Sero-Epidemiology of HBsAg among Health Workers in a South-eastern Nigerian Health Center: Challenges in Diagnosis

Abstract
Hepatitis B virus infection represent a major public health problem responsible for acute and chronic viral infections, HBV-related liver disease or hepatocellular carcinoma. Sub-Saharan Africa is worse hit by HBV infection and its related morbidity and mortality. Despite the burden of HBV in the region, the challenges confronting people living with HBV include making diagnosis and provision of their comparable estimates. This study aimed at analyzing the sero-prevalence of HBsAg among health workers and possible challenges in assessment of HBV prevalence in a resource-limited sub-Saharan African country. Blood samples from 275 consented health workers were tested for HBsAg using third generation ELISA. Data analysis was obtained using SPSS version 20. HBsAg screening was performed on a consecutive sample of 275 respondents (95 males and 180 females with M/F ratio of 1:1.9) aged 18-59 years who voluntarily turned up for the survey. Mean age ± SD was 36.1 ± 9.6 years. The overall prevalence of HBV was 1.5% (4/275). The prevalence in females 3/180 (1.7%) was insignificantly higher than males 1/95 (1.1%) (P>0.05).

In relation to age group, the younger age group (< 20 years) has significantly higher prevalence (1/5 (20%) compared to other age groups (P=0.02). Blood transfusion and vaginal discharge (STD) were the highest predisposing factors to HBV infection with ORs of 5.9 and 4.2 respectively. This study, although limited by small sample size, showed a reduced prevalence of HBV among the health workers. The result from this study may not be a true reflection of the prevalence of HBV in south-eastern Nigeria, hence the need to involve larger population size in subsequent study. There is need to institute elaborated serological and virological diagnostic markers for the diagnosis of HBV infection in our health institutions. Also, targetted group health education and national guidelines for hepatitis B prevention and treatment must be provided by the Federal Ministry of Health, Nigeria. These interventions will go a long way to curb the prevalence of HBV infection to the barest minimum.

Keywords: Seroepidemiology; HBsAg; Health Workers; Diagnosis; South-eastern Nigeria

Introduction
Infection with hepatitis B virus (HBV) is one of the major public health problems worldwide [1,2]. About one-third of the world’s population (approximately 2 billion people) has serological evidence of infection with hepatitis B virus (HBV) [2,3]. There are well over 350 million people with chronic HBV infections globally with annual mortality rate of 0.6-1 million from chronic liver disease, including liver cirrhosis and hepatocellular carcinoma [2,4]. HBV infection endemicity varies greatly depending on geographical region but majority of those infected (approximately 45%) of the global HBV population) are in developing countries especially in Asia and Africa [2,5]. Nigeria is a hyper-endemic area [4,6,7] with infected population of 18 million though the carriage rates vary widely between 2-20% [7-11] depending on the population studied but with a median of about 10.3% [7], though much higher figures have been reported [12-14]. However, the epidemiological scenario of HBV has been changing rapidly over the last two decades globally, possibly due to immunization program initiated by the World Health Organization [15].

HBV is a member of the Hepadnaviridae family, genus orthohepatodnavirus [16,17]. It is a partially double-stranded enveloped DNA virus with about 3200 nucleotides in its genome. HBV is the smallest known DNA virus [18] and the only pathogenic hepadna virus in which the proteins and genome were clearly identified and characterized. The hepatitis B surface antigen, HBsAg (formerly known as “Australian antigen”) was first recognized in 1968 in an Australian aborigine [19]. In 1970, Dane and colleagues first described the 42 nm particles that are the hepatitis B virions. The viral nature of Dane particles was confirmed by the detection of an endogenous DNA-dependent DNA polymerase within their core. HBV infected cell produce multiple virus related particles [20]. Electron microscopy of HBsAg positive serum reveals three morphologic forms: the 22-nm size spheres which are actually the HBsAg. They are the most
numeros existing separately from the whole virion [21]. These are followed by the 20 nm diameter filamentous forms and the 200 nm variable length. The third forms are the double shelled particles with diameter of 42 nm. These are the complete and intact infective virions, the Dane particles [22,23].

