Surveillance of Hepatitis B biomarkers in Borno state, Nigeria

Background: Hepatitis B virus infection is a major global health problem of public health importance. The World Health Organization (WHO) African Region has reported an approximately 100 million people with chronic hepatitis B virus (HBV) infection and Nigeria has been classified as endemic.

Methods: A total of four hundred and ten (410) sera were collected from patients attending various hospitals in Borno state. A self-structured questionnaire was used to obtained demographic data which included age and sex, social status (e.g. trader, farmer, civil servant or student), pregnancy status, blood donor type. Serum samples were obtained for qualitative detection of HBsAg using rapid chromatographic immunoassays with test kits from ABON (China) having sensitivity, specificity and accuracy of 99%, 97% and 98.5% respectively.

Result: This is a first-phase result of a surveillance of hepatitis B biomarkers in Borno state Nigeria. The proportion of male (248:60.5%) was higher than female (162:39.5%). Out of the 410 samples assayed, overall prevalence of 7.1%, 2%, 0%, 47.1% and 45.6% for HBsAg (Hepatitis B surface antigen), HBsAb (anti-HBs), HBeAg (Hepatitis B envelop antigen), HBeAb (Anti-HBe) and HBcAb (Anti-HBcore) respectively were obtained. A total of 24.1% (7/29) of the HBsAg positives were negative for all other biomarkers suggesting new cases in the acute phase. The later comprise of 2 pregnant women, 1 blood donor, 3 farmers and 1 civil servant. Sixteen (3.9%) of the 410 samples were negative for all the biomarkers. Of these, 11(68.8%) were pregnant women, 1 private blood donor and 2 students and 2 civil servants. Gender-based prevalence of HBsAg among male was 8.5% (21/248) and 4.9% (8/162) among female. Out of 29/410 (7.1%) HBsAg positives, 89.7% (26/29) were between 15–34 years old. Out of 150 blood donors, the prevalence of HBsAg was 8.7% (13/150) while among 50 pregnant women it was 8% (4/50). HBsAg positivity proportion was (10/29) among students; civil servants (7/29); traders (6/29) and farmers (6/29).

Conclusion: Hepatitis B infection continues to be a public health hazard due to existence of susceptible individuals and emerging new cases as observed in this study. Therefore, routine vaccinations at all levels should be invigorated while continuous surveillance of the infection is maintained until population immunity can be achieved.

Key words: Surveillance, biomarkers, blood donor, pregnant women, social status, Borno state.

Introduction

According to World Health Organization (WHO) report, about 100 million persons in African Region have chronic hepatitis B virus (HBV) infection, and all countries in the Region have an intermediate (2%–7%) or high (≥ 8%) population prevalence of chronic HBV infection (WHO, 2016). Individuals with chronic infection have a 15%–25% estimated lifetime risk of developing liver cancer or cirrhosis, dependent upon age at infection (WHO, 2016). An estimated 100 million people are chronic carriers of HBV in Africa with the carrier rate ranging from 9% to 20% in sub-Saharan Africa (Ott *et al.,* 2012; WHO, 2015). About 70%–90% of infants infected before 1 year of age will develop chronic HBV infection, compared with 20%–50% of those infected between 1-5 years of age, and with 5%–10% of those infected after 5 years of age (Breakwell *et al.*, 2017). About 90% of babies born to hepatitis B surface antigen (HBsAg) positive (a serologic marker of chronic HBV infection) and hepatitis B e antigen (HBeAg) positive (a marker of infectivity) mothers become chronically infected, compared with about 35% of babies born to HBeAg-negative chronically infected mothers (Breakwell *et al.,* 2017).

Hepatitis B virus is commonly spread by percutaneous or mucosal exposure to infected blood and other body fluids with numerous forms of human transmission. The virus has been detected in peripheral mononuclear cells, tissues of pancreas, spleen, kidney and skin, faces, and fluids such as saliva, semen, sweat, breast milk, tears, urine, and vaginal secretion (Aba and Aminu, 2016).

