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RESEARCH ARTICLE
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STUDY OF CHRONIC HEPATITIS B VIRUS INFECTED PATIENTS ('E' NEGATIVE) WITH VIRAL LOAD AROUND 10,000 COPIES/ML AND NORMAL OR BORDERLINE AST/ALT ELEVATIONAT A TERTIARY CARE HOSPITAL IN CHENNAI

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Abstract

Background: This study was conducted to examine patients who were chronically infected with the hepatitis B virus ('e' negative), with a viral load of around 10,000 copies/ml and normal to borderline elevated AST/ALT levels.

Methods: 43 chronic HBV-infected patients at Southern Railway Headquarters Hospital, Aynavaram, Chennai, participated in this hospital-based cross-sectional study from June 2011 to May 2013, with written informed consent from study participants and approval from the institutional ethics committee.

Results: When performing a correlation analysis, at the 0.05 level (2-tailed), the correlation is significant; at the 0.01 level (2-tailed), the correlation is significant. The minimum observed test value minus one is the smallest cut-off value in the ROC curve (PCR coordinates), while the maximum observed test value plus one is the greatest cut-off value. The remaining cut-off values are the averages of two consecutively ordered observed test values. There is at least one tie between the positive actual state group and the negative actual state group in the ROC curve's alt coordinates. a. The greatest observed test value plus one is the biggest cut-off value; the smallest cut-off value is the least observed test value minus one. The averages of two consecutively ordered observed test values are used to determine all other cut-off values. The test result variable(s): ALT has at least one tie between the positive actual state group and the negative actual state group in the area under the ROC curve comparison between alt and PCR. There could be bias in the statistics. a) Assuming nonparametric data; b) True area = 0.5 is the null hypothesis.

Conclusion: In order to distinguish between inactive carriers and e-negative chronic HBV infection, liver biopsy is essential in patients with normal AST/ALT and HBV DNA levels of approximately 105 copies/ml. Since ALT seems to have a stronger correlation with necro-inflammatory activity, periodic monitoring of it is essential.

Keywords: Chronic Hepatitis B Virus, Infected Patients ('e' negative), Viral Load, Normal or Borderline AST/ALT Elevation

INTRODUCTION

Infection with the HBV (Hepatitis B Virus) is a worldwide public health concern. An effective vaccine is available, yet 350 million people globally are thought to be chronically infected with HBV. Every year, almost 5,000 000 people pass away from liver illness linked to HBV.[1] HCC (Hepatocellular Carcinoma) is the fifth most common malignancy in humans, while hepatitis B is the tenth largest cause of mortality worldwide.[2] Around 350 million of the over 2000 million people on the planet today who have experienced HBV infection at some point in their lives are chronic carriers of the virus, according to a WHO survey. Approximately 75% of the global populace resides in regions with high endemicity. Approximately 25% of the 4 million acute clinical cases of HBV that arise each year end up as carriers. Cirrhosis, primary cancer, and chronic active hepatitis account for one million deaths annually.[3] Although South Asia, which includes India, has been classified as having an intermediate endemicity, the region's sheer population size is responsible for a significant portion of the global pool of HBV carriers.[4] Hepatitis-B is a serious health issue in India, where the incidence of HBsAg is intermediate, at 2-7%.[5,6] A common quote for the overall carrier rate is 4.7%. Based on a meta-analysis.[7,8] Over 40 million (four crore) HBsAg carriers are thought to exist in India. Hepatitis B virus (HBV) infection is meso-endemic in India, where newborns have a 0.04% lifetime risk of getting a chronic infection.[9] More than 100,000 Indians lose their lives to HBV-related ailments each year.[10,11] The pathogenesis of chronic HBV infection is dynamic. Five phases make up the natural history of chronic HBV infection; these phases are detailed in depth under the evaluation of literature, but they are not always sequential.

