Fetal pathology in intrauterine death due to parvovirus B19 infection

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Objectives To study the pathological features of fetuses dying because of parvovirus B19 infection, with particular reference to the presence of hydrops; to assess the usefulness of immuno-chemistry as a screening method for the detection of parvovirus infection at post-mortem examination.

Design Review of clinical, sonographic, serological and pathological data; immunohistochemical staining of post-mortem tissue.

Sample Cases of intrauterine fetal death occurring during the 18-month period January 1993 to June 1994 inclusive, referred for post-mortem examination to the Pathology Department, Royal Victoria Infirmary, Newcastle upon Tyne.

Results Eleven cases of fetal death due to parvovirus infection were identified. Seven fetuses were less than 18-week size. Three fetuses showed conspicuous hydropic change. One of the 11 cases was detected for the first time by retrospective immunochemical screening. Of cases originating from the Newcastle district, parvovirus infection was responsible for about 10% of all non-malformed fetal deaths occurring between 10 and 24 weeks of gestation referred for pathological examination.

Conclusions During the period of study, parvovirus infection was a relatively common cause of mid-trimester fetal death. Many fetuses dying because of this infection are not noticeably hydropic, and the possibility of parvovirus infection should be considered in any case of intrauterine fetal death. Immunochemistry can be used to confirm the histopathological diagnosis, and may be of particular help where there is advanced autolysis; immuno-histochemical screening may detect occasional cases not initially identified by examination of routinely stained tissue sections.

INTRODUCTION

Human infection with parvovirus B19 leads to a variety of clinical manifestations, including erythema infectiosum (fifth disease), arthropathy, aplastic crises in individuals with an underlying haemolytic disorder, and intrauterine fetal death[1,2]. Infected fetuses are described typically at autopsy as being hydropic, with characteristic inclusions in the nuclei of erythroid precursor cells[3-5]. Parvovirus infection occurs in regular epidemics, the most recent during 1993 to 1994[6] (I. Mellor, PHLS Communicable Disease Surveillance Centre, personal communication). This paper sets out our observations on the pathological features of fetuses dying due to parvovirus infection, with particular reference to the sensi-tivity and usefulness of hydrops as a marker for such infection.

METHODS

The pathology department at the Royal Victoria Infirmary provides a local perinatal pathology service for the Newcastle upon Tyne district (5300 deliveries/year), and a referral service for the former Northern Region of England (40,000 deliveries/year). During the 18 months of this study, from January 1993 to June 1994, post-mortem examinations were carried out in approximately 270 cases of intrauterine fetal death (miscarriages and stillbirths); all were carried out personally or under the supervision of one pathologist (C.W.). All deaths which had been attributed to parvovirus infection were identified by review of autopsy report files and the clinical, sonographic, autopsy and serological findings reviewed.

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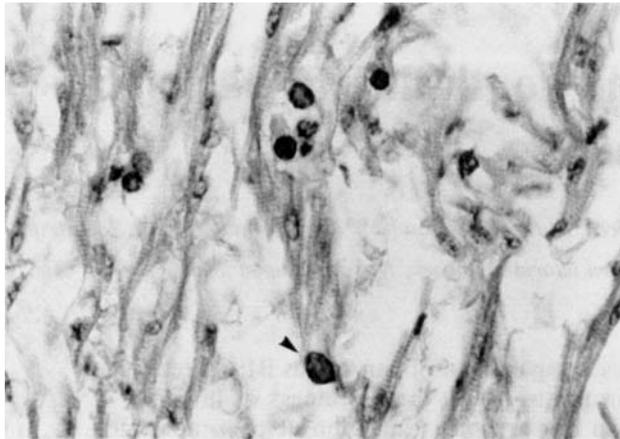
Fig. 1. Haematoxylin and eosin stained section of myocardium showing numerous intravascular cells with nuclear inclusions typical of parvovirus infection (one is arrowed); more darkly staining chromatin is pushed to the periphery of the nucleus.
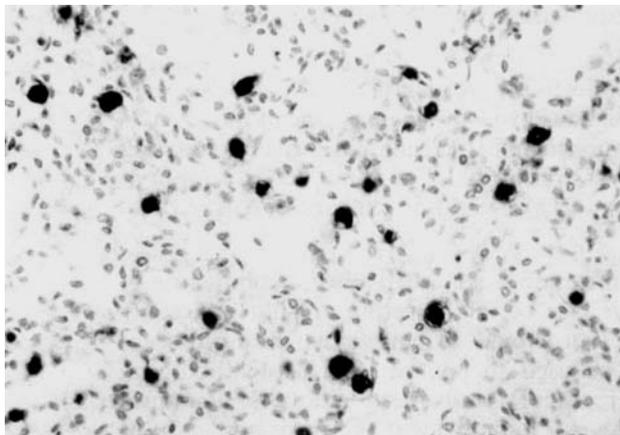

Fig. 2. Fetal lung tissue showing very heavy immunohistochemical labelling of intravascular cells using antibody R92F6.

