

HBeAg negative chronic hepatitis B in South Asia

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ABSTRACT

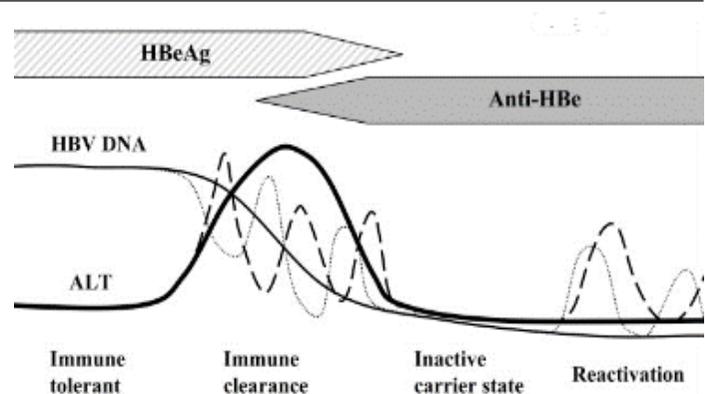
Chronic Hepatitis B (CHB) infection has an intermediate prevalence rate in South Asian countries, except for Nepal and Sri Lanka where it has a low rate. HBeAg negative CHB infection has been found to be more pronounced in this part of the world. As described previously, CHB infection has two phases with HBeAg positive and HBeAg negative based on ALT, DNA & biopsy. The latter was previously described to have a benign course. The later guidelines have distinguished HBeAg negative status into two categories with an inactive phase and a reactivation one. In this review article we have highlighted HBeAg Negative status burden in South Asian countries and shown that HBeAg negative CHB infection may not be as benign as previously thought. Furthermore, mutations at precore and basal core promoter region may contribute to disease severity in HBeAg negative CHB infection.

Keywords: CHB; HBeAg negative; HBeAg positive.

INTRODUCTION

There have been substantial changes in the understanding of CHB infection during last few decades. The previous concept of HBV infection as biphasic disease with HBeAg positive and HBeAg negative phase as active and inactive stages respectively has been radically changed¹. With the replacement of conventional hybridization technique for detection of HBV DNA by more sensitive PCR assay with wider dynamic range, it is now appreciated that HBeAg negative chronic HBV infection is not a homogeneous group and all HBeAg negative CHB infections are not essentially benign or inactive^{2,3}. HBeAg negative phase has now been classified into either an inactive carrier phase characterized by persistently normal ALT level, low HBV DNA level with inactive histology or a phase of reactivation where ALT, DNA levels fluctuate and active necroinflammation reappear at histology⁴ (Figure 1). Further, the concept of occult HBV infection phase has now been able to draw sufficient interest to be incorporated into new classification system as proposed by EASL⁵.

It is being increasingly recognized that HBeAg negative Chronic Hepatitis B may lead to progressive liver damage and culminate into liver cirrhosis, decompensation and hepatocellular carcinoma⁶⁻⁸. Chronic HBV infection is a lifelong disease, and as yet no treatment modalities can eradicate CCC (Closed Covalent Circular) DNA in infected hepatocytes, thus it is likely that active viral replication and progressive liver damage can reappear in



Figures 1. Natural history Chronic Hepatitis B Infection

inactive HBV carriers after seroconversion. Long term follow up studies have made it apparent that the HBV carrier state after seroconversion is not stable and reactivation frequently occurs over due course of time^{6,9}. The HBeAg negative phase is recognized to have a variable course with fluctuating ALT and DNA levels¹⁰. Therefore periodic follow up for reactivation with serial ALT and HBV DNA is required. But at times liver biopsy may be the only way to ascertain if patient really is an inactive carrier, particularly in those with age more than 40 and ALT levels 1-2 times the upper limit of normal as recommended by current guidelines^{11,12}. However, strictly following these recommendations does not make physicians immune to pit falls and as much as one fifth of apparently inactive carrier as defined on the basis of ALT and HBV DNA levels may have significant histological findings¹³.

Epidemiology of Chronic Hepatitis B virus infection in South Asia

South Asian countries share similar epidemiologic profiles, with most of the countries bearing intermediate prevalence¹⁴⁻¹⁶

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for HBV infection except for Nepal¹⁷ and Sri Lanka¹⁸ where it is particularly low. However, regional and ethnic variations in prevalence are noted even within the same country^{14,17}. Low prevalence of HBeAg positivity among pregnant women makes perinatal transmission unlikely to be a major contribution to the carrier pool^{14,15,17}. Further, it is observed that carrier pool is completed at an early age during childhood and adolescence¹⁴. Thus the most important route of transmission of hepatitis B virus appears to be horizontal during early childhood or adolescence¹⁴⁻¹⁷.

