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Profiles of Hepatitis B Virus Serological Markers among Asymptomatic Population in Anambra State, Southeastern Nigeria

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Abstract

Hepatitis B Virus (HBV) infection is apparent in endemic countries affecting millions of people. Further, the asymptomatic nature of the pathogen is a major public health concern. This study was designed to assess the burden of HBV by exploring the serologic markers of infection among consenting asymptomatic community dwellers in two cities in southeastern Nigeria.

A total of 405 blood specimens were tested for HBsAg, anti-HBs, HBeAg, anti-HBe, total anti-HBc and anti-HBc-IgM using ELISA technique. Overall, 14 (3.5%) of the participants had detectable HBsAg out of which 1 (7.1%) had HBeAg and 13, anti-HBe. Two of the HBsAg positives (14.3%) had detectable anti-HBc-IgM. A total of 144 (35.5%) had detectable anti-HBc, even as 65 (57.0%) of them had the marker as the only serologic evidence of HBV exposure. Thirty-seven (9.1%) participants had anti-HBs only although all of them were born before the start of the childhood HBV vaccination. Altogether, 224 (57.3%) had no detectable serological markers of HBV infection or immunity and were obviously at risk of HBV infection.

This study described various patterns of HBV serologic markers of infection in the study population and probable risk of virus spread. Our results support the need for urgent intervention and implementation of measures to control the spread of HBV infection in Nigeria.

Keywords

HBV; Serological markers; Asymptomatic; Community dwellers; ELISA; Anambra; Nigeria

Introduction

Hepatitis B virus (HBV) infection is one of the most common infectious diseases in the world and has infected 2 billion people globally; leaving an estimated 248 million as chronic carriers [1,2]. Infection with HBV may lead to acute or chronic conditions with consequences such as liver cirrhosis and hepatocellular carcinoma (HCC) overtime. It has been estimated that 70 – 90% of the population with chronic HBV infection have normal liver function [3,4]. This group of people are said to be apparently healthy HBsAg carriers (asymptomatic) forming a major component of the general population in endemic countries of Africa [5,6]. Due to its largely asymptomatic nature, chronic viral hepatitis is a silent epidemic, and most people are unaware of their infection.

Epidemiological studies carried out in different parts of the world show that the characteristics of the population, such as sanitary conditions, lifestyle, hygiene, risk and socioeconomic factors are related to large variations in the frequency and prevalence of HBV infection [7,8]. The major route of transmission of HBV in endemic regions like Nigeria is perinatal from Hepatitis B e Antigen (HBeAg)-positive mothers or through early horizontal transmission from close contacts with immediate family members [5,9]. However, this virus can also be spread via the use of contaminated blood and blood products, organs transplant from infected donors and unprotected sexual intercourse with an infectious person. Significantly, hepatitis B is a vaccine preventable infection, and WHO has recommended vaccination for several population groups ranging from infants to high risk adults [10].

Hepatitis B virus has different serological markers of infection which include Hepatitis B surface antigen (HBsAg) and its corresponding antibody (anti-HBs), antibody to HBcAg (anti-HBc), Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) [11,12]. Precisely, HBsAg beyond six (6) months of first detection, is a marker of chronic infection [13], while, presence of anti-HBs demonstrates immunity to HBV either by vaccination or via resolved infection by the virus. Furthermore, presence of antibody to the core antigen indicates an exposure to HBV irrespective of whether it is a recent or resolved infection [14]. It is possible to establish the infection or immunity status of a person in routine diagnostics with these three markers [12]. Additionally, HBeAg correlates with the virus infectivity and the risk of progression to cirrhosis in chronic carriers, while its corresponding antibody often connotes less infectious state [13].

Due to the fact that HBV infection is associated with either one or multiples of these serological markers and this signifies different phases or stages of the infection [12] we therefore aimed in this study to assess the prevalence of ongoing or resolved HBV infection by investigating various serological markers among asymptomatic community dwellers of Anambra State in southeastern Nigeria.

