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Hepatitis B Vaccination Outcomes and Broad Spectrum of Hepatitis B Infections in Nigeria: An Evidence-Based Picture

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Abstract

Background: Current evidence-based data on Hepatitis B vaccination coverage and outcomes in Nigeria are limited and these raise serious concern among stakeholders in clinical settings with respect to prevention of hepatitis B and safety of transfusion recipients. This study evaluated hepatitis B vaccination coverage and outcomes among apparently healthy blood donors in Ekiti State, Nigeria. Methods: Hepatitis B viral markers, including hepatitis B surface antibody (HBsAb), were serologically screened in four hundred and seventy prospective blood donors using NOVA 5-in-1 HBV rapid one-step multi-test kit and the results were interpreted using descriptive statistics of SPSS version 21. Results: Out of the four hundred and seventy (470) blood donors screened, 85 (18.1%) and 385 (81.9%) were vaccinated research participants and unvaccinated research participants (VRP and UVRP) respectively. Male: Female ratio and mean age (±SD) of VRP were 1:1 and 26.5 ± 6.8 while those of the UVRP were 1.6:1 and 26.9 ± 7.7. Evidence of successful vaccination was observed in 2.55% and 4.68% of VRP and UVRP respectively. Broad spectrum HBV infection totaling 11.05% based HBV markers seroprevalence was observed among the UVRP compared to the VRP with 0.85% seroprevalence. Conclusion: Low hepatitis B vaccine coverage among blood donors poses serious threats to public health including recipients of blood transfusion and hepatitis B vaccination programme should be re-strategized to cover the grass roots. Low HBV infection among the vaccinated research participants compared to the unvaccinated group showed long-term protection despite HBsAb loss. Post-vaccination testing is recommended for specific categories of individuals.

Keywords: Hepatitis B Virus, Vaccination, infections, Broad spectrum, Blood donors, Evidence-based picture

1. Background

Vaccination against hepatitis B virus (HBV) infection has been proved to be the sure way to courtail the scourge of the silent killer viral infection [1-5]. Hepatitis B has consistently remained a challenging and global health problem over the years affecting 2 billion people worldwide with 350 million individuals having chronic hepatitis B infections in spite of the availability of effective vaccination programmes for infants and adults due to multi-factorial obstacles [6-7]. Studies have reported that an estimate of more than 1 million people die on yearly basis while nearly 2 people die each minute from HBV infection[6, 8-9]. A global data showed that annually, a minimum of 27 million children do not have access to basic package of HBV vaccine and close to a quarter of children under five years' mortality is due to vaccine preventable infectious diseases including hepatitis B [10]. Published findings have showed liver cirrhosis, hepatocellular carcinoma and other liver-related disorders to be the dire consequences of hepatitis B infection since the first report [11-17]. A highly effective and inexpensive hepatitis B recombinant DNA vaccine has been available since 1982 following first issued recommendations by the Centers for Disease Control and Prevention (CDC) which were not implemented in Nigeria until 1995. All infants beginning at birth, children and adolescents not previously vaccinated and unvaccinated adults at risk for hepatitis B infection were the recommended target recipients [4]. In Nigeria, several authors have demonstrated the positive impacts of HBV vaccine initiatives on the prevention of chronic hepatitis B virus infection since implementation [7, 17-21]. The discovery and licensing of the HBV vaccine and subsequent establishments of vaccination programmes have led to great breakthroughs in the fight against the scourge of hepatitis B infections in high endemic regions including the prevention of vertical transmission of the virus through infected mother [17] and inadvertent transmission through blood donors [13].

Previous literatures reported that vaccinees who received complete (three) doses of HBV vaccine demonstrated evidence of optimal immune protection compared to those who had incomplete doses (single or two doses). Maximum immune protection is demonstrated by the detection of hepatitis B surface antibody or HBsAb greater than or equal to ten international unit per litre (≥ 10 IU/L) [22]. Immunization of infants at birth, older children and adults against hepatitis B has been through the administration of either plasma-derived or recombinant vaccine. Previous studies from the United States of America showed that infants who received HBV vaccine demonstrated up to twenty to thirty years of immune protection against chronic hepatitis B virus infection (i.e. HBsAb level ≥ 10IU/L) [23]. Thus, many of the adults (blood donors inclusive) with evidence of immune protection who are not recently vaccinated or unaware of their vaccination status have been vaccinated against hepatitis B infection from birth.