Clinically, HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, are the important markers in the diagnosis of Hepatitis B virus infection. HBsAg is the first marker to appear in the blood in acute hepatitis B, being detected 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms. Simultaneous with or shortly after the disappearance of HBsAg, antibody to HBsAg, (HBsAb) is found in the blood. Its appearance heralds complete recovery, and its presence provides lifelong immunity (Forbi *et al*., 2008; Brooks *et al*., 2010).

Antibody to HBcAg (HBcAb or anti-HBc) appears shortly after HBsAg, , roughly at the time that serum ALT begins to rise. Anti-HBc also remains elevated for life and is a useful marker for previous HBV infection. HBcAg itself does not circulate freely in the serum of such infected persons. HBeAg is seen in the blood before the onset of clinical disease and after the appearance of HBsAg. It generally disappears within about 2 weeks, while HBsAg is still present. Anti-HBe appears shorly after the disappearance of the antigen and detectable for up to 2 years or more after resolution of the hepatitis. The presence of HBeAg in the serum correlates with a period of intense viral replication and hence maximal infectivity of the patient (Willey *et al*., 2011).

Although there is a safe and effective vaccine for HBV infection, Nigeria continues to be a hyperendemic area for HBV infection (Ugwuja, 2010) because new cases of HBV are still being reported annually (Agbede, 2007; Baruch, 2015). This makes HBV infection in Nigeria still a public health issue (Akani, 2005). Hence, there is need for continuous surveillance of the infection in every part of the country.

Therefore, the objectives of this study were to determine (i) the prevalence of hepatitis B infection (ii) the immune status to hepatitis B among patients (iii) the susceptibility rate, and finally (iv) to detect hepatitis B genome using molecular technique. However, the result of the first three objectives is presented herein.

2.0 Materials and Methods

2.1 Study area

This research was carried out in hospitals in northern and southern Borno State, Nigeria. In the south, the town/villages covered were Biu, Shaffa, Yimirshika and Mirghan. While in the north, it was majorly Maiduguri, which is also called Yerwa by locals, and lies on the geographical coordinates of 11° 50' 42" N, 13° 9' 35" E. It is the capital and the largest city of Borno State in north-eastern Nigeria. The city sits along the seasonal Ngadda River which disappears into the Firki swamps in the areas around Lake Chad. The indigenes are predominantly Kanuri by tribe.

2.2 Ethical clearance

Ethical approval for the study was obtained from the Ethical Board of the hospitals.

2.3 Study design

A cross-sectional, hospital –based design was utilized.

2.4 Exclusion criteria

Any patient who decline consent was excluded from the research.

2.5 Inclusion criteria

Any patient who gave consent was included in the research.

2.6 Specimen Collection and Processing

Three milliliter (3mls) of blood sample was aseptically collected by venipuncture from patients. The blood samples were transferred into clean plain tubes and allowed to clot at room temperature before centrifuging at 300rpm for at least 5 mintues. The serum which was separated from the whole blood was aseptically aspirated into a labeled sterile container and kept or stored frozen at -20^{o}c until needed.

2.7 Assay Procedure

The HBV 5-Parameter rapid test device was used. All materials and specimen (serum) were brought to room temperature, and the test device was removed from the sealed foil pouch. The test device was placed on a flat horizontal surface and was labeled with specimen identity number. Pipette dropper was used to draw up the sample and holding it vertically, 2–3 drops (60-90µl) of sample were dispensed into each sample well. The result was read at 15 min after adding the sample. Interpretation was according to manufacturer's instruction.

3.0 Results

3.1 Prevalence of Hepatitis B biomarkers

The result of analysis of four hundred and ten (410) samples show a varying prevalence of 7.1%, 2.0%, 0%, 47.1% and 45.6% for HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, respectively. Seven (24.1%) of the 29 HBsAg positive were negative for all other biomarkers suggesting they

are new cases in acute phase. A total of 16(3.9%) samples were negative for all the biomarkers and are therefore susceptible to infection with hepatitis B virus (Table 1).