Methodology

Study location

This cross-sectional community-based study was carried out at Onitsha and Nnewi communities in Anambra State. The two communities serve as commercial nerve centers of the State with people trouping in daily for business activities from neighboring towns, villages and states across the country. Population of these cities during the business hours is usually more than ten times of the residents, thus, making it difficult to ascertain the exact population. Apart from Christianity which is the major religion in the area, some residents are adherents of African Traditional Religion (ATR). Apart from Igbo, the native language, many residents communicate in

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Pidgin English. These communities have access to primary health care facilities from which the residents and immigrants from neighboring settlements and towns benefit.

Enrolment of participants

A total of 405 consenting participants (M=194, F=211; age ranged 15-70 years and median age=26.4 years) were enrolled for the study in August, 2013 . The study relied upon availability of participants in their houses, workplace and willingness to be involved in the study. The main assumption for using this approach was based on the premise that the target population is homogeneous and is likely to share similar characteristics and life style. Health education messages relating to HBV prevalence and prevention were provided to each prospective participant prior to their enrolment in the study. Subsequently, a structured questionnaire was used to capture the socio-demographic information of the target population. Blood sample was collected from each consenting participant by a trained phlebotomist. Ethical approval for the study was granted by the Anambra State Ministry of Health, Awka (MH/PHD/MISC/1). All the participants were well informed on the nature, purpose and processes involved in the study prior to their enrolment. Participation was voluntary while the non-consenting participants were excluded from the study thereby maintaining the principle of autonomy. Verbal consent was used to enroll the participants into the study. Confidentiality, privacy and anonymity of the information for each participant were guaranteed for the sample provided.

Sample collection

Five milliliters of blood was collected from each participant by venipuncture. The blood sample was then dispensed into an appropriately labeled sterile container without any preservative or anticoagulant. Subsequently, the samples were transported to the laboratory at about 4-8°C in a cooler with frozen ice packs. Serum was separated from other blood components by low-speed centrifugation at 500g for 5 minutes and subsequently removed using a sterile disposable pipette. Two aliquots from each serum were made per sample in labeled sterile cryovials and stored at −20°C until ready for analysis. Laboratory analysis was carried out in the Department of Virology, and the Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria.

ELISA screening for HBV serological markers

All 405 samples were screened for HBsAg, anti-HBs, HBeAg, anti-HBe, total anti-HBc and anti-HBc-IgM using Enzyme Linked Immunosorbent Assay (ELISA) test kits (Diagnostic Automation/ Cortez Diagnostic, California, USA). All assays were carried out according to manufacturer's instructions while the optical density was read using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA) and the results interpreted accordingly.

Results

Overall, 14 (3.5%) of the participants had detectable HBsAg out of which 1 (7.1%) had HBeAg and 13 anti-HBe. Two of the HBsAg positives (14.3%) had detectable anti-HBc-IgM. In all, 144 (35.5.8%) of the participants had detectable total anti-HBc, while anti-HBs was detected in 97 (24.0%) (Tables 1 and 2).

The participants were subsequently grouped into 2 serological profiles: A (HBsAg positive) and B (HBsAg negative) (Figures 1 and 2). Profile A was subdivided into 3 subgroups, and B into 5 subgroups based on serologic markers present. Only one patient within profile A had detectable anti-HBc and HBeAg, thus, she was categorized as subgroup A1. A total of 12 patients with anti-HBe and anti-HBc were categorized as subgroup A2 while 1 participant with anti-HBc, anti-HBe and anti-HBs was categorized as A3. Within profile B, 130 (33.2%) of the 391 HBsAg negative participants had anti-HBc. Among these 130 were 6 (15.3%) subjects with detectable anti-HBc and anti-HBe who were categorized in subgroup B1. Sixty-five participants with anti-HBc only (16.6 %) were categorized into subgroup B2; and 59 others (15.1 %) with anti-HBs, anti-HBc and or without anti-HBe as B3. Subgroup B4 has 37 participants with detectable anti-HBs only, while 224 other participants without any detectable HBV serological markers categorized as B5 (Figure 2).