There are about four major serological subtypes (adr, adw, ayr and ayr) and seven genotypes (A-H) of HBV. Each of the HBV genotypes has a distinctive geographical distribution which provides a valuable epidemiological marker for identifying the source of a particular infection [24]. For example: Genotype A (mainly found in North West Europe, North America and Central Africa), B and C (South East Asia including China, Taiwan and Japan); D (southern Europe, India and the Middle East); E (West Africa); F (South and Central America); G (France and USA); and H (Central and South America). All HBV subtypes share one common antigenic determinant “a”. Thus, antibodies to the “a” determinant confer protection to all HBV subtypes.

The serologic and virologic markers elaborated during HBV infection may include: HBsAg, anti-Hbc, anti-HBs, HBeAg, anti-HBe and HBV DNA. Among these immunologic and virologic markers, HBSAg is the first marker detectable in serum following HBV infection [21]. It appears late in the incubation period (4th-10th week) declining to undetectable levels in 3-6 months. Its presence indicates infectivity of blood rarely persisting beyond six months except in chronic infections. When HBSAg disappears in the period of convalescence (“window” period), antibody to hepatitis B surface antigen (anti-HBs) becomes detectable in serum and remains in the serum for longer period of time. High titre of anti-HBs is an indication of immunity [25].

Anti-HBc is readily detectable in the serum after appearance of HBsAg but weeks or months before anti-HBs is detected. Anti-HBs is usually not present until HBsAg has disappeared and a variable period of weeks separate disappearance of the latter and appearance of the former creating a window period in which only anti-HBc represents serological evidence of current or recent HBV infection. Most cases of isolated anti-HBc represent hepatitis B infection in the remote past. Anti-HBc of the IgM class predominates during the first six months while IgG anti-HBc is usually isolated in the rare patient with chronic hepatitis B whose HBsAg is below the sensitivity threshold of contemporary immunoassays (a low-level carrier). Generally, in persons who have recovered from hepatitis B, anti-HBs and anti-HBc persist for long time.

HBeAg may be detected in the serum concurrently or shortly after appearance of HBsAg. Its appearance coincides with high levels of virus replication reflecting presence of circulating intact virions and detectable HBV DNA. The exception is in patients with precore mutations who do not synthesize hepatitis B e antigen (HBeAg). In self-limiting HBV infections, HBeAg becomes undetectable shortly after elevation of aminotransferase activity, but before the disappearance of HBsAg, anti-HBe becomes detectable, coinciding with the period of relatively lower infectivity and clinical resolution of infection. Although the presence of HBeAg reflects continued viral replication, HBeAg is only a qualitative marker while HBV DNA is quantitative indicator.

Generally, in persons who have recovered from hepatitis B infection, anti-HBs and anti-HBc persist for long time. The temporal association between the appearance of anti-HBs and resolution of HBV infection as well as the observation that persons with anti-HBs in serum are protected against re-infection with HBV suggests that anti-HBs is the protective antibody. Therefore, strategies for prevention of HBV are based on providing susceptible persons with circulating anti-HBs [21,25].

In hepatitis B primary infection, HBsAg is detectable in the serum within the fourth and sixth weeks of incubation period. This is followed by rise in total anti-HBc antibody titre in the serum. There may or may not be a rise in circulating HBeAg. A high HBV DNA may be recorded. At high HBV DNA replication rate (i.e. 109-1010) the HBV infection becomes highly contagious. However, decrease in HBsAg correlates with onset of T-cell mediated immunity response. Also, when present, correlates with onset of elevated liver enzymes. Traditionally, conversion to anti-HBs antibody signal cure. Sometimes HIV DNA may persist for years to lifetime.

The various mode of transmission of HBV include unprotected sexual intercourse, intravenous drug abuse, transfusion of infected blood, horizontal transmission in childhood, perinatal transmission from infected mother to the baby and occupational exposure especially among health-care providers. According to centre for disease control 1992 report, heterosexual intercourse and transfusion of hepatitis B infected blood rank highest among the risk factors responsible for HBV transmission in adulthood while perinatal transmission from infected mothers ranks highest in newborns in endemic regions. This study aimed at determining the seroprevalence of Hepatitis B surface antigen among the staffs of Federal Medical centre, Umuahia, a South eastern Nigerian Tertiary Hospital. Also, possible risk factors and challenges in diagnosis of HBV in this region will be explored in this study.

