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Brief communication (Original)

Frequency of hepatitis B envelope antigen-negative chronic hepatitis B virus infection in untreated patients from three cities in Pakistan

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Background: Hepatitis B envelope antigen (HBeAg)-negative chronic hepatitis B virus (CHB) infection is a clinical entity refractory to treatment and has implications for disease outcome.

Objectives: To determine the frequency of HBeAg-negative CHB in untreated hepatitis B surface antigen (HBsAg)-positive patients.

Methods: We conducted this cross-sectional study of untreated HBsAg-positive patients from 3 cities in Pakistan for more than 6 months.

Results: Of 495 patients, 276 (47.7%) had detectable hepatitis B virus (HBV) DNA (mean $5.3 \pm 1.96 \log_{10}$ copies/mL), 81 (16.4%) were HBeAg positive and 414 (83.6%) were HBeAg negative. All 81 (100%) HBeAg-positive patients had detectable HBV DNA. Frequency of HBeAg-negative CHB infection was 155 (31.3%) among HBsAg-positive patients. One hundred and sixteen (74.8%) of the HBeAg-negative patients with CHB infection were in the age range of 15–35 years.

Conclusions: HBeAg-negative patients constitute a considerable proportion of patients with CHB infection. HBsAg-positive patients, especially with younger age, should be thoroughly investigated for this entity to avoid the devastating long-term complications of this disease.

Keywords: Chronic hepatitis B, hepatitis, hepatitis B surface antigen, Pakistan

Globally, hepatitis B virus (HBV) infection is a major health problem with variable geographical distribution. The rates of chronic hepatitis B virus (CHB) infection vary from 0.1%–20% in different areas of the world; and are 0.1%–2% in areas of low prevalence. In areas of intermediate prevalence, e.g., Mediterranean countries, Eastern Europe, the Indian subcontinent, and Singapore the prevalence is 3%–5%. Pakistan falls into the intermediate zone [1-3]. The reported prevalence of HBV surface antigen (HBsAg) in the general population of Pakistan is 2.5% [4].

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In the Asia-Pacific region, CHB is mostly acquired perinatally or during childhood [5]. The virus has infected 2 billion individuals worldwide, of whom over 3 million have become chronic carriers [6]. HBV infection can lead to cirrhosis, hepatic decompensation, and hepatocellular carcinoma. It has achieved the tenth position among major deadly blood borne pathogens [7].

HBV infection is a major health issue in Pakistan. The routes of transmission of HBV are use of infected blood and blood products, blood contaminated surgical instruments, utensils, razors, combs, syringes, piercing needles, and tooth brushes. Sexual and perinatal transmission has also been documented [4]. HBV is a hepatotropic virus which belongs to the *Hepadnaviridae* family. The hepatitis B virion or Dane particle, is 42 nm with an outer envelope (of

HBsAg) surrounding the nucleocapsid, which contains the small genome.

The HBV genome has a high replicative property and can undergo mutations. Naturally occurring mutations occur in the precore and core promoter regions. The most common precore mutation is a point mutation at nucleotide 1896 of the HBV genome (G1896A). The core promoter variant encompasses a dual mutation at A1762T and G1764A [8]. The mutations downregulate production of hepatitis B envelope antigen (HBeAg) [9].

Precore mutations result in severe liver disease, fulminant hepatitis, and are also found in inactive carrier state patients. Core mutations have been associated with increased risk of hepatocellular carcinoma.

HBeAg is a marker of viral replication. Infectivity appears soon after the appearance of HBsAg. As the patient recovers, seroconversion to anti-HBe occurs. Seroconversion is mostly associated with a decrease in serum HBV DNA levels. However, sometimes following acute exposure with the absence of HBeAg and appearance of anti-HBe, the patient also has high levels of HBV DNA and continues to have active liver disease. These are the patients in whom HBV has undergone mutations that prevent decreased production of HBeAg. HBV DNA is a marker of viremia and infectivity, and it may be detected 2–3 weeks before the appearance of HBsAg, and may remain detectable even after HBsAg seroconversion to anti-HBs. Some HBsAg-positive patients, who are HBeAg negative and anti-HBe positive with detectable HBV DNA in their serum, are designated as harboring precore mutants because of mutation at the basal core promoter and precore regions. They are designated HBeAg-negative CHB patients. This group of patients are commonly encountered in clinical practice and require aggressive treatment. They are difficult to treat and their infection may lead to chronicity and complications. To our knowledge, no study to determine the frequency of HBeAg-negative patients with CHB among HBsAg-positive patients has been conducted in Pakistan, so we sought to determine the prevalence of this type of patient.

