Minor Salivary Gland Biopsy in Neonatal Hemochromatosis

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Background: Neonatal hemochromatosis (NH), a rare disorder seen in newborns, is defined as liver failure with extrahepatic iron deposition that spares the reticuloendothelial elements. This disorder is considered the pathologic end point of a variety of diseases that result in prenatal liver failure, and mortality without aggressive treatment is common. However, ready diagnosis remains a problem. A liver biopsy specimen showing siderosis is not specific for hemochromatosis and may be risky in patients with coagulopathy.

Objective: To describe a safe and effective method for diagnosing NH that uses lower-lip minor salivary gland biopsy and can be readily performed even in the most severe cases of coagulopathy under local anesthesia.

Methods: Eleven neonates with suspected NH were identified. After informed consent, a biopsy specimen of lower-lip tissue was taken under local anesthesia by the otolaryngology team.

Results: Ten of the 11 neonates had minor salivary gland tissue present (or detected) by initial frozen-section analysis. One of the 11 patients required a second biopsy owing to a lack of sufficient minor salivary gland tissue on the initial specimen, underscoring the importance of frozen-section analysis. Six of 7 neonates with NH had positive biopsy findings and the seventh had a false negative. There were 4 true negatives. Three of 7 children with NH survived, 1 requiring liver transplantation and 2 with medical treatment only.

Conclusion: Minor salivary gland biopsy is a safe and effective way to quickly diagnose NH, a rapidly progressive, often fatal condition.

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REPORT OF A CASE

An African American male infant was transferred to our institution at age 42 days with ascites and coagulopathy. He was...
born full-term via cesarean section secondary to placenta previa, and his immediate neonatal course was uncomplicated. He was discharged home at age 3 days.

At age 2 weeks, he was noted by his mother to be increasingly icteric, but she did not seek medical attention. At age 3 weeks, he brought to a local emergency department with a 5-day history of nonbilious vomiting and lethargy. Evaluation for sepsis and/or meningitis was performed, and the child was noted to have thrombocytopenia, increased liver function, and a direct bilirubin level of 13.6 mg/dL (233 µmol/L). At age 25 days, he was transferred to the referring institution because his cerebrospinal fluid culture was growing Flavobacter.

The child continued to be treated for meningitis at this second institution, but it was noted there that the child's synthetic liver function was not improving. He had received multiple transfusions of packed red blood cells, platelets, and fresh frozen plasma.

At age 42 days, this child was transferred to our institution for further evaluation and treatment of idiopathic liver failure. On arrival, further history revealed no family history of liver disease, no history of genetic diseases in the family, and no consanguineous relationships. On physical examination, it was noted that the child had hepatomegaly: the liver extended 4 cm below the costal margin. Initial laboratory values revealed a platelet count of 47 × 10^3/µL, a total bilirubin level of 17.9 mg/dL (306 µmol/L), a direct bilirubin level of 11.9 mg/dL (204 µmol/L), an albumin value of 2.7 g/dL, a prothrombin time of 31.0 seconds, a partial thromboplastin time of 72.0 seconds, and an international normalized ratio of 4.2.

Comprehensive diagnostic evaluations for neonatal liver failure were performed, including, but not limited to, alpha 1–antitrypsin phenotype, sweat chloride, urine organic acids and plasma amino acids, and serologic tests for congenital infections (toxoplasmosis, rubella, cytomegalovirus, herpesvirus, and syphilis), urine-reducing substances, and screening iron studies (ferritin, iron, and total iron-binding capacity) for NH. Two days after admission, the child’s iron studies were consistent with NH: ferritin level, 798 ng/mL; serum iron, 168 µg/dL (30.1 µmol/L); and total iron-binding capacity, 241 µg/dL (43.2 µmol/L). A lip biopsy was then performed.

The results of the lip biopsy were confirmatory for the diagnosis of NH, and the child was listed for transplantation. In addition, he then started medical therapy for NH, which included chelation and antioxidant treatments with phytonadione (vitamin K), d-α-tocopheryl polyethylene glycol 1000, vitamin E, mucomyst, proton, selenium, actigall, and deferoxime.

After 2 weeks of therapy, significant improvement was noted: the total and direct bilirubin levels were reduced to 11.1 mg/dL (190 µmol/L) and 7.2 mg/dL (123 µmol/L), respectively; the prothrombin time improved to 18.8 seconds (international normalized ratio, 1.8). Chelation therapy was discontinued, and antioxidant therapy was continued. After 3 weeks of treatment the child was discharged under treatment with ursodeoxycholic acid, d-α-tocopheryl polyethylene glycol 1000, vitamin E, and a medium-chain triglyceride containing predigested formula.

