Gestational Alloimmune Liver Disease in Cases of Fetal Death

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Objective To determine whether alloimmune liver disease can be identified as a cause of fetal death.
Study design This is a retrospective examination of the autopsy tissue of 6 stillborn fetuses and 2 extreme preterm infants (gestational age, 20 to 34 weeks) drawn from families referred for suspected neonatal hemochromatosis. Thirteen appropriate nondisease controls and 8 cases of neonatal acute liver failure with known etiology were also examined. Liver sections were immunostained using anti-human C5b-9 complex.
Results All of the study cases had died with no preceding evidence of fetal distress. Histopathology showed findings of acute liver injury, including global hepatocyte necrosis with minimal reticulum collapse and no fibrosis. Hepatocytes in cases stained strongly positively for C5b-9 complex, suggesting premortem IgG complement–mediated liver injury. Hepatocytes in acute liver failure case controls did not demonstrate a similar mechanism of liver injury.
Conclusions Alloimmune liver disease is sometimes associated with fetal death. *(J Pediatr 2011;159:612-6)*.

Neonatal hemochromatosis (NH), defined as the coexistence of severe liver disease and siderosis of extrahepatic tissue,[1-4] is a phenotype that apparently results from fetal liver injury.[1,2,5] Recent evidence suggests that most cases stem from gestational alloimmune liver disease (GALD).[6,7] Hepatocyte damage in GALD is mediated by fetal complement via classical activation of the terminal complement cascade.[8] The presence in hepatocytes of C5b-9 complex, the terminal complement cascade (TCC) neoantigen formed in assembly of the membrane attack complex (MAC), indicates TCC activation on the plasma membrane. This finding is unique to the cases of NH among cases of severe neonatal liver diseases and thus provides an alternative approach to diagnosing GALD in cases without the extrahepatic siderosis characteristic of NH.

In a small fraction of GALD cases with the NH phenotype, the liver demonstrates acute hepatocyte injury and minimal fibrosis.[8] These findings suggest that in some circumstances, materno-fetal alloimmunity may cause an acute form of fetal liver injury, as opposed to the subacute or chronic disease more commonly associated with NH. They further suggest that tissue siderosis may develop very soon after the onset of fetal liver disease if the liver injury is severe. It follows that materno-fetal alloimmunity could produce severe, acute liver injury in the fetus, possibly resulting in fetal acute liver failure (ALF). If this occurred in a brief time frame, extrahepatic siderosis might not have time to develop, and then the diagnosis of NH could not have been made based on standard criteria. We analyzed autopsy tissue specimens from stillborn fetuses and extremely preterm infants to investigate whether global hepatic necrosis due to GALD was the probable cause of death.

Methods

This study involved a retrospective analysis of autopsy tissue. The cases were an unselected sample of tissues provided by families referred for consultation regarding the need for gestational therapy to prevent recurrent NH.[7] The cases comprise all nonmacerated postmortem liver specimens from stillborn fetuses and live-born infants of gestational age ≤34 weeks (**Table I**). For comparison, we used specimens from autopsies of 11 newborns of 24 to 39 weeks gestational age, as described previously.[8,9] These newborns all died of perinatal asphyxia due to immaturity, meconium aspiration, or other causes. We also reviewed the electronically available records of stillbirths of 18 to 34 weeks' gestational age with postmortem examination performed in our institution between 2008 and 2010. We identified 2 (22 weeks and 28 weeks gestation) with no liver maceration, an unknown family history, and no autopsy evidence of liver disease for inclusion as comparison cases. Finally, we searched the autopsy records of Children's Memorial Hospital for the years 1996 to 2010 and identified 8 case controls in whom ALF was considered the primary cause of death based on clinical and/or autopsy grounds. These were all term infants who died within the first 3 months of life, with all but 1 dying in the first 2 weeks of life (**Table II**). The collection and study of these samples were approved by the Children's Memorial Hospital Institutional Review Board by exemption.

ALF	Acute liver failure
GALD	Gestational alloimmune liver disease
MAC	Membrane attack complex
NH	Neonatal hemochromatosis
TCC	Terminal complement cascade

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The authors declare no conflicts of interest.