Material and methods

This was a 6-month cross-sectional study conducted in 3 regional centers of the Pakistan Health Research Council, Islamabad. Patients with HBsAg positivity for more than 6 months and without interferon or nucleoside/nucleotide therapy were included in this study. Ethical approval was obtained from the Institutional Review Ethics Board (IREB) of the Postgraduate Medical Institution (PGMI) Hayatabad Medical Complex, Peshawar, before commencing the study. After obtaining written informed consent from the patients, demographic information from the included patients, family history of hepatitis B, and risk factors for disease transmission were collected. Informed consent for children was obtained from their parents or an appropriate proxy. For illiterate patients, verbal informed consent was documented after explaining the details of the study to the patient witnessed by another literate person to avoid undue influence from a “white coat” effect.

We recruited 495 patients and random serum sampling for HBeAg and anti-HBe were performed using an enzyme-linked immunosorbent assay (ELISA). Serum alanine transaminase (ALT) levels were determined using a Microlab 300 analyzer (ELITech Group, Puteaux, France) and HBV DNA of all samples were detected using a real-time PCR assay on a Cepheid SmartCycler (Sunnyvale, CA, USA). HBeAg negative, anti-HBe positive patients with detectable HBV DNA were designated as HBeAg-negative CHB. HBV DNA levels were categorized as detectable and undetectable, and correlated with the presence or absence of HBeAg.

SPSS for Windows (version 16.0; SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Qualitative data are presented in the form of frequencies and percentages, while quantitative data are presented as mean ± SD. A chi square test was applied to determine associations for categorical data (counts). Statistical significance was calculated for both qualitative and quantitative data using OpenEpi (Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2013/04/06, accessed 2016/07/09).

Results

We included a total of 495 patients with diverse ethnicities who were HBsAg positive for more than 6 months in the present study. Of the total, 164 patients were recruited from Peshawar, 165 from Multan, and 166 from Karachi. Mean age, ethnicity, sex, and family history of HBV of the patients is presented in **Table 1**.

Table 1. Epidemiological characteristics of patients (n = 495)

Variables	Peshawar n = 164	Multan n = 165	Karachi n = 166	Total n = 495
Age (years) (mean ± SD)	27.6 ± 11.0	31.7 ± 10.7	31.0 ± 10.3	30.1 ± 11.0*
Ethnic distribution of patients				
Pathan	142 (86.6)	7 (4.2)	19 (11.4)	168 (33.9)
Saraiki	–	113 (68.5)	17 (10.2)	130 (26.3)
Urdu speaking	–	24 (14.5)	40 (24.1)	64 (12.9)
Punjabi	–	19 (11.5)	21 (12.6)	40 (8.1)
Sindhi	–	–	38 (22.9)	38 (7.7)
Balochi	–	2 (1.2)	17 (10.2)	19 (3.8)
Other ethnic groups†	22 (13.4)	–	14 (8.4)	36 (7.3)
Sex				
Male	112 (68.3)	119 (72.1)	109 (65.7)	340 (68.7)
Female	52 (31.7)	46 (27.9)	57 (34.3)	155 (31.3)
Marital status				
Married	91 (55.5)	125 (75.8)	127 (76.5)	343 (69.3)
Single	73 (44.5)	40 (24.2)	39 (23.5)	152 (30.7)
Family history of HBV infection				
Yes	72 (56.7)	76 (46.1)	65 (39.2)	213 (43.0)
No	92 (43.3)	89 (53.9)	101(60.8)	282 (57.0)

*****$P > 0.05$, †Afghani Pathans, Bengalis, Hindkowans, Persians, Chitralis, and Baltis.

HBeAg serology, HBV DNA detectability, HBeAg-negative CHB numbers, and residency are presented in **Table 2**.

