Characterization of HBeAg-Negative Chronic Hepatitis B in Western Brazilian Amazonia

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The present study was conducted with 55 patients native from western Brazilian Amazonia, who were HBV-DNA positive after seroconversion of HBeAg. It is a descriptive case study, with the patients separated into two groups: with hepatitis and without hepatitis on histological examination. The aim of the present study was to describe the clinical and molecular characteristics of patients who are chronic carriers of HBsAg. The prevalence of hepatitis was 63.64%, with a predominance of males (41.82%) and a mean age of 42.5 years, occurring mostly in natives of the southeast sub-region (32.73%). Time was a variable proportional to the course of the disease and the most frequent symptoms were: dyspepsia, asthenia and loss of libido with the majority of the patients having history of prior contact with HBV or positive family history. Splenomegalia was the most frequent sign (40%). Among the tests, platelet count, serum albumin and prothrombin activity were significant in the diagnosis of hepatitis. Alpha-fetoprotein was greater in patients with hepatitis, and hepatocellular carcinoma was detected in 3.63% of the patients with hepatic cirrhosis. Three types of HBV genotypes were diagnosed: A, D and F in the samples amplified for gene S. Genotype A (AA) was observed in 54.54% of the cases with hepatitis, in contrast to other studies showing the predominance of genotype F in this region. We observed mutations in 36.36%, with a predominance of the mutations in the core promoter region (31.81%), due to the greater prevalence of genotype A in this study.

Key-Words: HBeAg-negative chronic hepatitis B, western Brazilian Amazonia, mutations.

Western Brazilian Amazonia is considered a region of high endemicity for hepatitis B virus (HBV), where a large part of the population is infected in perinatal period and during infancy. The prevalence of chronic carriers of HBV surface antigen (HBsAg) in these areas varies between 5% and 15% and approximately 50% to 95% of the population show serologic evidence of past infection with HBV [1,2,3]. Various factors are implicated in the development and outcome of HBV infection, but the progression to chronic hepatitis is proportionally greater in individuals infected by the vertical route or during the perinatal period [4]. Chronic hepatopathies related to HBV are seen in two forms: HBeAg positive and HBeAg negative. The HBeAg- negative chronic forms are represented by two groups of patients: chronic inactive HBsAg carriers and chronic hepatitis B (CHB) patients. The distinction between these two forms is difficult, particularly in patients with biochemical and intermittent virologic activity. It is estimated that in the world there are approximately 350 million inactive HBsAg carriers, and of these, 7% to 30% are infected with mutant forms of HBV, which despite the presence of anti-HBe antibody continue to be HBV-DNA positive [5]. Chronic HBsAg carriers who show HBeAg-negative/anti-HBe-positive serologic status and have serum HBV-DNA detectable by PCR and active hepatic disease characterized by elevated aminotransferases and hepatic histology demonstrating chronic hepatitis with or without cirrhosis are defined as HBeAg-negative CHB patients. These patients who are chronically infected with HBV show fluctuations in aminotransferase levels due to the presence of residual wild strains or due to the presence of HBV strains with mutations in the precore and core promoter regions. These patients progress to cirrhosis in an insidious and subclinical manner with elevated risk of developing hepatocarcinoma [6]. The aim of the present study was to describe the clinical and molecular characteristics of HBeAg-negative CHB patients in natives from western Brazilian Amazonia and who were seen at the hepatitis outpatient clinic of FMTAM.

Material and Methods

Study Design

A descriptive case study of HBeAg-negative chronic hepatitis was conducted in the period of January to December of 2005 in the viral hepatitis outpatient clinic of FMTAM. The patients were natives from western Brazilian Amazonia who were chronic HbsAg carriers, with HBeAg-negative/anti-HBe-positive serologic status and HBV-DNA positive by PCR. Excluded from the study were patients who were infected with human immunodeficiency virus (HIV) or hepatitis C and delta, were alcoholics, used immunosuppressive or hepatotoxic drugs in the preceding six months, were diabetics, had hypertension, were pregnant or puerperant, or had comorbidities of auto-immune nature. The research protocol was authorized by the Committee of Ethics in Research of FMTAM and followed the guidelines defined in Resolution 196/96 of the National Council of Health of the Brazilian Ministry of Health. The participants selected were informed about the objective, advantages and possible risks of the procedures and their participation was authorized by signing an informed consent form. From these patients, demographic, clinico-epidemiological and laboratory data were collected and entered in a databank during consults. In the period of clinical

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follow-up, a percutaneous liver biopsy was performed on each patient selected for study. During the consults, a 15-mL blood sample was drawn and centrifuged, and the plasma was aliquoted, stored at -20ºC and later sent to the Biotechnology Laboratory of the Universidade Federal do Amazonas for amplification, sequencing and HBV genotyping.

Determination of HBV-DNA

HBV-DNA was determined in the selected samples based on the methodological procedures described by Karasawa [7]. The extraction of viral DNA was carried out with the use of hydrated and buffered phenol, and the primers utilized in the amplification reactions were: P1 5´- TCA CCA TAT TCT TGG GAA CAA GA-3´, S1 5´- CGA ACC ACT GAA CAA ATG GC-3´ for the first PCR reaction, and P2 5´-TGC CTC TCA CAT CTC GTC AA-3´, S1 5´- CGA ACC ACT GAA CAA ATG GC-3´ for the second reaction of semi-*nested* PCR.

HBV Genotyping

The positive samples were amplified by PCR utilizing the same sense and antisense primers used in the second reaction of semi-*nested* PCR of the gene S, P2 5´-TGC CTC TCA CAT CTC GTC AA-3´ and S1 5´- CGA ACC ACT GAA CAA ATG GC-3´. DYEnamicä ET Dye Terminator Cycle Sequencing Kit (Mega BACE ä) from Amershan Bioscience was utilized according to the manufacturer's instructions. The positive samples were submitted to sequencing using an automatic sequencer "MegaBace 1000" from Amershan Pharmacia Biotech as described in the original method [8].

