REVIEW

HEPATITIS B: EPIDEMIOLOGICAL, IMMUNOLOGICAL, AND SEROLOGICAL CONSIDERATIONS EMPHASIZING MUTATION

Marcelo El Khouri and Vera Aparecida dos Santos

The global prevalence of hepatitis B virus is estimated to be 350 million chronic carriers, varying widely from low (<2%, as in Western Europe, North America, New Zealand, Australia, and Japan) to high (>8% as in Africa, Southeast Asia, and China). The overall prevalence in Brazil is about 8%. There are currently 7 genotypic variations, from *A* to *G*, and also 4 main surface antigen subtypes: adw, ayw, adr, and ayr. There has been great interest in identifying the geographic distribution and prognosis associated with the various genotypes and subtypes. Although the serologic test is highly sensitive and specific, it does not detect cases of mutant hepatitis B, which is increasingly common worldwide due to resistance and vaccine escape, antiviral therapy, and immunosuppression, among other causes. Alterations in surface, polymerase, X region, core, and precore genes have been described. The main mutations occur in surface and in core/precore genes, also known as occult hepatitis, since its serologic markers of active infection (HBsAg) and viral replication (HBeAg) can be negative. Thus, mutation should be suspected when serologic tests to hepatitis B show control of immunity or replication coincident with worsened clinical status and exclusion of other causes of hepatitis.

KEY WORDS: **Hepatitis B. Edidemiology. Serology. Mutation.**

The hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family and the *Orthohepadnavirus* genus. It measures 40 to 42 nm, has an external surface envelope made up of proteins, lipids, and carbohydrates, and has a double-stranded circular DNA surrounded by a hexagonal core region. Although the virus is very resistant to heat and other physical agents, infected plasma can be inactivated by heating it for 5 hours at 60°C. Hepatitis B virus infection may cause acute hepatitis (leading to cirrhosis), fulminant hepatitis with massive hepatic necrosis, an asymptomatic condition (with or without progression of the disease), or be a gateway to infection by the hepatitis Delta virus.[1]

The worldwide prevalence is estimated to be around 350 million chronic carriers,[2] with an annual incidence of 300,000 new cases in the United States of America (USA).[3] The overall prevalence estimates vary widely from low (2%), as is found in Western Europe, North America, New Zealand, Australia, and Japan;[5,6] intermediate, as is found in Eastern Europe (2% - 7%) and India (4%);[5] high

(>8%), as found in Africa, Southeast Asia, and China (15% and 20%), to very high, as found in Tibetans in Nepal (61%).[4] Recent studies have shown an incidence of 6.6% in Istanbul, Turkey,[8] 5.1% in Korea,[9] 4% in Singapore,[10] and 0.005% in Guangdong Province, China.[11] In Latin America, the highest rates were found in the Dominican Republic (21.4%) and in Brazil (7.9%), followed by Venezuela (3.2%) and Argentina (2.1%). The lowest ones were found in Mexico (1.4%) and Chile (0.6%).[12]

Among the Brazilian regions, there are variations from 66.1% in Western Amazon,[13] which is considered highly endemic for HBV,[7] 40% in Rio de Janeiro,[14] 21% in Manaus, and up to

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1.2% in Fortaleza. A study carried out in the Afro-descendant community in Mato Grosso do Sul revealed a prevalence of 42.7%,[15] and a prevalence of 50.7% was found in individuals with clinical evidence of hepatitis in Goias.[16]

According to data from the Brazilian Ministry of Health in the report "Indicadores de Dados Básicos – Brasil 2001" (IDB-2001),[17] there was an incidence of 7087 new cases in 2000, 540 of those in the state of São Paulo. According to data from the National Immunization Program, there was a vaccine coverage of 2,710,493 inhabitants, with 23,157,823 doses administered in the whole country in 1999.[18]

