Predicting High HBV DNA Levels among Reproductive-Aged Chronic Hepatitis B Women

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ABSTRACT

Mother-to-child transmission (MTCT) is a major route of hepatitis B virus (HBV) transmission. However, little is known about the clinical features of the key population involved with MTCT. This study aimed to characterize the clinical features and identify predictive factors of high serum HBV DNA levels (HBV DNA >200,000 IU/mL) among reproductive-aged chronic hepatitis B (CHB) women. This was a retrospective study performed in a tertiary hospital in Malaysia. Female, reproductive-aged, CHB patients in hospital care between 2009 to 2018 were identified from the medical records. Electronic medical records of the patients were reviewed to capture information on demographics, clinical features, and liver status. A total of 262 eligible CHB women (median age=37 years, IQR=12) were studied. Of this, 62.5%, (n=160) had high HBV DNA levels >200,000 IU/mL. A total of 202 patients had a recorded liver status of which 16 (7.9%) had advanced fibrosis, 38 (18.8%) had liver cirrhosis and 13 (6.4%) had hepatocellular cirrhosis. Predictors of high HBV DNA levels were HBeAg positive and abnormal ALT. HBeAg positive increased the risk of high HBV DNA levels by 9.99 times (adjusted OR=9.99; 95%CI=5.50, 18.13; p<0.001) compared to HBeAg negative patients. Abnormal ALT levels increased the risk of high HBV DNA levels by 1.87 times than those with normal ALT (adjusted OR=1.87; 95%CI=1.02, 3.45; p=0.015). In conclusion, current findings suggest that HBeAg positive and abnormal ALT levels may act as a guide in determining HBV transmission prevention strategies in female reproductive-aged patients.

Key Words: *Chronic hepatitis B; HBV DNA; HBeAg; factors; reproductive-aged women.*

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INTRODUCTION

Hepatitis B virus (HBV) is one of the major causes of viral hepatitis [1-3], cirrhosis, and hepatocellular carcinoma throughout the world [4]. More than 250 million people worldwide are reported to be infected with chronic hepatitis B virus (CHB) including approximately 65 million women who are in their reproductive age [5]. From this, perinatal transmission is estimated to contribute towards approximately half of the global CHB disease burden [6]. Approximately 90% of infants exposed perinatally are reported to become chronically infected [7]. Furthermore, HBV infection during their early ages is a risk factor for CHB and development of liver complications including cirrhosis and hepatocellular carcinoma (HCC) later in life [7]. Mother-to-child transmission (MTCT) [8, 9], acquired vertically via prenatal or intrauterine transmission, natal transmission i.e. transmission during delivery, and horizontally via postpartum transmission, are major routes of HBV transmission in the Asia-Pacific [10]. This emphasizes that

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measures focusing on reproductive-aged women should be taken to eradicate MTCT efficiently.

Passive-active hepatitis B immunization program, HBV vaccine, and hepatitis B immunoglobulin G (HBIG) soon after birth followed by two additional vaccination at six months reduce MTCT rates from 90% to less than 10% [10]. Although generally effective, approximately 10% of infants born to mothers with hepatitis B envelope antigen (HBeAg) positive and high HBV DNA levels, fail this management [11, 12]. Both high HBV DNA levels and positive HBeAg are known as significant risk factors for MTCT [12]. For these women, the use of antiviral therapy, as well as infant immunization, are potentially beneficial in reducing the risk of MTCT [13]. As such, current regional guidelines recommend consideration of maternal antiviral tenofovir [13], to be given during the third trimester of pregnancy among patients with HBV DNA >200,000 IU/mL to further reduce perinatal transmission [5, 13]. Although there have been contradicting results [14], the generalizability of the findings was limited due to immunization adherence challenges. Recently, reports show that only 87% of infants received complete HBV vaccination during their first year, while only 46% received timely birth-dose vaccination [15].