Determination of Precore and Core Promoter Mutations

The analyses of the sequences were carried out by comparison of sequences obtained from GenBank utilizing the program *Blast* with those already known of different HBV genotypes. These analyses were performed using the free software packages *BioEdit* 7.0.0 and Mega 3.1. The edition and alignment of the nucleotide sequences obtained were performed in the program *Editor* and *BioEdit*. After edition of the sequences utilizing the option *Graphic view*, the localization and identification was carried out of the principal mutations of the two genes used as reference sequences of the different genotypes of HBV, obtained from *GenBank*. The same sequences were used in genotyping analysis. The numbering adopted was based on the sequence of access number X51970.

Statistical Analysis

The data were recorded using free software on spreadsheets of Open Office, and the statistical analyses of the data were processed by Epi-Info 2000. Statistical tests for association and correlation were used, including the chi-square test, Fisher's exact test and Pearson's correlation. We adopted 5% as the level of significance for the statistical tests [9].

Results

Diagnosis of HBeAg-Negative CHB

Fifty-five chronic HBsAg carriers were studied, with HBeAg-negative/anti-HBe-positive serologic status and serum HBV-DNA positive by PCR. The prevalence of HBeAg-negative CHB was 63.64%, while36.36% of the patients were diagnosed as inactive HBsAg carriers.

Demographic data

It was observed in this study with hepatitis B patients a prevalence of males of 41.82%, with a ratio of 1.9:1 in relation to women. The mean age of the patients with hepatitis was 42.7 ± 15.8 years and of the carriers was 31.35 ± 10.25. The most frequent occupation recorded in this study was autonomous, which was recorded for 52.72% of the patients with hepatitis and 34.55% of the carriers, and the most common place of residence of the patients with hepatitis was the southeast sub-region (32.73%) while the most frequent origin of these patients was the central sub-region of western Brazilian Amazonia (56.36%) (Figure 1 and Table 1).

Forms of Clinical Presentation and Outcome

The most frequent form of presentation of the HBeAg-negative hepatic disease observed in this study was asymptomatic (78.18%), where decompensated chronic hepatic disease was observed in 21.82%.

In patients with hepatitis with less than five years of development, 25.45% showed the asymptomatic form and 10.90% showed signs of decompensated hepatic disease. In this same group of patients, in the period equal to or greater than five years, signs of decompensated hepatic disease were observed in 7.27%, and the asymptomatic form was observed in 20% of the cases.

In the carriers, in the period of less than five years of disease, 25.45% showed the asymptomatic form, and 1.81% showed signs of decompensated hepatic disease. In this same group of patients in the period equal to or greater than five years, the asymptomatic form was observed in 7.27%, and signs of decompensated hepatic disease in 1.81% (Figure 2).

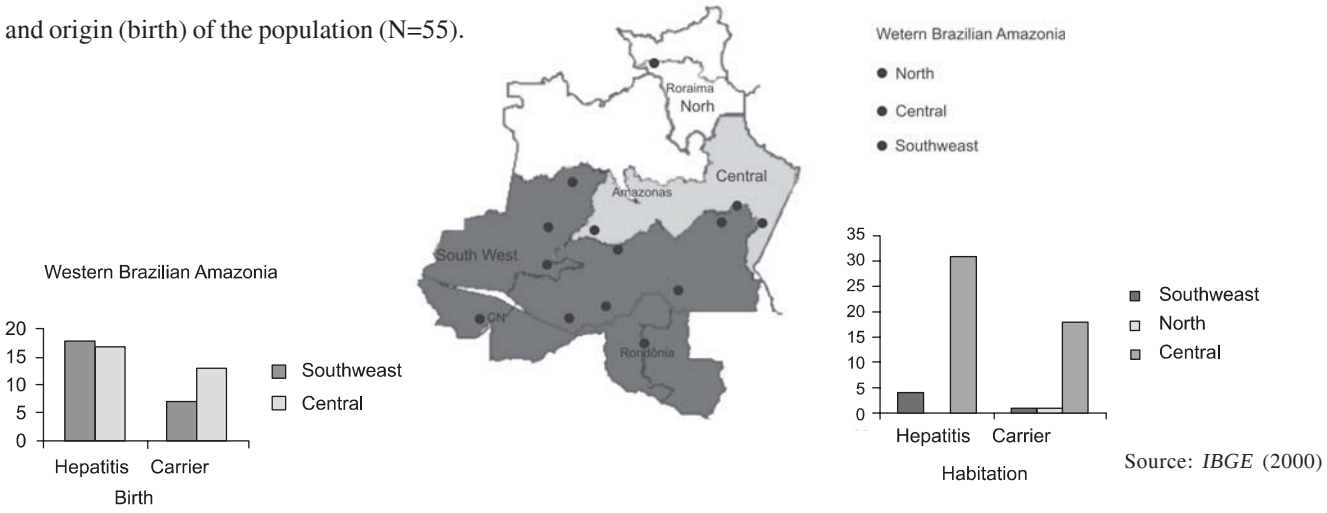
In relation to time to development of disease, the most frequent in the study was one to five years, for 45.46% (n = 25/55). Both hepatitis and carriers patients reported knowing of their disease in the period of one to five years, with respective incidences of 30.91% and 14.55% (Figure 3).

Clinico-Epidemiological Manifestations

In the patients with hepatitis, the most frequent symptoms were: dyspepsia and asthenia, 41.81% and 38.18%, respectively. Loss of libido was observed in 21.80%. Among carriers, 25.45% were asymptomatic, while 16.36% showed asthenia and 5.46% anorexia (Figure 4).

The physical signs of major occurrence in the population of patients with HBeAg-negative hepatitis were: splenomegalia and palmar erythema, 40% and 25.45%, respectively. Among the carriers, a normal physical

Figure 1. Sub-regions of Western Brazilian Amazonia, demonstrating the distribution of the areas of citizenship (habitation) and origin (birth) of the population (N=55).