In each case, the diagnosis of parvovirus infection had been made by identifying the typical eosinophilic or amphophilic intranuclear inclusions in intravascular cells (Fig. 1), followed by immunohistological confirmation using a monoclonal antibody (R92F6; Novocastra Laboratories, Newcastle upon Tyne, UK) which recognises parvovirus B19 capsid proteins[7]. Immunolabelling was performed by a standard streptavidin–biotin (SAB) technique, using 3 μ sections of formalin-fixed, paraffin-embedded lung tissue; primary antibody (ascites) at a dilution of 1 : 500; secondary antibody (biotinylated rabbit anti-mouse; Dako Ltd., High Wycombe, UK) at a dilution of 1 : 500; Dako SAB kit reagents as tertiary; and diaminobenzidine as chromogen. Using this method, parvovirus infected cells are identified by granular brown staining of both the nucleus and cytoplasm (Fig. 2). As negative controls for each case a duplicate section was stained, omitting the primary antibody. In addition, each run included a lung section from a previously identified case of parvovirus infection as a positive control. All sections were counterstained with haematoxylin.

In an attempt to determine whether immunochemistry might be a more sensitive method than routine haematoxylin/eosin staining for identifying fetal parvovirus infection in cases of unexplained spontaneous abortion, all 10 to 24-week size non-malformed fetuses referred for autopsy during the period of study were identified retrospectively, and lung sections from each case (n = 132, including four sets of twins) stained immunochemically using the R92F6 antibody. The sections were then examined independently by two histopathologists (C.W. and S.A.H.).

RESULTS

During the period of the study, 10 cases of fetal parvovirus infection had been diagnosed prospectively on routine autopsy examination of cases of intrauterine fetal death (five each in 1993 and 1994). The clinical and pathological features of these fetuses are presented in Table 1 (cases 1 to 10); the diagnoses were supported by serological data.

In addition to confirming the diagnosis made on routine histological examination, retrospective immunochemical staining using the R92F6 antibody identified one additional case of parvovirus infection (case 11) involving an 11-week sized fetus. Immunolabelled blood cells were numerous in the lung section. Review of the original haematoxylin/eosin sections from this case revealed poor tissue preservation due to considerable autolysis; very occasional, somewhat fragmented nuclear inclusions were apparent on close scrutiny, which had not been noticed when the sections were originally examined. Both pathologists examining the immunohistochemically stained sections identified independently the same positive cases.

The gestation of the 11 fetuses ranged from 11 to 26 weeks; seven were less than 18-week size. Only three fetuses had hydrops at autopsy; in these cases hydrops had also been specifically noted on antenatal ultrasound. In the remaining eight pregnancies there was no evidence on ultrasound or macroscopic examination of oedema in excess of the mild degree often noted in association with intrauterine death; in one case the fetus had been largely disrupted by suction termination.

During the 18 months of the study, autopsies were carried out on 66 nonmalformed fetuses

Table 1. Clinical and pathological details of cases of parvovirus infection.

Case no.	Maternal age (years)	Gestation at time of IUD* (weeks)	Pregnancy history	Ultrasound findings	Serology¶ Gestation	IgM	IgG	Maceration	Hydrops/ oedema	Other pathological findings	Abnormal placental findings
1	32	12	MA	IUD	11	+	+	Yes	No	—	(fragmented)
2	26	18	MA	IUD	18	+	+	Yes	Mild nuchal oedema	Excess hepatic iron deposition	—
3	25	11	MA	IUD	8	+	−	Yes	No	—	(uterine cast)
					12	+	+				
4	30	23	TOP on basis of ultrasound findings	Hydrops	15	−	−	No	Generalised hydrops	Fatty adrenals; excess hepatic iron deposition; periventricular white matter infarction	Heavy (430 g), pale, bulky
					23	+	+				
					23 (F)	+	ND				
5	28	17	Maternal rash MA	IUD	26	±	+	Yes	No	Fatty adrenals	(fragmented)
6	23	26	Stillbirth	Ascites, pleural effusions, IUD	21	+	+	Yes	Pleural effusions, ascites	Right ventricular dilatation, fatty adrenals	Heavy (500 g), pale, bulky
7	20	21	MA	IUD	21	+	+	Yes	No	Right ventricular dilatation, pulmonary hypoplasia, fatty adrenals	—
8	32	11	MA	IUD	8	+	−	Yes	NA†	—	(fragmented)
					11	+	+				
9	25	17	MA	Hydrops	14	+	−	Yes	Generalised hydrops	—	—
					18	+	+				
10	25	14	Maternal rash MA	IUD	11	+	ND	Yes	No	—	—
11	31	11	SA	—	11	ND	ND	Yes	No	—	—

* Gestation assessed from fetal measurements; † suction termination; ¶ serology: maternal, except F = fetal; ± = equivocal; ND = not done; IUD = intrauterine fetal death; MA = missed abortion; SA = spontaneous abortion; TOP = termination of pregnancy; FBS = fetal blood sampling; NA = not assessable.