Table 1: Prevalence of Hepatitis B biomarkers in Borno State, Nigeria

	Hepatitis B Biomarkers				
Overall Prevalence	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb
Number of positive	29	8	0	193	187
Prevalence (%)	7.1	2	0	47.1	45.6
Susceptible cases					
Number of negative	16	16	16	16	16
Prevalence (%)	**3.9**				
New cases					
Number of positive	7/29	0	0	0	0
Prevalence (%)	24.1	0	0	0	0

3.2 Gender-based distribution of Hepatitis B biomarkers

Figure 1 presents stratification of the 410 samples. It shows 248 male and 162 female. The prevalence of HBsAg among male was 8.5% (21/248), less than one percent (0.8%: 2/248) were positive for HBsAb while 36.3% (90/248) was positive for HBcAb (evidence of past exposure). Out of the 162 female, 8(4.9%) were HBsAg positive while 3.7% (6/162) expressed protective antibody i.e HBsAb. More than half (53.1%: 86/162) of the female had HBcAb suggesting previous exposure to hepatitis B virus.

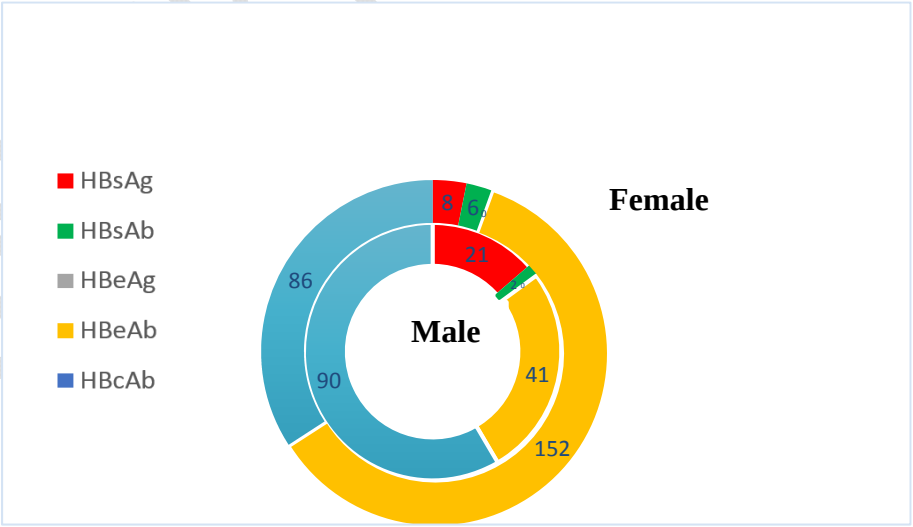

Figure 1: Number of Positive Hepatitis B Biomarkers based on Gender

3.3 Proportion of Hepatitis B Biomarkers according to age

The distribution of hepatitis B biomarkers is presented in Figure 2. Thirteen (13) each, of those between 15–24 years and 25–34 years were positives while 2 who fell within 45–54 years and 1 among those 55-64 years were positive. None of those in the 35–44 years was positive. In addition, the proportion of those HBcAb positive was observed to decline with increase in age: 15–24 years (74), 25–34 (69), 35–44 years (19), 45–54 years (18) 55-64 years (7). The proportion of those observed to have protective antibody (HBsAb) varied with age. However, HBsAb was observed to be absent among those in 45–54 years and 55-64 years.