The virus is transmitted especially through bodily fluids, such as semen, saliva, sweat, tears, or breast milk. During pregnancy, the virus may be transmitted to the fetus by crossing the placental barrier or also through cellular transmission.[19] Although there is evidence of HBV infection in animals, there is no evidence of zoonotic transmission.[20]

The primary risk categories for infection by HBV are transfusions, blood derivates, dialysis, needle accidents among health-care professionals, intravenous drug abuse, and among homosexual individuals. Data published in 2002 showed an active infection with HBV of about 1.7% of 3556 heath-care workers in Chandirgarh, China.[21] In Lagos, Nigeria, a study in 2000 found a prevalence of active infection by HBV of 25.7% in surgeons compared to 15% in administrative staff (control group).[22]

Vertical transmission is common in endemic regions such as Africa and Southeast Asia.[3] A report from CDC (Centers for Disease Control and Prevention) in the United States estimated an incidence of 0.60% among pregnant white non-Hispanics, 0.97% among black non-Hispanics, 0.14%

among Hispanics, and 5.79% among Asians. A factor associated with increased rates was not receiving prenatal care.[23] Another study conducted in Saudi Arabia found HBV causing 15% of the sporadic acute viral hepatitis in pregnancy.[24] Infants who acquire HBV perinatally have up to a 90% risk of developing HBV infection.[25]

Figure 1 shows the HBV genome. Proteins of greater importance produced by the HBV are:

- Nucleocapsid core protein (HBcAg), which is not detectable in the serum;
- Long polypeptide "e" (AgHBe), which is transcribed from the core and precore genomic regions during active viral replication;
- Envelope glycoprotein (HBsAg) indicating active infection;
- A DNA polymerase with reverse transcriptase activity used by the virus upon cell infection;
- A protein from the X region that is necessary for replication and transcriptional transactivation of the viral genes and has a key role in the development of hepatocellular carcinoma (HCC).

Envelope glycoprotein contains a constant and group-specific epitope designated "a" and at least 2 variable epitopes, which are group-specific: "d" or "y" and "w" or "r", allowing for the identification of 4 antigenic

subtypes: adw, ayw, adr, and ayr. The ad subtypes are frequently found in asymptomatic individuals, whereas in the acute form the ay and ad subtypes may be found. A study in Goias (2002) found in individuals with clinical evidence of hepatitis a laboratory confirmation of ay and ad subtype prevalences of 23.5% and 62.7%, respectively.[16] The ay subtype is found in drug abusers and dialysis patients.

Currently, enzyme immunoassay methods (EIA) are available for subtype determination. These assays have several applications such as for large epidemiologic studies and as a substitute for nucleotide sequencing, which is not adapted for large-scale
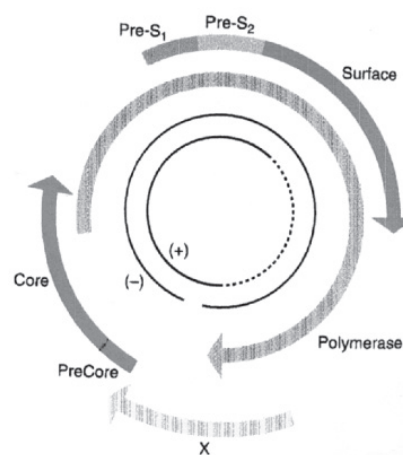
Figure 1 - Representation of DNA- Hepatitis B virus structure: adapted from Robbins, Pathologic Basis of Disease, 1999, pg 858.

Figure 2 - Global vaccination coverage against H hepatitis B virus. World Health Organization, 2003.