At age 116 days, his total albumin level was 3.6 g/dL, and his prothrombin time was 15.3 seconds. At age 1 year, his laboratory values were as follows: bilirubin level, 0.2 mg/dL (3 µmol/L); platelet count, 200 × 10^3/µL; albumin, 3.3 g/dL; and prothrombin time, 12.1 seconds. The child continues to do well and is thriving.

## METHODS

### PATIENTS

Eleven neonates who presented with suspected neonatal liver failure were included in the study. The sex, gestational age, and presenting signs and symptoms are listed in the Table.

Patients 6 and 7 were siblings. On presentation, the pediatric otolaryngology service was consulted. The mean gestational age of the neonates at the time of biopsy was 35.2 weeks (range, 30-40 weeks). Following informed consent by the patient's parent, the decision to proceed with the biopsy was made. The pathologist was not blinded to the patient's clinical status. Review of the patients' charts and pathology specimens was approved by the institutional review board at the Mount Sinai School of Medicine.
The slides were stained immediately with both hematoxylin-eosin and Perl iron stain for hemosiderin. The biopsy specimen tissue was fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 µm. Wound closure was done before the wound was closed and was successful at obtaining salivary tissue. All patients tolerated the procedure well, and there were no complications. Despite 9 of the 11 patients demonstrating coagulopathy with prothrombin times elevated above 20 seconds, there were no intraoperative or postoperative bleeding complications, and no patients required supplemental blood products.

Seven of the 11 patients were ultimately diagnosed as having NH. Six of these 7 had positive biopsy findings, with only 1 specimen (from patient 7) testing as a false negative (Table). The diagnosis of NH was established at the autopsy in patient 7. One of the 7 patients with NH (patient 3) underwent liver transplantation and is alive at age 21 months. The remaining 5 patients were treated medically, and 2 survived. Patients 1, 2, 6, and 7 died at age 120, 7, 26, and 25 days, respectively, and patients 4 and 5 are currently alive at ages 12 and 14 months, respectively. Five biopsy findings were negative, 4 of which were from patients where NH was excluded on clinical grounds (patients 8, 9, 10, and 11).

In all cases, the hematoxylin-eosin slides showed no histopathologic changes or evidence of hemosiderin deposition. Under Perl iron stain, hemosiderin accumulation, when present, was observed only within the epithelial cells of the minor salivary glands. The presence of hemosiderin deposition was graded from 0 to +4 as follows: 0, no hemosiderin identified; 1+, delicate and rare hemosiderin granules in occasional epithelial cells, often visualized only with ×40 magnification; 2+, slightly coarser and more frequent distribution of hemosiderin granules; 3+, increased but focal hemosiderin deposition; 4+, generalized hemosiderin deposition within most cells. Of note, most positive biopsy specimens were graded as 1+ or 2+, reflecting that the presence of hemosiderin was relatively subtle. Although this is a small series, there did not appear to be any correlation between age and degree of positivity.

Postmortem examination of 4 patients (patients 1, 2, 6, and 7) confirmed the diagnosis of NH with severe hepatic fibrosis/cirrhosis and siderosis of hepatic and widespread extrahepatic parenchymal tissues that largely spared cells of the reticuloendothelial (monocyte-macrophage) system. The antemortem lip biopsy specimen in 3 of these 4 patients with NH had shown the presence of hemosiderin deposition within salivary gland epithelium. The lip biopsy specimen of patient 1 is shown in Figure 1. The 1 false-negative case (patient 7) had obtained a 2-day-old preterm infant born at 30 weeks’ gestation. Three weeks passed before this patient’s autopsy demonstrated widespread iron deposition in the tissues. The autopsy of the fifth patient who died (patient 9), whose antemortem lip specimen had been negative, demonstrated massive hepatic necrosis suggestive of a viral cause and no evidence of NH.

Ten of the 11 specimens showed evidence of salivary tissue on the initial frozen-section analysis. One patient required immediate repeat biopsy because the frozen section did not reveal salivary tissue. The second biopsy was done before the wound was closed and was successful at obtaining salivary tissue. The incidence of hemosiderin deposition within salivary gland epithelium. The lip biopsy specimen of patient 1 is shown in Figure 1. The 1 false-negative case (patient 7) had obtained a 2-day-old preterm infant born at 30 weeks’ gestation. Three weeks passed before this patient’s autopsy demonstrated widespread iron deposition in the tissues. The autopsy of the fifth patient who died (patient 9), whose antemortem lip specimen had been negative, demonstrated massive hepatic necrosis suggestive of a viral cause and no evidence of NH.