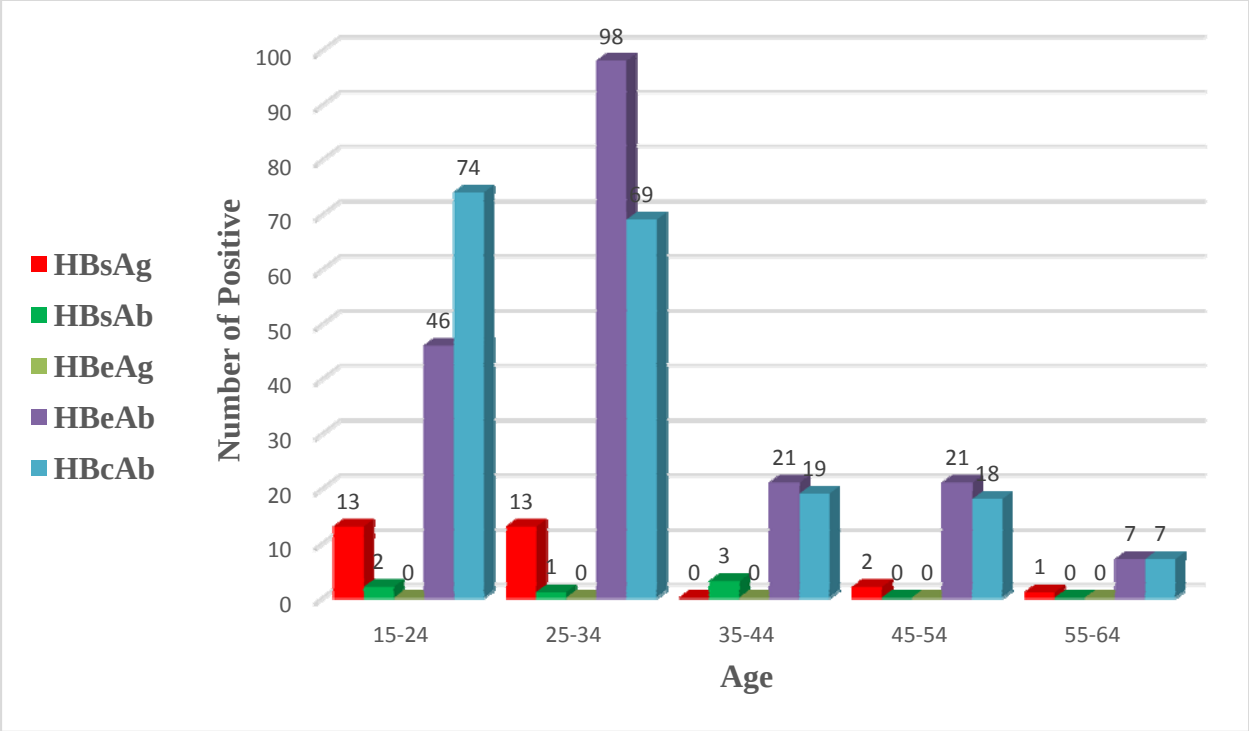

Figure 2: Distribution of Hepatitis B Biomarkers according to Age in Borno State, Nigeria

3.4 Hepatitis B Biomarkers among Blood donor

The 150 blood donors in this study were stratified into either private or commercial donors (Figure 3). The prevalence of HBsAg was 8.7% (13/150) among this cohort. None of the donors had protective antibody (i.e. HBsAb) while the proportion of HBcAb was higher (40) among private donors than commercial donors (21). One (1) male private donor was found to be susceptible to HBV infection (i.e. seronegative for all biomarkers).

