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Hepatitis B Virus Serological Markers in a Rural Community in Southeastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TOCF, MOA, IMI, CEO and ASB designed the study. Authors MOA, IMI, CEO and AA collected the sample. All authors acquired the reagents, laboratory and analyzed the data, wrote the first draft, revised and read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Due to the current blood safety algorithm in Nigeria which excludes only Hepatitis B surface Antigenaemia (HBsAg) positive individuals from blood donation, this study was therefore designed to investigate HBV markers of infection in a rural population in southeastern Nigeria.
Study Design: It is a cross sectional community-based study.

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Place and Duration of the Study: This study was carried out in Awuda village in Nnobi town of southeastern Nigeria in August 2013.
Methodology: A total of 92 consenting participants were enrolled for the study. The participants were screened for HBsAg, HBeAg, Anti-HBc IgM, Anti-HBc, Anti-HBe and Anti-HBs using ELISA technique and classified into different serological profiles indicative of infection stages.
Results: Respondents' mean age was 26.3(SD +11.5) years and 58 of them were females while 34 were males. An overall prevalence for HBsAg was 1.1% (1/92). The same HBsAg positive individual also had detectable anti-HBe and anti- HBc IgM. Analysis of the results showed 3 (3.3%) of the study participants were positive for both Anti-HBe and Anti-HBc. Also, 12 (13.0%) participants were positive for only Anti-HBc and Anti-HBs antibody. Another 9 (9.8%) participants were positive for only Anti-HBs while 51 (55.0%) had no serological marker for previous exposure to either HBV or HBV vaccine. Altogether, 31(33.7%), 1(1.1%) and 21(22.8%) participants were positive for HBc, HbcIgM and HBs antibodies respectively.
Conclusion: This study has demonstrated that at least 1 out of every 3 people in the studied community might have serological evidence of present or past HBV infection. The current dependence of blood safety algorithms which excludes only HBsAg positive individuals is not enough to guarantee safety of blood and/or blood products. More studies are needed to further investigate the theoretical basis of the algorithm.

Keywords: HBV markers; serological profiles; safety algorithm; rural; Nigeria.

1. INTRODUCTION

Hepatitis B virus (HBV) is the etiologic agent of hepatitis B virus infection. It is a partially double stranded DNA virus which belongs to the family *Hepadnaviridae* [1]. The main virion components include the envelope enclosing the capsid inside which is DNA and polymerase protein. The HBV genome is about 3.2 kb and has 4 ORFs (P, X, C and S) from which 7 proteins are translated. HBV is primarily spread through contact with infected blood and blood products, sexual intercourse and via mother to child [2].

Hepatitis B virus infection is a major public health concern worldwide. Over two billion of the world population are currently infected with HBV and 350 million persons being chronic carriers, with about 4.5 million new infections occurring each year; an approximately 600,000 people die yearly due to the consequences of acute or chronic hepatitis B infection [3]. Based on the prevalence of HBsAg the world can be divided into three major geographical areas: high endemic areas with over 8% of carriers in the general population, intermediate endemic areas with 2 to 8% carriage rate in a given population and low endemic areas in which the carriage rate is less than 2% [4,5]. Sub-Saharan Africa is considered to be a region of high endemicity with an average carrier rate of 10 - 20% in the general population [6]. Previous studies in Nigeria showed HBV carriage rate with a range of 9 to 39% [7-10].

Hepatitis B virus can manifest both clinically and in an asymptomatic manner, and sometimes with unusual serological patterns [11]. These serologic markers include hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), antibody to HBcAg (anti-HBc), hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe). It has been shown that at least one serologic marker is present during the different phases of HBV infection [12,2,13]. Studies have shown that the infection or immunity status of a person can be established with combined analysis of HBsAg, anti-HBc and anti-HBs markers in routine diagnostics [13,14]. Specifically, the presence of HBsAg is indicative of ongoing HBV infection, thus all HBsAg-positive persons are considered infectious [13]. Also, anti-HBc appears with the onset of acute hepatitis or after an unnoticed clinically silent HBV infection. If HBV infection is completely under immune control, the HBsAg disappears, but the anti-HBc remains, and anti-HBs usually appears as a sign of immunity. Furthermore, complete recovery from acute or chronic hepatitis B or successful immunization is mostly demonstrated by the presence of detectable anti-HBs. However, HBsAg and anti-HBc remain positive in chronic HBV infection [14].

