Paraná, Raymundo; Andrade, Zilton; Freitas, Luiz Antônio R de; Prata, Aluizio; Kay, Alan; Barberino Santos, João

Virological and Histological Re-evaluation of Labrea Hepatitis


Sociedad Argentina de Gastroenterología
Buenos Aires, Argentina

Available in: http://www.redalyc.org/articulo.oa?id=199317374011
Virological and histological re-evaluation of Labrea hepatitis

Raymundo Paraná, Zilton Andrade, Luiz Antônio R de Freitas, Aluizio Prata, Alan Kay, João Barberino Santos

1 Gastro-Hepatology Unit – Federal University of Bahia. Brazil. - 2 CPqGM-Oswaldo Cruz Foundation, Bahia. Brazil. - 3 Tropical Medicine Unit, Federal University of Uberaba. Brazil. - 4 INSERM Unité 871, Lyon. France. - 5 Tropical Medicine Unit, University of Brasilia. Brazil.

Acta Gastroenterol Latinoam 2008;38:284-290

Correspondencia: Raymundo Paraná
Serviço de Hepatologia, Av. Juracy Magalhães Jr 2096 sala 510
Salvador-Ba 41920000, Brasil.
E-mail: unif@svn.com.br

Summary
Background: a peculiar form of fulminant hepatitis known as Labrea hepatitis, probably related to hepatitis B and D, has been reported in Brazilian Amazon as early as the 1930s. Methods: we reviewed the post-mortem liver biopsies of 9 patients with Labrea Hepatitis. Immunostaining for HBV and HDV antigens were performed. Results: we found several important characteristics in the liver tissues: 1) moderate hepatocellular necro-inflammation, 2) hepatocellular ballooning, 3) ballooned hepatocytes with fat droplets surrounding the nucleus (morula-like cells or spongiocytes) 4) mild to moderate necrosis and/or mild portal septal fibrosis. Hepatitis B surface antigen (HBsAg) was identified in 7 of the 9 cases and was concentrated in the Morula-like cells. Hepatitis B core antigen (HBcAg) was present in 5 cases, mostly in the hepatocyte's nucleus. The hepatitis D virus antigen (HDV Ag) was present in 5 cases, mostly in the cytoplasm and concentrated in the Morula-like cells. Conclusion: labrea hepatitis is a fatal disease mostly affecting isolated communities in the Amazon. Evidence implicates HBV and HDV in the etiology of this disease, but this hypothesis needs to be confirmed with genotyping and sequencing research on HBV DNA and HDV RNA extracted from the liver and sera of these patients.

Key words: labrea Hepatitis, Fulminant Hepatitis, Hepatitis D, Hepatitis B, Amazonia.

Reevaluación viológica e histológica de la hepatitis de Labrea

Resumen
Introducción: la hepatitis B y D son endémicas en América del Sur. En esta región fueron descriptas formas fulminantes de hepatitis relacionadas con la co-infección HVB-HVD. En Brasil es llamada Hepatitis de Labrea. Métodos: nuestro grupo estudió en forma retrospectiva 9 casos de Hepatitis de Labrea submetidos a necropsia durante los años 70. En el estudio histológico del hígado fueron realizadas inmunotinciones para el HVB y el HVD. Resultados: las peculiaridades más importantes encontradas en el tejido hepático fueron: 1) solamente necro-inflamación hepatocelular;moderada; 2) balonización hepatocelular; 3) presencia de hepatocitos balloonizados conteniendo gotas de grasas rodeando el núcleo (células similares a mórulas o espongiocitos) y 4) leve a moderada necrosis y leve fibrosis portal septal. El antígeno de superficie (HBs Ag) estuvo presente en 7 de los 9 casos. El HBs Ag estuvo fuertemente concentrado en las células similares a mórula. El HBc Ag estuvo presente en 5 de 9 casos, la mayoría en el núcleo de los hepatocitos. El HDV Ag estuvo presente en 5 casos, la mayoría en el citoplasma, concentrado también en las células similares a mórula. Conclusión: la hepatitis de labrea es una enfermedad mortal que usualmente compromete familias de comunidades aisladas del Amazonas. Existen evidencias asociando cepas peculiares del HVB y HVD en la etiología de esta enfermedad, datos que precisan ser confirmados. La extracción del DNA y el RNA del HVD del hígado, así como la obtención del suero de estos pacientes para genotipificar y secuenciar estos virus será necesario para resolver este enigma.

Palabras clave: hepatitis de Labrea, hepatitis fulminante, hepatitis D, hepatitis B, Amazonia.