Methodology

This cross sectional study was conducted at the Department of Hematology, Federal Medical Centre, Umuahia, Abia state Nigeria during the period of June, 2013 after duly obtaining ethical clearance from the same hospital. A total of 275 staffs of Federal Medical Center, Umuahia who gave consent to participate in the study were recruited. The inclusion criterion was that the participant must be a staff of Federal Medical Centre, Umuahia. Participants who were recently immunized against HBsAg were excluded from the study. The bio-data which include personal and demographic information such as age, sex as well as medical history regarding risk factors and all other relevant information were collected with the aid of pretested self-administered questionnaire to the consenting partidant who met the inclusion and exclusion criteria. Each participant completed a serially numbered questionnaire administered by the researcher after being duly informed on the intended study. The study was at no cost to the partidants. Confidentiality of participant information was also maintained.

Five millilitres (5ml) of venous blood was collected aseptically from each participant and emptied into a sterile labelled plain vacutainer tube. Blood was allowed to clot by standing at room temperature and then spun in a centrifuge at 2500 rpm for 5 minutes to separate the serum. The serum was disposed in a clean dry glass tube and used to test for HBsAg using third generation

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National AIDS Control Organisation (NACO) approved enzyme-linked immunosorbent assay (ELISA); CTK Biotech, onsite rapid HBsAg test kit (LOT:F1214H1). The test was done following manufacturer’s instructions and the results interpreted and documented accordingly. Every reactive sample was re-tested for confirmation before labelling it seropositive.

Statistical Analysis

Data obtained were entered and analyzed with SPSS version 20. The association between categorical variables was tested using Chi square (X²) test. P<0.05 is considered statistically significant.

Results

A total of 275 respondents who were health care providers were recruited for the study. The age range of respondents was 18-59 years with mean age of 36.1 ±9.6. Females (n=180; 65.5%), constituted the majority, while males accounted for 95 (34.5%) with female to male ratio of 1:1.9. Five (1.8%) of the respondents were less than 20 years while 34 (12.4%) were above 50 years. The highest number of respondents was in the 30-39 (n=104; 37.8%) and 20-29 (n=74; 26.9%) years age brackets. Majority of the respondents were married (n=169; 61.5%), while single respondents accounted for 92 (35.5%). Ten (3.6%) of the respondents were widowed. Those with tertiary education (n=232; 84.4%) were the highest respondents, followed by secondary (n=33; 12.3%) and primary (n=10; 3.6%) education respectively (Tables 1 & 2).

Table 1: General characteristics of staff in the study.

| Characteristics          | Male N = 95 N (%) | Female N = 180 N (%) | Total N = 275 N (%) |
|--------------------------|-------------------|----------------------|---------------------|
| Age of Respondents (Years) |                   |                      |                     |
| < 20                     | 0                 | 5 (1.8)              | 5 (1.8)             |
| 20 - 29                  | 18 (18.9)         | 56 (31.1)            | 74 (26.9)           |
| 30 - 39                  | 43 (45.3)         | 61 (33.9)            | 104 (37.8)          |
| 40 - 49                  | 21 (22.1)         | 37 (20.6)            | 58 (21.1)           |
| 50 - 59                  | 13 (13.7)         | 21 (11.7)            | 34 (12.4)           |
| Total                    | 95 (100)          | 180 (100)            | 275 (100)           |
| Marital Status           |                   |                      |                     |
| Single                   | 33 (34.7)         | 63 (35.0)            | 96 (35.0)           |
| Widowed                  | 2 (2.1)           | 8 (4.4)              | 10 (3.6)            |
| Married                  | 60 (63.2)         | 109 (60.6)           | 169 (61.4)          |
| Highest Level of Education |                 |                      |                     |
| Primary                  | 6 (6.3)           | 4 (2.2)              | 10 (3.6)            |
| Secondary                | 10 (10.5)         | 23 (12.8)            | 33 (12.3)           |
| Tertiary                 | 79 (83.2)         | 153 (85)             | 232 (84.4)          |
| Total                    | 95 (100)          | 180 (100)            | 275 (100)           |