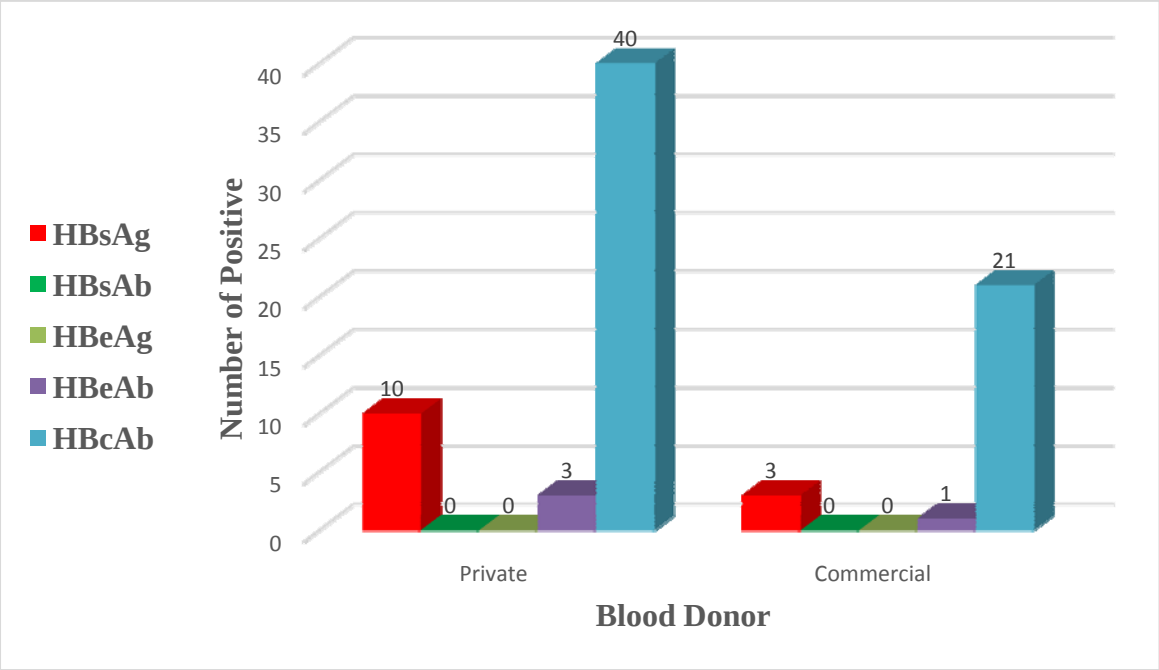

Figure 3: Hepatitis B Biomarkers distribution among Blood Donors in Borno State, Nigeria

3.5 Distribution of Hepatitis B Biomarkers in Pregnant women

The 410 samples in this study comprised of 50 pregnancy women in different trimester (Figure 4). The prevalence of HBsAg in this cohort was 8% (4/50). None of those in the 2nd trimester tested positive for HBsAg. However, it is important to report that 62.5% (5/8) of those susceptible to hepatitis B virus infection were pregnant women in different trimester (2 each in the 1st and 2nd trimester and 1 in the 3rd trimester).