In South America, a peculiar form of fulminant hepatitis known as Labrea hepatitis, or Labrea black fever, has been described since the 1930s.1,2 To date, outbreaks of the hepatitis with clinical and histopathological features similar to the first documented cases continue to cause deaths in the Amazon regions, mainly among Amerindians.3
The histopathology of this rare disease has been studied only since the 1970s after the work of Barberino Santos. The separate characterizations of Labrea Hepatitis were consolidated because most cases were reported in the county of Labrea, Alto Purus, in the state of Amazonia. A minority of Labrea hepatitis cases were reported in other areas of the Brazilian Amazon.\textsuperscript{4,5}

Labrea hepatitis has been described epidemiologically as a unique form of fulminant hepatitis, mainly present in the western Amazon regions. Outbreaks of the disease occur periodically among isolated American populations and sometimes in non-Americans as well. The hallmark of this disease is the presence of ballooned hepatocytes with cytoplasm filled with fat droplets surrounding the nuclei.\textsuperscript{6}

Building on Barberino Santos’ research, we reexamined 9 cases of Labrea hepatitis in light of new reports on similar diseases in other South American countries and using immunostaining techniques for HBV and HDV.

**Material and methods**

We reviewed the histology of 9 cases of Labrea hepatitis diagnosed in the 1970s. Liver tissue samples from these patients had been fixed in 10% formalin solution and stored in ambient temperature until they were examined in this study.

Liver samples were embedded in parafin and stained with hematoxylin & eosin (H&E), reticulin, picrosirius red, and periodic acid-Schiff stain.

Because of the stability of immunostaining for HBV and HDV markers in liver tissues collected as much as 5 decades before, we tested for AgHBs as well as AgHBc using the indirect immunoperoxidase technique with alkaline phosphatase as recommended by the Brazilian Pathology Society.\textsuperscript{7,8}

The HDV antigen was measured with direct fluorescence using a monoclonal anti-HDV antibody as described before.\textsuperscript{9}

**Results**

**Clinical and histological features**

All 9 cases presented similar clinical and histopathological features with a range of intensity. Remarkable characteristics included: 1) moderate hepatocellular necro-inflammation, 2) hepatocellular ballooning, 3) fat droplets surrounding the nuclei in ballooned hepatocytes (morula-like cells or spongicocytes), 4) mild to moderate necrosis, and 5) mild porto-septal fibrosis. (Figures 1 and 2).

**Tabla 1.** Demographics data of nine cases of labrea fever.

<table>
<thead>
<tr>
<th>Case Nº</th>
<th>Identification</th>
<th>GENDER</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MNA</td>
<td>female</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>APL</td>
<td>male</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>PRS</td>
<td>male</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>BIP</td>
<td>male</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>MFS</td>
<td>female</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>IQF</td>
<td>female</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>MSS</td>
<td>male</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>LFN</td>
<td>male</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>ARS</td>
<td>male</td>
<td>11</td>
</tr>
</tbody>
</table>

**Figure 1.** Hematoxylin & eosin and picrosirius red showing ballooned hepatocytes with morula-like cells, little inflammation, and moderate fibrosis (40x).

**Figure 2.** H&E staining showing inflammation anparenchima detrabeculation.
Other histological features were spot coagulation necrosis, lobular inflammation, and parenchyma detrabeculation, which were similar to histological features described in eclampsia. (Figure 3) In one case, the features of ballooning, apoptosis, and cholestasis were especially pronounced.

In several cases, periportal necrosis was more pronounced, and portal and septal fibrosis were present. (Figure 4).

**Figure 3.** H&E staining showing abundant morula-like cells (40x).

**Figure 4.** H&E staining showing portal inflammation with mild peace-meal necrosis (40x).

In 2 cases, the clinical pictures were compatible with a chronic hepatitis with varying degrees of portal and parenchyma inflammation; however, morula-like cells were always identified.

All 9 patients were under 20 years old and lived in Labrea City or nearby areas. According to the medical records, they presented an icterohemorrhagic fever followed by encephalopathy and death. All had gastrointestinal bleeding. The patients died within 1 to 7 days of hospitalization. The necropsy showed cerebral edema and hemorrhage in the gastrointestinal tract and other organs.

**Tissue HBV and HDV markers**

HBsAg was identified in 7 of the 9 cases. The HBsAg was dispersed primarily in the hepatic parenchyma, but also concentrated in the Morula-like cells. (Figure 5).

The HBcAg was present in 5 of the 9 cases and was seen primarily in the hepatocyte's nucleus, but a mild stain in cytoplasm was observed in two cases. (Figure 6).

The HDV Ag was present in 5 cases, mostly in the cytoplasm of the hepatocytes and, like the other antigens, was also concentrated in the Morula-like cells. (Figure 7).

**Figure 5.** Immunoperoxidase for HBsAg showing antigen to be more concentrated in morula-like cells (20x).

**Figure 6.** Immunoperoxidase staining showing HBcAg in nucleus and cytoplasm of hepatocytes (20x).
**Discussion**

The clinical characteristics of Lebrea hepatitis, including the young age of patients, familial clustering, and rapid fatality have been well documented since the disease was first described. In the nine patients we studied, all died within a few days and most presented signs and symptoms consistent with hemorrhagic fever, although hepatic encephalo-
The etiology of the disease has not been well established, but studies have implicated the hepatitis B and D viruses. From the 1970s to 1990s, both viruses were found to be involved in fulminant hepatitis in many countries, but severe hepatitis D cases have clinical and histopathological characteristics similar to fulminant hepatitis caused by other hepatotropic viruses. The unique epidemiological, clinical, and histopathological features separate Lebrea hepatitis from other fulminant hepatitis related to hepatotropic virus infections.

