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Serological profile of chronic hepatitis B carriers – A tertiary care experience

Swaathy Ramamurthy[1], Rathinam Suresh[2], Purushothaman Ganesan[3]

[1,2,3]Senior Assistant Professor, Department of Microbiology, Government Medical College, Omandurar Government Estate, Chennai, Tamil Nadu, India

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ABSTRACT

Background: Hepatitis B virus (HBV) infection in India has prevalence of around 40 million chronic HBV (CHB) carriers; 1 to 2 lakhs reported deaths annually due to its complications, such as cirrhosis and hepatocellular carcinoma. Most are asymptomatic, serving as the source of this highly transmissible infection. These patients must be evaluated and monitored regularly, for prediction of their prognosis to reduce their morbidity and mortality. **Aims and Objectives:** In this study, asymptomatic CHB carriers are evaluated, to assess the risk factors, and to determine replication markers of HBV and coinfection with hepatitis D virus. **Materials and Methods:** In this cross-sectional study, 123 asymptomatic CHB carriers were subjected to hepatitis B serological assays such as hepatitis B surface antigen, hepatitis B "e" antigen, anti-hepatitis B "e" antibody, and anti-HDV antibody by enzyme-linked immunosorbent assay. **Results:** Majority (70%) belonged to the age group of 21–50 years. Replicative carriers (HBeAg+) belonging to the age group below 40 years constituted 75% (P=0.04). The most common risk factor in the study group was frequent therapeutic injections (36.6%) followed by family contacts (33.3%). Significant part of family contacts was replicative carrier (P=0.025). Serological profile showed that 83.3% were HBe-seroconverted, with only16.2% showing HBeAg positivity. No patient showed co/super-infection with HDV. **Conclusion:** Preponderance of hepatitis B carrier status in young adult males and four fold increased incidence in family contacts than general population necessitates stringent screening of young adults and family contacts. Furthermore, this study mandates regular evaluation of chronic hepatitis B carriers by serological assays for better therapeutic management.

Key words: Hepatitis B; Asymptomatic carriers; Serological profile; HBeAg

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INTRODUCTION

Hepatitis B virus (HBV) is a deoxyribonucleic acid (DNA) virus, belonging to Hepadnaviridae family.[1] It is the most common cause of viral hepatitis causing chronic infection, along with hepatitis C. It is unique among the blood borne viruses and gains exceptional importance due to its peculiar character of high transmissibility, making it 100 times more infectious than human immunodeficiency virus (HIV).[2]

HBV infection and its sequelae are major health problems globally. About 350 million (5%) people worldwide are estimated to be chronic HBV (CHB) carriers, of which 75% of the global pool is found in Asia. About 15–40% of the CHB carriers have the propensity to suffer complications.[3] India has intermediate endemicity, with the prevalence of around 40 million CHB carriers, and about 1 to 2 lakhs reported deaths annually due to its complications, such as cirrhosis and hepatocellular carcinoma (HCC). HBV infection is the etiology of about 80–90% primary hepatic carcinoma in India.[4]

Chronic hepatitis B (CHB) infection has a varied clinical spectrum, ranging from asymptomatic state to cirrhosis and its complications. The natural history of HBV infection is extremely complex, with variable course, affected by viral, host, and environmental factors. The host factors include, age of the patient at the onset of infection, male gender, host immune response to the virus and the viral factors being, HBV genotype, level of viral replication, and HBV mutations.

Address for Correspondence:
Dr. Swaathy Ramamurthy, Senior Assistant Professor, Department of Microbiology, Government Medical College, Omandurar Government Estate, Walaja Road, Triplicane, Chennai - 600 002, Tamil Nadu, India. **Mobile:** +91-9842397954. **E-mail:** drswaathyr@gmail.com

The other environmental factors are coinfection with hepatitis D, hepatitis C and HIVs, alcohol abuse, diabetes mellitus, obesity, etc.[5] CHB carrier state is defined by the persistence of hepatitis B surface antigen (HBsAg) in patient's serum, for more than 6 months. The propensity to CHB carrier state is more in case of children, who tend to acquire infection perinatally, and it accounts to 90% of those infected, whereas, in adults, it is far less, between 5% and 10%.[6] The carrier state is marked by the presence or absence of hepatitis B "e" antigen (HBeAg), anti-hepatitis B "e" antibody (anti-HBe), and hepatitis B virus DNA (HBV DNA).