Currently, HBV DNA levels is an essential parameter, among others, used to guide CHB management [16]. However, the accessibility to HBV DNA quantification tests is limited due to costs and the need for special equipment and laboratory, posing as barriers in resource-constrained countries [16, 17]. Therefore, in the past decade, to improve CHB management, efforts were made to explore alternative tests to HBV DNA quantification such as point-of-care screening tests, nucleic acid tests, dried blood spot tests, and HBsAg titers. However, there is yet an alternative cost-effective test that has been established to improve management in resource-constrained areas [16, 17]. At present, clinical features of CHB; alanine aminotransferase (ALT), and HBV DNA levels are often used to describe the extent of disease among CHB patients [11, 12, 17]. However, there is a paucity of data regarding clinical features among perinatal transmission CHB reproductive-aged women.

Hence, the study aimed to describe the demographics, clinical features, and predictors of high HBV DNA among the multi-ethnic Malaysian CHB women of reproductive years. This is due to the need to explore alternatives to the HBV DNA quantification test used to determine eligibility for antiviral prophylaxis.

MATERIALS AND METHODS

Study design

This was a retrospective study performed in a tertiary hospital in Malaysia. Medical records from the 1st of January 2009 to the 31st of December 2018 were screened and patients included through convenient sampling. The inclusion criteria of the study were women between reproductive ages of 15 to 49 years old [18], HBsAg seropositive on first hospital presentation and for at least six months after, with a one year follow up. Those that were excluded were patients with co-infections of other types of viral hepatitis or human immunodeficiency virus, concurrent liver diseases (e.g.: autoimmune hepatitis, Wilson's disease), previous organ transplant, malignancy - except hepatocellular carcinoma (HCC), and patients with incomplete data.

Ethical approval

Ethical approval was obtained from the Medical Research Ethics Committee, Ministry of Health, Malaysia (ID: NMRR-18-3365-45185), and National University of Malaysia (ID: UKM PPI/111/8/JJEP-2019-091). An informed consent waiver was approved in view of a non-intervention, retrospective study.

Data collection

Electronic medical records were used to systematically identify patients using the diagnosis keyword "Hepatitis B" and then filtered by gender. Patients' demographics (age, ethnicity, and family history of CHB) were recorded. Clinical features of patients during their first presentation to the hepatology ward or clinic were also recorded, and divided into two parts: laboratory data and liver status. Laboratory data included serum alanine aminotransferase (ALT) levels (normal value ≤33 U/L, abnormal value >33 U/L) on the first presentation to hospital, HBeAg status, and HBV DNA viral load (high HBV DNA when >200,000 IU/mL) [11, 12]. Several methods were used to determine patients' liver disease status as described in Table 1 [19].

Table 1: Classification of liver status [19]

Liver Status	Description
Advanced Fibrosis	Liver Biopsy: METAVIR scored F3 or Modified HAI F5 or Liver Stiffness: >9kpa (ALT ≤33 U/L); >12kpa (ALT >33 U/L) Without evidence of portal hypertension sign, irregular liver margin from radiological imaging or platelet counts less than 150,000.
Liver Cirrhosis	Liver Biopsy: METAVIR scored F4 or Modified HAI F6 or Liver Stiffness: >9kpa (ALT ≤33 U/L); >12kpa (ALT >33 U/L)

	with evidence of portal hypertension sign, irregular liver margin from radiological imaging or platelet counts less than 150,000.
Hepatocellular carcinoma	Hepatocellular carcinoma confirmed by 4-phase computed tomography (CT) scan.
None of the above	Did not fill up any criteria as above

Data analyses

Statistical analyses were performed using IBM® Statistical Package for Social Sciences version 23.0 (SPSS, Chicago, IL). Numerical variables were presented using mean and standard deviation (SD) for normally distributed data while median and interquartile (IQR) ranges were additionally presented for non-normally distributed data. Associations were determined by Pearson's chi-square test, Fischer's exact Test, Mann Whitney U Test or T-test wherever appropriate. Predictive factors of high HBV DNA was assessed using univariate and multivariate logistic regression. Variables with a $p < 0.25$ in univariate logistic regression were included into the multivariate model [20]. A median split [20], was performed to categorize measured continuous data. For ethnicity, the Chinese population was set as a comparator to the non-Chinese population, as the former was found to be the population with the highest CHB prevalence. All tests were two-sided and a $p<0.05$ was considered as statistically significant.