Lebrea hepatitis was firstly reported in Brazilian Amazon during the 1930s by Costa, but its histopathology was first studied in depth in the 1970s by Barberino Santos, who lived in this area, treated many patients, performed autopsies, and collected tissue samples from various organs. The liver samples he collected were studied by expert pathologists who described the histopathological features of the disease.

Although the disease was first reported and described in Brazil, similar diseases were reported in Ecuador, Peru, Colombia, and Venezuela, and those cases were also found to be related to HBV and HDV infections.

In recent years, researchers have observed that most cases had positive serology to HBV and/or HDV infections. Despite strong argument in favor of HDV superinfection in HBV carriers as the etiology of the disease, some cases had no serological markers for these viruses.

In one study, the serum sample from one patient who died from Lebrea hepatitis but had no serological markers for HBV and HDV, induced a severe HDV hepatitis when it was inoculated in a Woodchuck carrying the Woodchuck hepatitis virus. This data strongly argue in favor of an HBV/HDV etiology for Lebrea hepatitis.

Labrea hepatitis causes a 100% mortality within a few days after the patient becomes symptomatic. It is therefore possible that the patient may not have enough time to express serum virological markers for co-infection. In cases of superinfection, which is common, HBV repression by HDV may be responsible for the false-negative serology in some patients. In addition, tissue samples were usually collected post-mortem and used for immunostaining after a long period of storage. We cannot rule out the possibility that technical problems in immunostaining due to long-term liver tissue preservation resulted in negative antigen findings. Autolysis was seen in 5 of the 9 liver samples examined in this study.

In the 1980s, cases similar to Lebrea hepatitis were reported in Central African Republic. French researchers reported the same etiological association with HBV/HDV. The histological features of these African cases of fulminant hepatitis were also similar to Lebrea hepatitis.

Among the histopathological features of Lebrea hepatitis, morula-like cells are considered the hallmark of this mysterious disease. These cells are so named because of their morphology. Some researchers have called them spongiocytes.

We observed only mild to moderate hepatocellular necro-inflammation, which differed from classic fulminant viral hepatitis. Some of the liver injuries appeared inconsistent with the clinical severity of the disease. Some cases presented mild to moderate necrosis and a varying degree of portal fibrosis which could be the result of having been a HBV carrier before.

Similar aspects were also described in experimental models of Woodchucks carrying the Woodchuck hepatitis virus. When inoculated with sera from patients of a fulminant hepatitis in Central African Republic, the animals developed a similar disease as the humans and all died in a few days. In contrast, the control animals inoculated with sera from a European patient who died from the classic fulminant hepatitis D developed acute hepatitis D but survived. The presentation of the hepatocytes differed between animals that had received the African and the European innocula.

The histopathology of Lebrea hepatitis needs to be clarified. Morula-like cells are observed in other diseases such as Raye’s syndrome, eclampsia and acute liver failure in pregnancy, and its cause is related to mitochondrial damage. However, ballooning is the most pronounced in Lebrea hepatitis. It is possible that mitochondrial damage is induced by the virus or an unknown toxic co-factor, but there are no data so far to clarify the observations.

There is additional evidence to support a possible cytopathic effect of specific strains of HBV and HDV. Isolates from infected animals was shown to carry mutations in the HDV genome. A more pathogenic mutant Delta strain cannot be ruled out.

Other authors have suggested that the Genotype III of HDV is more cytopathic than other HDV genotypes, especially if the patient is a superinfected
HBV genotype F carrier. It was also reported that the Genotype III HDV is prevalent in Brazilian Amazon.44,45

Furthermore, steatosis has been associated with a direct cytopathic effect of other hepatotropic viruses such as Hepatitis C virus (HCV) genotype 3. High HCV viral load is related with more pronounced liver steatosis without other risk factors for fatty liver disease.46

Another characteristic of Lebrea hepatitis is the abundant expression of HBsAg and HDV Ag in the cytoplasm of the morula-like cells, which is rare in humans.47 Direct toxic hepatocyte damage is a well-known phenomenon in patients HBV or HCV after liver transplantation, but in those cases steatosis is not usually present.48

Generally, HDV superinfection has been shown to strongly reduce HBV replication, and thus a display of HBsAg or HbcAg in hepatocyte nucleus would not be expected in HDV superinfection.49 However, in our study, the HDV superinfection did not appear to have suppressed HBV.

In conclusion, Lebrea hepatitis is a fatal disease that usually affects those living in isolated communities. There is evidence to implicate special HBV and HDV strains in the etiology of this disease, but this hypothesis needs to be confirmed. HBV-DNA and HDV-RNA extracted from liver and sera of these patients for genotyping and sequencing will help solve this puzzle.

References