1. The immune-tolerant phase, which is the initial stage of chronic HBV infection, is marked by low aminotransferase levels, strong HBV replication, and minimal to no histologic activity. It is also known as the HBeAg-positive phase.
2. HBeAg positivity, a comparatively lower level of replication (as indicated by lower serum HBV DNA levels), an increased or fluctuating level of aminotransferases, moderate to severe liver necro-inflammation, and a faster rate of fibrosis progression are the characteristics of the "immune reactive HBeAg-positive phase." Seroconversion marks the end of this phase. This stage is also known as "e" positive CHB or "e" positive chronic hepatitis B.
3. HBeAg seroconversion results in the production of the corresponding antibody (anti-HBe), which is typically linked to the infection's transition from a high-replication phase to an inactive phase that essentially has normal liver histology and little to no residual viral replication (referred to as the "inactive HBsAg carrier state").[12,13] Not all patients who lose HBeAg and seroconvert to anti-HBe antibodies, however, experience a long-lasting reduction in the activity of liver disease and HBV replication.
4. Depending on the HBV genotype and other variables, a variable percentage of HBeAg-negative and anti-HBe-positive patients maintain or re-establish high serum HBV-DNA levels and chronic or sporadic elevations in alanine aminotransferase activity. These individuals have replication-competent HBV variants that lack the ability to create HBeAg because of mutations in either the basic core promoter region or the pre-core region of the HBV genome.[14] This type of CHB is also known as anti-HBe-positive or HBeAg-negative CHB; it is a potentially serious and progressive liver disease that frequently results in the development of cirrhosis and HCC.[15-19]
5. A liver biopsy and blood sample containing HBV DNA may reveal low-level replication even in patients who develop neutralizing antibodies (anti-HBs) and remove HBsAg from their blood (HBsAg negative phase).

There is continuous discussion on how to distinguish between inactive carrier and HBeAg-negative CHB (Chronic Hepatitis B) patients with normal ALT levels. It's still unknown what the HBV DNA cut-off value is to distinguish between these two CHB stages. In fact, serum ALT fluctuates greatly in patients with HBeAg(-) chronic hepatitis B, and 20%–30% of these patients with histologically

confirmed chronic hepatitis have normal ALT at presentation.[20] This cut-off level may fluctuate from community to population and may change over time based on the host immunological status and other exogenous factors, given the diverse natural history of chronic hepatitis B virus infection as well as genotype and mutation variations. This depends on a variety of factors, including the host's CD4 immune response, the viral components (HBV genotypes and mutations in the core promoter and precore regions), the environment (alcohol use), and the host.[21]

AIMS AND OBJECTIVES

1. To investigate the biochemical, clinical, and demographic characteristics of individuals with chronic HBV infection who test negative for HBeAg and have a viral load of about 10,000 copies/ml (2,000 IU/ml).
2. To determine whether the 10,000 copies/ml (2,000 IU/ml) cut-off value for HBV DNA could accurately differentiate between "e" antigen-negative chronic HBV infection and carriers who are not active.
3. To evaluate the connection between histopathology and the levels of HBV DNA and biochemical profiles.

MATERIALS & METHODS

43 chronic HBV-infected patients at Southern Railway Headquarters Hospital, Aynavaram, Chennai, participated in this hospital-based cross-sectional study from June 2011 to May 2013, with written informed consent from study participants and approval from the institutional ethics committee.

Inclusion Criteria

- Both genders
- 15 years and above
- HBsAg (+)
- HBeAg (-)
- Anti-HBe (+)
- Anti-HBcIgM (-)
- Anti-HBc total (+)
- BMI < 35
- HBV-DNA PCR up to 1,00, 000 copies/ml [preferably around 10, 000 copies/ml]
- Normal/<2ULN AST and ALT

Exclusion Criteria

- Current alcohol use > 30 g/d
- Hepato-toxic drug intake
- Morbid obesity
- Hepatocellular carcinoma
- Hepatitis C co-infection
- HIV co-infection
- De-compensated liver disease
- Bilirubin level > 3 mg/dL (25.6 μmol/L)
- INR > 1.5
- Platelet count < 50,000 / mm^3
- Albumin level < 3.0 g/dL
- Ascites
- Bleeding oesophageal varices
- Hepatic encephalopathy
- Grossly elevated AST/ALT [>2 times ULN]
- HBV DNA PCR more than 1,00, 000 copies/ml

Statistical Methods

The data were analysed using InStat software (trial version), Version 3. 10 (copyright 1992-2009), GraphPad Software, Inc.), SPSS Statistics [originally, Statistical Package for the Social Sciences, later modified to read Statistical Product and Service Solutions] (full version), Version 17.0.0 (copyright IBM Inc.) and Analytics Genie Android application by Asia Analytics Inc. (formerly SPSS China).