Magnitude of HBeAg negative Chronic Hepatitis B infection in South Asia

The predominant genotype seen in this region being D¹⁸⁻²⁰ and the major mode of transmission being horizontal, it can be extrapolated that a substantial proportion of the HBV infected population bears HBeAg negative status. Limited numbers of community- based reports are available from this region in this regard, most of them being from India, Bangladesh and Pakistan (Table 1). HBeAg negative subsets were found in 90%, 73% and 35.6% - 57.3% of chronic HBV infected populations in India, Pakistan and Bangladesh respectively¹⁹⁻²². It appears that the proportion of the HBeAg positive subset is seen to be higher in hospital based studies as compared to community based

studies. This is likely due to referral bias or incidentally detected young job seekers attending the tertiary healthcare facilities where these studies are conducted. A subgroup of these patients would have HBeAg negative chronic hepatitis B warranting therapeutic interventions and the remaining who are supposedly healthy carriers are at risk of reactivation and developing chronic hepatitis B over period of time. The exact percentage of these HBeAg negative Chronic HBV infected individuals having active disease i.e. negative CHB is difficult to estimate given the paucity of data, however, a large community based study carried out in Kolkata found that only 4% of HBeAg negative CHB infected individuals qualify the diagnosis of active disease based on HBV DNA level and ALT²¹.

HBeAg negative phase may harbor significant liver disease

Long term follow up studies have proved that HBeAg negative inactive carrier can progress to HBeAg negative CHB over time; the figure may be as high as 24 % at 4 years⁹. The cumulated probability of an ALT flare among HBeAg negative individuals who initially had normal ALT was found to be 10.8% and 47.3% at 5 and 10 years respectively²³. These observations underline the fact that the HBeAg negative phase is not a stable disease and inactive carriers may develop HBeAg negative CHB over time, thus regular careful follow up is mandatory.

Table 1. HBeAg negative status among chronic HBV infection in South Asia

Country	Frequency	Reference no.
India	90%	(22)
	56.5%	(13)
Pakistan	73%	(19)
Bangladesh	57.3%	(20)
	35.6%	(21)
Nepal	85.6%	Unpublished Data

Table 2. Reported prevalence of high HBV DNA in HBeAg negative chronic HBV infection in South Asia

Country	Frequency	Remarks	Reference no.
India	15.5%	+ve by Hybrid assay	(22)
	59.68%	HBV DNA > 10 ⁴ cps/ml	(13)
Bangladesh	74.6%	HBV DNA > 10 ⁴ cps/ml	(25)

The outcome of HBeAg negative chronic hepatitis B infected patients has been well documented. In an Italian study, as much as 54% of these patients progressed to cirrhosis during mean follow up period of 4-5 years⁸. However, in the above study they did consider HDV co-infected individuals among whom the probability was nearly 80%. Similarly, another long term follow up report demonstrated that among HBeAg negative patients with undetectable DNA by hybrid assay, 26.9% developed liver cirrhosis²⁴. Older age and bridging necrosis at baseline together with persistently detectable HBV DNA by hybrid assay were recognized as risk factors for progression to cirrhosis. There are only a few reported studies from South Asia where histology has

been assessed among HBeAg negative patients and none have follow up biopsies. A few studies have noted high HBV DNA levels (> 10⁴ copies/ml) among HBeAg negative patients (Table 2). A large hospital based follow up study from India found that 85% of HBeAg negative subjects had HBV DNA > 10⁴ copies and 59.68% had fibrosis ≥ 2¹³. In a Bangladeshi study, 74.6% of HBeAg negative patients had HBV DNA > 10⁴ copies/ml²⁵, Mahtab et al. reported histological activity index (HAI score)>8 in 20.8% and fibrosis of ≥ 2 in 28.3% in HBeAg negative patients from Bangladesh²⁶. These figures indicate that active disease with significant liver disease is found in a substantial

proportion of HBeAg negative CHB infection. Further, HBeAg negative individuals have more severe liver disease as compared to HBeAg positive ones^{21,26}. But contrary to these hospital based studies, Choudhary et al. reported that only 15.5% of HBeAg negative subsets had HBV DNA detectable by the hybridization method in a large community based study from Kolkata²¹.

