FETO-PLACENTARY PATHOLOGY IN HUMAN PARVOVIRUS B¹⁹ INFECTION(1)

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SUMMARY
In view of the scarce references concerning the histological data in congenital parvovirus human B19 infection, we intend to provide a description of the pathological features observed in six autopsies. The virus was detected by DNA hybridization (ISH-DBH), PCR and electronmicroscopy (EM) in paraffin-embedded feto-placentary tissues. These cases constitute a subset from 86 Non Immunologic Hydrops Fetalis (NIHF) cases, in which a systemic complex of inflammatory/degenerative lesions of unknown etiology was visualized by optical microscopy. In one case a syphilitic process was detected, typefying a double infection. All fetuses showed a similar pathology - hydrops, hepato-splenomegaly, lung hypoplasia and erythroblastemia, the specific histological feature being the presence of intranuclear inclusions in the erythroid progenitors, in the erythropoietic visceral tissue and in blood marrow. Complex cardiopathy allied to abnormal lung lobulation and polysplenia were observed once; in 2 cases endocardial fibroelastosis was diagnosed. The pulmonary lesions were represented by dysmaturity allied to interstitial mononuclear infiltration. The hepatic consisted of cholestasis, portal fibrosis, canalicular proliferation, hemossiderosis, focal necroses and giant cell transformation. The central nervous system lesions were predominantly anoxic although the autolysis impaired a correct diagnosis. KEYWORDS: Non immunologic hydrops fetalis; Intruterin infection; Human parvovirus B¹⁹; Morphological study; Virus detection.
INTRODUCTION

We report the pathological findings from a series of six cases of Non-Immunologic-Hydrops Fetalis (NIHF) consecutive to intrauterine parvovirus B_{19} infection, which was unsuspected clinically but documented by autopsy. Diagnosis was based in (1) morphological examination (gross and optical microscopy - OM), (2) DNA detection (in-situ - ISH, dot-blot hybridization -DBH), nested polimerase chain reaction (PCR) and (3) electron-microscopy (EM) for viral identification in formalin-fixed paraffin-embedded fetoplacentary tissues.

MATERIAL AND METHODS

A series of fetal and neonatal autopsies performed at Instituto Fernandes Figueira - FIOCRUZ, Rio de Janeiro - Brazil was retrospectively investigated for the presence of NIHF and the correlated pathology. The clinical charts, operative description of the gross and microscopic pathology as well as photographic documentation have been reviewed; reexamination of tissues and confirmation of the pathologic diagnoses were made in each case. Among 3111 pediatric autopsies performed in a standard fashion (1954-1992), 86 cases of NIHF were reviewed; placentas were available in all cases.

Data collected in 30 autopsies of NIHF from the original 86 showed a systemic complex of feto-placentary inflammatory/degenerative lesions, in different combinations. This constellation of lesions was similar to that described in congenital rubella and posteriorly observed in other viral infections. We named it - "Intrauterine Systemic Infection (IUSI)" - of unknown etiology.

Formalin-fixed paraffin-embedded lung and liver tissues from this subset of cases of NIHF as well as 5 control-cases were examined for the presence of human parvovirus B_{19} by DNA hybridization, as described previously. Using ISH with a biotynilate probe one positive case was detected. Using 32-P-labelled probes in a DBH assay format, five further positive cases were obtained and confirmed by PCR assay and direct electron-microscopy (EM). It is noteworthy that one control-case, which was originally diagnosed as syphilis (typical lesional complex and evidentiation of Treponema pallidum in several organs), was included in this group of five cases, therefore substantiating the dual infection.

Tissues available included placenta, liver, heart, lungs, bone-marrow, kidneys, adrenals, pancreas, spleen, thymus, skeletal muscle, brain and bone. Occasionally a case was lacking one of these organs, but lungs, liver, brain, spleen, adrenals, pancreas, thymus, bone-marrow were examined in each case. The most reliable tissue for histology was lung followed by brain, placenta and heart.

For electron-microscopic observation the tissues were minced and the supernatants were stained with 2% phosphotungstic acid.
RESULTS

A summary of clinical history and characteristic features of each case are presented in Table 1; macroscopic and microscopic data, allied to methods and results of virological detection, are summarized in Table 2.

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Neg. - Negative
Pos. - Positive
W. - Week

**TABLE 1**

Summary of the characteristics of the cases
Pathological Findings - Gross features: five of the six fetuses presented evidence of maceration; in all cases feto-placental hydrops was observed (Fig.1), aside effusions in one or more body cavities and subcutaneous edema. Hepato-splenomegaly and pulmonary hypoplasia were a constant. In the baby who lived for an hour (Case V) icterus was detected. Only one fetus (Case I) exhibited visceral malformations, represented by cardiopathy (atrio-ventriculare commune), abnormal lobulation of one of the lungs and polysplenia.
In case II a syphilitic infection was suggested by the evidentiation of osteochondritis, periostitis allied to the gross aspect of the lungs (pneumonia alba) and maternal serology (VDRL 1/64).