The national guidelines on blood safety recommends that individuals must be HBsAg negative in order to be eligible for blood donation [15]. Also, according to Mast et al. [13], it has been recommended that individuals must be

screened for HBsAg to determine their eligibility for HBV vaccination. However different studies [16-18] have confirmed higher prevalence of anti-HBc than HBsAg. It has also been noted that isolated anti-HBc (anti- HBc as the only serologic marker of infection) is becoming more frequent [5]. This raises complicated issues when serologic testing for HBV is being considered.

Isolated anti-HBc can represent a number of conditions: it could be (a) interpreted as a resolved HBV infection, with loss of antibody to hepatitis B surface antigen (anti-HBs) [19]; (b) An indication of occult chronic HBV infection, with levels of the hepatitis B surface antigen (HBsAg) below the limits of detection [19,20]; Or (c) that the individual was infected but yet to develop anti-HBs (window period) [14]; (d) A false-positive test result. Thus, the clinical and public health importance of isolated anti-HBc remain uncertain [21]. However, the continual dependence on HBsAg alone to evaluate the burden of HBV infection may be underestimating the actual burden of the disease.

More importantly, in a bid to safeguard the blood and blood products supply and prevent mother-to-child transmission, various algorithms have been developed to determine which individual or combination of HBV components and/or host response should be used to identify HBV infected and infectious individuals. In Nigeria, the recommended algorithm permits any individual that is HBsAg negative to donate blood. This assumes that such individuals either have no history of HBV infection and if otherwise, are no longer infectious and consequently pose no risk to the Nations blood supply. This study was therefore designed to investigate markers of HBV infection in a rural population in southeastern Nigeria with a view at describing HBV serological profiles in a resource limited setting and the ability of the current HBV blood donation safety algorithm used in Nigeria to exclude HBV infectious individuals from the Nations blood and blood products supply.

2. MATERIALS AND METHODS

2.1 Study Location

This cross-sectional community-based study was carried out in Awuda village in Nnobi town, Anambra state, southeastern Nigeria. Awuda community is a border village between Nnobi and Nnewi, a major city in Anambra State. Apart from Christianity which is the major religion in the area, some residents are adherents of African Traditional Religion (ATR). The population of the community is estimated to be about 8000 inhabitants with only about 1/10 of this population residing in the community as majority of the indigenes have gone to other major cities in search of greener pastures. These emigrants usually return home during festivities and interact with the ones residing in the community. The residents of the community are majorly into petty trading and peasant farming. Apart from Igbo, the native language, many residents communicate in pidgin english. Awuda community has a primary health centre where community members seek for health care services. The general hospital located in Nnobi town equally renders medical services to the people of Awuda community and two other communities that make up the town.

2.1.1 Enrolment of participants

A total of 92 consenting participants were enrolled for the study in August, 2013. The study was predicated upon availability of participants in their houses, and willingness to be involved in the study. The main assumption for using this approach hangs 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 being enrolled in the study. Subsequently, a structured questionnaire was used to capture the participants socio-demographic information including history of vaccination. 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 (AD3/479/349). The nature, purpose and processes involved in the study were well explained to the participants prior to their enrolment. Participation was voluntary, and individuals who did not consent were excluded from the study to observe the principle of autonomy. Particpants were assured of the confidentialty, privacy, and annomity of the information and sample provided.

2.1.2 Sample collection

Five milliliters of blood was collected from each participant by venepuncture. 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 500xg 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.

2.2 HBsAg ELISA Screening Test

All the 92 samples were subjected to HBsAg, HBeAg, Anti-HBc IgM, Anti-HBc, Anti-HBe and Anti-HBs specific Enzyme Linked Immunosorbent Assay (ELISA) using corresponding ELISA kits (Diagnostic Automation/Cortez Diagnostic, California, USA). The sensitivity and the specificity for the test was 100%. The assays were performed according to manufacturer's instructions. The optical density was read at 450nM using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA) and the results were also interpreted according to the manufacturer's instructions.