Note: Mean age; Median age 37.2±8.9 35.5±9.8 36.1±9.6

Four (1.5%) out of the two hundred and seventy five respondents were HBsAg seropositive. The female gender recorded the highest prevalence rate (3/180; 1.7%) compared to male counterpart (1/95; 1.0%), but this was not statistically significant (p>0.05) (Figures 1 & 2). The age groups <20 and 40-49 years have the highest prevalence rates of 1(20.0%) and 2(3.5%) respectively. There was no HBV infection recorded in the age groups 30-39 and 50-59 years. The highest prevalence rate of HBV was found in respondents with secondary (1/33; 3.0%) compared to tertiary (3/233; 1.2%) education (Table 2).

The clinical variables with the highest prevalence rates of HBsAg were previous blood transfusion 1(6.7%) and vaginal discharge 1(6.2%) followed by previous intravenous drug use 1(1.3%) and surgery 1(1.2%) respectively. However, these were not statistically significant. There was no HBsAg seropositive respondent with a past history of STI, hepatitis and scarification/tattoo marks from this study (Table 3).

Discussion

In our study, females were the predominant respondents, constituting 180(65.5%), with male: female ratio of 1:1.9. This is at variance with the ratio of 1.3:1 reported by Sirisena, et al. [7] in a similar study in an urban community in Jos, but similar to a report from Turkey where 62.7% were female donors while 37.3% were male [26]. The gender ratio of this study represents apparently healthy individuals who voluntarily turned up for the survey. It is not a good representation of the male:female ratio.
in blood donation in Nigeria. Majority of our respondents were married healthy adults, between 30 to 49 years with mean age ± SD of 36.1 ± 9.6 years. This agrees with mean age of 32.58 ±10.24 reported by Buseri, et al. [27].

Table 2: Socio-demographic characteristics by HBsAg status.

| Variable          | HBsAg Positive n = 4 N (%) | HBsAg Negative n = 271 N (%) | Total | FT  | p value |
|-------------------|-----------------------------|-------------------------------|-------|-----|---------|
| Sex               |                             |                               |       |     |         |
| Male              | 1(1.1)                      | 94(98.9)                      | 95(34.5) | 0.16 | 1.0     |
| Female            | 3(1.7)                      | 177(98.3)                     | 180(65.5)|     |         |
| HIV               |                             |                               |       |     |         |
| Negative          | 2(1.0)                      | 202(99.0)                     | 204(74.2) | 4.29 | 0.21    |
| Positive          | 0                           | 25(100)                       | 25(9.1)      |     |         |
| Unknown           | 2(4.3)                      | 44(95.7)                      | 46(16.7)      |     |         |
| Level of Education|                             |                               |       |     |         |
| Primary           | 0                           | 10(100)                       | 10(3.6)      | 1.66 | 0.5     |
| Secondary         | 1(3.1)                      | 32(96.9)                      | 33(12.0)      |     |         |
| Tertiary          | 3(1.3)                      | 229(98.7)                     | 232(84.4) |     |         |
| Marital Status    |                             |                               |       |     |         |
| Single            | 2(2.1)                      | 94(97.9)                      | 96(35.0) | 2.23 | 1.0     |
| Married           | 2(1.2)                      | 167(98.8)                     | 169(61.5)     |     |         |
| Widowed           | 0                           | 10(100)                       | 10(3.5)      |     |         |
| Age Group (Years) |                             |                               |       |     |         |
| <20               | 1(20.0)                     | 4(80.0)                       | 5(1.8)       | 0.02 |         |
| 20-29             | 1(1.4)                      | 73(98.6)                      | 74(26.9)      |     |         |
| 30-39             | 0                           | 104(100.0)                    | 104(37.44)    |     |         |
| 40-49             | 2(3.5)                      | 56(96.5)                      | 58(20.88)     |     |         |
| 50-59             | 0                           | 34(100.0)                     | 34(12.24)     |     |         |