Source: *IBGE* (2000)

Cities: Porto Velho, Tarauaca, Boca do Acre, Labrea, Humaita, São Paulo de Olivença, Fonte Boa, Carauari, Boa Vista, Manaus, Manacapuru, Parintins, Itacoatiara, Tefe and Coari.

Table 1. Demographic characteristics of the population

Demographic characteristics	Hepatitis	Carriers	p
N of patients	35	20	
Gender (% males)	41.82%	18.18%	<0.05
Age (years, mean ± standard deviation)	42.57 ± 15	31.35 ± 10	<0.05
Autonomous work	52.72%	34.55%	NS***
Citizenship *	32.73%	23.65%	NS***
Origin**	56.36%	32.72%	NS***

*Southeast sub-region of western Brazilian Amazonia: Porto Velho (RO), Tarauaca, Boca do Acre, Labrea, Humaita, São Paulo de Olivença, Fonte Boa and Carauari (AM). ** Central sub-region of western Brazilian Amazonia: Manaus, Manacapuru, Parintins, Itacoatiara, Tefe and Coari (AM). ***NS = not significant.

examination was the most frequent finding, observed in 20% (Figure 5).

The most frequent clinico-epidemiological antecedents of the patients with hepatitis were: past history of jaundice in 45.45% and family history of hepatitis in 36.36%. Among the carriers, the most frequent clinico-epidemiological antecedents were: coming in contact with HBV and hepatitis in the family, which showed the same frequency of 18.18% (Figure 6).

Hematological and Biochemical Analyses

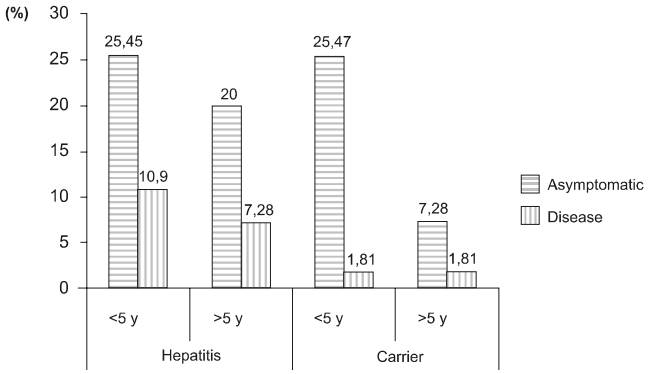
Hematological and biochemical tests revealed lower platelet counts and prothrombin activity in the patients with hepatitis, 119.029 ± 63/σL and 67.79 ± 23%, respectively. They also showed higher values of AST and ALT, 91.3 ± 120 IU/L and 90.3 ± 120 IU/L, respectively. Alpha-fetoprotein levels were higher in patients with HBeAg-negative CHB, 14.03 ± 26 IU/mL (Table 2).

Table 2. Distribution of the means and standard deviation of the hematological and biochemical tests of patients with hepatitis and HBV carriers (n = 55)

Test	Hepatitis	Carriers	p
Platelets, nº/σL	119,029 ± 63	214,950 ± 58	<0.05
Total bilirubin, mg/dL	1.583 ± 2	1.152 ± 0.9	NS*
Prothrombin activity, %	67.79 ± 23	89.90 ± 13	<0.05
Albumin, g/dL	3.280 ± 0.8	3.855 ± 0.4	NS*
AST, IU/L**	91.30 ± 120	42.20 ± 42	<0.05
ALT, IU/L***	90.30 ± 120	57.0 ± 59	<0.05
AFP, IU/mL****	14.03 ± 26	4.925 ± 0.3	<0.05

*Not significant; **Aspartate aminotransferase; ***Alanine aminotransferase; ****Alpha-fetoprotein.

Figure 2. Distribution of the study population based on clinical presentation and time of development of infection in hepatitis and carriers patients



(1) Disease: They are patients who visited the hepatitis outpatient clinic of FMTAM because they showed signs and symptoms of hepatic decompensation after infection with HBV. (2) Asymptomatic: They are patients who were HBsAg positive, HBeAg negative, diagnosed at time of blood donation, after acute hepatitis, during pre-operative check-up or during pre-natal examinations. $p < 0.05$ (patients with hepatitis). $p > 0.05$ (carriers).

Figure 3. Distribution of the population based on the tendency of development curves shown by patients with hepatitis and HBV carriers (n = 55)

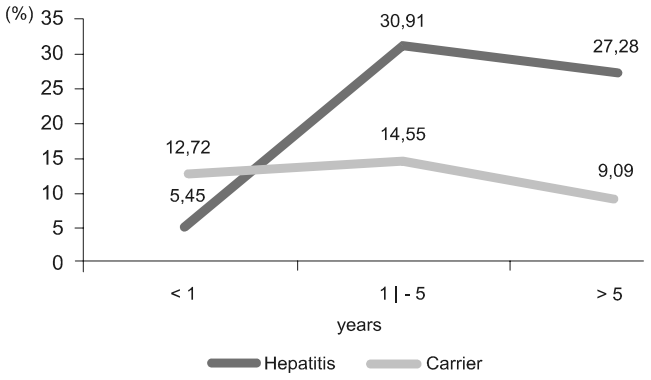


Figure 4. Distribution of the population based on symptoms shown by the patients with hepatitis and HBV carriers (n = 55).