2.3 Serological Profile Definitions

Four different serological profiles (each divided into groups; Fig. 1) have been described as regards HBV infections. Profile A and B describe acute HBV infections that have resolved. The HBV isolate responsible for profile A is actively expressing HBeAg while that for profile B is an HBeAg negative. Profile C is that of an individual immunized with HBV vaccine and profile D is that of an individual that has never been exposed to neither HBV nor its vaccine. Individuals that are HBV infected (Fig. 1 A and B), vaccinated (Fig. 1C) or unexposed (Fig. 1D) will be at different stages of the profiles. Therefore, during a cross-sectional study, which is what happens during screening of blood donors or pregnant women, subjects will be at different stages of infection.

3. RESULTS

Overall prevalence for Hepatitis B surface Antigenaemia was 1.1% (Table 1). The same HBsAg positive individual also had detectable Anti-HBe and Anti- HBc IgM and this furthermore categorized its serological profile into group AII (Fig. 1A). Three (3.3%) of the 92 people screened were positive for both Anti-HBe and Anti-HBc and consequently fell into group AIII of the serological profile (Table 1 and Fig. 1A). None of the members of the population belonged to group AIV of the serological profile. In all, four (4.3%) participants belonged to serological profile A.

Though none of the members of the population belonged to group BII of the serological profile, 16 (17.4%) belonged to group BIII (Tables 1 and 2 and Fig. 1B) by being positive for only Anti-HBc. Twelve (13.0%) belonged to group BIV (Tables 1 and 2 and Fig. 1B) because they are positive for only Anti-HBc and Anti-HBs. Therefore, 28 (30.4%) members of the population had serological profile that fit into group B.

Furthermore, 9 (9.8%) members of the population (Table 1 and Fig. 1C) had profile indicative of group C and all belonged to group CIII by being positive for only Anti-HBs. Of the nine people in this group, six (66.7%) claimed not to have been vaccinated against HBV (Table 3). Finally, 51 (55.0%) participants in the population had no serological marker for previous exposure to either HBV or HBV vaccine. This category of people represent a sum of groups AI, BI, CII and D (Table 2 and Figs. 1A, B, C and D).

4. DISCUSSION

4.1 Algorithm for Estimating HBV Prevalence

Most studies [9,22-25] have reported the prevalence for HBsAg in the region with figures varying significantly from 1% to 20%. In this light, Nigeria is categorised as highly endemic for HBV with HBsAg prevalence >8% [26]. The HBsAg prevalence of 1.1% detected in this study may be low but it falls within the range previously reported. However, this value does not appropriately describe the burden of HBV infection in this population. The reality in this regard is that an approximately one-third (34.8%) of the population has serological evidence of past or present exposure to HBV (all those in groups A and B; Fig 1 and Table 2) and this questions the appropriateness of estimating HBV disease burden by HBsAg alone considering the issue of occult HBV especially in endemic regions. This practice downplays the public health significance of the risk HBV circulation poses to the population as a whole.

4.1.1 Algorithm for screening blood donors

This problem tapers into the safety of blood and blood products in the region. Currently, on the

premise that only people that are HBsAg positive are infectious, the algorithm gives opportunity to individuals negative for HBsAg to donate blood in Nigeria. This premise has been shown to be faulty [11]. As a matter of fact, studies have shown that once HBV infects a host cell, the virus remains lodged in hepatocytes even when Anti-HBs antibody can be detected in the blood of an HBV infected individual [27]. Consequently, immunosuppression usually results in reactivation of HBV secretion from hepatocytes and HBsAg positivity of blood from such people. In this light, some developed countries, refuse any person with serological evidence of natural HBV infection from blood donation [11,28,29]. In this study 32 (34.8%) members of the population had serological evidence of past or present HBV infection and only one (1.1%) would have been excluded from donating blood. The remaining 31 people with serological evidence of natural HBV infection would have been allowed by the current blood safety algorithm in Nigeria to donate blood,

Table 1. Distribution of serological profiles of HBV markers of infection in Awuda community of southeastern Nigeria