In the consortium of CHB carriers, most of them are asymptomatic, diagnosed only incidentally, during routine antenatal and pre-operative screening and screening of family contacts of HBV-positive patients, blood donation, etc.[7] Moreover, those diagnosed not only represent only tip of an iceberg but also serve as the source of this highly transmissible infection, contributing to the rapid expansion of the infection in the community.

Furthermore, this asymptomatic phase of CHB is not static and may progress to severity. Hence, these patients must be evaluated with serological and biochemical parameters and monitored regularly, for prediction of their prognosis. Hence, proper diagnosis and follow-up of these patients reduces their morbidity and mortality and curtails the spread of infection as well.

Coinfection of HBV with hepatitis D virus (HDV) is 5% worldwide, with Asia being one of the high prevalence areas.[8] HDV coinfection is said to increase the severity of infection and accelerates its progression to cirrhosis. Hence, it can be evaluated, in CHB carriers as one of the predictors of prognosis.

The diagnosis of CHB infection is based on serological assays for markers of viral replication, such as, HBeAg, Anti-HBe, and molecular assays, for detection of HBV DNA by quantitative polymerase chain reaction, supplemented by liver function tests, particularly liver enzymes, alanine and aspartate amino transferases (ALT, AST), and radiological work up if required.

In this study, asymptomatic CHB carriers are evaluated by recording detailed history, followed by serological assays for HBeAg and anti-HBe, and also for anti-HDV.

Aims and objectives

In this study, asymptomatic CHB carriers are evaluated, to assess the risk factors, and to determine replication markers of HBV and co-infection with Hepatitis D virus.

MATERIALS AND METHODS

It is a cross-sectional study, conducted in Government Medical College and Hosptial, Omandurar Government Estate, Chennai-02, for a period of 6 months (March 2022–August 2022).

Study is conducted after obtaining Institutional Ethics Committee approval, and study population is selected according to the following criteria:

Inclusion criteria

Patients of all age groups and both sexes, who tested positive for HBsAg in their serum, on two separate occasions, at an interval of 6 months were included the study.

Exclusion criteria

Patients diagnosed as CHB who is on treatment with antiviral drugs; patients with history of chronic liver disease or decompensated liver disease; alcoholic patients and those on hepatotoxic drugs; patients with diabetes mellitus, congestive heart disease, and obesity; and patients testing positive with anti-HCV were excluded from the study.

Informed consent was taken from all the patients, after explaining about the study and detailed history of the patients recorded using a preformed questionnaire.

Under aseptic precautions, 5 mL of blood collected form median cubital vein by venepuncture, and blood samples were then transferred to microbiology diagnostic laboratory immediately and were then processed for HBsAg, HBeAg, anti-HBe, and anti-HDV detection by enzyme-linked immunosorbent assay.

Statistical analysis

Statistical analysis was done using SPSS software version 25 (the Statistical Package for the Social Sciences). Association between categorical variables was done by Pearson's Chi-square test.

RESULTS

The study population includes 123 chronic hepatitis B carriers showed following demographic and serological profile distribution.

Age and sex distribution

The age-wise distribution of the patients in the study group shows that majority of the patients belong to age group of 21–50 years (70%), with majority of 30 members each in 21-30 years and 41-50 years categories (Table 1). The age of the patients ranges from 6 to 76 years, with a mean of 39.7. Among 123 patients under study group, 75 (61%)

were male and 48 (39%) were female (Table 1). Among females, five of them were antenatal mothers.

Risk factors distribution

The risk factors for transmission of hepatitis B infection analyzed in the study group are – transfusion of blood and blood products, surgery, and other invasive diagnostic and therapeutic procedures. Frequent therapeutic injections and lifestyle behaviors such as tattooing and sexual promiscuity are also other contributing factors. The distribution of risk factors in the study population shows that frequent therapeutic injections are the most common, contributing to 36.6%. History of HBV positivity in family members is present in 41 (33.3%) patients, mostly mothers, then their spouses, followed by their siblings (Table 1).

Serological profile of study population

The serological assays for detection of HBsAg, HBeAg, anti-HBe, and anti-HDV (total) were done. The assay for HBsAg was done to confirm the seropositivity of HBsAg. The serological markers of HBV replication HBeAg and Anti-HBe assays revealed that 19 (15.4%) patients were positive for HBeAg alone, and 103 (83.3%) patients in the study group were positive for anti-HBe alone. Both HBeAg and anti-HBe were found to be positive in one patient (0.8%). The serology of anti-HDV was done to assess the coinfection of hepatitis D virus in the study groups which turned out to be nil (Table 1).