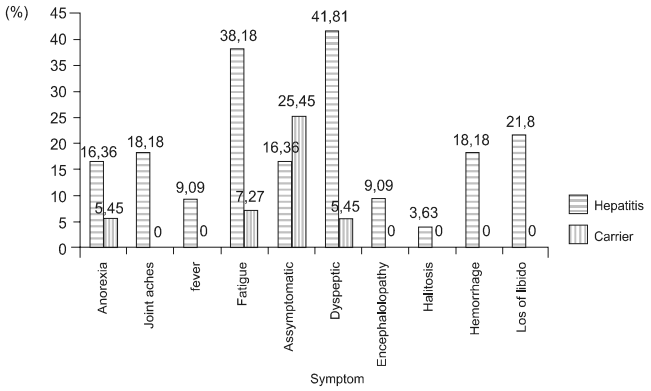


Figure 5. Distribution of the population based on physical examination shown by the patients with hepatitis and HBV carriers (n = 55).

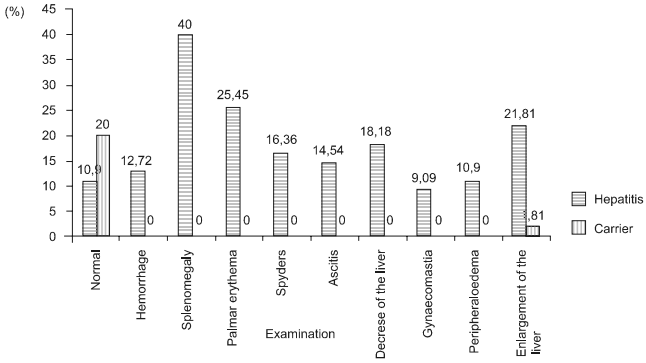
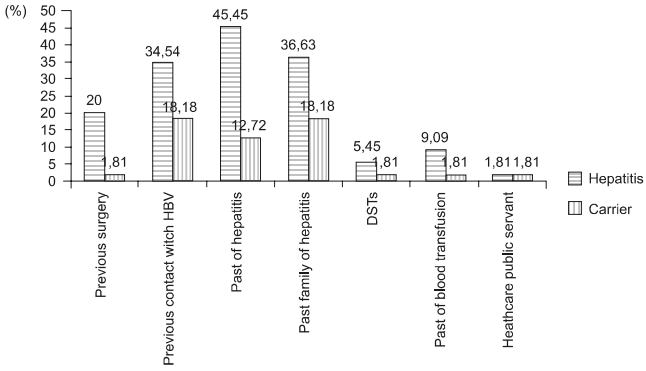


Figure 6. Distribution of the population based on clinico-epidemiological antecedents shown by patients with hepatitis and HBV carriers (n = 55)



(1) Healthcare professional (PAS).

Determination of HBV-DNA by PCR

HBV-DNA was determined by PCR in the 55 serum samples of HBsAg-positive patients selected to participate in the study with HBeAg-negative/anti-HBe-positive serologic status.

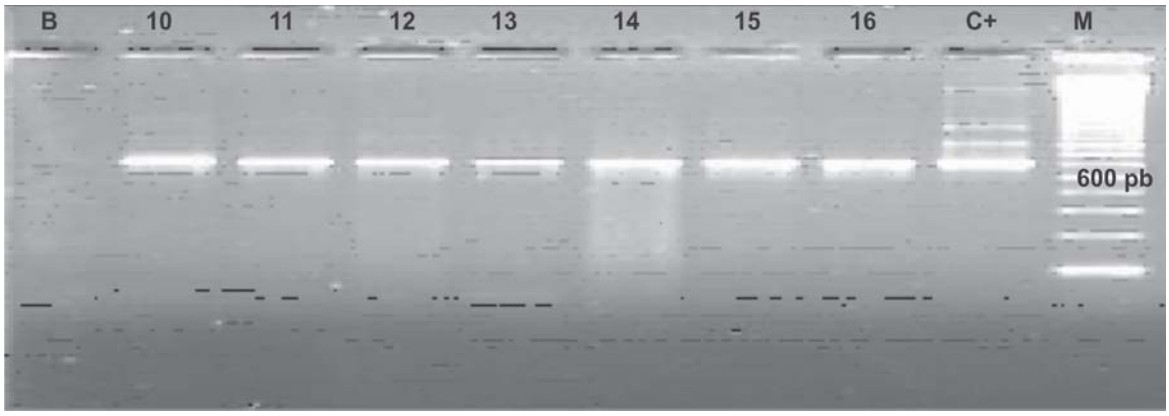
Amplification of Gene S

Of the 55 serum samples of HBV-DNA positive by PCR, amplification of gene S was possible in 40% of the samples, where 25.45% were from patients with HBeAg- negative CHB and 14.54% from HBV carriers. The samples were considered to be positive if they showed a band of 600 bp (Figure 7).

Amplification and Sequencing of the Precore and Core Promoter Regions

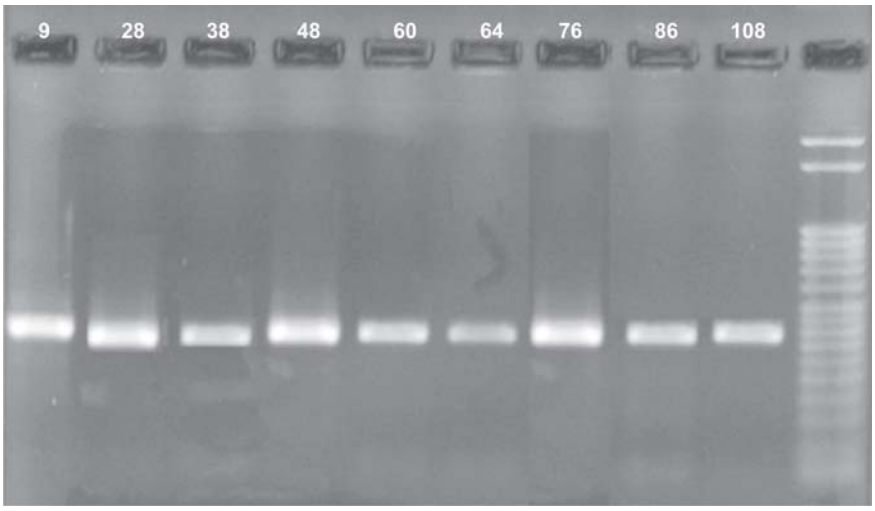
Viral DNA extracted from the 22 samples sequenced was processed utilizing the same sense and antisense primers, using the second reaction of the *nested* PCR of gene C, where samples considered positive had a band of 300 bp (Figure 8).