Serological profiles of HBV infection by age show highest (62.8%) susceptibility for HBV infection (B5) among age group 31-40 years while lowest rate (41.5%) among age group >50 years. Highest rates for detectable isolated anti-HBs in profile B4 (25.0%) were observed in age groups <20 years. Profile B3 had highest and lowest rates (21.6% and 5.1%) among age groups 41-50 and <20 years respectively. Also, highest rate (39.6%) for profile B2 was recorded in age group >50

Table 1: Distribution of serological markers of HBV infection by age among residents in Anambra State, Nigeria.

Age Range (yrs)	No Tested	HBsAg (%)	HBeAg (%)	Anti-HBe (%)	Anti-HBc (%)	Anti-HBc-IgM (%)	Anti-HBs (%)
<20	39	0(0.0)	0(0.0)	0(0.0)	4(10.3)	0(0.0)	11(28.2)
21-30	129	4(3.1)	1(25.0)	9(7.0)	40(31.0)	1(25.0)	30(23.3)
31-40	129	9(7.0)	0(0.0)	13(10.1)	46(35.7)	1(11.1)	25(19.4)
41-50	55	1(1.8)	0(0.0)	3(5.5)	24(43.6)	0(0.0)	17(30.9)
>50	53	0(0.0)	0(0.0)	0(0.0)	30(56.6)	0(0.0)	14(26.4)
Total	405	14(3.5)	1(7.1)	25(6.2)	144(35.5)	2(14.3)	97(24.0)

Table 2: Serological profiles of HBV infection by age group among the study population in Anambra state, Nigeria.

Age range	No tested	HBsAg Positive			HBsAg Negative				
		A1	A2	A3	B1	B2	B3	B4	B5
<20	39	0(0.0)	1(2.6)	0(0.0)	0(0.0)	1(2.6)	2(5.1)	10(25.6)	24(61.5)
21-30	129	1(0.8)	3(2.3)	0(0.0)	2(1.6)	15(11.6)	19(14.7)	15(11.6)	72(55.8)
31-40	129	0(0.0)	8(6.2)	1(0.8)	3(2.3)	18(14.0)	17(13.2)	5(3.9)	81(62.8)
41-50	55	0(0.0)	1(1.8)	0(0.0)	1(1.8)	10(18.2)	12(21.6)	6(10.9)	25(45.5)
>50	53	0(0.0)	0(0.0)	0(0.0)	0(0.0)	21(39.6)	9(17.0)	1(1.9)	22(41.5)
Total	405	1	12	1	6	65	59	37	224
		14			391				

