ORIGINAL ARTICLES

VIROLOGICAL CHARACTERIZATION OF HEPATITIS B VIRUS INFECTION AT A MAJOR GASTROENTEROLOGY CLINIC IN ACCRA, GHANA

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Abstract

Introduction *:* Hepatitis B is prevalent in sub-Saharan Africa. Adverse outcomes of infection include cirrhosis and hepatocellular carcinoma with prognosis worse in endemic areas. Hepatitis B treatment guidelines recommend treatment in patients with active chronic inflammation or liver cirrhosis to reduce risk of disease progression. However, there is as yet no national hepatitis B treatment program in Ghana. This study risk-stratifies new patients by serology and viremia at the tertiary centre in Accra.

Methods : Retrospective study of new patients referred with chronic hepatitis B at the Korle-Bu Teaching Hospital, Accra. Serologic data were obtained from medical records using standard data collection form. Means, medians, linear range (±SD) were presented for continuous variables and frequencies for categorical variables.

Results : Overall, 387 patients with hepatitis B were reviewed. Of the 255 patients with serology, 209 (82.0%) were HBeAg-negative. Serum ALT was elevated, > 40 IU/mL, in 38.5%. HBV DNA > 2,000 IU/mL in 52.7% (n = 167). ALT > 40 IU/mL and HBV DNA > 2,000 IU/mL in 23.3% (n = 150). Patients with ALT > 40 IU/mL were more likely to have HBV DNA > 2,000 IU/ml, P=0.001. In patients with liver ultrasound (n=51), liver cirrhosis and hepatocellular carcinoma were diagnosed in 8 and 9 patients respectively.

Conclusions : Liver cirrhosis and hepatocellular carcinoma were evident at presentation in some patients. Furthermore, 23.3% had ALT > 40 IU/mL and HBV DNA > 2,000 IU/mL, associated with increased risk of liver-related complications. In achieving current hepatitis B guidelines, there is the need for a sustainable national treatment program for eligible patients in Ghana.

Keywords : *Hepatitis B, Ghana, ALT, viremia*

Introduction

It is estimated that there are more than 240 million hepatitis B (HBV) carriers in the world[1]. The prevalence of HBV carriers varies from 0.1-2% in low prevalence areas (United States, Western Europe), to 10-20% in high prevalence areas such as Sub-Saharan Africa[2, 3]. The reported prevalence of HBV infection ranges from 12-15% from blood donors in Ghana[4]. The rate of progression from acute to chronic HBV infection is approximately 90% for peri-natally acquired infection as pertains in Sub-Saharan Africa[4]. The sequelae of chronic HBV infection varies from an inactive carrier state to the development of cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) [5]. The prognosis is worse in HBV-infected patients from endemic areas[5].

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Liver cancer is the leading cause of cancer deaths in males and the third in females among Ghanaians based on a ten-year review of autopsies and hospital mortality in Korle-bu Teaching hospital, the largest tertiary center in Ghana[6]. A case-control study on HBV sero-prevalence among patients with cirrhosis of the liver in Ghana showed that the risk of cirrhosis of the liver was strongly associated with HBV status[4].

Treatment is therefore needed in patients with chronic HBV to reduce risk of transmission to others and long-term complications such as cirrhosis and hepatocellular carcinoma[7]. The European Association for the Study of the Liver (EASL) updated guidelines in 2012 suggest patients with chronic HBV be considered for treatment when they have HBV DNA levels greater than 2000 IU/mL and have serum ALT levels above the upper limit of normal[8]. The American Association for the Study of Liver Diseases (AASLD) and Asia-Pacific Association for the study of the Liver (APASL) recommend that treatment may be initiated once a diagnosis of HBeAg-negative chronic HBV (ALT > 2 x ULN and HBV DNA > 2000 IU/mL) or HBeAg-positive chronic HBV (ALT > 2 x ULN and HBV DNA > 20,000 IU/mL) is established[7, 9].

Although widespread HBV screening occurs in endemic sub-Saharan countries, there is as yet no

countrywide HBV treatment program in Ghana and investigations such as HBV DNA and anti-viral medications are expensive for many patients. It would therefore be important to risk-stratify chronic HBV patients by serology and viremia to guide therapy in a sub-Saharan country like Ghana. This study presents the biochemical, serological and virological characteristics of patients with HBV at the major teaching hospital and treatment center in Accra, Ghana.