RESULTS

			AST	**ALT**	**PCR**	**HAI**	**Fibrosis**
Spearman's Rho	AST	Correlation Coefficient	1.000	.540	-.025	.497	.637*
		Sig. (2-tailed)	.	.057	.936	.084	.019
		N	13	13	13	13	13
	ALT	Correlation Coefficient	.540	1.000	.319	.818**	.779**
		Sig. (2-tailed)	.057	.	.288	.001	.002
		N	13	13	13	13	13
	PCR	Correlation Coefficient	-.025	.319	1.000	.618*	.428
		Sig. (2-tailed)	.936	.288	.	.025	.145
		N	13	13	13	13	13
	HAI	Correlation Coefficient	.497	.818**	.618*	1.000	.816**
		Sig. (2-tailed)	.084	.001	.025	.	.001
		N	13	13	13	13	13
	Fibrosis	Correlation Coefficient	.637*	.779**	.428	.816**	1.000
		Sig. (2-tailed)	.019	.002	.145	.001	.
		N	13	13	13	13	13

Table 1: Correlation Analysis

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Coordinates of the Curve

Test result variable(s): PCR

Positive if Greater Than[a]	**Sensitivity**	**1–Specificity**
270.00	1.000	1.000
275.50	1.000	.875
1015.00	1.000	.750
1850.00	.800	.750
2012.00	.800	.625
2282.00	.600	.625
2668.50	.600	.500
3056.50	.400	.500
3408.00	.400	.375
4194.00	.400	.250
5344.00	.200	.250
6586.00	.000	.250
10011.00	.000	.125
12701.00	.000	.000

Table 2: Co-Ordinates of ROC Curve–PCR

The maximum observed test value plus one is the largest cut-off value, while the minimum observed test value minus one is the least.

The averages of two consecutively ordered observed test values are used to determine all other cut off values.

Coordinates of the Curve

Test result variable(s): ALT

Positive if Greater Than or Equal To[a]	Sensitivity	1–Specificity
8.00	1.000	1.000
11.00	1.000	.875
14.00	1.000	.750
15.50	1.000	.500
16.50	.800	.375
19.00	.600	.375
22.00	.400	.250
23.50	.000	.125
25.00	.000	.000

Table 3: Co-Ordinates of ROC Curve-ALT

The test outcome variable(s): There is at least one tie in ALT between the groups representing the positive and negative actual states.

The maximum observed test value plus one is the largest cut off value, while the minimum observed test value minus one is the least. The averages of two consecutively ordered observed test values are used to determine all other cut off values.

DISCUSSION

Distribution of the Sample

Despite having a small sample size (n = 43), a major portion of the sampled data passed the Kolmogorov-Smirnov test for normality, indicating that the sampled population is likely to be Gaussian or very close to it and may therefore be somewhat extrapolated to a larger population. The Dallal and Wilkinson approximation to Lilliefors' approach (Am. Statistician, 40:294-296, 1986) was used to obtain the p-value. GraphPad Software, Inc.'s InStat Software (trial version), Version 3.10, was used to perform the normalcy test and calculate the p-value.

Baseline Characteristics of the Patients

Of the 43 cases, 19 were female and 24 were male. The BMI, albumin, PT, and AFP of men and women were nearly the same. Men were marginally older, while women's average sickness duration was greater. Males had somewhat greater levels of HBV DNA as well as ALT and AST than females did.

Mode of Diagnosis

46.5% of the 43 patients received their diagnosis by accident—that is, when they were examined while being admitted to a ward for a separate ailment. Twenty-nine percent of the patients received a diagnosis during the pre-procedure workup, thirteen percent during contact screening, two percent throughout camp, two percent during the master health check-up, and four percent during voluntary blood donation.

Contact Screening

Just 76.7% of the 43 patients' contacts had undergone appropriate screening, and only 72.1% of the patients' contacts had received all recommended vaccinations. For a variety of reasons, including physical separation, family conflict, not having a railroad plan, etc., the remaining 27.9% of patients did not vaccinate their families.

Risk Factor Analysis

Of the 43 patients, 58.14% had previously undergone surgery, 37.2% had previously undergone dental work, 16.2% had previously received a blood transfusion, 16.2% had previously had a

household contact who tested positive for HBsAg, 13.9% had previously undergone invasive procedures, and just 2.3% had previously had a tattoo.