Age group (years)	No tested	Gender		HBV serological profiles						
		Male	Female	HBsAg +ve(%)	HBeAg +ve(%)	HBeAb +ve(%)	HBcIgM +ve(%)	Total HBcAb +ve(%)	Isolated AntiHBc +ve(%)	Anti-HBs (HBsAb) +ve(%)
≤20	4	1	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)
21-30	29	10	19	1(3.4)	0(0.0)	2(6.9)	1(3.4)	6 (20.7)	2(6.9)	8(27.6)
31-40	28	16	12	0(0.0)	0(0.0)	2(7.1)	0(0.0)	14(50.0)	5(17.9)	7(25.0)
41-50	14	4	10	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(35.7)	4(28.6)	3(21.4)
>50	17	3	14	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(35.3)	4(23.5)	3(17.6)
Total	92	34	58	1(1.1)	0(0.0)	4(4.3)	1(1.1)	32(34.8)	16(17.4)	21(22.8)

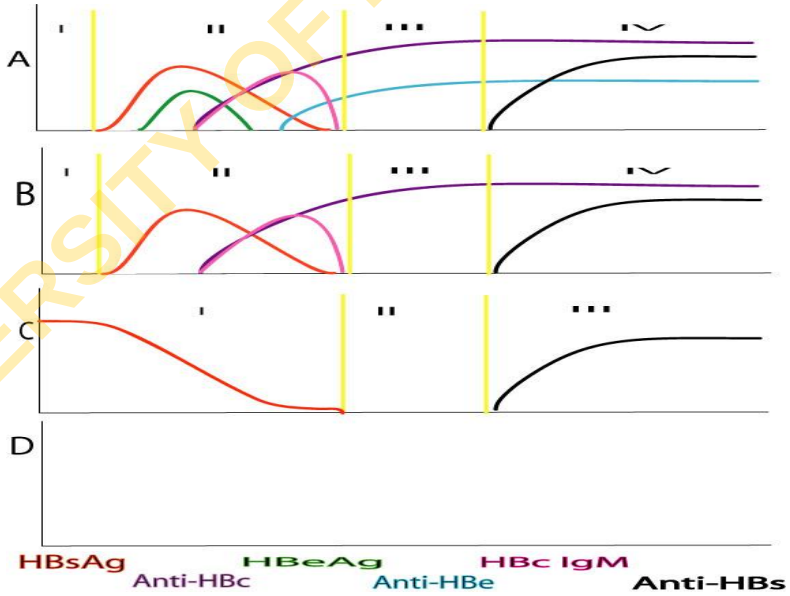

Fig. 1. Prevailing paradigms on HBV serological profiles. Four different serological profiles are presented each divided into groups. Profile A and B describe acute HBV infections that have been resolved. The HBV isolate responsible for profile A is actively expressing HBeAg while that for profile B is an HBeAg negative mutant. Profile C is that of an individual immunized with HBV vaccine and profile D that of an individual that has never been exposed to neither HBV nor its vaccine. The individual markers are colour coded as indicated by the key at the bottom of the figure e.g. HBeAg is green

Table 2. Serological classification of the individuals screened and the differences between their virological and algorithmic interpretations

S/N	Group	Markers positive	Virologic interpretation	Qualified to donate blood by the Nigerian Algorithm	Number of subjects in group
1	AI	NONE	Recently infected	Unexposed and fit to donate	X_1
2	AII	HBsAg, HBeAg /Anti-HBe, HBc IgM/ Anti-HBc	Recently exposed and infectious	Exposed and not fit to donate	1
3	AIII	Anti-HBe, Anti-HBc	Exposed and probably infectious	Unexposed and fit to donate	3
4	AIV	Anti-HBe, Anti-HBc, Anti-HBs	Exposed, recovered and immune	Unexposed and fit to donate	0
5	BI	NONE	Recently infected	Unexposed and fit to donate	X_2
6	BII	HBsAg, HBc IgM/ Anti-HBc	Recently exposed and infectious	Exposed and not fit to donate	0
7	BIII	Anti-HBc	Exposed and probably infectious	Unexposed and fit to donate	16
8	BIV	Anti-HBc, Anti-HBs	Exposed, recovered and immune	Unexposed and fit to donate	12
9	CI	HBsAg	Recently vaccinated	Exposed and not fit to donate	0
10	CII	NONE	Unexposed	Unexposed and fit to donate	X_3
11	CIII	Anti-HBs	Vaccinated and immune	Unexposed and fit to donate	9
12	D	NONE	Unexposed	Unexposed and fit to donate	X_4