Methods

Study design

This study utilized a retrospective design to recruit new patients with chronic hepatitis B infection at the Gastroenterology Unit of the Korle-Bu Teaching Hospital in Accra, Ghana from 2010 to 2014.

Study participants

Patients were eligible for inclusion if they were chronically infected, HBsAg (Hepatitis B-surface-Antigen)-positive on two occasions more than 6 months apart or were HBsAg and HBcIgG positive[10]. Hepatitis B core-IgM positivity indicated acute infection[10]. HBsAg positivity with the Core HBsAg rapid test were confirmed serologically using ELISA. The study was performed in compliance with relevant laws and conducted in accordance with the ethical standards of the Declaration of Helsinki.

Study variables, data collection and statistical analysis

Demographic and clinical data were obtained from medical records of patients using standard data collection form. Demographic data included age, sex, ethnicity[11] and hometown by regional groupings. These comprised of the Northern belt (Northern region, Upper West, Upper East), Middle belt (Ashanti, Brong-Ahafo, Volta) and Southern belt (Western Central, Eastern). Biochemical data included liver function tests (LFTs). Data collated were hepatitis B serology (hepatitis B-s-Antigen, hepatitis B-e-Antigen, HBeAg; hepatitis B-e Antibody, HBeAb; Hepatitis B-core IgG, HBcIgG; Hepatitis B-core IgM, HBcIgM) by ELISA and serum hepatitis B DNA determined using COBAS® TaqMan® Analyzer. The lower limit of detection of the Roche TaqMan assay was 20 IU/mL and the linear range 20 – 170,000,000 IU/ml. All data extracted from medical records had no patient identifiable information. Data were subsequently entered into Microsoft Access database. Statistical analyses were performed using Software SPSS 16 program. Means, medians, linear range (±SD) were presented for continuous variables and frequencies for categorical variables.

Results

Baseline characteristics of study participants

Overall, 387 new patients with hepatitis B were reviewed at the Gastroenterology Clinic between 2010 and 2014. The mean age of patients was 35.6yrs, ranging from 11 – 80 years, (SD 12.42). Two hundred and nineteen (56.6%) were males and 168 (43.4%) females. Table 1 illustrates the distribution of patients by clinical presentation, demographics, serological and virological characteristics. Two hundred and fifty-two (65.5%) were asymptomatic, 35 (9.0%) presented with jaundice, 29 (7.5%) weight loss and 19 (3.6%) extra-gastro-intestinal symptoms such as headache, arthralgia, numbness; Table 1.

Of the 255 patients with Hepatitis B serology, 210 (82.4%) were HBeAg-negative and 45 (17.6%) HBeAg-positive. All patients (100.0%) were HBsAg-positive on two occasions more than 6 months apart and were HBcIgG positive on serologic profile establishing chronicity. In addition, 11 (4.3%) were HBcIgM positive suggesting acute-on-chronic hepatitis B.

Two hundred and seventy-eight (278) patients had liver biochemical tests. Median ALT was 32 IU/L (range 2 – 4176 IU/L; mean ALT 76.8 IU/L; SD 278.01). Serum ALT was elevated above the upper limit of normal (ULN), > 40 IU/mL, in 38.5% (n=107).

Of the study participants, one hundred and sixty-seven (167) had HBV DNA quantification. The median Hepatitis B viral DNA titre was 2,537 IU/mL. Eighty-eight (52.7%) had HBV DNA > 2,000 IU/mL while 79 (47.3%) had < 2,000 IU/mL. Sixty-three (37.7%) and 104 (62.3%) had HBV DNA > 20,000 IU/mL and < 20,000 IU/mL respectively. Of the 150 patients with liver biochemistry and HBV DNA, 23.3% (n=35) had both ALT > 40 IU/mL and HBV DNA > 2,000 IU/mL.