Seroprevalence of HBsAg in this study was 1.5%. This value is lower than 7.5%, 15.1% and 14.3% earlier reported by Onoja, et al. [28], Egah, et al. [29], and Uneke, et al. [30] respectively among blood donors in Jos, North central Nigeria. It is also lower than 13.22% reported by Fasciola, et al. [31] in Ibadan, 7.50% by Salawu, et al. [32] in Ile-Ife, south western Nigeria and 5.6% reported by Okocha, et al. [33] in Nnewi, South eastern Nigeria. Findings in some Asian part of the world such as Karnataka in India showed a prevalence of 3.2% in pre-transfusion blood donors [34]. However, this value was in keeping with 1.7% reported by Arora, et al. [35] in Southern Haryana and higher than 0.3% reported by Nwokeolu, et al. [36] among blood donors in Umuahia, South eastern Nigeria. These findings are in keeping with previous observations that in Nigeria, the prevalence of HBsAg seropositivity increase as one migrates from the south to the North though the reason is yet to be clearly understood.[37,38] This, therefore, calls for an in-depth study of the characteristics of the different serotypes and genotypes of HBV found in the various geopolitical zones of Nigeria.

The study showed that prevalence of HBsAg positivity was higher in females than males. The percentages were 1.7% versus 1.1%. However, this was not statistically significant (P=1.0). Similar pattern in a similar study was obtained by Okocha, et al. [33] from Nnewi, where the prevalence was 5% and 8.5% in males and females respectively. However, in the study, the difference was statistically significant (P=0.04) while the odd ratio showed males were 0.5 times less likely to be HBsAg positive than females. Similarly, Sirisena, et al. [7] recorded a statistically higher prevalence of HBsAg in females (13.0%) compared to their male counterpart (8.2%) from their study in Jos, Nigeria.

The effect of age on the prevalence of HBV infection was remarkable in this study. It showed a reduction in the prevalence of HBsAg with advancing age with the highest in those aged <20 years (20%) and the least in those above 50 years (0%). This relationship was found to be statistically significant (P=0.02). This finding was similar to that obtained by Okocha, et al. [33] where they found a decreasing prevalence of HBsAg positivity with advancing age (i.e, 18.6% in those <20 years and 2.9% in
those >50 years) with a statistically P trend (P=0.005). However, this pattern tend to differ in similar study by Sirisena, et al. [7] in North central Nigeria where the prevalence tend to follow a non-uniform pattern with the highest prevalence in the age group above 60 years (21.4%) while the least prevalence was between 31-40 years (6.1%). In our study, no HBsAg seropositivity was recorded between age group of 30-39 years.

Table 3: Distribution of clinical history among staff by HBsAg results

| Variable                        | HBsAg Positive n = 4 N (%) | HBsAg Negative n = 271 N (%) | Total     | FT  | p value |
|---------------------------------|---------------------------|------------------------------|-----------|-----|---------|
| Past History of STI             |                           |                              |           |     |         |
| No                              | 4(1.5)                    | 268(98.5)                    | 272(98.9) | 0.05| 1.0     |
| Yes                             | 0                         | 3(100)                       | 3(1.1)    |     |         |
| Past history of Vaginal Discharge|                           |                              |           |     |         |
| No                              | 3(1.2)                    | 256(98.8)                    | 259(94.2) | 2.73| 0.21    |
| Yes                             | 1(6.2)                    | 15(93.8)                     | 16(5.8)   |     |         |
| Past History of Genital Ulcer   |                           |                              |           |     |         |
| No                              | 4(1.5)                    | 267(98.5)                    | 271(98.5) | 0.06| 1.0     |
| Yes                             | 0                         | 4(100)                       | 4(1.5)    |     |         |
| Past History of Dental/Surgery  |                           |                              |           |     |         |
| No                              | 3(1.5)                    | 192(98.5)                    | 195(70.9) | 0.03| 1.0     |
| Yes                             | 1(1.2)                    | 79(98.8)                     | 80(29.1)  |     |         |
| History of IV Drug Use          |                           |                              |           |     |         |
| No                              | 3(1.5)                    | 196(98.5)                    | 199(72.4) | 0.01| 1.0     |
| Yes                             | 1(1.3)                    | 75(98.7)                     | 76(27.6)  |     |         |
| History of Scarification/Tattoo |                           |                              |           |     |         |
| No                              | 4(1.5)                    | 262(98.5)                    | 266(96.7) | 0.14| 1.0     |
| Yes                             | 0                         | 9(100)                       | 9(3.3)    |     |         |
| History of Blood Transfusion    |                           |                              |           |     |         |
| No                              | 3(1.2)                    | 257(98.8)                    | 260(94.5) | 3.0 | 0.20    |
| Yes                             | 1(6.7)                    | 14(93.3)                     | 15(5.5)   |     |         |
| Past History of Contact- Jaundice|                           |                              |           |     |         |
| No                              | 4(1.8)                    | 223(98.2)                    | 227(82.5) | 0.86| 1.0     |
| Yes                             | 0                         | 48(100)                      | 48(17.5)  |     |         |
| Past History Hepatitis          |                           |                              |           |     |         |
| No                              | 4(1.5)                    | 259(98.5)                    | 263(95.6) | 0.19| 1.0     |
| Yes                             | 0                         | 12(100)                      | 12(4.4)   |     |         |
| Past History of Jaundice        |                           |                              |           |     |         |
| No                              | 4(1.5)                    | 270(98.5)                    | 274(99.6) | 0.02| 1.0     |
| Yes                             | 0                         | 1(100)                       | 1(0.4)    |     |         |