RESULTS

Demographics and clinical characteristics

A total of 300 individual reproductive-aged women with CHB were identified. Of these, 262 were included in the study (Table 2). A total of 189 (72.1%) patients in their reproductive age were given hepatitis B treatment. Among these, 134 (51.1%) patients were treated on their first presentation to the hospital due to an active disease while 55 (20.9%) patients were treated due to disease flare, after their first visit.

Table 2: Demographic and clinical profile characteristics of the study population (n=262)

Characteristics	Value
Age, year, median (IQR)	37.00 (12.00)
Ethnicity, n (%)	
Chinese	146 (55.7)
Indian	5 (1.9)
Malay	97 (37.0)
Other	14 (5.3)
Family history of CHB, n (%)	
Unknown	87 (33.2)
Known	175 (66.8)
ALT, U/L, n (%)	
Normal (≤ 33 U/L)	98 (37.4)
Abnormal (> 33 U/L)	164 (62.6)
HBeAg*, n (%)	
Positive	158 (60.5)
Negative	103 (39.5)
HBV DNA*, IU/mL, n (%)	
HBV DNA ≤ 200,000	96 (37.5)
HBV DNA > 200,000	160 (62.5)

Abbreviations: IQR=Interquartile range; SD=Standard deviation
*6 missing HBV DNA data (HBV DNA records untraceable), 1 missing HBeAg data (HBeAg showed "borderline" in status)

Liver status

A total of 202 patients had recorded liver status (Table 3). Among the remaining 202 patients with available liver fibrosis staging data, patients with advanced fibrosis and liver cirrhosis were significantly older with a median age of 44.0 years for both complications (advanced fibrosis, IQR=8, p=0.02; liver cirrhosis, IQR=10, p<0.001) compared to those without advanced fibrosis and liver cirrhosis (median age=37.0, IQR=12; median age=37, IQR=12, respectively). A significant association was found between ethnicity and incidences of cirrhosis (p=0.001). A posthoc analysis comparing individual ethnicities found that the Malay ethnicity (median=21, IQR=55.3) was significantly associated with cirrhosis compared to the Chinese (median=13, IQR=34.2, p=0.002). No other significant findings were observed.

Table 3: Liver status of the study population (n=202)

Liver status	Value
Liver stiffness, kpa, mean (SD)	7.51(7.32)
Fibrosis staging, n (%)	
Advanced fibrosis	16 (7.9)
Liver cirrhosis	38 (18.8)
Hepatocellular carcinoma, n (%)	13 (6.4)

Abbreviations: SD=Standard deviation

Predictors of high HBV DNA

A univariate and multivariate logistic regression was performed, in which both HBeAg positive status and abnormal ALT were found to be significant predictors for high HBV DNA levels. HBeAg positive women had a 9.99-fold higher risk of showing high HBV DNA levels compared to those who were HBeAg negative (AOR=9.99; 95% CI=5.50, 18.13; p<0.001). Women with abnormal ALT levels also had a 1.87-fold higher risk for a high HBV

DNA level compared to those with normal ALT levels (AOR=1.87; 95% CI=1.02, 3.45; p=0.015) (Table 4).

Table 4: Predictive factors of high serum HBV DNA levels (n=256)

Univariate logistic	Wald (df)	COR (95% CI)	p-value
Age <37 years old	13.57 (1)	2.75 (1.61, 4.72)	<0.001
Chinese ethnicity	2.22 (1)	0.68 (0.40, 1.13)	0.676
Known family history of CHB	2.58 (1)	1.57 (0.91, 2.73)	0.108
Abnormal ALT	5.93 (1)	1.92 (1.14, 3.24)	0.015
HBeAg positive	58.73 (1)	10.00 (5.56, 18.08)	<0.001
Multivariate logistic	**Wald (df)**	**AOR (95% CI)**	**p-value**
Abnormal ALT	4.06 (1)	1.87 (1.02, 3.45)	0.044
HBeAg positive	57.25 (1)	9.99 (5.50, 18.13)	<0.001