Figure 7. Electrophoresis in a 1.5% agarose gel of the product of the second reaction of semi-*nested* PCR amplification of gene S of HBV



M. Molecular marker on the right is a 100 bp ladder.
B Reagent control to verify purity of reagents.
C. Positive control of the manufacturer with sensitivity of $7x10^3$ copies per mL.

Figure 8. Electrophoresis in a 1.5% agarose gel of the product of the second reaction of *nested* PCR of gene C of HBV



Genotyping of HBV

The genotypes most prevalent in this study were: genotype A (AA) in 81.81%, genotype F (F2) in 13.63% and genotype D (D1) in 4.54%.

Prevalence of the HBV Genotypes and Geographic Origin

In this study, it was observed that genotype A (AA) of HBV was the most prevalent in the samples from the central and southeast sub-regions of western Brazilian Amazonia with a frequency of 81.82%, while genotypes F (F2) and D (D1) were less prevalent, respectively 13.64% and 4.54% in the same sub-regions (Table 3).

Prevalence of Precore/Core Promoter Mutations

Mutations were observed in the precore and core promoter regions of gene C in 36.36% of the patients. Mutations $G_{1896}A$ of the precore region of HBV were observed in 4.54% of the carriers and mutations $G_{1764}A$ and $A_{1762}T$ of the core promoter region were observed in 31.81% of the patients with HBeAg-negative CHB.

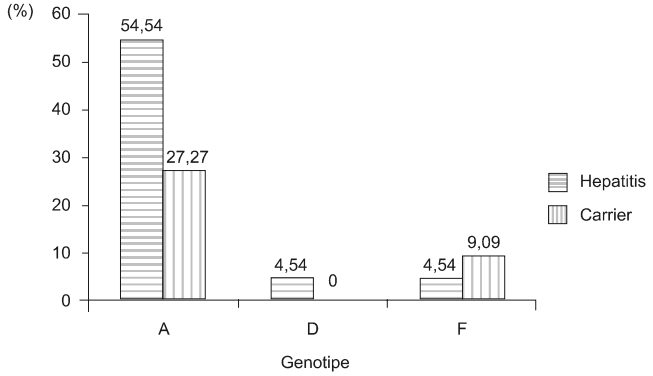
Prevalence of HBV Genotypes and Hepatocellular Lesion

The prevalence of patients with hepatitis with genotype A (AA) was 54.54%, where 4.54% showed cirrhosis associated with hepatocarcinoma. The prevalence of patients with hepatitis with genotypes D (D1) and F (F2) was 4.54% for both genotypes. There was an association with hepatocarcinoma in the patients with genotype F (F2) hepatitis. The prevalence of carriers with genotype A (AA) was 27.27%, while 9.09% of the patients had genotype F (F2) (Figure 9).

Table 3. Geographic origin in western Brazilian Amazonia of the samples of patients who were HBV-DNA positive and HBeAg negative based on the genotype and subgenotype of HBV (n = 22/55)

Genotype	Sub-regions of western Amazonia							
	Southeast		Central		North		Total	
	N	%	N	%	N	%	N	%
A	9	40.90	9	40.90	0	0.00	18	81.82
F	2	9.09	1	4.54	0	0.00	3	13.64
D	0	0.00	1	4.54	0	0.00	1	4.54
Total	11	49.99	11	49.98	0	0.00	22	100

Figure 9. Relation of the HBV genotypes and degree of hepatic lesion (n = 22/55)



Discussion

During the study period, 55 native patients from western Brazilian Amazon region having serum HBV-DNA positive by PCR after seroconversion of HBeAg were followed up. There was a prevalence of 63.64% of HBeAg-negative CHB and 36.36% of the patients were in remission with regard to clinical signs and histological hepatitis, despite the presence of HBV-DNA in serum. Cirrhosis was observed in 9.09% and an association with hepatocarcinoma was demonstrated in 3.63% of the biopsies.

Some authors who followed patients after seroconversion of HBeAg observed higher rates of clinical and histological remission and lower percentage of patients with serum anti-HBe and HBV-DNA positive, but with incidence of cirrhosis and hepatocarcinoma similar to those found in this study [10]. Meanwhile, other authors studying the prevalence of these cases observed that in the last decades there has been a gradual increase in the prevalence of HBeAg-negative CHB [6]. However, a more recent multi-center study of the world prevalence of HBeAg-negative CHB showed an increased prevalence of HBeAg-negative disease in chronic HBsAg carriers (33%) [11]. The elevated prevalence of HBeAg-negative CHB obtained in this study confirms the progressive tendency of the prevalence of HBeAg-negative CHB, possibly influenced by the type of prevalent transmission and by the high endemicity of the area studied [11].

It was observed in this study that in the group of patients with hepatitis, 41.82% were males and 21.82% females, but that in the group of carriers the two sexes showed the same distribution, 18.18%. In the male population, we found that 69.69% had hepatitis, and 30.30% were carriers.

A study evaluating the importance of gender in HBV infection [12] demonstrated that there was a predominance of males with HBeAg-negative CHB, being shown hepatitis with marked necroinflammatory activity in more than 50% of these patients, confirming the incidence found in the present study (69.69%). Other authors evaluating seroconverted patients observed lower levels of annual variation in males, mainly in populations where HBV transmission was usually horizontal during infancy, while perinatal transmission was infrequent in these patients. In regions with a vertical transmission pattern, the frequency of seroconversion of HBeAg shows much lower levels of annual variation [13]. In the present study, the rates of onset in males in the population with hepatitis were relatively higher, confirming previous studies, which suggested that horizontal transmission during infancy or in adulthood was more frequent in females, therefore explaining the lower levels of hepatitis in relation to males who had vertical or perinatal transmission as the predominant form, showing as well higher rates of hepatitis and less variation of annual levels of HBeAg seroconversion.