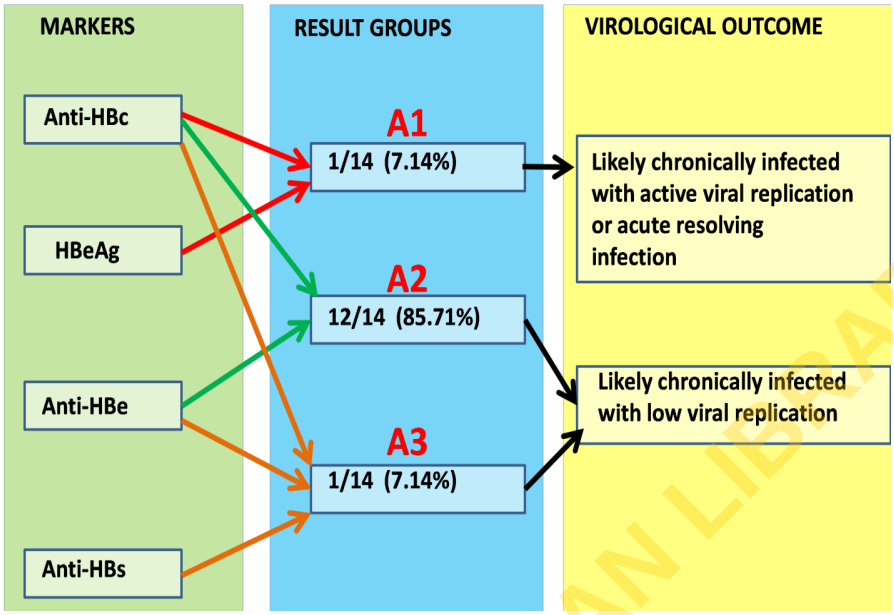

Figure 1: Profile A: Serological profile of HBsAg positive participants in the two communities in Anambra state, Nigeria.

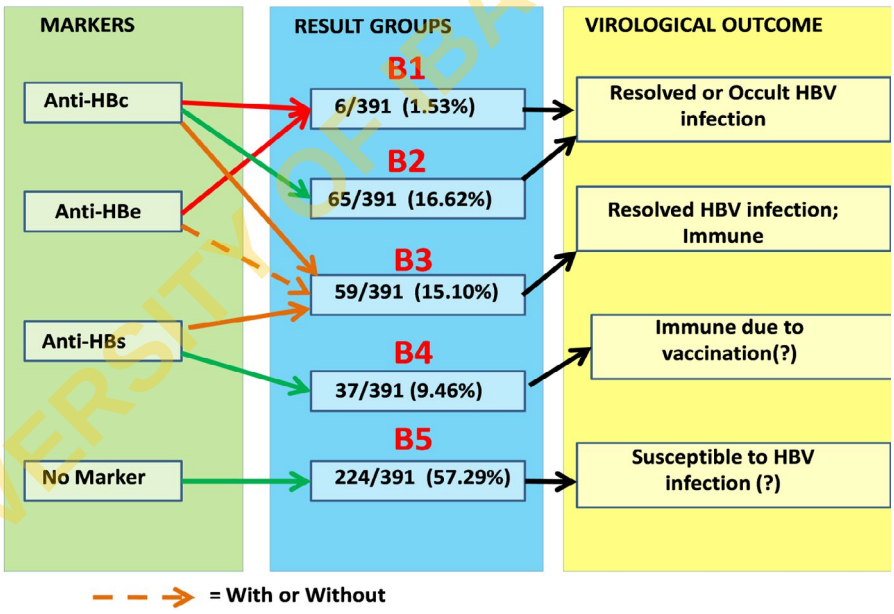

Figure 2: Profile B: Serological profiles of HBsAg Negative participants in the two communities in Anambra state, Nigeria.

years and the lowest (2.6%) amongst 21-30years. Profile A (HBsAg-positive) with the majority categorized into A2 recorded highest rate (6.2%) in age group 31-40 years and the lowest (1.8%) in 41-50years (Table 2).

Discussion

An overall rate of 3.5% for HBsAg observed among the study population (Table 1) is within the range of 1.1%-4.3% previously reported from different parts of Nigeria [15-17], but lower than the 5.7%-12.3% range reported [18-20] in southern and northern regions of the country. Many factors may be adduced for the difference in rates, nevertheless, the overall prevalence of 3.5% HBs-antigenaemia detected in this study may not represent the true picture of HBV infection in southwest Nigeria considering the earlier findings reported from this region [21,22].

The rate of 7.1% (1/14) for HBeAg found in this study (Figure 1) is within the range of 6.4- 8.9% [6,8,9,16] found among blood donors in Nigeria. This rate is however lower than 26.7% (4/15) and 30.3% (10/33) reported by Anaedobe et al. [7] and Bayo et al., [23] among other cohorts, respectively. The HBeAg has been described both as an indicator of active HBV replication, and as an immunomodulator that reduces the capability of children born to HBsAg and HBeAg

positive mothers to clear the virus, thus making them chronic carriers and consequently, reservoirs of HBV in different populations [24]. The participant with profile A1 tends to be highly infectious with active viral replication, since HBeAg is a marker of high infectivity [24,25]. He also poses a high risk of possible transmission not only to his sexual partners but to his household.

