantly larger proportion of women were anti NS-1 IgG positive compared to the children ($n = 13/18$, 72% versus $n = 5/18$, 28%; $p = 0.022$; table 1).

In the 54 age matched control children, 11 were antiparvovirus B19 (IgG) positive (seroprevalence 20%). The mean titre in 11/54 IgG positive sera of controls was 68 IU/l. There was no statistical difference between antiparvovirus B19 IgG of study children and controls ($p > 0.05$; table 2).

Clinical and hematological findings

In the children no sequelae of fetal parvovirus B19 disease were reported and there was no history of neurological symptoms or episodes of arthralgia. Red and white blood cell counts were normal for age and no haematological disorders were evident.

Five mothers had a history of arthralgia related to the infection. Joint symptoms did not last longer than three months and none of these patients had signs of chronic arthritis.

DISCUSSION

In this study, the serostatus of children affected by severe fetal parvovirus disease with consecutive hydrops formation was investigated. We also tested their mothers in order to characterise differences between the fetal and the maternal immune response. There was no correlation between the increased rate of maternal NS-1 IgG positive results and arthropathy. NS-1 IgG has been related to prolonged viraemia, which may be a result of pregnancy associated immunosuppression.⁵ In pregnancy, host defence is modified by a shift of the immune response towards a TH2-type profile, which is associated with immunological tolerance.^{4,6,7} It has been hypothesised that this allows protection of the fetal allograft. Cytokines may play an important role in controlling quantity and quality of B cell antibody production during pregnancy.^{6,12} Since neutralising antibodies may contribute to parvovirus B19 elimination, a TH2 dominance in pregnancy may be associated with the synthesis of ineffective (for example, asymmetric) antibodies and prolonged viraemia, which in turn may activate anti NS-1 production.^{12,13} Our results confirm other reports of a high prevalence of maternal NS-1 IgG in pregnancies complicated by parvovirus B19 infection.⁵ In this study we show that a proportion of fetuses with hydropic disease express anti NS-1 persisting for years. However, in the children significantly fewer sera were anti NS-1 positive compared to their mothers. An association between chronic arthritis and parvovirus B19 infection is controversial; however, persistent virus particles have been observed in synovial fluid/tissue.¹⁴ Although increased prevalences have been observed, the role of NS-1 antibody in the pathogenesis of arthritis or other persistent infection is not understood completely.¹⁴ In this study, the occurrence of NS-1 antibody was not associated with chronic arthritis.

At the time of testing, none of the NS-1 positive children in our study had joint symptoms, and of five women with acute infection associated arthralgia only two were NS-1 positive. These findings, although derived from a small cohort, underline previous observations questioning the association of NS-1 antibodies and chronic rheumatoid arthritis.¹⁵

The levels of IgG against structural proteins in this study were significantly increased in maternal sera compared to their children. Obviously the infection has been controlled in all women, since all were IgM and PCR negative. Methods for sensitive nested PCR have been described elsewhere.¹⁶ For ethical reasons we were not able to study bone marrow, which is a limitation of our study as cases of persistent myeloid infection may have been missed. However, none of the children and their mothers showed evidence of a haematological disorder resulting from apoptosis of parvovirus B19 infected erythroid lineage cells. The molecular structure and functional competence of detected IgG antibodies has not been evaluated. It is hypothesised that reduced neutralising activity of maternal IgG antibodies necessitates higher levels of specific IgG for infection control and definitive virus elimination, which may explain higher maternal levels.

In children aged 1–9 years the seroprevalence of specific antiparvovirus B19 IgG is positively correlated with age and rises from approximately 15–50%.^{17–20} These data are confirmed by the seroprevalence of 20% in our control group. Isolated lymphocytes of convalescent adults exhibit a type 1

immune response to parvovirus B19 antigen.²¹ In contrast, IFN γ production has been shown to be significantly reduced in childhood.²¹ In the hydropic fetus, disease may be self limiting by rapid formation of anaemia and consecutive extinction of cells permissive for viral replication. A relatively large proportion of fetuses infected >20 weeks of gestation survive without development of severe hydrops.^{22–25} Moreover, postnatal viraemia has been observed rarely in neonates following intrauterine infection.^{26–28} A terminated interval of viraemia may contribute to the reduced levels of anti VP1/VP2 IgG. Also, the observed IgG levels were higher than reported from other studies of uncomplicated pregnancies,²⁹ indicating an immune response different to that in cases with fetal hydrops.

In conclusion, in children having survived severe fetal parvovirus B19 disease, we did not find serological signs of persistent infection. In their mothers, the high prevalence of anti NS-1 (72% v 28%) and increased levels of antiparvovirus B19 IgG (mean 197 v 48 IU/ml) may retrospectively reflect prolonged viraemia during pregnancy, whereas in the fetus viral replication may be self limited. However, since this study has been based on single time point tests, our results may serve as a basis for serial, follow up studies in order to characterise the maternofetal immune response and duration of viral persistence in detail.

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