In patients presenting with liver ultrasonography (n=51), liver cancer and liver cirrhosis was evident in 9 and 8 patients respectively, figure 1. Alpha Feto-protein (AFP) levels ranged from 0.1 - > 50,000 ng/mL, SD 8245.1, n=79. Seventy-two-percent (n=57) had AFP less than 10 ng/mL with 13% (n=10) AFP levels greater than 500 ng/mL. The median AFP was 4.3 ng/mL.

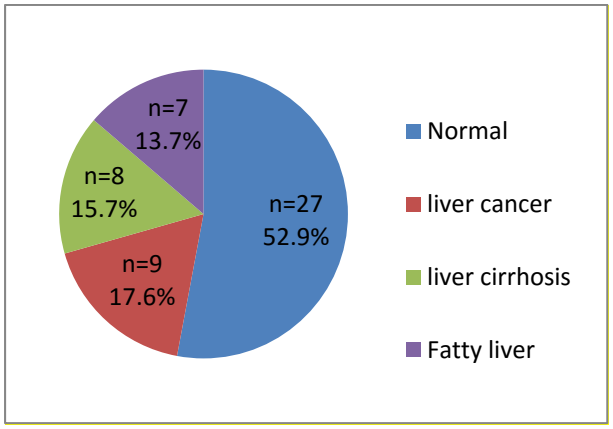

Figure 1. Distribution of ultrasonographic diagnoses at presentation in patients with chronic hepatitis B (n=51)

Table 1: Baseline clinical, demographic and serological characteristics of patients with Chronic Hepatitis B

Clinical presentation	**n**	**(%)**
Asymptomatic	252	(65.1)
Abdominal pain	14	(3.6)
*Constitutional symptoms	11	(2.8)
Anorexia	6	(1.6)
Weight loss	29	(7.5)
Abdominal distension	12	(3.1)
Leg swelling	3	(0.8)
Jaundice	35	(9.0)
Vomiting	1	(0.3)
Haematemesis	1	(0.3)
**Extra-gastrointestinal	23	(5.9)
Total	387	(100.0)
Ethnicity		
Akan	174	(45.0)
Ga-Adangme	54	(14.0)
Ewe	57	(14.7)
Guan	5	(1.3)
Gurma	31	(8.0)
Mole-Dagbani	38	(9.8)
Grusi	2	(0.5)
Mande	8	(2.1)
Other	15	(3.9)
Not indicated	3	(0.8)
Total	387	(100.0)
Occupation		
Professionals	77	(19.9)
Technicians	25	(6.5)
Clerks	35	(9.0)
Sales/service worker	32	(8.3)
Agriculture/fishery	11	(2.8)
Craft & related trade workers	17	(4.4)
Drivers, mechanical operators	27	(7.0)
Elementary occupations	76	(19.6)
Other	87	(22.5)
Total	387	(100.0)
Serologic profile		
HBeAg +	46	18.0
HBeAg -	209	82.0
HBeAB +	203	79.6
HBeAB -	52	20.4
HBcIgM +	11	(4.3)
HBcIgM -	244	(95.7)
HBcIgG +	255	(100.0)
HBcIgG -	0	(0.0)
HBV DNA		
20 – 2,000 IU/mL	79	(47.3)
2000 – 20,000 IU/mL	24	(14.9)
>20,000 IU/mL	64	(38.3)
Total	167	(100.0)

*constitutional symptoms (e.g. fever, malaise, lethargy)

**Extra-gastrointestinal(e.g.headache, arthralgia, palpitations, anxiety)

Table 2. Factors associated with Hepatitis B viremia in chronically infected patients at KBTH, Accra