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screening and not applicable on samples from nonviremic HBV carriers.[26]

There are 7 genotypic variations for HBV, named from *A* to *G*,[1] which have been associated with geographic distribution and with the progression of hepatic disease. The current tendency is to identify the more prevalent type to correlate to clinical findings. In southern Taiwan, the most common genotypes found were *B* (60%) and *C* (34%), the latter being associated with more severe liver disease.[27] However, the life-long risk of progression to advanced fibrosis and development of HCC may not differ among genotypes *B*- and *C*-related chronic liver disease.[28] A retrospective study done in Amsterdam from 1992 to 1997 associated genotype *A* with homosexual men and genotype *D* with intravenous drug users.[29]

The mechanism of injury after infection of the hepatocytes by HBV is not clearly established. Some evidence indicates that cytotoxic T cells directed towards the HBcAg antigen that is expressed on the cell surface may lead to hepatocyte destruction.

The asymptomatic incubation period varies from 4 to 26 weeks, followed by acute disease lasting from a few weeks to 6 months. In the post-transfusional infections, the incubation period may vary from 12 to 14 weeks.

The serologic markers are:

1. HBsAg—appears before the onset of symptoms, peaks during overt disease, and declines after 3 to 6 weeks;
2. HBeAg—appears in the serum indicating active viral replication. There is a significant correlation of HBeAg with HBV DNA (through PCR) and DNA polymerase;
3. Anti-HBc IgM—detectable in the serum shortly after the onset of symptoms, along with the elevation of serum transaminases and HBsAg and HBeAg. Anti-HBc IgM represents the first sign of an immune response after infection by the HBV, since it comes before the detection of anti-HBs and anti-HBe. Over ensuing months, IgM is replaced by anti-HBc IgG. If HBsAg or HBeAg cannot be detected, anti-HBc may the only serologic marker that points towards a present or past HBV infection, as well as potentially contaminated blood. Thus, anti-HBc is a serologic marker for contact with HBV.
4. Anti-HBe—detectable in sera soon after the decline of HBeAg, indicating the peak of acute disease and suggesting that the disease is near remission, with titers declining after 20 weeks;
5. Anti-HBs—its levels do not decline until acute disease is over and may even last for a lifetime, conferring immunity.

In adults, 90% to 95% of HBV infections have a spontaneous remission, with anti-HBc and anti-HBs detected in serologic tests for a long period. In asymptomatic carriers, HBsAg may be present alone, without viral replication and hepatic damage. Patients with a chronic evolution (5% - 10%) may present with a persistent chronic hepatitis of a self-limiting nature or, as in 3% of the cases, with active replication and poor outcome, characterized by the presence of HBsAg, HBeAg, HBV DNA, and usually total anti-HBc. Progressive liver damage may occur, leading to cirrhosis and HCC, increasing the risk of death.[30] An Indian study found HBV infection in 70% of the cases of chronic hepatitis, 80% of hepatic cirrhosis, and 60% of HCC.[5] Nevertheless, these situations can be simply avoided with mass vaccination program.[31]

Two types of products are available for prophylaxis against HBV infection. Hepatitis B immune globulin (HBIG) provides temporary protection (ie, 3 to 6 months) and is indicated only in certain postexposure settings, and hepatitis B vaccine provides long-term protection against HBV infection and is recommended for both preexposure and postexposure prophylaxis.[73]

Hepatitis B immune globulin is prepared from plasma known to contain a high titer of antibody against HBsAg (anti-HBs). In the United States, HBIG has an anti-HBs titer of >100,000 by radioimmunoassay. The human plasma from which HBIG is prepared is screened for antibodies to HIV; in addition, the process used to prepare HBIG inactivates and eliminates HIV from the final product. There is no evidence that HIV can be transmitted by HBIG.[79,80]

Vaccination is highly effective. Hepatitis B vaccines are composed of the HBsAg polypeptide that is produced in yeasts by the recombinant DNA technique or is artificially synthesized, and they are packaged to contain 10 to 40 ug of HBsAg protein/mL after adsorption to aluminum hydroxide (0.5 mg/mL); thimerosal (1:20,000 concentration) is added as a preservative.[73] The recommended series of 3 intramuscular doses of hepatitis B vaccine induces a protective antibody response with 90% to 95% efficacy in preventing HBV infection and its chronic sequelae. Currently licensed hepatitis B vaccines include Recombivax HB® and Engerix-B®.[75] Initially administered to higher risk populations, such as health-care professionals, contacts to chronic carriers, newborns from infected mothers, and homosexual men, the vaccine protected many individuals but had little impact on the total number of infected persons. Thus, a pediatric vaccine was manufactured that was given along with the MMR vaccine to children in the high-risk groups, with a WHO recommendation for all countries to integrate hepatitis B vaccination into their universal childhood vaccination pro-