Analysis of the population with hepatitis revealed a mean age of 42.5 ± 15.48 years, while the mean age of the population of carriers was lower, 31.3 ± 10.25 years. Some authors who have evaluated patients with hepatitis confirm the findings of this study [13].

The analysis of age groups in the population in relation to the presence or not of hepatitis within the same age bracket demonstrated that there is greater probability of an individual having hepatitis in the older age groups (= 65 years), while there is greater probability of an individual being a carrier in the younger age groups (13 to 26 years). The relative risk for worsening of disease in patients with hepatitis in accordance with increase in age is 0.6 times greater in age = 40 years than in individuals younger than 40 years. In carriers, the relation is inverse, individuals younger than 40 years have a greater chance of not contracting hepatitis than individuals = 40 years (95% CI: 0.4595; 1.0029).

In this study, no association was observed between occupation and the probability of having hepatitis, in

accordance with the literature, which points out that in areas of high endemicity the transmission of HBV through work accident is not the most frequent form of transmission, and that in these areas perinatal transmission, propagation via hematophagous insects and horizontal transmission are the most frequent forms of transmission [14]. However, despite that this type of transmission is not common in these areas, it has been documented by some authors that the transmission of HBV can occur mainly among healthcare professionals infected by carriers of HBeAg-negative liver disease [15].

With respect to the citizenship of the patients with hepatitis in this study, it was revealed that the sub-region of greatest prevalence of cases was the southeast sub-region with 32.73%, and the analysis of the origin of this population showed that most of the patients were from the central sub-region with 56.36%, while among the patients with origin in the southeast sub-region there was a significantly higher prevalence of hepatitis cases (80%). These findings demonstrated a heterogeneous distribution of cases of HBeAg-negative CHB in western Amazonia with areas of high, intermediate and low endemicity.

In Brazil, various studies of the prevalence of HBV infection have demonstrated its variability, as it is a country with a large geographic area and cultural, ethnic and socioeconomic diversity, [2,16,17]. The present study confirms the high prevalence of HBeAg-negative disease, conferring to this region an important epidemiological role in the natural history of HBV.

Forms of Presentation and Time of Development

In this study was significant the relation between time *versus* consultation reason in the group of patients with hepatitis, indicating that there is a relation between these two independent variables; while in the group of carriers it does not exist. This confirms the data found by other authors that the HCB HBeAg negative is a late manifestation of a chronic evolution disease [18].

The analysis of the time evolution of the hepatic disease in this study revealed that the time was a variable directly proportional in relation to clinical evolution of the disease. A review of the line of tendency of patients with hepatitis and positive patients suggests that with the evolution of the disease, the number of cases of positive patients will decline gradually, while the number of patients with hepatitis will increase, confirming previous studies on the global expansion of HCB HBeAg negative [6].

Clinico-Epidemiological Manifestations

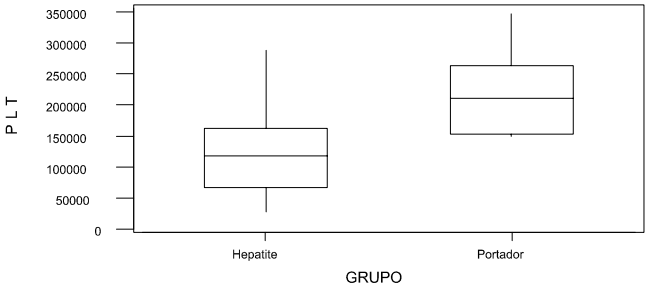
During the follow-up period, the most prevalent symptoms in the patients with hepatitis were: dyspepsia, asthenia and loss of libido, while the most common in carriers was the asymptomatic form, where this condition is generally discovered during blood donation or in the evaluation of elevated ALT levels in routine examinations, similar to those reported in the literature [18].

The analysis of the clinico-epidemiological antecedents showed that the majority of the patients with hepatitis and the carriers had prior history of contact with HBV or positive family history. This is in line with observations made in other studies that reported that there are few patients who recall their acute episode of hepatitis, making it likely that intra-family acquisition of the virus in the first years of life is the most common form of transmission of HBeAg-negative disease [5,19].

Splenomegalia was the most frequent finding (40%) in the patients with hepatitis, suggesting complications of chronic infection with HBV, confirming the findings of other authors [20].

The box plots of the platelet counts demonstrate a marked decrease in platelets in the patients with hepatitis, where 75% showed a platelet count lower than 150,000/σL and the contrary occurred in the HBV carriers (Figure 10).

Figure 10. Box plots utilized to compare the patients with hepatitis and carriers in relation to platelet count (n = 55)



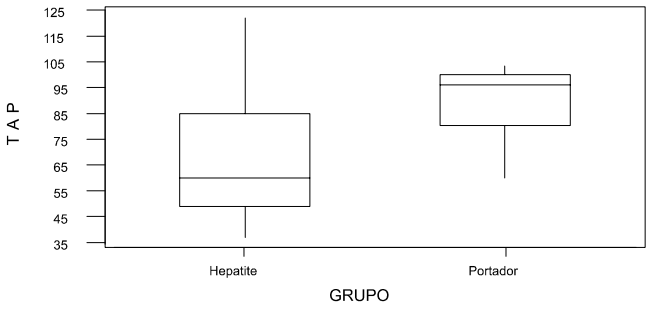
$p < 0.05$.

Various authors confirmed these findings, calling attention to the nonspecific character in relation to the cause of the event [21,22,23]. Other studies found an alteration in platelet adhesion function, which is evidenced earlier than aggregation defects, that in general are delayed, as demonstrated in the test of platelet function in patients with chronic hepatic insufficiency [24,25].