Characteristic	HBV DNA < 2000IU/mL (n/%)	HBV DNA ≥ 2000IU/mL (n/%)	Total	P-value T test
Age (years)				0.192
< 20	4 (26.7)	11 (73.3)	15	
21-30	26 (42.6)	35 (57.4)	61	
31-40	32 (58.2)	23 (41.8)	55	
41-50	8 (42.1)	11 (57.9)	19	
51-60	9 (60.0)	6 (40.0)	15	
>60	0 (0)	2 (100.0)	2	
Sex				0.095
Male	43 (42.2)	59 (57.8)	102	
Female	36 (55.4)	29 (44.6)	65	
Regional belt				0.106
Southern	36 (48.0)	39 (52.0)	75	
Middle	15 (34.9)	28 (65.1)	43	
Northern	26 (55.3)	21 (44.7)	47	
Other	2 (100.0)	0 (0.0)	2	
HBeAg status				0.001
Positive	4 (16.7)	20 (83.3)	24	
Negative	65 (55.3)	57 (46.7)	122	
Liver function tests				
Total bilirubin				0.306
Normal (< 20umol/l)	49 (50.0)	49 (50.0)	98	
Elevated (> 20umol/l)	21 (41.2)	30 (58.8)	51	
Alanine aminotransferase				0.001
Normal (5-40 U/L)	57 (55.3)	46 (44.7)	103	
Elevated >ULN	13 (27.1)	35 (72.9)	48	
Aspartate aminotransferase				<0.0001
Normal (5-40 U/L)	60 (56.1)	47 (43.9)	107	
Elevated >ULN	10 (22.7)	34 (77.3)	44	
Albumin				0.113
Normal (35-50 g/L)	65 (48.1)	70 (51.9)	135	
Low < LLN	4 (26.7)	11 (73.3)	15	

Factors associated with Hepatitis B viremia in Hepatitis B infected patients

Table 2 describes the demographic and serological factors associated with detectable HBV > 2,000 IU/mL. HBeAg-positive patients were more likely to have detectable viremia (> 2,000 IU/mL); HBeAg-positive (83.3%) vs HBeAg-negative (46.7%), P=0.001. Patients with elevated serum ALT above ULN had higher prevalence of detectable HBV > 2,000 IU/mL, (72.9%), in comparison with patients with ALT within normal limits, (44.7%) P=0.001. An elevated serum AST above ULN was associated with detectable HBV > 2,000 IU/mL, P < 0.0001. Elevated serum bilirubin did not reliably predict HBV viremia > 2,000 IU/mL, P=0.306.

Table 3. Factors associated with Hepatitis B E-antigen status in chronically infected patients at KBTH, Accra

Characteristic	HBeAg Positive	HBeAg Negative	Total	P-value T test
Age (years)				0.006
< 20	7 (50.0)	7 (50.0)	14	
21-30	8 (14.5)	47 (85.5)	55	
31-40	3 (6.4)	44 (93.7)	47	
41-50	4 (26.7)	11 (73.3)	15	
51-60	2 (14.3)	12 (85.7)	14	
>60	0 (0)	1 (100.0)	1	
Sex				0.580
Male	16 (17.8)	74 (82.2)	90	
Female	8 (14.3)	48 (85.7)	56	
Regional belt				0.592
Southern	10 (14.5)	59 (85.5)	69	
Middle	6 (18.2)	27 (81.8)	33	
Northern	7 (16.7)	35 (83.3)	42	
Other	1 (50.0)	1 (50.0)	2	
Liver function tests				
Total bilirubin				0.196
Normal (< 20umol/l)	12 (13.8)	75 (86.2)	87	
Elevated (> 20umol/l)	10 (22.7)	34 (77.3)	44	
Alanine aminotransferase				0.009
Normal (5-40 U/L)	10 (11.0)	81 (89.0)	91	
Elevated >ULN	12 (29.3)	29 (70.7)	41	
Aspartate aminotransferase				0.027
Normal (5-40 U/L)	12 (12.4)	85 (87.6)	97	
Elevated >ULN	10 (28.6)	25 (71.4)	35	
Albumin				0.523
Normal (35-50 g/L)	19 (16.1)	99 (83.9)	118	
Low < LLN	3 (23.1)	10 (76.9)	13	
HBV DNA				0.001
<2000 IU/mL	4 (5.8)	65 (94.2)	69	
>2000 IU/mL	20 (26)	57 (74.0)	77	

Factors associated with Hepatitis B E-Antigen status in Hepatitis B infected patients

Table 3 illustrates the demographic and serological factors associated with HBeAg positivity. There was an increased prevalence of HBeAg-negative chronic hepatitis B with increasing age above 20 years, P=0.006. Elevated serum ALT above ULN was associated with increased prevalence of HBeAg positivity in comparison with patients with ALT within normal limits P=0.009. Similarly, an increase in prevalence of HBeAg was seen in patients with elevated serum AST above ULN (P=0.027) and HBV DNA > 2000 IU/mL (P=0.001) respectively.