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grams by 1997.[73] It is known that childhood vaccination can protect children against fulminant hepatitis and HCC[1]; however, to prevent perinatal HBV transmission, the first dose of vaccine should be administered within the first 24 hours after birth.[73,74] Unpublished data from the CDC from 2003 shows that approximately 21% of HBV-related deaths result from infection acquired in the perinatal period and 48% from infection acquired in early childhood (age <5 years). Therefore, vaccination of infants and children is the highest priority for hepatitis B vaccination programs. Some examples of vaccination program effectiveness include the Canadian results, with a HBV prevalence of 0.0038% during the period 1993 to 1995[33], and the United States results, with a decline of 76.1% after 11 years of the program, having rates of 0.033% in 1998.[3] In The Gambia, the prevalence of chronic infection among children declined from 10.0% to 0.6% after implementation of universal infant hepatitis B vaccination.[76] Similar declines in prevalence of chronic infection associated with infant and childhood hepatitis B vaccination have been demonstrated in China, Indonesia, Senegal, and Thailand, and among Alaska Natives.[77,78] According to the WHO estimate, a total of 151 (79%) of 192 member states had adopted universal childhood hepatitis B vaccination policies. In the 6 WHO regions, the proportion of children aged <1 year who were vaccinated fully was 65% in the Western Pacific Region, 58% in the Americas Region, 45% in the European region, 41% in the Eastern Mediterranean region, 9% in the Southeast Asia region, and 6% in the African region. Even so, only 32% of children aged <1 year were vaccinated fully with the 3-dose hepatitis B vaccination series.[75]

Serologic tests carried out after immunization are intended to evaluate immunity, identifying nonresponsive or weak-response individuals. A response is considered absent whenever anti-HBs titers are less than 10 UI/L and weak when titers are less than 100 UI/L[36]. Immunized people may lose anti-HBs antibodies over variable courses of time, and initial antibody concentration after immunization may allow the prediction of the period during which the person will be immune as well as the scheduling of a date for revaccination.

Recent innovations have included the 2-dose, 10 mg Recombivax HB® regimen for those who are 11 to 15 years old and Twinrix®, a combination hepatitis A and B vaccine.[81] Goals for global hepatitis B vaccination are for the vaccine to be introduced in all countries by 2007, for coverage with the 3-dose hepatitis B vaccination series to reach 90% by 2010,[72] and for the total control in first half of the 21st century.[1]