Age-wise distribution of 155 HBeAg-negative patients with CHB (**Figure 1**) shows that out of 155, 116 (75%) were in the age range of 15–35 years, whereas 25% of were older than 35 years.

Ethnic distribution (**Figure 2**) shows that HBeAg-negative CHB infection was prevalent among Pathans (43 patients), followed by Saraikis (41 patients), and Urdu speaking patients (26 cases).

HBeAg negative patients with or without detectable DNA were significantly older than HBeAg-positive patients. Serum ALT levels of HBeAg positive patients were significantly higher than ALT levels of HBeAg-negative patients, regardless of their HBV DNA status. Similarly, HBV DNA loads (log_{10} copies/mL) of HBeAg-positive patients tended to be higher than patients with HBeAg-negative CHB, but the difference was not significant (**Table 3**).

Table 2. Biochemical, serological, and virological characteristics of patients (n = 495)

Variable	Peshawar	Multan	Karachi	Total	*P*
n	164	165	166	495	
Alanine transaminase (IU/L)					
Mean ± SD	38.0 ± 26.1	37.0 ± 60.4	43.67 ± 36.1	39.6 ± 43.3	>0.05
HBV DNA detected by PCR	80 (48.8%)	65 (39.4%)	91 (54.8%)	236 (47.7%)	–
HBV DNA (log_{10} copies/ml)					
Mean ± SD	5.5 ± 2.2	5.1 ± 2.1	5.2 ± 1.7	5.3 ± 1.7	>0.05
HBeAg positive	37 (22.6%)	19 (11.5%)	25 (15.1%)	81(16.4%)	–
HBeAg negative	127 (77.4%)	146 (88.5%)	141 (84.9%)	414 (83.6%)	–
HBeAg negative without detectable DNA	84 (51.2%)	100 (60.2%)	75 (45.2%)	259 (52.3%)	–
HBeAg negative with detectable DNA	43 (27.5%)	46 (29.7%)	66 (42.6%)	155 (31.3%)	–

HBV, hepatitis B virus; HBeAg, hepatitis B envelope antigen

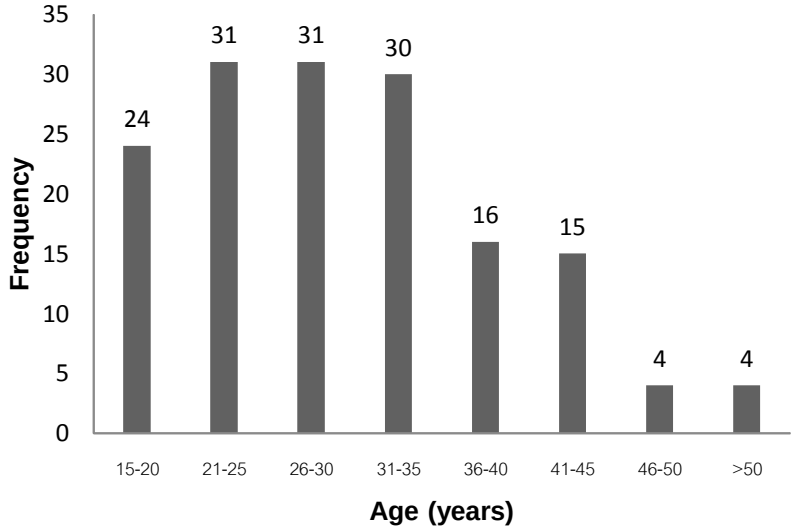

Figure 1. Age distribution of hepatitis B envelope antigen-negative patients with chronic hepatitis B virus