Overall, the placentas had increased weight, being bulky, pale and edematous.

**Histological Findings** - Sections of formalin-fixed paraffin-embedded feto-placentary tissues from all cases were filled in the department. The routine histological stain was hematoxylin-eosin; for the revision, special stainings were made (Giemsa Papanicolaou, periodic acid of Schiff (PAS), trichromic of Gomori). Sections of the heart were also stained with orcein for elastic fibers and sections of the liver and brain were stained by Pearl's Prussian blue for ferric iron, and by Kossa's method for mineralized deposits. In case 2 the silver impregnated material (Levaditi’s method) was reviewed and in the liver, lungs, adrenals and pancreas myriads of organisms were observed.

In all cases OM revealed the presence of intranuclear eosinophil-inclusions and margination of the chromatin in erythroblasts; in some of them a popcorn profile (Figs. 2, 3, 4) with blebs of basophilic material projecting from the nuclear surface is mentionwhile. The majority of these cells containing inclusions clearly layed within the visceral and placentary blood vessels and was also visualized in extramedullary visceral foci. The cells were more frequent in the lungs, liver, brain and placenta; in the bone-marrow of the femur and temporal bone they were also seen (Cases II, III) in spite of advanced autolysis.
Fig. 2 - Capillary of the lung: erythroblastic inclusions with margination of the chromatin and blebs on the nuclear surface (Case II). H. E. x 1200.

Fig. 3 - Bone marrow of the temporal bone. Identical inclusions are observed (Case III) exhibiting a "popcorn like" appearance (arrow). H. E. x 1200.
In the lungs diffuse interstitial or focal round cell infiltration was a common finding.

Focal round cell infiltrates were present in the myocardium; rarely areas of coagulative necrosis of myofibers were observed. Microscopic features of fibroelastosis beneath the ventricular endocardium were detected in 2 out of 6 babies (Cases I, VI) (Fig. 5).

Although advanced autolysis impaired detailed examination of the hepatic parenchyma, marked periportal fibrosis and bile duct proliferation were common findings; cholestasis was prominent, diffuse (Fig. 6). In case 3 irregularly disseminated foci of mineralization
were identified; the staining with Prussian blue revealed the presence of uniform sparse ferric iron (Cases V, VI).

![Liver - Portal - space with irregular prolongations, mild canaliculare proliferation and mononuclear infiltration. Erythropoietic parenchymal foci. H.E x 560.]

Brain tissue was always examined, in spite of the autolysis. Round cell infiltration of the leptomeninge was observed as well as marked erythroblastemia. In the cortical and subcortical areas, mainly in the basal nuclei, mineralized plates in the vessel walls or in the nervous tissue were observed. These features were more extensive in Cases VI and VII. In the white substance cellular groups of cells simulating glial nodes were present (Cases V, VI); chronic ependimitis was also present.

Pathological features were detected in the placentas of the six cases; congenital parvovirus B19 infection was strongly suggested by placental histology. Hydropic villus was present in all cases aside villous tissue dysmaturity, villitis (Fig. 7), and intervillitis. The most striking abnormality was a vasculitis affecting all the fetal circuit, represented by swelling, fragmentation or necrosis of endothelial cell nuclei; in the basal decidua foci of lymphoplasmocitoid cells were sparsely seen.
EM by negative staining detected viral particles in the supernatant of infected tissues, size 20 nm diameter.

DISCUSSION

In face of the paucity of data relative to detailed pathological findings in congenital parvovirus B<sup>19</sup> infection<sup>13</sup>, we intend to provide a morphological study (gross and OM examination) of six cases of NIHF. These cases were a subset from 86 autopsies of NIHF of a larger perinatal series in which a complex of systemic feto-placentary inflammatory/degenerative lesions was observed<sup>9</sup>. This complex of lesions was similar to the one described in some viral infections, but the etiologic agent could not be detected by the morphological examination alone. A combination of old (reexamination of routinely microscopic paraffin-embedded sections) and new technology (ISH-DBH-PCR and EM) established the presence of B<sup>19</sup> in a number of fetal organs where this virus preferentially infected erythroblasts. As several authors consider<sup>2,10,16</sup>, these techniques can be used to confirm B<sup>19</sup> infection, but the starting point is the recognition of inclusion-bearing erythroid cells in the feto-placentary tissues as well as in parenchymatous cells of fetal tissues. PORTER et al.<sup>15</sup> and MARK et al.<sup>11</sup> accentuated that histology of feto-placentary tissues is as sensitive as PCR and less labor-intensive, emphasizing that routine histology is an easier and more reliable method of diagnosing fetal parvovirus infection. As only four of six placentas had typical parvovirus inclusions it seems that the morphological examination of this organ alone is insufficient for diagnosis of parvovirus infection.