The 11 of the 12 participants in profile A2 (Figure 1) could be cases of chronic infection with low viral titers. Presence of anti-HBe may suggest good prognosis and is indicative of controlled viral replication in these individuals in profile A2, since persons with anti-HBe tend to have lower viraemia [25]. One might assume that patients in this subgroup are mostly inactive carriers, and thus may not transmit the infection to others. However, mutations in the regions of the viral genome that codes for HBeAg can result in absence or decreased levels of detectable HBeAg, but this may not alter the sequeale of chronic infection [13] It has been reported that HBeAg-negative HBV mutants prevail in the general population of HBV carriers [26]. One of these 12 participants in profile A2 (Figure 1) was also positive for anti-HBc-IgM, thus, might be a case of acute infection. However, several studies have shown that many patients with chronic hepatitis B have low to moderate titers of anti-HBc-IgM [27], especially during acute exacerbation of chronic HBV infection [28].

In profile A3 is a participant with both HBsAg and anti-HBs serologic markers. This may be a case of chronic HBV infection as previous report has shown that in less than 1% of times chronic HBV carriers can be positive for both HBsAg and its corresponding antibody and has suggested that such patients be categorized as infectious [14]. Anti-HBs in absence of HBsAg indicates protection against HBV and may be acquired either through infection or vaccination [12]. Although, vaccinated individuals with anti-HBs are protected against clinical apparent hepatitis B, asymptomatic breakthrough infection in vaccinated population has been demonstrated [29]. The presence of HBsAg in this participant could theoretically also portray breakthrough infection but vaccination and pre-existing anti-HBs are not known for this person. It is more likely that this is a chronic HBV carrier with a detectable but insufficient anti-HBs [30] response. It is worth mentioning that though breakthrough infections caused by immune escape mutants have been demonstrated, they have not been shown to represent a major problem yet [31,32]. Further, the qualitative presence of anti-HBs alone after vaccination but not enough to confer HBV immunity as the titer must be up to 10mIU/mL. The levels might drop to less after 5 to 10 years post vaccination [33] and may need booster doses.

The 71 members of profiles B1 and B2 (Figure 2) could possibly be in the window period in which anti-HBs is yet to develop but have cleared the HBsAg below detectable limit of the test kit [34]. However, they could also be candidates for occult HBV infection. Studies have shown that individuals with detectable anti-HBc with or without anti-HBs but negative for HBsAg can be viraemic at a low level [35-37]. However, the majority of HBV particles in occult infected subject is not infectious [38]. The rate of isolated anti-HBc (B2) recorded among those who were exposed to HBV was 57.0 % (65/114) (Figure 2). Isolated anti-HBc may represent resolved infection with the loss of anti-HBs, occult chronic HBV infection with levels of HBsAg below detectable limit, or a false positive reaction [39]. It has been suggested that such individuals be regarded as non-immune to HBV and should be considered for vaccination.

Thirty-seven of the total 391 (9.5%) HBsAg negative participants had detectable anti-HBs (B4), with highest rate in age group <20 years. This may be due to unspecific reaction since childhood HBV immunization started in 2004 in Nigeria, thus this adult population may not have benefitted from the program.On the other Anti-HBs alone without previous vaccination seems to be relatively frequent.

This study found a rate of 57.3% (224/405) of the population belonging to subgroup B5 (Figure 2 and Table 2) with no detectable marker to confirm previous exposure to HBV infection or vaccination. Thus, are at risk of being infected, they therefore are of public health concern to successful control of HBV infection, since if infected could transmit the virus.

Conclusion

This study described various HBV serological markers of infection among the study population and their virological significance. Our results confirm the need for urgent intervention and implementation of measures to control the circulation of HBV infection in Nigeria.

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