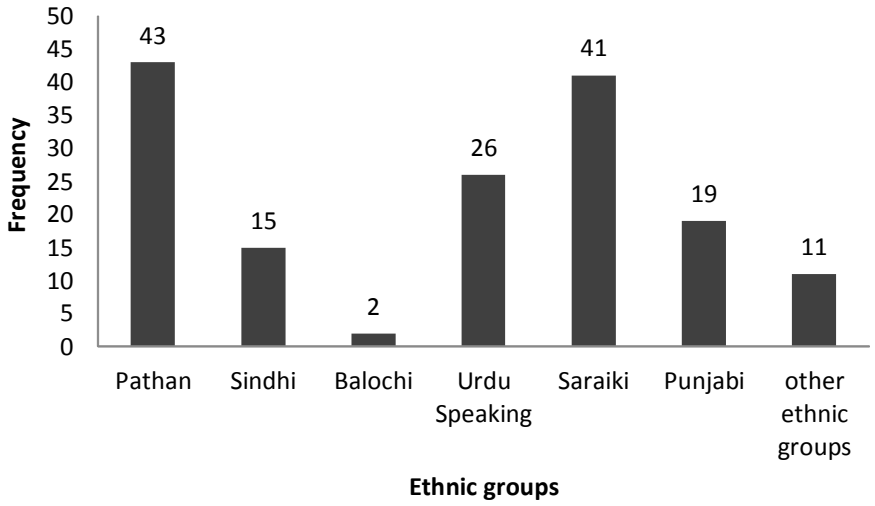

Figure 2. Ethnic distribution of hepatitis B envelope antigen-negative patients with chronic hepatitis B virus. Other ethnic groups include Afghani Pathans, Hindkowans, Bengali, and Balti

Table 3. Age, serum alanine transaminase level, and viral load of the patients with different serological conditions (N = 495)

Variable	HBeAg negative without detectable DNA	HBeAg-positive patients	HBeAg negative with detectable DNA	*P*
n	259	81	155	
Age (years)				
Mean ± SD	31.2 ± 11.4	25.3 ± 10.6	30.6 ± 9.9	<0.01
Alanine transaminase (IU/L)				
Mean ± SD	32.9 ± 24.4	61.5 ± 49.6	35.4 ± 17.3	<0.01
Hepatitis B virus DNA (log_{10} copies/mL)				
Mean	–	6.9 ± 1.7	4.4 ± 1.4	>0.05

HBeAg, hepatitis B envelope antigen

Of 81 HBeAg-positive patients, 49 had elevated serum ALT levels, while the levels were normal in 32 patients. Of the 414 HBeAg-negative patients, 105 had elevated ALT levels, while the levels were normal in 309 patients. A chi square test showed a significant relationship and suggests that HBeAg-positive patients were at 4 times higher risk of having increased ALT levels compared with HBeAg negative patients (OR = 4.51 (2.7 < OR < 7.4), $P < 0.01$).

Of 236 patients with detectable HBV DNA, 101 patients had elevated serum ALT, while 135 had normal ALT levels. Of the total 259 patients with undetectable DNA levels, 53 had elevated ALT levels while 206 patients had normal ALT levels. Significant association was observed and patients in whom HBV DNA could be detected were almost 3 times more likely to have elevated ALT levels compared with those in whom HBV DNA was undetectable (OR = 2.90 (1.9 < OR <4.3), $P < 0.01$).

Of 81 HBeAg- positive patients, 76 had elevated HBV DNA levels and only 5 patients had low HBV DNA levels. Of 155 HBeAg-negative patients, 80 had elevated HBV DNA levels, while 75 had low levels of HBV DNA. A chi square test suggested that HBeAg-positive patients had significantly increased HBV DNA compared with HBeAg-negative patients (OR = 14.3 (5.5 < OR <37.2), $P < 0.01$).

Discussion

Our study focused at occurrence of HBeAg-negative CHB infection among HBsAg-positive patients with various ethnicities from 3 cities in Pakistan. Although the presence of HBeAg is a strong predictor of viral replication and infectivity, anti-HBe antigen and HBV DNA can be detected simultaneously in HBeAg-negative patients with chronic HBV. Because of the serious consequences of this disease, it is imperative to obtain epidemiological information to identify patients for appropriate early management.