This study revealed that there is no association between total bilirubin level and whether or not the patient has hepatitis or is an HBV carrier, because total bilirubin is not a good marker of hepatic dysfunction, as it is common to find hepatopathies with normal total bilirubin values. However, when increased it confers a poor prognosis for these patients, confirming the findings of other authors [21,26].

The box plots demonstrate that 75% of the patients with hepatitis have prothrombin activity level less than 85%, while 75% of the carriers have an activity level greater than 85%. Despite the significance in the study, as a liver function test alone it is not sensitive nor specific, but in patients with chronic hepatic disease it can be used together with other tests as a noninvasive manner of determining the diagnosis and prognosis of the patients, as has also been described by other authors [27] (Figure 11).

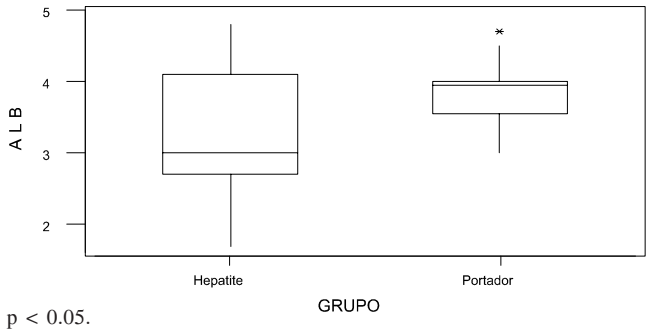
Figure 11. Box plots utilized to compare the hepatitis and carrier groups with regard to the level of prothrombin activity (n = 55)



$p < 0.05$.

The distribution of albumin in the box plots shows that, in 75% of the patients with hepatitis, albumin was less than 4 g/dL, while in the carriers 75% showed an albumin above 3.5 g/dL (Figure 12).

Figure 12. Box plots utilized to compare the patients with hepatitis and carriers in relation to albumin levels (n = 55)



$p < 0.05$.

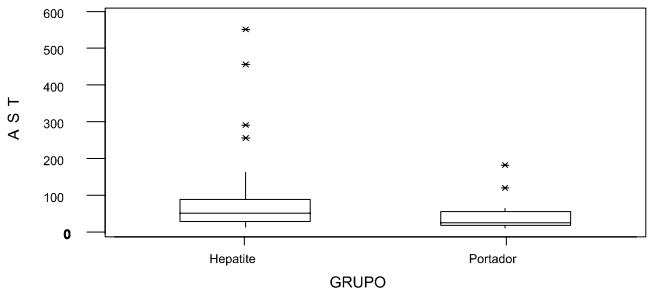
Despite the results being significant, there is a limitation to the use of albumin alone as an indicator of hepatic synthesis, due to its low specificity as also described by other authors [21].

The box plots of aspartate and alanine aminotransferase levels were similar for the patients with hepatitis and for the carriers and no association was found between the groups (Figures 13 and 14).

The results obtained in this study were not significant, in agreement with studies by other authors who confirmed the low specificity of these markers in the diagnosis of chronic hepatic disease [21,28].

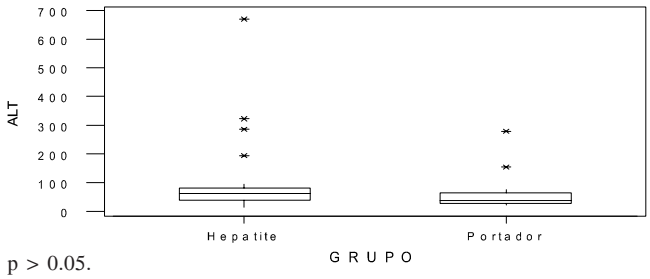
Other authors have studied the relation of AST/ALT ratio and chronic viral hepatopathies, suggesting that this ratio when greater than one is related to a diagnosis of cirrhosis, with a specificity of 94% and sensitivity of 44% to 75% [26]. In this study we observed a positive ratio of 86.66% when correlating AST/ALT >1 and the presence of hepatitis with advanced fibrosis, revealing specificity rates similar to those in the literature.

Figure 13. Box plots utilized to compare the patients with hepatitis and carriers in relation to aspartate aminotransferase levels (n = 55)



$p > 0.05$.

Figure 14. Box plots utilized to compare the patients with hepatitis and carriers in relation to alanine aminotransferase levels (n = 55)



$p > 0.05$.

Mean values of alpha-fetoprotein in this study were 14.03 ± 26 IU/mL in patients with hepatitis and 4.92 ± 0.3 IU/mL in carriers, with a significant association between this marker and the presence of hepatitis, where hepatocarcinoma was detected in 3.63% of the patients with hepatitis and cirrhosis, confirming studies of other authors who showed that alpha-fetoprotein is an important laboratory test in the screening for hepatocellular carcinoma (CHC), but its elevation is not obligatory in CHC since 20% to 30% of the cases have normal values of this protein [29]. Other authors observed a similar incidence of CHC in HBsAg-positive patients after HBeAg seroconversion [10]. In this study, with respect to risk factors for CHC, the prevalence of this cancer was higher in males, with a mean age of 35.50 ± 13 years, natives from the southeast and central sub-regions and being HBV carriers with cirrhosis. These findings are similar to those reported by other authors for areas of high prevalence of HBV and suggest that in this study the patients possibly had gotten infected with HBV by transmission through family contact during the perinatal period [30,31].

HBV-DNA was determined by PCR based on the methodological procedures described by Karasawa [7] in the 55 serum samples of the patients selected for study. Three genotypes of HBV were found: A, D and F in 40% of the

samples where sequencing of gene S was successful. Genotype A subgenotype A1 (AA) was the most common, found in 81.81%, followed by genotype F subgenotype F2 with 13.63% and genotype D subgenotype D1 with 4.54%.