0022-3476/$ - see front matter. 10.1016/j.jpeds.2011.03.048

Table I. Cases: Clinical characteristics and liver immunohistochemistry

Case	Gestational age, weeks	Birth status	Sibling with NH	Extrahepatic siderosis	C5b-9 staining
1	22	Live birth	Yes	No	4+
2	22	Stillbirth	No	No	4+
3	34	Stillbirth	No	Yes	4+
4	30	Live birth	Yes	Yes	4+
5	22	Stillbirth	No	Yes	4+
6	20	Stillbirth	No	Yes	4+
7	20	Stillbirth	No	No	4+
8	21	Stillbirth	No	Yes	4+

Histology and Immunohistochemistry

From paraffin-embedded liver tissue specimens, 5-μM sections were obtained for histology (hematoxylin and eosin, trichrome, reticulum [modified silver], and Perls' Prussian blue staining) and immunohistochemistry analyses. The immunohistochemistry techniques used have been reported previously.[8,9] In brief, sections were treated with monoclonal antibody to human SC5b-9 neoantigen (TCC-neoantigen, monoclonal antibody to human SC5b-9 neoantigen; Quidel, San Diego, California), followed by treatment with an appropriate biotinylated antibody (Vector Laboratories, Burlingame, California), followed by development with VECTASTAIN ABC reagent (Vector Laboratories) according to the manufacturer's instructions. Sections processed without primary antibody were considered controls for nonspecific reaction.

Quantification of TCC-Neoantigen Expression in Hepatocytes

Liver sections stained for TCC-neoantigen were photographed to determine the fraction of hepatocytes displaying MAC. Random nonoverlapping 200× magnification images were obtained of hepatic parenchyma from each liver section stained for TCC-neoantigen; 3 to 5 images per specimen were acquired to ensure adequate sampling. Because the specimens often contained few intact hepatocytes, a semiquantitative scale for positive staining was used rather than the quantitative approach reported previously.[8] The total number of identifiable hepatocytes in the images and then the number either showing or not showing positive staining (depending on which condition was not dominant and thus easier to tally) were tallied. The proportion of positively stained hepatocytes was calculated, and a staining scale of 0 to 4+ was applied, with 0 = no staining, 1+ = <25% positive hepatocytes, 2+ = 25%-50% positive hepatocytes, 3+ = 50%-75% positive hepatocytes, and 4+ = >75% positive hepatocytes.

Results

Eight cases were acquired from centers in the United States (n = 5) and Australia (n = 3) (Table I). The indication for family referral was a positive family history of NH in siblings in 2 cases and suspicion of NH in 6 cases, 3 because of hepatic siderosis detected on autopsy and 3 because of recurrent stillbirths in a maternal sibship. Two of these patients were full siblings (cases 2 and 3), and the remainder were unrelated. In addition to these siblings, 4 cases studied (cases 1, 4, 5, and 8) had 7 stillborn maternal siblings that were not studied because no postmortem examination was performed (n = 4) or the tissues were too macerated to allow examination (n = 3).

No signs or symptoms of fetal distress preceding the terminal event were reported, and in no case was the cause of death discovered on postmortem examination. Postmortem examination revealed anasarca (hydrops fetalis) in 3 cases (2, 3, and 7), one with reported edema detected on ultrasound a few days before fetal death. The mean gestational age of the cases was 23.5 ± 5.2 weeks. Six cases were stillborn, and 2 died within minutes after abrupt spontaneous labor. Our reexamination of the 8 cases found extrahepatic siderosis and an anatomic diagnosis of NH in 5 cases, including 3 of the 4 cases with unexamined stillborn maternal siblings (cases 4, 5, and 8).

Hepatic Histopathology

Most cases of NH have histopathology that is most consistent with subacute or chronic injury.[1,2,5,8,10] In contrast, our cases demonstrated evidence only of acute liver injury. Global,

Table II. ALF case controls: Clinical characteristics and liver immunohistochemistry

Case control	Age at death	Autopsy diagnosis	C5b-9 staining
CC-1	12 days	HSV acute hepatic necrosis	1+
CC-2	9 days	Echovirus liver necrosis	1+
CC-3	3 months	Adenovirus ALF	1+
CC-4	12 days	Enterovirus hepatic necrosis	2+
CC-5	9 days	HSV acute hepatic necrosis	1+
CC-6	13 days	HSV acute hepatic necrosis	1+
CC-7	1 day	Ischemic hepatic necrosis	1+
CC-8	0 days	Maternal HELLP syndrome	1+