Abbreviations: CI = confidence interval; df = degree of freedom; COR = crude odds ratio; AOR = adjusted odds ratio

DISCUSSION

The incidence of CHB among reproductive-aged women with a known family history has often been reported, reflecting the common route of HBV vertical transmission in our region [6]. Most interesting is the larger number of CHB among Chinese patients, despite the ethnically Malay majority in Malaysia [21]. This has been similarly reported among Malaysians in another work, which was followed by the Malays and Indians [21, 22]. Such findings may depict that transmission remained within the same family and ethnicity, suggesting that it is more likely to have occurred perinatally, further supporting the rationale of focusing on MTCT prevention as an important HBV transmission prevention strategy, whilst reducing complications such as liver diseases.

Liver complications were observed in older reproductive patients and among Malays. Similar trends of liver complications have been observed in other adult CHB patients [17, 23]. With advancing age, rates of seroconversion have been found to increase at a 10% rate [24]. This is concerning as HBeAg positive in the presence of high HBV DNA poses a high MTCT risk [25], and leads to patients developing liver complications requiring antiviral treatment, clearly observed in the current work. This, however, may also be due to the complicated cases encountered, as the study site was a national liver referral center in Malaysia. Interestingly, Malays had a higher occurrence of liver cirrhosis, although this may be due to other indirect contributing factors. Other established host and environmental risk factors for the development of liver cirrhosis include other viral hepatitis, non-alcoholic fatty liver diseases, autoimmune liver disease, and excessive chronic alcohol intake [26-28]. As such, further investigation is required to explain such an association.

The current work demonstrated that both positive HBeAg and abnormal ALT levels were predictors of high HBV DNA levels among women of reproductive ages. Positive HBeAg detected in patients with high HBV DNA can potentially be explained by the natural course of CHB infection observed during the immune tolerance phase of CHB, a specific phase that is found primarily in patients with perinatal acquired HBV infection [29]. The immune tolerance phase is characterized by high levels of viral replication [30]. As the disease progress, the continued presence of HBeAg-positive hepatic cells will eventually stimulate T-cell recognition, which develops over a long period of time [31, 32]. Hence, patients who were infected perinatally can remain in the immune tolerance phase for an extended period, spanning from childhood to early adulthood [30], which is usually absent or short-lived in CHB infection acquired through horizontal infection [33]. Some patients in the current work belong to this immune tolerance phase as observed through their family history of CHB. In addition, a correlation between HBeAg and HBV DNA levels in treatment-native CHB Asian patients has previously been reported [33]. However, HBeAg quantification is not yet standardized nor recommended by guidelines. The current work is one of the first to have indicated a potential correlation between the recommended HBeAg serostatus and high HBV DNA levels in this specific sub-population.

On the other hand, the rise in ALT levels may indicate inflammation of the liver cells, which may be caused by high viral loads. However, ALT abnormalities are also commonly associated with factors such as alcohol, drug, or herb-induced factors [34]. Unfortunately, due to the retrospective nature of the study, accurate records of other potential confounding factors are limited. Thus, future prospective studies are needed to control such confounding factors to further explore and confirm its predictive value.

To that end, it should be noted that there were a few limitations to the study. Firstly, patients were referred to the liver referral center from all over the country, which uses different laboratory machines and assays. Bias can potentially occur in such cases where each individual laboratory may have machines of different sensitivities for detection and quantification of serum HBV DNA, ALT, and HBeAg. As this is a retrospective study, such discrepancies could not be avoided. Therefore, the generalization of the study should be done with caution. However, these findings are useful preliminary data that may guide future research of larger sample sizes and better study designs. Exploration of these potential alternative tests is particularly valuable in guiding antiviral prophylaxis for MTCT prevention. However, considering the natural course of CHB infection and the current focus on reproductive-aged women, these findings may only be

applicable in areas where MTCT is the main route of CHB transmission.

CONCLUSION

In conclusion, the current work indicates the potential use of HBeAg and ALT status as an alternative to HBV DNA quantification in this specific population, both of which are a cheaper and less labor-intensive option. This is especially beneficial in determining patients that require a more drastic approach in treatment in cases where HBV DNA levels may not be available.

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