Note: $X_1 + X_2 + X_3 + X_4 = 51$

Table 3. Hepatitis B Virus vaccination status by mental recall of people with serological evidence of vaccination

Lab Id	HBV vaccination	HBsAg	HBeAg	HBeAb	HBcIgM	Anti-HBc	AntiHBs
IW002	YES	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
IW016	YES	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
IW030	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
OZ011	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
NW003	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
MN001	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
MN010	YES	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
MN014	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE
MN024	NO	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE

consequently putting the truly unexposed others at risk of HBV infection via a medical procedure.

4.1.2 The true picture of the serologically unexposed

The result of this study shows that 51 (55.4%) of the 92 individuals screened in this study have no marker of HBV infection. These people are deemed fit to donate blood for medical procedures. However, Fig. 1 shows that the fact that an individual is negative for all serological markers of HBV infection does not mean that the person has not been exposed to HBV. As a matter of fact, several people in groups AI and BI would be assumed to belong to group(s) CII

and/or D. However, a good number of people in groups AI and BI can be distinguished from those in groups CII and D by HBV DNA detection. Several cases of HBV DNA detection in people who have been exposed to HBV but have no serological marker of HBV infection have been described [1,21,30,31]. Such people are said to have occult HBV infection. Occult HBV infection is a major public health problem especially in resource-limited settings where sensitive techniques such as polymerase chain reaction (PCR) are unavailable and selogical assays have to be relied upon for making major health-related decisions and policy [32].

4.2 HBeag Positive and HBeag Negative Isolates Circulation and Implications for Infectivity, Vertical Transmission and the Incidence of Chronically Infected Hbv Reservoirs

The prevalence of HBeAg negative isolates is another significant issue raised by the findings of this study. Of the 32 (34.8%) members of the population that have evidence of past or present HBV infection, 87.5% (25/29) were infected by HBeAg-negtive HBV isolates. The HBeAg has been described both as an indicator of HBV replication, high titre, and as an immunomodulator that reduces the capacity of children born to HBsAg and HBeAg positive mothers to clear the virus. Thus, making them chronic carriers and consequently, reservoirs of HBV in populations. Hence, the recommendation to screen pregnant women for HBsAg and HBeAg in a bid to both monitor their viral load and simultaneously stem the tide of HBV reservoirs in the population. In this regard, the preponderance of HBeAg negative isolates might indicate a significant reduction, looking forward, both in replicative fitness and the incidence of HBV reservoirs in the human population.

Studies [11,25,33] have however also shown, that HBeAg negative isolates, tend to replicate, in some cases, even better than HBeAg positive isolates. This might account for their increased transmissibility, evidenced in their increased prevalence in this population. Therefore, clinicians should tilt more to the side of caution, when interpreting HBeAg results especially for HBsAg positive pregnant women. What the increased circulation of HBeAg negative isolates means in futuristic terms for the incidence of HBV reservoirs in the human population needs more rigorous analysis.

4.2.1 HBV vaccines, knowledge gap, immunological response to vaccination and time of examination

The results of this study showed that 6 out of the 9 people that showed serological evidence of vaccination do not remember being vaccinated (Table 3). On the other hand, only three of the 13 people that claimed to be vaccinated have corroborating serological profile (data not shown). This raises questions about the knowledge the people have with respect to HBV vaccines, the vaccination process and the need to increase awareness not only on HBV and its vaccine but also on the details of the vaccination process to resolve all ambiguities.

5. CONCLUSION

The authors are aware that due to small number of samples screened in this study, the results should be extrapolated with caution. However, one very crucial insight can be gleaned from the findings of this study. The disease burden of HBV in Nigeria is significantly bigger than captured by the prevalence of HBsAg in the population. At least one out of every three people in the studied community might have serological evidence of present or past HBV infection. The current blood safety algorithm which excludes only HBsAg positive individuals from blood donation is not enough to guarantee safety of blood and/or blood products. Additional tests for other HBV markers, and occult hepatitis should be considered while advocating for more studies to further investigate the theoretical basis of the current algorithm. Also, preventive measures through vaccination and public education are advocated.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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