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ACUTE EXACERBATION IN CHRONIC HEPATITIS B VIRUS INFECTION

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A case of an acute exacerbation of liver injury in a chronic HBV infected young male is reported. The correlation between the severe symptomatic hepatitis is done with the histopathologic findings of extense areas of bridging necrosis on the liver biopsy. The serological pattern for markers of HBV (HB_S Ag +, anti HB_S -, HB_e Ag -, anti HB_e +, anti HB_C IgG + and IgM -) confirm a chronic infection, and the authors propose that the episode of severe hepatitis relates to the recent spontaneous seroconvertion of HB_e Ag to anti HB_e. Other causes of hepatitis were excluded, and the control liver biopsy (6 months later) showed normalization of hepatic architecture and absence of markers of viral replication in tissue and serum. A review of literature is done in an attempt to elucidate the diagnostic possibilities in this case, with emphasis on new immunoassays useful in differentiating between acute hepatitis B and acute exacerbation of a chronic hepatitis by the same virus.

Key-words: acute exacerbation. HB_e Ag- anti HB_e seroconversion. Chronic HBV infection.

Chronic hepatitis B virus infection results in a spectrum of disease entities ranging from the most severe form of chronic active hepatitis to the asymptomatic carrier state of the Hepatitis B surface antigen (HB_S Ag). This wide range of liver injury among chronically infected hepatitis B virus (HBV) patients suggests a great degree of variability in the interaction between the replicating virus and the immune responses to the infected hepatocyte[7]. It is well known that HBV - specific liver cell injury, and subsequent viral clearance, is believed to be mediated mainly by cellular immune mechanisms[6].

Patients with chronic HBV infection can be generally divided into two groups:

A) Those with chronic liver disease (usually referred to as having chronic type B hepatitis [CHB]). These patients have markers of active viral replication, such as hepatitis B_e antigen (HB_e Ag) or HBV-DNA in serum or hepatitis B_C antigen (HB_C Ag) in the liver, in addition to abnormal serum alanine aminotransferase (ALT) activities and detectable HB_S Ag in serum and liver.

B) Those without liver disease, referred to as being in a "healthy" or "inactive" HB_S Ag carrier state. These patients have HB_S Ag, but without HBV-DNA or HB_e Ag in serum, or detectable HB_C Ag and HB_e Ag in the liver, having, usually, normal serum ALT levels, no active inflammation or hepatocellular necrosis in the liver biopsies[9].

Studies on the natural history of chronic HBV infection indicate that these two groups probably represent two stages of chronic HBV infection: either spontaneously or during treatment, patients with CHB can lose HBV-DNA and HB_e Ag from serum and then have a remission with subsequent appearance of antibody to HB_e Ag (anti-HB_e). In this case, the disease usually evolves from chronic hepatitis to the "healthy" HB_S Ag carrier state. Seroconversion from HB_e Ag to anti-HB_e is thus a "critical" event in the natural history of chronic HBV infection, and can occur directly after the acute phase (acute hepatitis) or it may happen years later, often following a flare-up of symptoms and increase in the ALT levels, or, in other words, presenting as an acute exacerbation in the evolution of a chronic hepatitis[9].

A large percentage of patients with chronic active hepatitis (CAH) show a ciclic pattern

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characterized by acute exacerbations of liver injury alternating with normal liver function, suggesting a dynamic interaction between the virus replication and the immune response[6]. Acute exacerbations in chronic B hepatitis are usually defined as episodes of abrupt elevation of ALT with or without significant symptom aggravation. Persistence of HB_e Ag after spontaneous flares is referred to as "abortive seroconversion"[9].

We describe a case of an acute exacerbation of liver injury in a chronic HBV infected young male who probably acquired the disease in the neonatal or early childhood period, and was asymptomatic before this episode of acute exacerbation. Others causes of hepatitis were excluded by the available commercial assays and a percutaneous liver biopsy especimen was analised using immunohistochemical techniques.