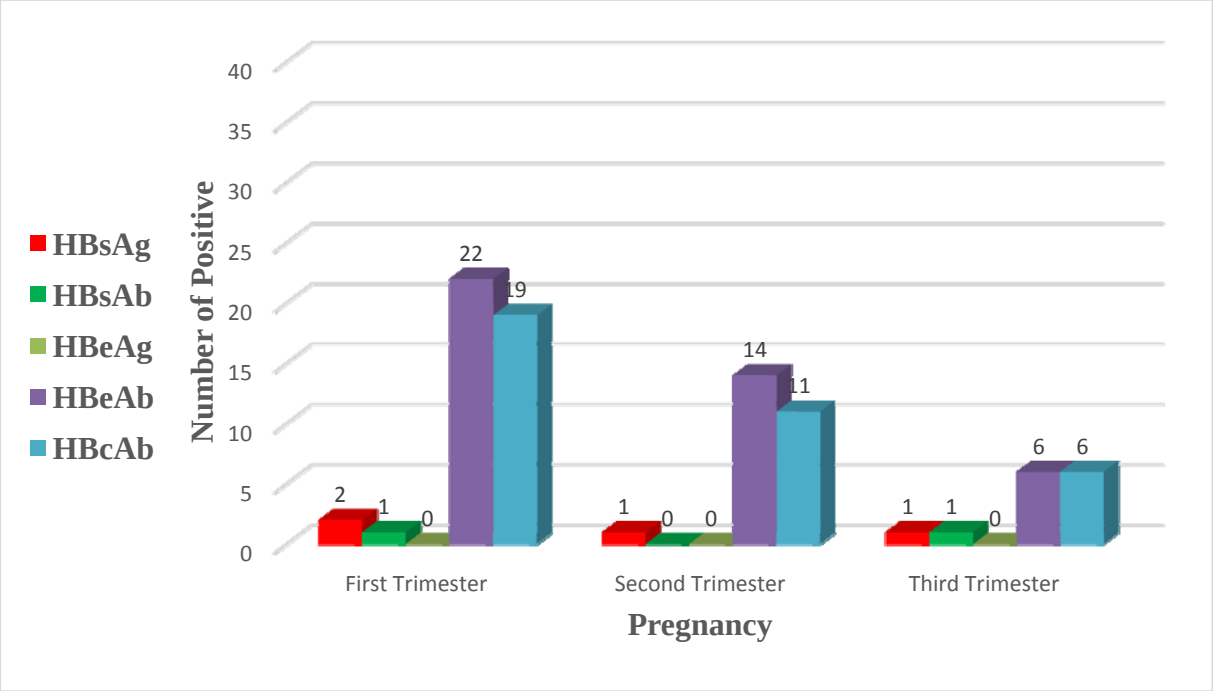

Figure 4: Distribution of Hepatitis B Biomarkers based on Trimester of Pregnancy

3.6 Distribution of Hepatitis B Biomarker according to Social Status

Based on social status stratification, the 29 HBsAg positive in this study was distributed among traders, farmers, civil servants and students in 6, 6, 7 and 10 proportions respectively. Students had the highest proportion (56) of HBcAb followed by traders (49), farmers (46) and finally civil servants (36). Worthy of note is the fact that one female civil servant and one male farmer were among the 16 susceptible to hepatitis B virus infection in this study (Figure 5).

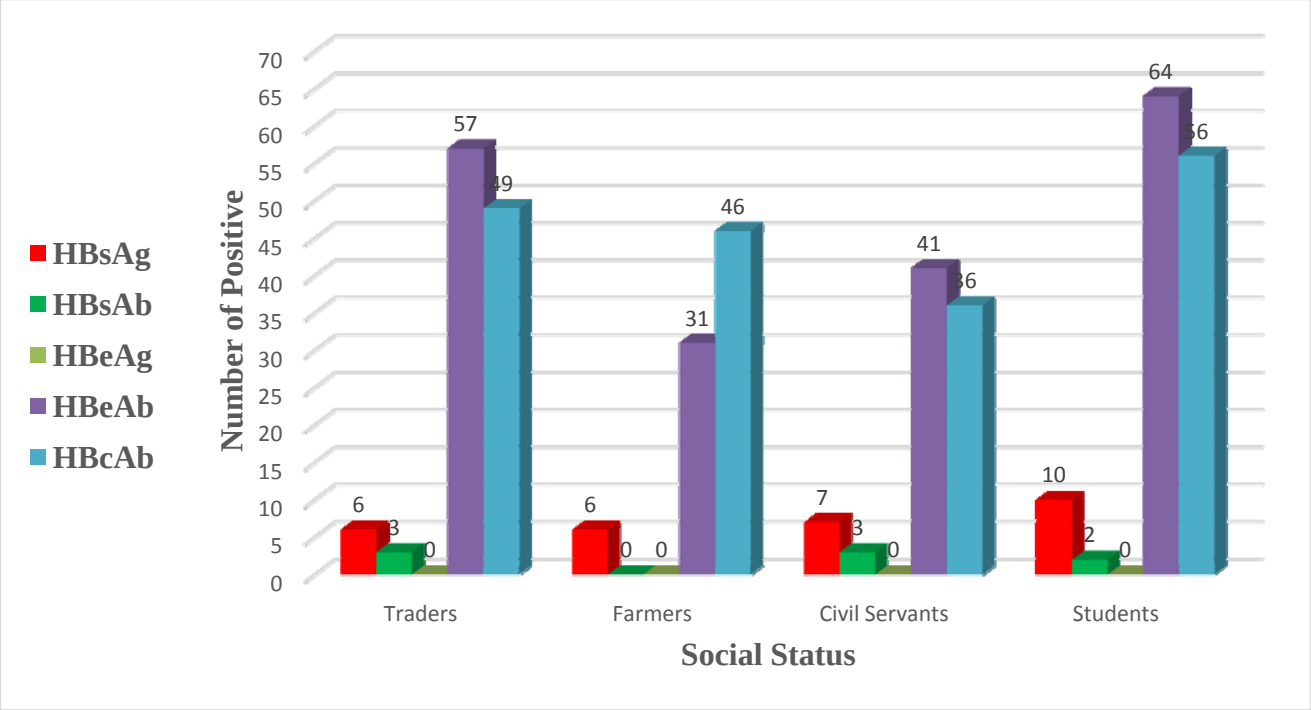

Figure 5: Distribution of Hepatitis B Biomarkers according to Social Status

Discussion

The prevalence of 7.1% HBsAg obtained in this study corroborates the classification of Nigeria as endemic for hepatitis B infection (Ugwuja, 2010; Musa *et al.,* 2015) According to Baruch (2015), 10-30 million people will become newly infected each year and approximately 2 people die each minute from hepatitis B. Hence, 24.1% (7/29) of the HBsAg positive new cases in the acute phase of infection shows that this scourge is in circulation among the population and lends credence to later assertion. The reasons for the emerging new cases is attributable to the existence of susceptible individuals yet among the populace; either unwillingness to submit to or poor vaccination coverage in the study area. Yet, Hepatitis B vaccine is 95% effective in preventing infection and its chronic consequences (WHO, 2001)