ROGERS (1992)<sup>17</sup> pointed out that in many cases examination of fetal organs from the autopsy has revealed many nucleated red blood cells with diagnostic inclusions regardless of the presence of inclusions in placental cells. BURTON & CAUL (1988)<sup>5</sup> noted that in order to increase the chance of detecting B<sup>19</sup> infection a very careful look for intranuclear
inclusion bodies should be conducted not only in erythroid cells but also in cells of other tissues.

Our case-reports support the view that human parvovirus B19 infection may result in severe damage of the fetal heart: inflammatory lesions and subendocardial elastosis. It is considered that the latter reflects the severity and chronicity of heart failure, probably related to delayed intrauterine infection. Myocardiopathy represented by eosinophilic damage without inflammation has been reported in association to parvovirosis; in this series the autolysis impaired a true judgement.

In case I a complex cardiac malformation was verified, allied to abnormal lung lobulation and polisplenia. It is the opinion of some authors that B19 can interfere with organ development, but BERRY et al. (1992) affirmed that, although paroviruses are teratogenic in animals, there is no evidence that B19 is a significant teratogen in man. USSER & DEMMILER (1996) consider that there have been few reports of congenital anomalies associated with B19, and that there is little evidence to suggest that the rate of congenital anomalies after B19 infection exceeds background rates in the population. Notwithstanding it is difficult to affirm that an association between uterine infection and congenital defects in the offspring could be more than a coincidence. It is possible that the presence of cardiopathy does not constitute causal relationship; probably there are other factors involved, as this mother also had congenital cardiopathy.

There have been isolated reports of hepatic damage due to B19; the hepatic lesions included giant-cell hepatitis, cholestasis, hemossiderin deposition, periportal fibrosis and bile duct proliferation. In our cases periportal fibrosis and bile duct proliferation were frequent; hemossiderin deposition was present in cases V, VI. In case III irregularly disseminated foci of mineralization were observed, a feature which permits considering the possibility of previous parenchymal necrosis, lesion described by ANAND et al. (1987). As METZMAN et al. accentuated (1989), these data suggest that parvovirus B19 should be added to the list of agents capable of causing hepatic disease manifest at birth. WHITE et al. (1995) proposed that recognition of combination of siderosis with fibrosis and bile duct proliferation permit identification of cases of fetal parvovirus B19 infection.

Overall the lesions in the brain are compatible with those described in chronic hypoxia, although chronic inflammatory infiltration of the leptomeninges, mild perivascular collections of lymphocytes and amorphous mineralized deposits allied to chronic ependimitis were observed. As CONRY et al. considered (1993), a report describing three infants with severe central nervous system abnormalities after maternal B19 infection emphasized the need for additional studies to determine whether fetal infection might cause brain damage.

We may conclude that investigation of parvovirus B19 by newly developed methods of molecular biology will enlighten many fetal and perinatal autopsies, specially those of macerated fetuses.
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RESUMO

Patologia feto-placentária na infecção pelo parvovírus humano B19

São escassas as referências aos dados histológicos relativos à infecção congênita pelo parvovírus humano B19. Apresentamos estudo morfológico de seis autópsias em que o vírus foi detectado por hibridização DNA (HIS-HDB), PCR e microscopia eletrônica (ME) em tecidos feto-placentários fixados em formol e incluídos em parafina. Estas autópsias integravam um grupo de 86 Hidropisias Fetais não Imunológicas (HFNI) que apresentaram à microscopia óptica complexo lesional sistêmico inflamatório/degenerativo de causa indeterminada. Em uma criança detectou-se processo sifilítico multivisceral com microorganismos, caracterizando infecção dupla. Os fetos exibiram quadro semelhante: hidropisia, hepato-esplenomegalia, hipoplasia pulmonar e eritroblastemia. O dado histológico específico consistiu em inclusão nuclear em eritroblastos do sangue, do tecido visceral eritropoético e medula óssea. Cardiopatia complexa, lobulação pulmonar anômala e poliesplenía foram observadas em um caso. Em dois corações evidenciou-se fibroelastose difusa. As lesões pulmonares se manifestaram por dismaturidade e processo crônico intersticial; as hepáticas revelaram colestase, fibrose portal, proliferação canalicular, necroses hepatocitárias focais irregularmente dispostas, transformação gigantocitária e hemossiderose. No sistema nervoso central predominaram as lesões anóxicas, embora a autólise não permitisse análise minuciosa.
REFERENCES


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