Discussion

In this study, the prevalence of HBeAg-positivity was low (18.0%) with majority of chronically infected patients being HBeAg-negative. This was similar to an earlier study on blood donors in Kumasi, Ghana where HBeAg-positivity was 13.3%[12]. The typical patient referred to the tertiary center in Korle-Bu, Accra was HBeAg-negative and HBeAb positive. HBeAg-negative chronic hepatitis B infection defines strains that are not producing the HBeAg, usually testing positive for HBeAb, hepatitis B viremia and demonstrating fluctuating or elevated liver function tests[13]. There is loss of immune tolerance against the wild type virus with clearance of HBeAg and subsequent selection of HbeAg-negative mutants[14]. There is an increased prevalence in males compared to females[15], as shown in Korle-Bu, Accra. At presentation, many are asymptomatic and identified usually by screening[13], as demonstrated in this study. The age range of this group of HBeAg-negative patients in most studies was 40 – 55 years, significantly higher than HBeAg positive patients[16, 17]. Although, no major differences exist in

clinical presentation, prognosis is relatively poor in comparison to HBeAg-positive patients[13], with an increased prevalence of advanced liver disease[18]. This may reflect the increased duration of infection and in some treatment-refractory disease. Forty-percent have been shown to have histologic cirrhosis at presentation in earlier studies[18,19]. A large cohort series demonstrated mortality and hepatocellular carcinoma formation within 4 years from diagnosis was 29% and 14% respectively, being higher than in HBeAg-positive chronic hepatitis B[13]. The younger age of diagnosis of HBeAg-negative chronic HBV (mean age in this study 35.6 years), suggests patients may be more susceptible to disease progression and the highlighted adverse outcomes.

The serum aminotransferases are sensitive indicators of liver cell injury and inflammation[20]. The most commonly measured are alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[21]. Elevated HBV DNA (> 2,000 IU/mL) and elevated ALT are among the most important determinants of risk of progression to liver cirrhosis[22-24]. Additionally, Elevated HBV DNA is a key predictor of hepatocellular carcinoma risk[22, 23]. In this study, we found that patients with detectable viremia > 2,000 IU/ml were more likely to have elevated AST or ALT above the upper limit of normal (ULN) than patients with viremia < 2,000 IU/mL, P < 0.0001. Additionally, HBeAg-positivity was associated with detectable viremia > 2000 IU/mL, P= 0.001. At presentation, 23.3% had both abnormal serum ALT > 40 IU/mL and elevated HBV DNA > 2,000 IU/mL, suggesting active chronic inflammation and an increased risk for liver-related complications. In patients with alpha-feto-protein levels, ten (10) out of 79 patients had levels greater than 500 ng/mL; high-risk for diagnosis of hepatocellular carcinoma in predisposed individuals[25].

In achieving international hepatitis B guidelines, this study emphasizes the need for a sustainable national screening and treatment program for eligible patients including periodic liver ultrasonography surveillance and public education in Ghana.

Limitations in our study included lack of comparative liver histology data, however it provides a basis for prospective evaluation of the temporal relationship between biochemical, serological, virological factors and the stage and grade of chronic hepatitis B in treatment-naïve and treated patients in an endemic area.

Conclusion

Liver cirrhosis and hepatocellular carcinoma were evident at presentation in some chronically infected patients. Furthermore, 23.3% had ALT > 40 IU/mL and HBV DNA > 2,000 IU/mL, associated with increased risk of liver-related complications. In achieving current hepatitis B guidelines, there is the need for a sustainable national screening and treatment program for eligible patients in Ghana.

Abbreviations

ALT: serum alanine Aminotransferase
AST: serum aspartate Aminotransferase
HBcIgG: Hepatitis B-c-IgG
HBcIgM: Hepatitis B-c-IgM
HBeAb: Hepatitis B-e-Antibody
HBeAg: Hepatitis B-e-Antigen
HBsAg: Hepatitis B-surface Antigen
HBV: Hepatitis B
HCC: Hepatocellular carcinoma
KBTH: Korle-Bu Teaching Hospital
ULN: Upper limit of normal
WHO: World Health Organization

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