The prevalence of HBsAg among pregnant women in this study was 8%. This is similar to 8.7% obtained in a previous study in Maiduguri (Oyinloye *et al.,* 2016) and 8.3% in Zaria (Luka *et al.,* 2008) but more than 4.3% and 3.9% in Port Hacourt and Zaria respectively (Akanni *et al.,* 2005 and Abah and Aminu, 2016). In this study, 16(3.9%) patients were negative for all the biomarkers which infer they are susceptible to infection by hepatitis B virus. This has significant public health implication because 68.8% of these susceptible patients were pregnant women (5 in first trimester, 3 each, in second and third trimester). When HBV infection occurs in the first or second trimester, about 10% of the neonates are affected by vertical transmission, and when it does occur in the third trimester, a higher percentage of 60–90% of the children may have the infection by vertical transmission (Nongo *et al.,* 2016). Also, according to WHO (2016) more than 686,000 people die every year due to complications from hepatitis B, including cirrhosis and liver cancer. Therefore, if any of these, especially those in the third trimester, were to contract the virus and the infection allowed to progress, there are chances of vertical transmission of the virus with the attendant fatal sequelae in the foetus.

In this study, we observed that 89.7% (26/29) of HBsAg positive individuals were between 15–34 years old. First, the reason for this observation may be because individuals in this group are sexually active therefore sexual promiscuity, marrying and remarrying and other vices might be high. This reason is corroborated by Ipinmisho, (2016) who reported that "In a country like Nigeria, HBV transmission occurs mostly during adolescence or young adulthood as a result of precarious and unprotected sexual activities". Secondly, high risk predisposing activities such as body incision, tattooing, self intravenous drug administration, exposure to needle prick/sharp object injury are rife among individuals in this age group. All the aforementioned predispose to viral infection and therefore may be responsible for the observed high proportion of the biomarker. This assertion also agrees with a report by Boakye, (2014) that drug users, people that pierce and tattoo their bodies and engage in unprotected sex without adequate knowledge about sexual negotiation and safe sex practices have a higher risk of getting infected with hepatitis B virus.

The prevalence of HBsAg among blood donor was 8.7% (13/150). This is less than 11.6% and 14.9% observed in previous studies in Maiduguri and Yola respectively (Harry et al. 1994 and

Olokoba *et al.,* 2009). These HBsAg positive individuals as well as the private blood donor that was found to be susceptible to infection being negative to all biomarkers are of dual clinical significance. First, if the HBsAg positive blood had been inadvertently transfused without screening (which is possible in some remote primary health centres) that would lead to horizontal transmission of the virus. Secondly, even the HBsAg negative blood cannot be considered absolutely negative until analysis shall confirm such blood were HBsAg DNA negative; this is because such HBsAg negative blood might just be in the window period of infection. Yet, blood is hardly screened for HBsAg DNA before transfusion in this part of the world.

In this study, an overall prevalence of 7.1% HBsAg across different cohorts, 2% anti-HBs rate, 24.1% new cases of hepatitis and 3.9% prevalence of susceptible individuals have been reported. This result will complement those of previous research that have been conducted in Nigeria with the view to establishing a robust data base which may serve to highlight the immune status of the populace thus acting as an impetus to invigorate existing vaccination policy in order to stem the rising tide of hepatitis B infection sequelae in Nigeria and Borno state in particular. Furthermore, this result has also highlighted prevalence of each biomarker of hepatitis B in Borno state, Nigeria.

Financial sponsorship

This work was sponsored by a Research Grant from Tertiary Education Trust Fund (TetFund) through the Centre for Research and Innovation, University of Maiduguri via a letter: Ref. No. TETFUND/DESS/UNIMAID/MAIDUGURI/RP/VOL II

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