Figure 3 shows a current algorithm for serological diagnosis of HBV used in Immunological Section of Central Clinical Laboratory Division of Clinic Hospital of University of São Paulo Medical School (HC-FMUSP). The serologic marker for contact is total anti-HBc (IgM and IgG). A negative test indicates no previous contact with HBV and thus excludes current infection. A positive test requires evaluation for anti-HBs and HbsAg, which will indicate either immunity or active infection. If the latter is confirmed, the presence of anti-HBe, HbeAg, and anti-HBc
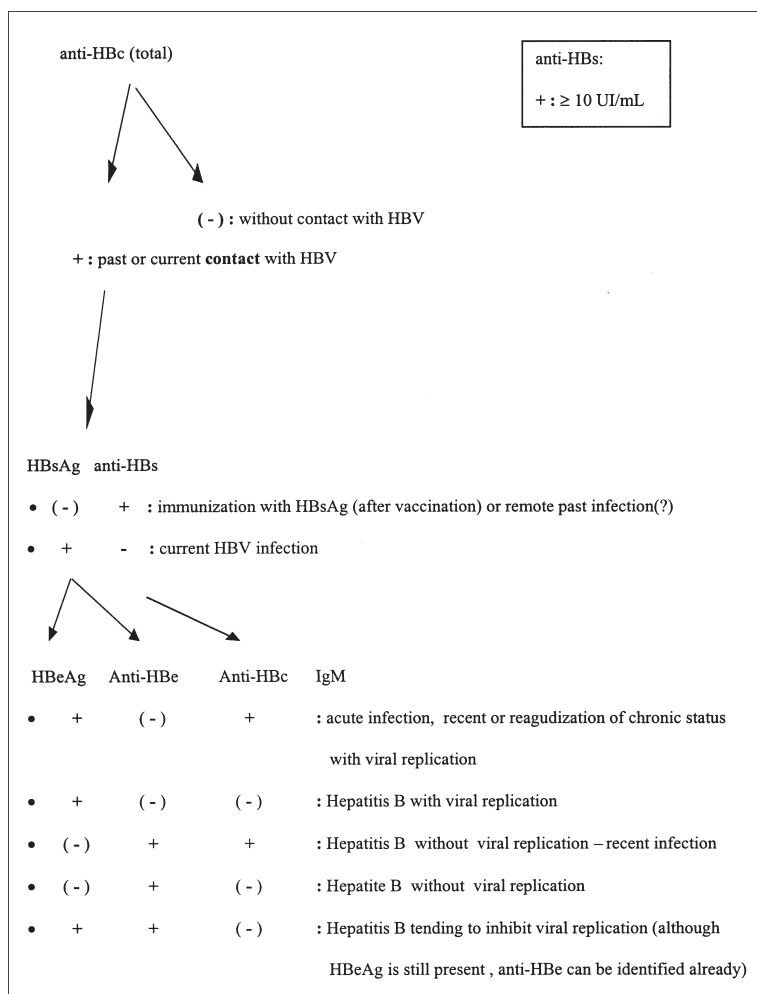

Figure 3 - Suggested algorithm for serologic diagnosis of Hepatitis B virus (HBV).

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(IgM) must be determined, which will indicate the probable evolution of the disease. Anti-HBs tests can be performed separately when the purpose is to evaluate the immunological status of a patient against HBV infection, ie, whether there was previous vaccination or not.

Although these EIA tests are highly sensitive and specific, their interpretation should be done considering clinical and epidemiological status. In cases with serologic markers indicating immunity or cessation of replication with other causes of hepatitis excluded, a genomic mutation of HBV—also known as occult hepatitis—has to be suspected. The presence of normal transaminase and low levels of HBV-DNA does not exclude the existence of resistant mutants.[37]

Mutations have been described in several regions of viral DNA, including surface gene, X gene, core gene, polymerase gene, and precore gene. The main mutations occur in the surface genes, which encode epitopes neutralized by antibodies, and in precore/core genes, which encode epitopes presented to CD8 T cells.[39]

Genetic transformation can be detected by the following tests: PCR (polymerase chain reaction),[39] bDNA (branched DNA assay),[40] PCR-RFLP (PCR followed by restriction length polymorphism assay),[41] msPCR (mutant-specific PCR),[42] CMSSA (competitive mutation site-specific assay),[41] or MSSA (mutant site-specific assay).[43]

Serologic assays do not reveal genomic transformation; PCR is more sensitive than bDNA;[44] CMSSA is more sensitive and specific than PCR-RFLP, mainly for detecting precore mutants; and msPCR is more sensitive and specific than other similar assays. In addition, other PCR variations are being tested to detect mutants. SP-PCR (sequence-specific PCR) and LDR-PCR (limiting dilution-cloning PCR)

are some examples, the latter having the greatest sensitivity for detecting variants in the surface antigen.[45]