(from 63 pregnancies with three sets of twins) dying between 10 and 24 weeks of gestation, whose mothers were from the Newcastle district. Eight (12·1%) of these fetuses had features of parvovirus infection. Fetuses referred from outside Newcastle were excluded from this assessment of the frequency of infection since they represent a selected population, in contrast to the more complete ascertainment of Newcastle cases.

DISCUSSION

Although in previously reported series of parvovirus-infected fetuses hydrops was an almost constant feature, these series are probably not generally representative of parvovirus cases. For example, the fetuses studied by Morey et al.[5] and Rogers et al.[8] were diagnosed retrospectively by reviewing cases of non-immune fetal hydrops. Similarly, the cases reported by Schwarz et al.[3] were identified by antenatal maternal serological investigations prompted by the presence of fetal hydrops (n = 12), maternal rash (n = 2), or contact with individuals known to have parvovirus B19 infection (n = 2). In the present study cases were identified prospectively (except case 11) by examining all intrauterine fetal deaths for parvovirus inclusions, irrespective of the clinical history, ultrasound findings or appearance of the fetus.

Conspicuous fetal hydrops was only present in three of 11 cases of parvovirus infection identified during an 18-month period. In the other eight cases, oedema was either not noticeable or of a mild degree consistent with the effects of intrauterine death; only in the three cases with marked oedema had a specific diagnosis of hydrops been made on ultrasound examination. Many of the other features identified at autopsy in this series are well recognised in cases of parvovirus infection and/or hydrops, including cardiac dilatation, accumulation of fat in the adrenal cortex, excess hepatic iron deposition and increased weight and bulk of the placenta[4].

In searching for features that might explain why hydrops was less frequent in our cases, it is perhaps relevant that in seven pregnancies the gestation at the time of fetal death (based on fetal size) was less than 18 weeks, with four fetuses of 11 to 12-weeks size. In a prospective study of pregnant women identified as having parvovirus infection because of a rash or contact with erythema infectiosum, the fetal loss rate was 16% (30/186), with 27 of the losses occurring before 21 weeks; B19 DNA was found in the tissues of six of 14 fetuses examined, but of these six only one was described as hydropic[9]. In the study by Schwarz et al.[3] noted above, in only one of 15 cases of IUD

had the death occurred before 18 weeks (range 14 to 33 weeks); the corresponding figures for the series of Morey et al.[5] and Rogers et al.[8] were two of 10 (14 to 29 weeks), and none of five (21 to 25 weeks). In all the cases examined here, very large numbers of blood cells appeared to be infected with parvovirus and it would seem reasonable to speculate that smaller fetuses might die relatively quickly because of overwhelming, parvovirus-induced anaemia. Larger fetuses may have greater haematological reserve which allows time for the development of hydrops; the pathogenesis of hydrops in parvovirus infection has been attributed to the anaemia, but other mechanisms such as placental oedema and obstruction of venous return due to ascites have been suggested as contributory factors[5].

Thus, the likelihood of hydrops developing may increase with the length of gestation, so that only by about 18 weeks of gestation nearly all infected fetuses will be hydropic. In some cases the oedema is transitory, resolving by the time of fetal death[5, 10]. It follows that the possibility of parvovirus infection should be considered in every case of intrauterine death, even when there is no conspicuous soft tissue oedema or effusions. During the 18 months of this study, which coincided with the most recent epidemic, more than 10% of all non-malformed fetal losses in the Newcastle district occurring between 10 and 24 weeks, and coming to autopsy, were due to parvovirus.

Immunochemistry is a useful confirmatory test when dealing with a fetus showing parvovirus-type nuclear inclusions. Case 11 in this series suggests that it may be more sensitive than examination of routinely stained sections as a screening method for identifying cases of parvovirus infection and that it may be particularly useful when there is advanced tissue autolysis. In studies of series of non-immune hydrops, no additional cases of parvovirus infection were identified by in situ DNA hybridisation[11] or by DNA amplification using the polymerase chain reaction[12] over those showing nuclear inclusions in routinely stained sections, although Morey et al.[13], using in situ DNA hybridisation, report consistent detection of small numbers of positive cells in one case where

viral inclusions were not readily identifiable. It would appear, however, that the majority of cases will be detected by scrutiny of relatively inexpensive, routinely stained histological sections.

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