CASE REPORT

HAF, a 15-year-old young male, natural from São Paulo - Brazil, looked for medical attention in April, 19th 1994, with a 2 week history of right upper quadrant pain, jaundice, dark urine and a 4 day history of lethargy and change in personality. He denied fever, headache, diarrhoea, and his past medical history was unremarkable. There was no history of digestive or hepatic symptoms in the past. He denied intravenous drug addiction, sexual activity, previous blood transfusions, recent travel, as well as previous use of medications or exposure to toxins. The physical examination revealed an acutely ill, well developed patient with jaundice, without signs of chronic liver disease such as spiders, hepatomegaly, splenomegaly or ascites. The cardiac and pulmonary examination was normal. He was lethargic, confused, but there were no focal neurologic deficits, seizures or meningismus. The laboratory findings at admission and the serologic pattern are listed below:

ALT = 1621 U/L (0-18 U/L)
Bilirubin total = 17.2mg/dL
conjugated = 12.3mg/dL
Protein total = 6.0g/dL
Albumin = 3.9g/dL
· α 1 globulin = 0.2g/dL
· α 2 globulin = 0.4g/dL
· β globulin = 0.5g/dL
· γ globulin = 1.1g/dL

AST = 467 U/L(0-18 U/L)
GGT = 20 U/L(4-28 U/L)
Alkaline phosphatase = 707 U/L
Sodium = 133mEq/L
Potassium = 4.4mEq/L
Urea nitrogen = 25mg/dL(10-45mg/dL)
Creatinine = 0.1mg/dL(0.6-1,4mg/dL)
Amylase = 46 U/L(0-120 U/L)
Glucose = 59mg/dL(70-110mg/dL)
Prothrombin time = 32"- 16%

Hb = 14.6g/dL Hct = 48%
WBC count = 7.800/mm^3
neutrophils - bands = 3%
segmented = 49%
eosinophils - 2%
basophils - 0%
lymphocytes - 44%
monocytes - 2%
Platelet count: 308.000/mm^3
anti HAV IgG + IgM -
anti HCV -
HCV-RNA (PCR) -
anti HDV -

HBs Ag + anti HBs -
anti HBc IgG + IgM -
HBe Ag - Anti HBe +

Thrombin time = 23"
Activated partial thromboplastin time=58"
factor V = 26" - 74%

anti-nuclear antibody -
anti smooth-muscle antibody -
anti-mitochondrial antibody -
anti-liver/kidney microsomal antibodies -
anti CMV = IgG + IgM -
Paul-Bunnell <1/7

Evolution. The patient was admitted to the intensive care unit and received supportive care for hepatic insufficiency, with prompt improvement in the level of consciousness. There were no bleeding complications, and the hepatic function improved to a level that an emergency hepatic transplantation was unnecessary. A percutaneous liver biopsy was done on May, 25th, 1994 and the hepatic tissue evidenced extense areas of bridging necrosis, either between portal zones or from portal zones to central veins. These necrotic areas had their reticulin framework colapsed and their hepatocytes had disappeared. The portal zones showed a moderate mononuclear cell infiltrate, without piecemeal necrosis or aggression to the bile ducts. A small number of neutrophils was also observed in the portal zones and necrotic areas. Ballooning degeneration, without evident aspect of ground-glass cell could also be noted in the preserved parenchyma. There was a discrete hypertrophy and hyperplasy of the Kupffer cells. No portal fibrosis was observed. The immunohistochemical technique revealed a small number of hepatocytes (less than 25%) positive to HB_s Ag and an absence of HB_c Ag or HDV Ag (antigen of the Hepatitis Delta Virus). At the time of the first biopsy, serum was collected and the PCR for HBV-DNA was negative.

Bridging necrosis found during acute viral hepatites indicates a serious lesion that can progress to postnecrotic cirrhosis or to "restitutio ad integrum". Because of this, another percutaneous liver biopsy was done 6 months after discharge (on December, 12th ,1994), and the specimen showed a practically normal hepatic architecture. The hepatocytes had no sign of degeneration or necrosis. The portal

zones were not expanded and evidenced a mild limphocytic infiltration, which was also ocasionally observed in the lobular spaces. The hypertrophy and hiperplasy of Kupffer cells remained discrete. A small strip of intralobular fibrosis could be observed.The immunohistochemical technique had similar results when compared to the first examination: few hepatocytes expressing HB_S Ag and an absence of HB_C Ag. The serological pattern for markers of HBV persisted exactly the same during the six month period of follow-up, with normalizaton of laboratory tests of hepatic function.

DISCUSSION

The serological pattern for markers of HBV, caracterized by the absense of detectable anti HB_C-IgM ruled out acute-phase infection by HBV[5], as well as the negative results of IgM anti-HAV, anti-HCV antibodies and PCR for HCV-RNA in serum, antibody to HDV (anti-HDV) and the negative result of immunohistochemical for HDV in the liver biopsy ruled out superinfections with other hepatotropic virus. The laboratory tests currently used for diagnosis of hepatitis E virus were not available at the time of patient illness : a serum sample was stored and tested retrospectively for antibodies to this new described virus, giving a negative reaction by the method of Elisa.