Surface antigen mutations occur in pre-S1/2 and S regions, also known as escape or immune escape mutations.[46] They are characterized by either positive HBsAg and anti-HBs or negative HBsAg with positive anti-HBs, occurring mainly in chronic hepatitis carriers.[48] An international study that involved investigators from CDC and WHO demonstrated a prevalence of 51% in variants of surface antigen among infants with chronic HBV infection.[45] Pre-S1 epitope has been possibly related to virus replication (positive HBeAg), even with the presence of anti-HBe. In turn, the pre-S2 epitope has not been related to virus replication levels.[49] But in the case of the *S* epitope, this mutation decreases or abolishes the HBsAg properties, probably inhibiting its transcription levels. This explains a persistence in the natural course of the HBV infection[50] coincidentally with having negative HBsAg and positive anti-HBs serologic results.[48] In addition, advanced hepatic fibrosis and diminished response to interferon alpha can occur.[51]

Precore/core gene transformations have generally been associated with chronic HBV infection,[38, 41, 52-58] having serologic results with either positive HBeAg[40-42,47,52,53,57-60] or negative HBeAg.[54] The latter have been more frequently reported,[46,61] also known as an e-minus mutation. Frequently, the mutation occurs after seroconversion to anti-HBe.[42]

Mutations that produce a stop codon in the precore region of HBV DNA are usually point mutations, with a single nucleotide (nt) substitution. Currently, mutations have been reported in nt 1762 (A to T), 1764 (G to A), 1835 (A to C), 1896 (G to A), 1857, and 1897 among others.[38,62,63] One of the most prevalent is the precore nt 1896 mutation.[38,62,63] Sero-

logic markers include positive HBeAg/anti-HBe[39,41,47,58] as well as negative HBeAg,[38,53,54] both in chronic hepatitis B patients. Precore mutations can also be related to fulminant or severe hepatitis, and their absence in HBeAg-positive patients indicates a good prognosis.[43,62]

Core mutations possibly alter the transcription factor-binding site, increasing the activity of core promoter related to HBV reactivation,[57] HCC, and hepatic cirrhosis. Investigators from China have reported that the presence of core promoter mutations were significantly more common in HCC patients than asymptomatic carriers and also were more common in patients with cirrhosis and chronic hepatitis.[64] The prevalence of both types of chronic hepatitis e-minus (e-CHB) mutations varies according to worldwide region. A literature review, which included 50 studies, found the highest rates in Far East and Mediterranean countries[54] – about 33%—followed by 15% in Asia Pacific and 14% in the USA and Northern Europe.[65] The precore stop-codon variant was detected in a median of 60% (range 0-100%) of HBeAg-negative patients overall, 92% in the Mediterranean, 50% in Asia Pacific, 27% in Gambia and 25%, and 24% in the USA and Northern Europe.[63,65]

The main causes of genetic variation have been related to the escape mechanism and to viral resistance and have been associated with:

1. Natural infection (mainly novel surface mutations)[48];
2. Vaccination (mutation in surface antigen[47,66-68]) or presence of antibodies against dominant B cell epitopes[69];
3. Antiviral therapy (activation of HBV genetic mutant variants during lamivudine treatment or chemotherapy or associated with acute liver disease[62,66,67,69]). A study carried out in India found a fre-

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quency of 29% of lamivudine–resistant HBV variants with mutations in the surface and polymerase genes 18 month after lamivudine therapy.[37] Another study relates the reactivation of HBV with a precore mutation (nt 1896) with cytotoxic chemotherapy, suggesting the need for a prophylactic use of lamivudine before and during the chemotherapy for patients with precore mutant varieties of HBV[62];

4. Immunosuppression associated with HBV infection (mutation in different types and positions: core, pre-S1/2, X gene[70]);

5. Chronic hepatis B followed by liver,[56-71] renal,[53,59] or bone marrow[56] transplantation;

In most of the cases, genetic variation occurs concomitant with wild strains, it rarely occurs isolated.[47,54]

Figure 4 suggests an algorithm for serologic diagnosis with suspicion of mutation, which must be confirmed with further exams, such as molecular typing.