Table 1: Demographic and serological profile of study population

Age-wise distribution of study population		
Age groups (in years)	**Numbers**	**Percentage**
<10	1	0.8
11–20	7	5.7
21–30	**30**	**24.3**
31–40	**28**	**22.7**
41–50	**30**	**24.4**
51–60	18	14.6
>60	9	7.3
Total	123	100
Gender-wise distribution of study population		
Gender		
Male	75	61
Female	48	39
Total	123	100
Risk factors distribution in study population		
Risk factors		
Blood transfusion	8	6.5
Surgery	17	13.8
Frequent therapeutic injections	45	36.6
Tattooing	13	10.6
Sexual promiscuity	10	8.1
Positive family history	41	**33.3**
Unknown	34	27.6
Serological profile of study population		
Serology		
HBeAg alone	19	15.4
Anti-HBe alone	**102**	**83.7**
Both HBeAg and anti-HBe	1	0.8

HBeAg: Hepatitis B "e" antigen

Baseline characteristics according to the HBeAg positivity

The study population is divided into two according to the age group as <40 years and more than 40 years and compared with HBeAg-positive and negative patients. It showed that 75% (15 out of 20) of patients showing HBeAg positivity belonged to age group less than 40 years, which is statistically significant (P=0.04) (Table 2).

The comparison of gender and risk factors distribution with HBeAg positivity shows that there is no significant association between them (Table 3). The history of HBV positivity in family members when compared with HBeAg positivity showed that 55% of the HBeAg-positive patients were found to be having family history of HBV infection. It is a significant association (P=0.025) (Table 4).

DISCUSSION

In our study, 123 chronic hepatitis B carriers are included as study population, who were asymptomatic and diagnosed incidentally during routine antenatal, and pre-operative screening and screening of family contacts of HBV-infected persons and also during blood donation.

Table 2: Association of age group with HBeAg positivity

Age group (in years)	**HBeAg**		**Total**
	Positive	**Negative**	
Numbers			
<40	15	52	67
>40	5	51	56
Total	20	103	123
Pearson Chi-square	**Value**	**Df**	**P value**
	4.06	1	**0.043**

HBeAg: Hepatitis B "e" antigen

Table 3: Baseline characters of study population according to HBeAg positivity

Characteristics	**HBeAg positive**		**HBeAg negative**		**P value**
	Nos.	**%**	**Nos.**	**%**	
Gender					>0.05
Male	12	16	63	16.7	
Female	8	84	40	83.3	
Risk factors					
Blood transfusion	1	11.1	8	88.9	>0.05
Surgery	3	17.6	14	82.4	>0.05
Frequent therapeutic injections	7	15.6	38	84.4	>0.05
Tattooing	2	15.4	11	84.6	>0.05
Sexual promiscuity	3	30	7	70	>0.05

HBeAg: Hepatitis B "e" antigen

Table 4: Association of positive family history with HBeAg positivity

HBeAg	Family history		Total
	No	Yes	
Positive			
Numbers	9	11	20
% within HBeAg group	45%	**55%**	100%
Negative			
Numbers	73	30	103
% within HBeAg group	70.9%	29.1%	100%
Total	82	41	123
Numbers			
% of Total	66.7%	33.3%	100%

HBeAg: Hepatitis B "e" antigen

	Value	Df	P value
Pearson Chi-square	5.045	1	**0.025**

The age of the patients under study ranged from 6 years to 76 years, with a mean of 39.7. About 70% of the patients belonged to age group of 21–50 years. There is a significant association between those with HBeAg positivity and age group below 40 years. Among the replicative carriers with the presence of HBeAg, 75% were below 40 years of age, than those who are seroconverted, with the presence of anti-HBe (P=0.04). This shows that replicative carriers with HBeAg positivity tend to be younger than those who are HBe-seroconverted (HBeAg negative). This finding is consistent with the other studies, such as Shanmugam et al.,[9] Dixit et al.,[10] and Chen et al.,[11] Borgaonkar and Shahapur.[12]

The gender distribution shows that the patients were predominantly males (61%), the rest being females (39%). Among females, five were antenatal mothers (4.1% of total study group). Similar preponderance of males is seen in studies such as Hamida et al.,[13] while Borgaonkar and Shahapur,[12] and Feindiri et al.,[14] show a female preponderance. There was no significant association between gender distribution and HBeAg positivity.