The serologic tests for intection with other non hepatotropic virus such as cytomegalovirus, Epstein-Barr virus were negative.

The absence of markers of viral replication of HBV virus (HB_e Ag and HBV-DNA) in serum and the presence of anti-HB_e, in addition to the absence of HB_C Ag in the liver indicates that active viral replication is subsiding and disease activity decreasing. Because those who have cleared HB_e Ag frequently have temporary exacerbations of hepatitis before the seroconversion, it has been suggested this event represents an "immune clearance" of hepatocytes containing actively replicating virus as well as HBc Ag/HBe Ag[6,7,9].

The nucleocapside antigens of HBV (in the context of HLA antigens) are the most important target for cytoxicity. Recent studies on T cell response to HBV antigens during acute exacerbations of CHB demonstrated that the T cell proliferative response to recombinant HB_C Ag and natural HB_e Ag was higher in patients with CHB than those in healthy HB_S Ag carriers, and that there was a further increase in T cell proliferative response to HB_C Ag/HB_e Ag during acute exacerbations. In the recovery from these acute exacerbations with HB_e Ag seroconvertion, the same study demonstrated a subsidence in T cell responses to the same antigens[9].

Studying the alterations in quantitative serum HBV-DNA, HB_e Ag (as indicators of viral replication) and anti-HB_e production, HB_e Ag specific immunocomplex formation (as indicators of an immune responsiveness) before, during and after injury in HB_e Ag positive patients with CAH, Maruyama et al[6] showed that the increasing levels of viral replication occurred before the peak ALT levels, and then precipitously declined as ALT levels peaked and subsided. The indicators of immune responsiveness (anti-HBe and HB_e Ag specific immuno complex) increased in parallel with HBV-DNA/HB_e Ag levels, with the exception of the anti-HB_e antibody, that peaked somewhat later than HBV-DNA/HB_e Ag. The ability to produce anti HBe reflects the immune competence of these patients with CAH, at least with respect to the HB_e Ag - specific helper T cells and B cells, and is consistent with the possibility that the liver cell injury is mediated by immune mechanisms.

Another diagnostic possibility in this case is the existence of HBV precore mutants. Variants of HBV that do not express HB_e Ag have recently been described[2,3]. The most common is the G-to-A switch at base 1896 of HBV-DNA precore region. The emergence of this precore mutants has been associated with fulminant hepatitis and severe liver disease in patients with anti-HB_e positive chronic hepatitis[1]. In these cases, other markers of viral replication (as HBV-DNA) are expected to be present, and that was not shown in the present report, making this hypothesis less probable.

In some circunstances, it can be difficult to distinguish between an acute HBV infection from an acute exacerbation of a chronic HBV infection. This is true because, in contrast to most viral infections, patients with acute and chronic HBV often produce both immunoglobulin (Ig)M and IgG anti-HB_C antibodies, and so, the quantitative difference in the levels of IgM anti-HB_C (higher level of IgM anti HB_C are generally produced during

acute phase infection) can be the only serological means of differentiating this situation, what is important in terms of prognosis and possible treatment modalities[7]. Recent studies have focused on the serology of chronic HBV infections, using assays capable of detecting antibody to HB_e Ag and HB_s Ag in the presence of circulating HB_e and HB_s Ag[7]. The use of these experimental immunoassays has modified our view of the serology of chronic HBV infection by showing that virtually all HBV chronic carriers with liver disease are positive for both HB_e Ag and anti-HBe for many years before the loss of HB_e Ag, and anti-HB_s production can be detected as HB_s Ag - specific immune complexes (ICs) in parallel with liver damage in CAH patients[7].