Apparently HBeAg negative carrier state: A wolf inside a sheep’s hide

Now since it is beyond doubt that HBeAg negative chronic HBV infection may have both active and inactive phases, the major concern that arises is the differentiation between inactive carrier and active disease. For this purpose, there is general consensus among different guidelines that serial ALT measurements and HBV DNA levels be done and classification into inactive carrier or HBeAg negative CHB be done accordingly^{11,12}. However, despite careful follow up with serial ALT and HBV DNA levels, it may not be possible to reliably separate inactive carrier from active disease. A substantial proportion of apparently inactive carriers were found to have significant histological changes at liver biopsy in different studies (Table 3)^{13,27}. Around one fourth to one fifth of these HBeAg negative patients who have ALT and DNA levels that define inactive carrier have histological findings and some of them warrant antiviral therapy.

Alanine transaminase has long been regarded as a marker of ongoing liver injury and most of the prominent guidelines recommend use of serial ALT levels for evaluation of chronic hepatitis B infection. Despite these widespread recommendations there are certain gray zones that need to be acknowledged especially in HBeAg negative CHB infection. Several studies have shown that normal ALT may not always indicate inactive disease state and some authors even recommend lowering the conventional cut off value for ALT from 40 IU/ml to 30 and 19 in males and females respectively²⁸. Alam et al. proposed that the ALT levels, particularly normal ones and those <2 times of upper limit of normal have poor predictive accuracy for predicting histology and cut off value of 2 times upper limit of normal (ULN) for starting antiviral therapy be lowered to 1.5 times ULN²⁹.

In an Indian study, Kumar et al. reported 21.8% of HBeAg negative patients had normal ALT at presentation and 67% of them had persistently normal ALT during follow up of 1 year. Surprisingly, 13.8% of these apparently healthy inactive carriers had fibrosis ≥ 2. Among patients with PNALT with HBV DNA <10⁴ copies/ml, twenty two percent had active liver

disease at histology. Even updated ALT cut off values didn’t have a good predictive accuracy in this regard. Among those who had intermittently elevated ALT levels, fibrosis ≥ 2 was seen in 63.9%¹³. A similar study from Bangladesh found 9.5% and 19% of patients with PNALT and HBV DNA <10⁵ copies/ml had moderate necroinflammation and fibrosis ≥ 2 respectively, warranting antiviral therapy²⁷. Another study conducted in Bangladesh among 141 inactive healthy carriers with normal ALT and HBV DNA < 10⁴ copies/ml found that 26 % had Histological activity index for necroinflammation (HAI Ni) > 7 and 12 % had fibrosis 3 and above. 7% of these apparently healthy inactive carriers had both moderate necroinflammation and significant fibrosis³⁰. In long term follow up of these apparently inactive carrier patients who were HBeAg negative and HBV DNA negative by hybrid assay progression to cirrhosis was noted at follow up biopsies; figure was as high as 16.6%- 33%^{8,24}.

These findings underline the fact that a handsome number of individuals have HBeAg negative CHB and these could have been misclassified if standard guidelines were followed. However it is also important to remember that only bridging necrosis is a well documented risk factor for progression to cirrhosis²⁴ and no long term follow up studies have actually showed that moderate necroinflammation and fibrosis ≥ 2 are associated with adverse long term outcome in chronic HBV infection. These recently assigned histological cut off values of HAI greater than 9 together with fibrosis 2 and above for initiating antiviral are solely arbitrary.

Thus evaluation of HBeAg negative CHB infection appears to be incomplete without histological assessment which is obviously invasive and associated with denial by both patients and healthcare providers. This has led to development of noninvasive markers for predicting histological findings (mainly fibrosis). A number of tools have been developed for this purpose but most of them have been suboptimal in performance except for liver stiffness measurement techniques like Transient Elastography, Acoustic Radiaton Force Impulse and Shear Wave Elastography.