The risk factors for assessing possible routes of transmission were analyzed by taking detailed history form the study population. The commonly found risk factors in the study population include, frequent therapeutic injections, transfusion of blood and blood products, surgery and other diagnostic/therapeutic invasive procedures, and lifestyle behaviors such as sexual promiscuity and tattooing. Of the risk factors analyzed, frequent therapeutic injections are the commonest found in our study population, present in 36.6% of patients, most of them hailing from rural areas. This is consistent with the findings of the study by Shanmugam et al.[9], done in Chennai, Tamil Nadu, which showed that exposure to unsterile instruments is found in majority of their study population. This finding is also supported by other studies done in various states of India, by Singh et al.[15], which showed that the use of inadequately sterilized syringes led to major viral hepatitis outbreak in Rajahmundry of Andhra Pradesh, a rural district of Haryana, and three villages in Gujarat.[16]

This emphasizes that in spite of the advent of sterilization techniques and strict use of single-use disposable syringes and needles for therapeutic injections in urban settings, the illicit use and improper sterilization of syringes and needles are still prevalent in rural areas, mostly by quacks. This contributes to increased transmission of hepatitis B in rural areas.

The frequency of other risk factors is as follows: surgery and other invasive procedures found in 13.8% of patients, transfusion of blood and blood products in 6.5%, sexual promiscuity in 8.1%, and tattooing in 10.6% of patients. The study by Shanmugam et al.[9], Chennai, also has reported similar findings, such as sexual promiscuity and tattooing in 8% each, blood transfusion, and surgery in 5% of their study population. The presence of family contacts with HBV infection was seen in 33.3% of study group. There is a significant association between those showing HBeAg positivity and the presence of positive family history. Of the patients showing positive family history, 55% were replicative carriers (HBeAg positive), greater than the seroconverted group, with P=0.025. The family contacts with HBV infection usually are their mothers, followed by their spouse and siblings. This association signifies that most of the patients with positive family history would have acquired their infection perinatally from their mothers: The other modes being horizontal transmission in the early childhood and sexual transmission.[17]

Furthermore, it is to be noted that considerable number of patients in study group are members within a family, which includes, married couples, mother and her child, father, and his two daughters or siblings within a family. These findings are supported by the study on "Intrafamilial transmission of HBV infection" by Hatami et al.[18], Iran, which states that occurrence of HBsAg positivity was 4 times more common among family members than the general population. Among the family contacts, the HBV positivity was more prevalent in mothers of index cases than their spouses. Thus, it supports the view that perinatal transmission is more efficient in contributing to the spread of infection than sexual transmission in developing countries. The other risk factors such as occupations in healthcare, public safety, hemodialysis, travel to highly endemic areas, and intravenous drug abuse were not found in our study population.

Regarding serological profile, the study population is constituted by chronic hepatitis B carriers, who have shown HBsAg positivity for more than 6 months. Hence, the

serological markers of viral replication, such as HBeAg, Anti-HBe, and serological marker of HDV co/super-infection (i.e.) anti-HDV (total) were assayed, to determine the status of replication, and the stage of CHB infection/coinfection with HDV. The other serological markers such as anti-HBs and anti-HBc antibodies were not assayed since they are not routinely recommended for diagnosis and monitoring of CHB carriers. Among 123 patients in study population, majority (83.3%) were found to be HBe-seroconverted, showing positive Anti-HBe. Only 20 patients (16.2%) were replicative carriers, showing HBeAg positivity, 19 being positive for HBeAg alone, whereas 1 (0.8%) patient showed positivity for both HBeAg and anti-HBe. This finding is consistent with the study by Shanmugam et al.[9], which showed replicative carriers to be 23.4% and the remaining majority belonging to HBe-seroconverted group, and similar findings are seen in Borgaonkar and Shahapur,[12] which shows replicative carriers to be 23.3%, rest 76.6% being HBe seroconverted group.

The unusual serology of one patient showing both HBeAg and Anti-HBe positivity may be explained by the fact that during seroconversion of HBeAg to anti-HBe, both the markers may be present in the patient's serum in a minimal amount. This can be detected by more sensitive assays. Similar finding with coexistence of HBeAg and anti-HBe in the serum of chronic hepatitis B carriers was reported in two studies done in Bangladesh, by Rabbi et al.,[19] and Hasan et al.,[20] with a frequency of 4.1% and 2.4%, respectively.