Correlation Analysis

For fibrosis and HAI, respectively, ALT had extremely high Spearman's Rho values of 0.779 and 0.818, both of which were highly significant at 0.01 levels (2-tailed). Another example of this can be seen in ROC analysis.

For HAI and fibrosis scores, HBV-DNA-PCR had medium Rho values of 0.618 and 0.428; only the former is significant at the 0.05 level (2-tailed). ROC analysis also shows that PCR is less accurate than ALT in terms of connection with the degree of inflammation (HAI score).

The Spearman's Rho for the fibrosis score and HAI was 0.816, and it was significant at the 0.01 level (2-tailed). This is a predicted result because the inflammatory and fibrotic alterations are correlated.

Hoofnagle JH, Lok AS, et al. found that in individuals with chronic hepatitis B who were positive or negative for HBeAg, there was no relationship between blood HBV DNA and histological grade.[22]

ROC Analysis

In the given case of an e-antigen negative chronic HBV infected patient, the area under the ROC curve values for ALT and HBV-DNA PCR were 0.7 and 0.525, respectively. This suggests that both ALT and PCR could reliably predict substantial liver impairment, with ALT being more reliable than PCR.

It has been discovered that no significant PCR cut-off value could be obtained from it since the false positivity rate always rises when the PCR value is decreased in order to boost sensitivity. A cut-off value of 2668 IU/ml would result in 50% specificity and 60% sensitivity. When it comes to predicting considerable necro-inflammatory activity in a liver biopsy, an ALT cut-off value of 16 would result in an 80% sensitivity and a 62.5% specificity. A cut-off value of 19 would produce a 60% sensitivity and a 62.5% specificity.

A cut-off HBV DNA level of 6.2x102 IU/ml would produce intermediate sensitivity (62.1%) and specificity (63.8%) in distinguishing HBeAg negative CHB from inactive carriers, according to research by Paras S, Sakshi G, et al. from Jaipur. It is important to note that serial ALT values, rather than histology, were used to distinguish between e-negative chronic HBV infection and inactive carriers. In addition, the patients were from a different geographic area [64], were younger (mean age 34.85 +/- 13.25 compared to our group's mean age 46.86 +/- 11.00), and were primarily male (M:F = 5.48:1 compared to our group's M:F = 1.26:1).

To distinguish "inactive carriers" from HBeAg-negative CHB, Manesis EK, Papatheodoridis GV, et al. from Greece proposed a cut-off HBV DNA level of 3x104 copies/mL (6000 IU/ml).[23]

In a recent study conducted in Japan, Seo Y, Yoon S, et al. determined that a level of <105 copies/ml (<2000 IU/ml) was appropriate for the diagnosis of "inactive carriers." However 20% of HBeAg-negative CHB patients were misdiagnosed.[24]

About 21% of HBeAg-negative individuals with PNALT and HBV DNA less than 105 copies/ml (2, 000 IU/ml) exhibited histologically active liver disease, according to Kumar et al. (fibrosis stage higher than 2, histological activity index [HAI] greater than 3). According to our research, 30.7% of patients with a normal ALT level at presentation and HBV DNA levels less than 20,000 IU/ml will have histologically active liver disease (HAI greater than 3 and/or fibrosis stage greater than 2).

CONCLUSION

A considerable percentage (30.7%) of HBsAg-positive and HBeAg-negative individuals with HBV DNA levels of about 105 copies/ml had necro-inflammation. There is no precise HBV DNA threshold or cut-off value that can be used to accurately distinguish between inactive carriers and HBeAg negative chronic hepatitis. Therefore, liver biopsy plays a vital role in distinguishing between inactive carriers and e-negative chronic HBV infection in individuals with normal AST/ALT and HBV DNA

at around 105 copies/ml. Since ALT seems to have a stronger correlation with necro-inflammatory activity, periodic monitoring of it is essential.

LIMITATIONS OF THE STUDY

1. Small sample size
2. Cross-sectional study (any categorization of chronic HBV infection may subsequently alter over several years due to the substantial variations in HBV DNA levels and ALT levels over time).[25]
3. Lack of genotype sequencing in order to identify HBV mutants.

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