Note: FT-Fisher's exact test used when and expected cell count is less than 5.

This study showed HBsAg prevalence decreased with increasing educational level with the highest prevalence in secondary population (3.1%) while the least was with tertiary population (1.3%). Although the difference was not statistically significant, it was in keeping with similar studies in China [37-39] where epidemiological serosurvey of hepatitis B in a population of children and adult 1-59 years of age following hepatitis B immunization showed highest prevalence among illiterate population (9.7%) while undergraduate population recorded 3.1%. In the same study, a multivariable logistic regression to identify factors that will affect prevalence of HBsAg in a population identified education as one of the independent variables that can predict the HBsAg status of an individual. Other independent variables include gender, location (urban versus rural community),
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We must acknowledge the limitations encountered in carrying out this research. The fact that the studied population is a selected group of individual from health institution may be contributory to the low prevalence of HBV. Therefore, this study population cannot be assumed to reflect the epidemiology of HBV of the general public. The study recorded a too small numbers of study population (275) and positive subjects (4), hence, a larger study would be preferable in future in order to make valid conclusions about the disease’s predisposing factors. The fact that only new infections were analyzed in this study by measuring only HBsAg of the patients sera was a limitation. HBsAg is detected within the fourth and sixth weeks of incubation period. Thus, one could assume that the number of HBV-infected individuals would be higher if more hepatitis B serologic and virologic markers (such as HBeAg, Anti-HBc, Anti-HBs, Anti-HBe and HBV DNA) were used in the study. This was not possible due to the financial implications. We hope in future studies we will include these markers to produce better results.

Conclusion/Recommendations

HBV is still a public health problem globally and Nigeria is an endemic zone. Although the study size was too small to make a valid conclusion, its prevalence in the south eastern Nigeria is relatively on the decrease. However, levels of education, blood transfusion practices, sexual habits and immunization status may be strong predictors of HBV status of any given population. It is, therefore, recommended that the federal ministry of health should scale-up the awareness of HBV to the public and establish national guidelines for hepatitis B prevention and treatment in Nigeria. In this guideline/policy, they should, as a matter of urgency, remove the financial barriers to access hepatitis B immunization in both children and adult population. By so doing, hepatitis B disease burden will be reduced to the barest minimum in Nigeria.

Challenges/Limitations

We must acknowledge the limitations encountered in carrying out this research. The fact that the studied population is a selected group of individual from health institution may be contributory to the low prevalence of HBV. Therefore, this study population cannot be assumed to reflect the epidemiology of HBV of the general public. The study recorded a too small numbers of study population (275) and positive subjects (4), hence, a larger study would be preferable in future in order to make valid conclusions about the disease’s predisposing factors. The fact that only new infections were analyzed in this study by measuring only HBsAg of the patients sera was a limitation. HBsAg is detected within the fourth and sixth weeks of incubation period. Thus, one could assume that the number of HBV-infected individuals would be higher if more hepatitis B serologic and virologic markers (such as HBeAg, Anti-HBc, Anti-HBs, Anti-HBe and HBV DNA) were used in the study. This was not possible due to the financial implications. We hope in future studies we will include these markers to produce better results.

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