These same experimental assays, when applied to acute HBV infection reveal that patients with CHB show significantly higher levels of free anti-HB_e, HB_e Ag/anti-HB_e ICs and HB_s Ag/anti-HBs ICs compared with acute HBV patients sera. Furthermore, the most significant and possibly the most useful difference in the serology of patients with acute and chronic HBV is the presence of a novel specificity of IgG anti-HB_c antibody designated as anti WHBc antibodies. Previous studies have indicated that the nucleocapsides of HBV and the Woodchuck hepatitis virus (another member of the Hepadnaviridae family) share a cross-reactive epitope(s). So, it is demonstrated that human anti-HB_c antibodies recognize Woodchuck HB_c Ag (WHB_c Ag), and that CHB patient sera show significantly higher levels of IgG anti-WHB_c compared with acute hepatitis B patient sera, without overlap values. The same authors propose a ratio of IgM anti-HB_c/IgG anti-WHB_c that could be performed in acute and chronic HBV infections in order to discriminate between acute hepatitis B from symptomatic chronic hepatitis B infection: an inverse correlation between IgM anti-HB_c and IgG anti-WHB_c values existed in both patients with acute and chronic HBV infection[7].

Recent studies on the prevalence of HB_s Ag subtypes (adr, adw, ayr and ayw) have demonstrated differences in their geographic distribution and their relationship with epidemiologic factors. More recently, HB_s Ag subtypes have been associated with HB_e Ag/anti HB_e status and may, consequently, influence the development of liver disease in the HB_s Ag carriers. For example, in a restricted geographic area of Japan, with a homogenous epidemiological/cultural background, adr carriers tend to be seroconverted to anti-HBe at an older age than adw carriers, and thus, adr carriers may develop chronic liver disease more frequently than adw carriers[8]. Data on HBs Ag subtypes prevalence in our geographic region (São Paulo - Brazil) are not available, so, conclusions based in the HB_s Ag subtype of our patient could not be done, and it was not determined.

Various average annual conversion rates for HB_e Ag to anti-HB_e have been reported in adults; these rates range from 2.3% to 25% in various parts of the world[4]. With respect to other factors affecting the clearance of HB_e Ag specifically in children, studies have shown that the presence of symptoms, a negative maternal HB_s Ag status, and the infection occurring later in life, are correlated with a higher HB_e Ag clearance rate[4].

The patient reported in this review was a 14 years old boy that denied the most important risk factors related to HBV infection, including sexual activity. His mother serum was obtained and the biochemical and serologic analysis revealed a normal hepatic function and a negative result for HB_sAg with a positive result for anti-HB_s and anti-HB_c IgG antibodies, proving a previous contact with HBV with adquired resistance to HBV infection. So, it is possible to assume that our patient had probably been infected during the perinatal period by his mother, or during early childhood. At least, it is possible to infer that sometime in her life, the patient's mother had active HBV replication, and was able to transmit HBV to her infants or household contacts.

In conclusion, we postulate that the acute and serious exacerbation of liver injury presented here is related to the immune clearance of hepatocytes expressing HBV nucleocapside antigens acumulated intracellularly after a period of increased replication. Probably, as the serum HB_e Ag concentration reached a threshold level, HB_e/HB_c Ag specific TH cells became activated, resulting in anti-HBe production. Similarly, HB_e/HB_c Ag - specific citotoxic T lymphocytes (CTL) were induced, favoring cell-mediated immunity and resulted in an efficient viral clearance mechanism of hepatocytes producing

nucleocapside antigens, but sparing the hepatocytes exclusively producing HB_S Ag, resulting in an improvement of clinical/laboratorial and histopathologic findings, with change to the "healthy" HB_S Ag carrier state.

RESUMO

Descreve-se um caso de exacerbação aguda sintomática em um paciente cronicamente infectado pelo VHB, mostrando-se correlação entre o quadro clínico grave (com insuficiência hepática transitória) e os achados histopatológicos de hepatite severa com extensas áreas de necrose em ponte. O perfil sorológico para marcadores do VHB (Ag HB_S +, anti HB_S -, Ag HB_e -, anti HB_e +, anti HB_C IgG + IgM -) confirmou infecção crônica, e os autores levantam a hipótese de que a hepatite tenha se correlacionado à recente soroconversão Ag HB_e para anti-HB_e. Outras etiologias possíveis foram descartadas e se contou com biópsia controle 6 meses depois, mostrando normalização da arquitetura hepática e ausência de marcadores de replicação viral no tecido e no soro. Revisa-se a literatura sobre o diagnóstico diferencial cabível nesta situação, dando ênfase a novos ensaios sorológicos úteis na diferenciação entre infecção aguda pelo VHB e exacerbação aguda de uma hepatite crônica pelo mesmo agente.

Palavras-chaves: Exacerbação aguda. Soroconversão $AgHB_e$-anti Hb_e; Infecção crônica pelo VHB.

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