In relation to the geographic distribution of the HBV genotypes, we observed in this study a varied occurrence of genotypes in accordance with the geographic situation of the patients, where there was predominance of genotype A (AA) in the southeast and central sub-regions of western Brazilian Amazonia.

Some authors studying the prevalence of diverse genotypes of HBV in the world found prevalence results different from those in this study, demonstrating a greater prevalence of genotype D in relation to genotype A and observing that the association of genotype, albeit possible, is rare [32]. In the USA, where the population shows various ethnicities, as in Brazil, all the genotypes occur [32,33]. Genotype A forms two large geographic groups: one of European and North American origin, A2 (AE), and the other of Afro-Asiatic origin, A1 (AA). Genotype D is the most widely spread of the genotypes and predominates in areas of the Mediterranean region and in India, currently showing four subgenotypes, D1, D2, D3 and D4. Genotype F is subdivided into two subgenotypes, F1 and F2, where subgenotype F1 is the most encountered in Central America and F2 principally in South America. Genotype F subgenotype F2 was identified primarily in Brazil [34]. Other authors [35] corroborate the present study with regard to the predominance of genotype A in Brazilian regions; however, in other studies, genotype F has greater frequency among isolated tribes of Amazonia who do not maintain contact with whites. In this same study, it was shown that contact with whites modified the prevalence of the genotypes favoring the predominance of genotype A [36,37]. These data suggest a change in the native genotypic profile of the region, possibly influenced by the migratory dynamics that has been occurring in western Amazonia since the end of the 19th century and beginning of the 20th century, characterized in this study by the increased prevalence of genotype A in relation to genotype F, seen as the native genotype of this region. These data corroborate the observations of other authors with respect to the geographic distribution of the genotypes, reflecting anthropologic and epidemiological findings relative to the migratory events inherent to the human race [38].

In the group of patients with HBeAg-negative CHB, the most prevalent genotype was genotype A (AA) with 54.54%, while the other genotypes found in this study, F (F2) and D (D1), showed the same low frequency of 4.54% in these patients. In the carriers there were no recorded cases of patients infected with genotype D1, but only patients infected with genotypes A (AA) and F (F2), respectively, 27.27% and 9.09%.

Various authors [39,40-42] in the world have related genotype with the degree of hepatocellular lesion, where those considered most aggressive are genotypes A and C which show higher scores of necroinflammatory activity and fibrosis, in concordance with the present study, including higher rates of cirrhosis and hepatocarcinoma. However, other authors after various analyses concluded that more important than the genotype would be the determination of the subgenotype in the characterization of the pathogenicity of HBV [43].

In this study after the sequencing of the precore and core promoter regions, mutations were seen in 36.36% of the HBV-DNA-positive samples of the patients with hepatitis and carriers with a predominance of the mutations in the core promoter region over mutations in the precore region, respectively, 31.81% and 4.54%. In the patients with hepatitis, mutations were observed in the core promoter T_{1762} region only in 9.09% and a double mutation A_{1764} with T_{1762} in 18.18%. With respect to the presence of mutations in the precore region, we observed that in the group of patients with hepatitis there was an A_{1896} mutation in 4.54%. In carriers, no mutations were observed in the precore region, but in the core promoter region we found an A_{1764} mutation only in 4.54%.

In relation to the presence of mutations in the precore region, no mutations were found with genotypes A (AA) and F (F2), but in patients infected with genotype D (D1), we found 4.54% with a mutation in the precore region. Core promoter mutations were observed in 31.81% of patients infected with genotype A (AA).

Studies on HBV mutations have shown that the precore variant results in the substitution of G_{1896} by A_{1896} in the core/precore regions, leading to the loss of translation of HBeAg, but maintaining the production of HBcAg, where this mutation is known as A_{1896}. The most likely mechanism of this mutation is the selection through immune response. Another type of mutation that also affects the production of HBeAg can be found in the core promoter region, A_{1762}-T_{1762} and G_{1764}-A_{1764}, corresponding to important nucleotides for the maintenance of secondary structure of the encapsidation signal, where these mutations are known as mutations T_{1762} and A_{1764} [44].

Other authors have shown that the frequency of mutations of HBV is estimated to be approximately 1.4 to 3.2 x 10^5 substitutions/nucleotide/site/year, this rate being ten times greater than that found in other DNA viruses. The mutation rate is influenced mainly by the clinical phase of the disease, such as immune tolerance, immune elimination and immunosuppression after liver transplantation, and by treatment with antiviral drugs. The precore and core promoter mutations are among the principal mutations of HBV. The precore mutation was detected in up to 95% of HBeAg-negative patients, but it is more prevalent in Mediterranean countries. There are few data with respect to core promoter mutations outside Asia where mean values of prevalence among HBeAg-negatives was found to be 77% [11]. Based on studies conducted in Campinas (Brazil), about 70% of HBsAg-positive patients are HBeAg negative, with elevated potential of mutations in the precore or core promoter region [45].

In the present study, the prevalence of mutations in the precore and core promoter regions were relatively low, 4.54%

and 31.81%, respectively, when compared with other studies [46]. These differences probably occurred due to the high prevalence of genotype A (AA) (81.81%), when compared with the prevalence of genotype A found in other studies, the negative correlation between genotype A and precore mutation A_{1896} is related to base pairing in the structure of the stem-loop in the pre-genomic sequence of encapsidation, but the basis for positive correlation between genotype A and the mutation of the core promoter region is not established [46].

This study showed an elevated prevalence of genotype A (AA) in the southeast and central sub-regions of western Brazilian Amazonia, contrasting with the low prevalence of genotype F (F2) in these areas. This genotype is described by various authors as a genotype native from western Brazilian Amazonia. The Amazon region has been undergoing migratory influences from other regions for decades, which could be contributing to the change in genotypic profile, necessitating more studies to confirm these epidemiological observations (Figure 15).

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