Although no comprehensive study has been conducted, a hospital based study in Islamabad [10] conducted on patients incidentally diagnosed with asymptomatic HBsAg, found 176/224 (78%) patients were HBeAg negative, and patients with elevated ALT levels were tested for HBV DNA, and 14/76 (18%) patients were found positive for HBV DNA. Another study [11], conducted on 100 Hepatitis B carriers, found 19 who were negative for HBeAg suggesting a frequency of 19% HBeAg-negative CHB. A third study [12], conducted on patients with detectable HBV DNA levels for genotyping and precore mutations, 15% of HBV DNA positives were found negative for HBeAg.

The frequency of HBeAg-negative CHB in our study (31.3%) was higher than that previously reported for Pakistan [10, 12]. The frequency of HBeAg-negative CHB was less than the reported 35.6% [13] and 39.7% [14] from Bangladesh. High prevalence of HBe-negative CHB may be attributed to the inclusion of HBV DNA detected patients in both studies. Studies of HBsAg-positive patients showed 17% occurrence of chronic HBV in Hong Kong [15] and 19.6% in Korea [16].

Variation in the prevalence of HBeAg-negative CHB may be the result of genotypic diversity, mode of transmission, and to some extent, the method used for the detection of HBV DNA. Although PCR is the main method employed to detect DNA, differences in the sensitivity of the methods may also contribute to variation in the prevalence of HBeAg-negative CHB. The high prevalence of HBV precore variants in Asia may be the result of the preponderance of genotypes B, C, and D.

In the present series, all HBeAg-positive patients harbored HBV DNA; which is in agreement with previous studies that found HBV DNA in 83%–100% of HBeAg-positive/anti-HBe negative patients and 26%–64% of HBeAg-negative/anti-HBe-positive patients [10, 17-20]. HBeAg-positive patients were usually younger than HBeAg-negative patients both with or without detectable HBV DNA, which was described by others [13, 14, 16].

HBeAg-negative CHB patients were divided into different age groups. We found that most of the HBeAg-negative patients with CHB were in the range 15–35 years and very few were older than 50 years. This shows that the number of HBeAg-negative people in Pakistan is increasing over time, and younger people are more affected. These findings are similar to those in a study conducted in Bangladesh, where a similar proportion of HBeAg-negative patients had CHB. Such patients are at a higher risk of developing CHB-related liver diseases. This finding may be attributed to the likely perinatal acquisition of infection by a majority of patients in this region [21] as is reported for other Asian countries. Most individuals in Pakistan are diagnosed incidentally during mandatory screening for HBV, HCV, and HIV before blood donation. This might be a reason for the high frequency of younger people in our study sample.

Overall, HBeAg negativity was observed in 83.6% of the patients in accordance with previous studies [15, 22]. Serum ALT and HBV DNA levels in HBeAg-positive patients were significantly higher than in HBeAg-negative patients in the present study. The same findings have been reported in other studies [17, 23]. ALT levels are highly dependent on HBeAg status and viremia, which causes liver inflammation and increased ALT levels. HBeAg positivity and high HBV DNA levels are associated with elevated ALT levels. An association between the presence of viral DNA and elevated ALT levels, and HBeAg positivity has been shown in a couple of studies [17, 20]. Serum ALT and HBV DNA levels of patients with HBeAg-negative CHB were significantly lower than those in HBeAg-positive patients This may be the result of the abrogation of precore protein, which results in downregulation of HBeAg, lowering the infectivity and affecting the viral load and liver inflammation. HBeAg-positive patients that seroconvert to HBeAg-negative status show sustained suppression of viral DNA replication [24].

Male preponderance in our study is not unusual and is consistent with other studies [10, 12, 14, 25]. A positive family history in a considerable number of patients suggests intrafamilial transmission of infection, both vertically or horizontally.

We conclude that HBeAg-negative patients with CHB constitute a substantial number of CHB patients. Mostly, these patients were younger and in their third decade of life. This demonstrates the need for early detection of these HBeAg-negative patients with chronic HBV among HBsAg-positive patients. They are younger and likely to develop severe liver disease as they age; some of which can be prevented by early management. A positive family history of HBV infection indicates intrafamilial spread, which mandates creating public awareness programs.

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Conflict of interest statement

The authors declare that they have no conflicts of interest in this research.

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