Neonatal hemochromatosis is a rapidly progressive, usually fatal disorder, for which prompt diagnosis is imperative to initiate medical management and steps for liver transplantation, the definitive treatment. The diagnosis, however, is notoriously difficult. Serum concentrations of iron in patients with NH are elevated, but this may re-

Surgical Procedure
The biopsies were performed in the neonatal intensive care unit with local anesthesia and did not require intubation. Under sterile technique, the lower lip of the neonate was anesthetized locally at the intended biopsy site with approximately 0.5 mL of 1% xilocaine with 1:100000 epinephrine. A vertical incision (perpendicular to the vermilion border) was then made on the mucosal surface of the lip immediately lateral to the midline. The deep tissue was gently dissected and a specimen taken using Iris scissors and forceps. A frozen section was done at that time to confirm the presence of minor salivary tissue. Hemostasis was achieved by direct pressure while waiting for the frozen section results. Once it was determined that the biopsy specimen was adequate, the wound was closed using 5-0 chromic gut sutures in an interrupted fashion (Figure 1). The tissue was sent for permanent section and special stains for iron.

Tissue Preparation
The biopsy specimen tissue was fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 µm. The slides were stained immediately with both hematoxylin-eosin and Perl iron stain for hemosiderin.

Results
Ten of the 11 specimens showed evidence of salivary tissue on the initial frozen-section analysis. One patient required immediate repeat biopsy because the frozen section did not reveal salivary tissue. The second biopsy was done before the wound was closed and was successful at obtaining salivary tissue. All patients tolerated the procedure well, and there were no complications. Despite 9 of the 11 patients demonstrating coagulopathy with prothrombin times elevated above 20 seconds, there were no intraoperative or postoperative bleeding complications, and no patients required supplemental blood products.

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Comment
Neonatal hemochromatosis is a rapidly progressive, usually fatal disorder, for which prompt diagnosis is imperative to initiate medical management and steps for liver transplantation, the definitive treatment. The diagnosis, however, is notoriously difficult. Serum concentrations of iron in patients with NH are elevated, but this may re-

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flect total body iron overload, which may nonspecifically reflect liver disease. Knisely reported that hyperferritinemia supports but does not establish the diagnosis of NH. Hypersideremia, hypersaturation of iron-binding capacity, and low iron-binding capacity are seen in NH but are, again, nonspecific and do not definitively confirm the diagnosis. Magnetic resonance imaging can noninvasively identify a siderosis pattern in the liver, heart, and pancreas. While this has the advantage of being able to identify the pathologic outcomes in NH in vivo, the interpretation of the scans can be difficult. Also, the patients are often too ill to undergo the procedure in the magnetic resonance imaging suite.

The lower-lip biopsy for minor salivary gland involvement has been anecdotally reported to be an effective means to establish the diagnosis of NH. We confirmed these findings by reporting a case series of 11 neonates who underwent this simple procedure at our institution. It is important to remember that frozen section is essential at the time of the biopsy to confirm that salivary gland tissue has been removed. This precludes the need for a repeat biopsy and the concomitant lost time, which can be critical in the management of these children.

While the technical aspects of the procedure are straightforward, the histologic diagnosis can be challenging. The identification of glandular iron deposition requires a specific stain for hemosiderin, which should be ordered at the time of accessioning to avoid an unnecessary delay in the diagnosis. Even with the use of a special stain, the amount of intracellular hemosiderin is often minimal, requiring the use of high-power magnification for accurate identification. Distinction between true pathologic iron accumulation and precipitation of stain may be difficult. Prematurity may raise the risk of a false-negative result, as was seen in our patient 7, because iron deposition may yet be evolving and may still be below histologic detection in the salivary glands. It has been reported by Hoogstraten et al that fetal liver disease may precede the typical accumulation of body iron seen in NH.

The importance of early diagnosis of NH by minor salivary gland biopsy cannot be overstated. The rationale for the biopsy is 2-fold. First, prognosis is invariably fatal without aggressive medical therapy and/or a liver transplant, and the management plan must be established early. These patients can occasionally survive with medical therapy, but the treatment has to be directed as soon as possible to optimize survival chances. Second, a positive diagnosis should enable the medical team to provide genetic counseling to the parents. Since NH has been reported in siblings in the literature and in our series, parental awareness of the possibility that this may occur in subsequent pregnancies may help to allow treatment to begin earlier.

In summary, untreated NH is a nearly uniformly rapidly progressing and fatal disease. The otolaryngologist should be prepared to aid his or her pediatric colleagues in establishing a diagnosis promptly to ensure that adequate treatment and preparation for a liver transplantation begins as soon as possible. The present study confirms that minor salivary gland biopsy is a safe, effective, and readily performed method of diagnosing this disease.

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