Mutations can therefore affect the natural course of HBV infection, viral clearance, and antiviral therapy response. The exact contributions of specific genetic variations, diagnosis, and proper therapy still have to be established.[47]
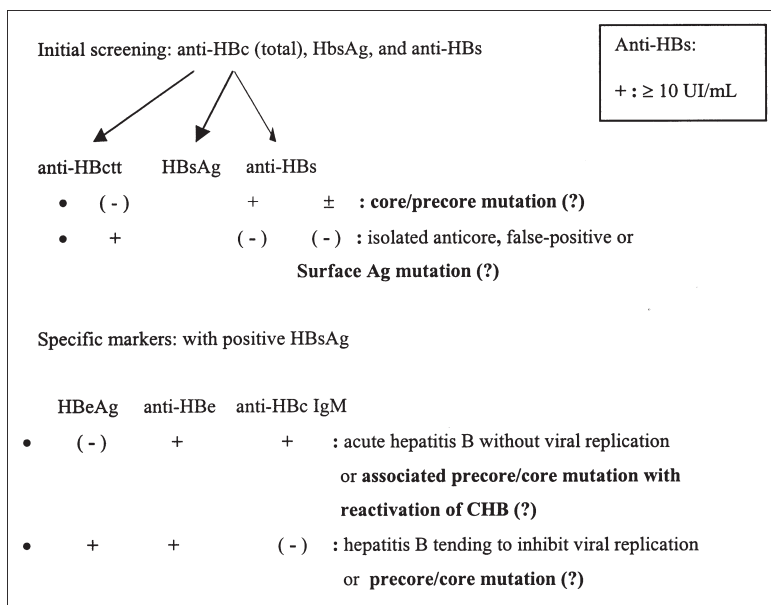
Figure 4 - Suggestion of algorithm for anomalous serologic markers. (suspicion of Hepatitis B virus (HBV) mutants).

RESUMO

Khouri ME e col. Hepatite B: considerações epidemiológicas, imunológicas e sorológicas com ênfase em mutação. **Rev. Hosp. Clí. Fac. Med. S. Paulo 59**(4):216-224, 2004.

A prevalência mundial do vírus da Hepatite B é estimada em cerca 350 milhões de infectados cronicamente, tendo distribuição bastante variada com prevalências baixas desde inferiores a dois por cento, como Europa Ocidental, América do Norte, Nova Zelândia, Austrália e Japão – até altas, superiores a oito por cento como encontradas na África, Sudeste Asiático e China. No Brasil, a prevalência média é em torno de 8%. São descritos atualmente sete variações genotípicas do HBV, nomeadas de A a G, e quatro subtipos principais de antígenos de superfície: "adw", "ayw", "adr "e "ayr", existindo um grande interesse em identificar quais os subtipos e genotipos mais prevalentes a fim de correlacioná-los com manifestações clínicas e distribuição geográfica. Apesar do diagnóstico sorológico ser normalmente bastante sensível e específico, este não detecta casos de Hepatite B mutantes, cada vez mais freqüentes atualmente devido a escape e resistência de vacinação, terapias antivirais, imunossupressão dentre outras. São descritas alterações genômicas no gene de superfície (envelope); gene X; gene do "core"; gene polimerase e gene "pré-core". As principais mutações ocorrem nos genes de superfície e nos genes "pré-core/core", podendo também ser conhecidas como *hepatite oculta*, uma vez que os marcadores de infecção ativa (AgHBs) e replicação viral (AgHBe) podem estar negativos. Assim, deve-se suspeitar de mutação viral nos casos em que a sorologia para a hepatite B indica imunidade ou parada da replicação com o quadro clínico evoluindo mal, excluído outras causas de hepatites.

UNITERMOS: **Hepatite B. Epidemiologia. Serologia. Mutação.**

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