Mutations affecting expression of HBeAg antigen in South Asia

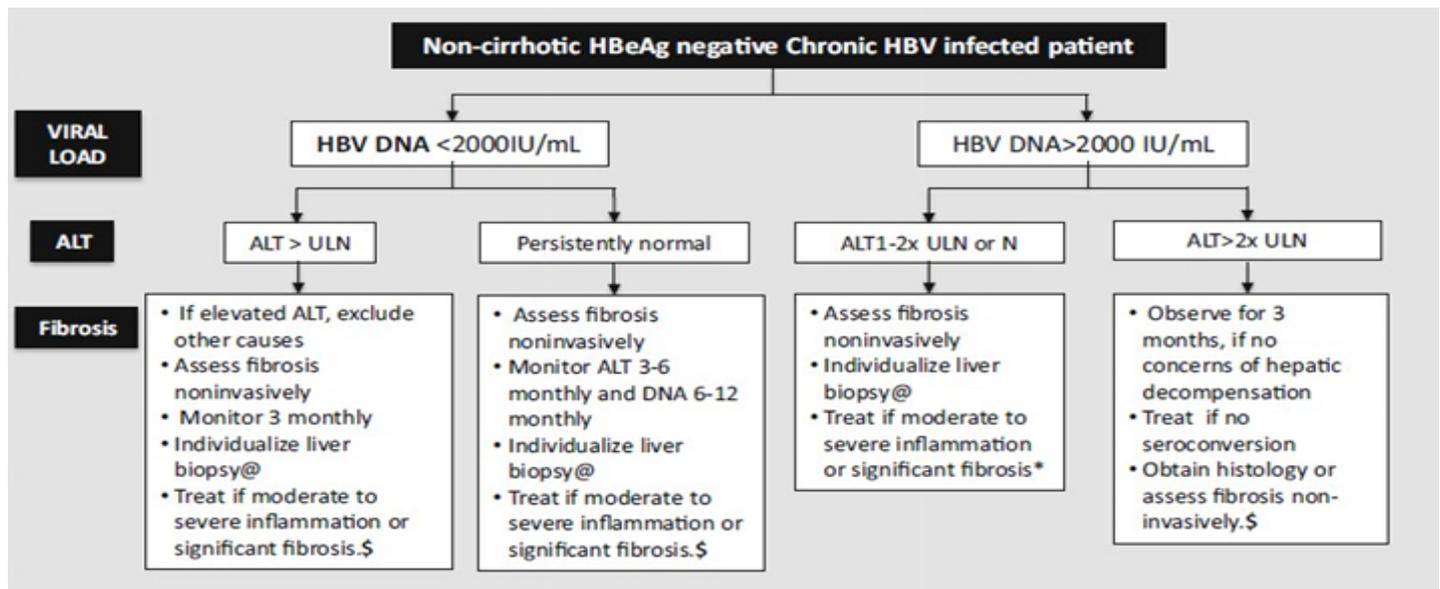
HBeAg negative chronic hepatitis B develops out of immune pressure, and underlying mutations at precore locus 1896 and Basal core promoter (BCP) region 1762 and 1764 need special mention. Mutations at these regions either stop production of HBeAg antigen or produce them at lower levels despite

Table 3. Histologic findings in apparently inactive HBV carrier

Country	HAI > 8	Fibrosis ≥ 2	Reference no.
Bangladesh	9.5%	19%	(27)
	26%	12%	(30)
India	22% (HAI > 3)	13.8%	(13)

Table 4. Frequency of precore and basal core promoter mutations in HBeAg negative chronic HBV infection in south Asia

Country Reference No.	Precore	BCP	Study Population
India (31)	40%	48.3%	Inactive carrier
(32)	33%	36.3%	Chronic hepatitis B and Decompensated CLD
(21)	8.6%	15.52%	eAg negative CHB infection (Population based study)
Pakistan (35)	31%	52%	eAg negative CHB infection HBV DNA +ve by PCR
(36)	23.4%	62%	eAg negative CHB infection HBV DNA +ve by PCR



@ Biopsy if non-invasive tests suggest evidence of significant fibrosis, ALT persistently elevated, Age >35 yr. or family h/o HCC or cirrhosis.