Thus, HBeAg is useful in diagnosis treatment of hepatitis B infection, but it cannot replace HBV DNA especially when we consider HBeAg-negative CHB patients. It can be used as a complementary test.

The serological assay of anti-HDV (total) to detect the presence of HBV coinfection with HDV turned out to be nil, which denotes that there was no co/super-infection of HBV with HDV in our study population. A study by Chakraborthy et al.,[21] done in New Delhi, India in 2005, reports that there is a transition toward declining prevalence of HDV in HBV-related liver diseases in India. No data on prevalence of HDV in South India seem to be available, as of now.

The study population was divided into three serology groups, according to the HBV replication markers as follows:

- Group I – HBeAg positive and anti-HBe negative – 15.4%
- Group II – HBeAg negative and anti-HBe positive – 83.7%
- Group III – Both HBeAg and anti-HBe positive – 0.8%.

The patients belonging to serological group I are categorized as replicative carriers and, hence, should be investigated further with liver enzymes and radiological work up and be managed accordingly.

The group II patients with HBeAg negativity may mostly be inactive carriers. But, they may be evaluated further with molecular studies like HBV DNA viral load, to rule out the possibility of precore mutation, where patients may still be replicative in spite of HBeAg negativity, which is attributed to the same mutation. Here comes the importance of molecular study, along with biochemical and radiological work up, which may guide in the appropriate management of the patients of this category.[22]

The third transient group with both HBeAg and anti-HBeAb positivity may be due to the possibility of patient being in the seroconversion stage.[23]

Limitations of the study

The limitations of this study are that it is a single centric study with a small sample size and lack of molecular level work up due to resource limitations. Clinical parameters ALT and AST are not considered. Although markers that reflect the behavior of virus in the host are studied, the whole range of markers which reflect and quantitate the host response have not been included in the study.

CONCLUSION

Asymptomatic chronic hepatitis B carriers are the majority among the hepatitis B-infected population and they represent the main reservoir, for silent transmission of infection in the community. They are studied less commonly, due to their seemingly benign nature, but complicated natural history of chronic hepatitis B infection makes them prone to risk of disease progression and complications. This study aimed to determine the common risk factors and also to evaluate their serological profile, for assessment of the stage of infection, and as a guide for their management. Furthermore, the infectivity of the patients was determined, so as to prevent the transmission of infection, by screening and vaccination of their family contacts. In this study, we found a male preponderance and also majority are young adults, with most common risk factor being frequent therapeutic injections, and also there was found to be a fourfold increased risk of infection in family contacts. These findings necessitate more stringent screening of young adults with high risk behaviors and family contacts. These the serological profile revealed that greater part of the study population was constituted by chronic inactive healthy carriers, who have a benign course, with possibility of HBsAg clearance in their later life. The serology of

HBeAg positivity was found to be enough for predicting the infectivity of replicative carriers. They have a high risk of transmitting the infection and also disease progression to CHB and its complications, such as cirrhosis and HCC.

The categorization of HBeAg negative chronic hepatitis B patients will be possible with estimation of HBV DNA levels, which is crucial for their effective management. One of the most common clinical dilemmas in chronic hepatitis B is the differentiation of HBeAg-negative chronic hepatitis B patients and inactive simple carriers, since both conditions have identical serological profile. The limitations of this study are that it is a single centric study with a small sample size and lack of molecular level work up due to resource limitations. Clinical parameters ALT and AST are not considered. Although markers that reflect the behavior of virus in the host are studied, the whole range of markers which reflect and quantitate the host response have not been included in the study.

Hence, furthermore, we recommend a multi centric vertical study with large sample size, with further investigations of molecular HBV viral load, biochemical and radiological parameters, for better understanding of the patients' clinical stages and thus guiding their pertinent management.

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Authors Contribution:
SR- Definition of intellectual content, Literature survey, Prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation, and submission of article; **RS-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **PG-** Design of study, statistical Analysis and Interpretation, Review of Manuscript.

Work attributed to:
Department of Microbiology, Government Medical College and Hospital, Omandurar Government Estate, Chennai, Tamil Nadu, India.

Orcid ID:
Swaathy Ramamurthy - https://orcid.org/0009-0004-2922-593X
Purushothaman Ganesan - https://orcid.org/0009-0006-7527-9490

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