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- Moderate to severe inflammation on liver biopsy means either Hepatic activity index by Ishak activity score >3/18 or METAVIR activity score A2 or A3
- Significant fibrosis on liver biopsy means F≥2 by METAVIR fibrosis score or Ishak fibrosis stage ≥ 3
- Liver stiffness ≥ 8 kPa (by Fibroscan) or APRI ≥1.5 indicates significant fibrosis; Liver stiffness ≥ 11 kPa (by Fibroscan) or APRI ≥2.0 indicates cirrhosis

Figure 2. Evaluation and treatment of HBeAg negative CHB infection

continued replication of virus, rendering it inefficient as a marker of replication and infectivity. Again, there are only a few studies from this region which estimate these mutations. The results are conflicting. Prevalence studies on mutation at precore and basal core promoter region mainly come from India and Pakistan (Table 4). About 48.3% of HBeAg negative inactive carriers were found to have the BCP mutation and 40% had mutation at precore region in northern India³¹. A community based study from Kolkata showed that the prevalence of these mutations in the studied population was relatively low at 30% and HBeAg negative CHB was found in only 4% of HBeAg negative subsets²¹. In a study done in patients with chronic Hepatitis B and decompensated chronic liver disease who were HBeAg negative, it was seen that 36% had double mutation at BCP region and 33% at precore region³². No relation between the presence of these mutations and the severity of liver disease was noted. Similarly, a study from Chandigarh reported that the BCP mutation was found to be significantly higher in inactive carriers and compensated cirrhosis compared to chronic hepatitis and decompensated

cirrhosis, again showing no relation between the severity of liver disease and the presence of these mutations³¹. However a study from Delhi found mutation in the BCP region at A1762T/G1764A but not precore region (G1896A) more common among patients with Hepatocellular carcinoma as compared to those with chronic liver disease without HCC³³. Overall, 92% of HBeAg negative chronic hepatitis B and decompensated chronic liver disease had some mutation at BCP and or precore region in the same study. Similarly Guptan et al. observed that patients with HBV related chronic liver disease harboring precore mutant virus had signs of decompensation compared to those with wild type virus³⁴.

In Pakistan, variable prevalence of precore and BCP mutations was seen among HBeAg negative individuals. Presence of precore G1896A or BCP A1762T/G1764A mutations or their combinations were noted in 62% - 81.6% of HBeAg negative subsets in different studies^{35,36}. Again no relationship between disease severity and presence of these mutations was observed³⁵. Overall, hospital based studies show relatively high prevalence of BCP and precore mutations in HBeAg negative population

and observations have been made that these mutations are more common in HBeAg negative subsets and those with Genotype D as compared to Genotype A. Chauhan et al. reported that HBeAg negative CHB infection had high HBV DNA among those with BCP mutations and TA 1-3 mutations but not in those bearing precore mutation³². However, a long term follow up studies in HBeAg negative inactive carrier revealed that the probability of ALT flare was related to presence of mutations at precore region²³. Contribution of these mutations in development of HBeAg negative CHB could not be exactly ascertained as substantial proportions of both inactive carrier as well as HBeAg negative CHB harbor them (Figure 2)^{31,35}. It can at least be inferred at the moment that even though mutations at precore and BCP regions are present in good proportion of inactive carriers, the likelihood of ALT flare and development of HBeAg negative CHB may be higher among those who harbor them.

CONCLUSIONS

Substantial proportions of Chronic HBV infected patients have HBeAg negative status in South Asia. HBeAg negative chronic hepatitis B is a well recognized phase, occurring in natural history of hepatitis B virus infection. It does not have a benign course as it seems. Differentiating inactive carrier state from HBeAg negative chronic hepatitis B is currently based on serial ALT and HBV DNA monitoring, however it seems inadequate and a good proportion of patients could be misclassified as inactive carrier despite having significant histological changes. Whether these histological changes (Moderate necroinflammation and fibrosis 2 and above) that are considered significant do really portray adverse long term prognosis is not yet known except for bridging necrosis. Further, fibrosis represents injury that has occurred in the past, and in HBeAg negative phase it may simply be residue of injury or activity that occurred during the immune active phase just prior to seroconversion. Therefore, in absence of ongoing necroinflammation, fibrosis alone may not be a good marker for initiation of antiviral therapy.

A good number of HBeAg negative patients who have persistently normal ALT and HBV DNA levels less than 10⁴ copies/ml are found to have significant necroinflammation and fibrosis. Even in the west, long term follow up studies have shown progression to cirrhosis among those with HBV DNA levels undetectable by hybridization methods. Whether these changes are solely accounted for by HBV infection or whether something else behind the scenes is responsible is yet to be seen. An open mind should be kept. This could be an area of further research.

Regarding mutations in precore and basal core promoter regions, it is seen more often in HBeAg negative group compared to HBeAg positive group. However definite association of these mutations with severity of liver disease is lacking based on the data available from South Asia. Epidemiology and course of disease in South Asia differs from that in east and from Pacific and Mediterranean region. We have more to explore to understand

this deadly menace.

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