Most of the pre-donation or post-donation HBV screening done on blood donors' samples and commonplace in Nigerian Health Institutions is based on hepatitis B surface antigen (HBsAg) detection thus failing to identify truly vaccinated and unvaccinated subjects[24-25]. This lack of evidence-based data on vaccination status of prospective blood donors (research participants or RP) often leads to wrong counselling and recommendation of vaccination to already protected donors and those with occult or chronic hepatitis B who are HBsAg-negative. This has often resulted in unnecessary associated expenditures, false security on immune protection while the silent disease is progressing towards chronicity and consequent cirrhosis or hepatocellular carcinoma. We hypothesized that there might be research participants who fell into the category of voluntary non-remunerated blood donors (VBD), replacement blood donors (RBD) and paid blood donors (PDBD) with evidence of successful vaccination and natural immunity, and others with acute, occult or chronic hepatitis B infections for whom HBV vaccination is no longer necessary. This study is meant to provide evidence-based data on vaccination status of research participants, HBV markers (hepatitis B surface antigen or HBsAg, hepatitis B surface antibody or HBsAb, hepatitis B envelope antigen or

HBeAg, hepatitis B envelope antibody or HBeAb, and hepatitis B core antibody total or HBcAb) seroprevalence among vaccinated and unvaccinated blood donors to assess the success of vaccination programmes in prevention of hepatitis B infections in Ekiti state, Nigeria.

2. Materials and Methods

2.1 Sample collection

Following written informed consent obtained from the prospective blood donors, about five millilitres (5mL) of whole blood samples were collected into tripotassium ethylene diamine tetra-acetic acid (K_3EDTA) blood collection tubes from four hundred and seventy apparently healthy blood donors between August, 2014 and November, 2015. Immediately, plasma samples separated from whole blood into plain containers were used for serologic analysis.

2.2 Study location

Study was carried out at the blood transfusion laboratory of the Federal Teaching Hospital, Ido Ekiti. Federal Teaching Hospital is a tertiary health institution strategically located at the heart of Ido/Osi Local Government and serving over 2.7 million people of Ekiti state as well as the neighbouring states (Ondo, Kwara, Osun and Kogi states).

2.3 Ethical Clearance

Ethical clearance for the study was obtained from the Ethics and Research Committee of the Federal Teaching Hospital, Ido Ekiti, Nigeria.

2.4 Administration of Questionnaires

Questionnaires were administered to blood donors to obtain essential demographic data and vaccination status.

2.5 Methodology

Serologic analysis for hepatitis B viral markers was carried out using NOVA 5-in-1 HBV multi-test rapid one-step enzyme immunoassay (EIA) kit. This is a lateral flow chromatographic enzyme immunoassay for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human serum or plasma. The HBsAg and hepatitis B envelope antigen (HBeAg) strips of the kit are antibody-based enzyme immunoassay; HBsAb (HBcAb) strips of the kit is an antigen-based enzyme immunoassay while the hepatitis B envelope antibody (HBeAb) and hepatitis B core antibody total (HBcAb) are based on the principle of competitive protein immunoassay.

Test Procedure: plasma samples following separation were labelled appropriately and corresponding numbers of HBV 260 test devices were removed from pouches. The pouches were opened at the notches and the test devices were removed and properly labelled with the assigned codes for the research participants. The cassettes were placed on a flat surface. Two to three (2-3) drops (equivalent to 60-90µl) of test samples were applied to each of the sample wells with the aid of vertically held droppers supplied with the kit. Air bubbles were avoided. The timer was started and the results were read in 15 minutes. No results were read after 15 minutes.

2.6 Assay detection Limits

Kit detection limits for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb total were 1picogram/litre (1pg/L), 30IU/L, 2 National Clinical Unit/millilitre (2NCU/mL), 2NCU/mL and 2NCU/mL respectively. .

2.7 Quality Control measures

Pre-analytical, analytical and post-analytical measures were taken to ensure accuracy and reproducibility of results. Positive and negative control samples were also run along with the test to further validate the results obtained from this study.

3. Results

3.1 Mean Age, Gender Ratio, Marital and Educational Status of Vaccinated and Unvaccinated Research Participants

Table I showed the results of the social demographic data of the research participants. Results showed that out of the four hundred and seventy research participants enrolled for the study, 85 (18.1%) and 385 (81.9%) were vaccinated research participants (VRP) and unvaccinated research participants (UVRP) respectively. The mean age and gender ratio of VRP were 26.5 ± 6.8

years and 1.2:1 while those of the UVRP were 26.9 ± 7.7years and 1.6:1. Approximately three-fifth (61.2% and 61.7% respectively) of VRP and UVRP were singles while more than one-third (37.6% and 37.1% respectively) of them were married individuals. Irrespective of vaccination status, more than 80.0% of research volunteers had tertiary education with VRP consisting of more 96.4% of them.

3.2 The Patterns of $HBsAb^+$ Seroprevalence in Research Participants Based on Vaccination Index and Blood Donor Category

Furthermore, we studied the pattern of $HBsAb^+$ seroprevalence in research participants based on vaccination index and blood donor category as shown in Table II. Based on the overall data, only 12 out of 85 VRP (2.55%) had evidence of successful vaccination as against 22 (4.68%) of the unvaccinated group (UVRP) thus yielding cummulative $HBsAb^+$ seroprevalence of 7.23%. Based on blood donor categories, of the overall 85 VRP, 78 (21.49%), 6 (8.33%) and 1 (2.86%) were VBD, RBD and PDBD respectively. Of these, 9 (2.48%), 2 (2.78%) and 1 (2.86%) of the VBD, RBD and PDBD respectively demonstrated successful vaccination/immunization. On the other hand, among the unvaccinated group based on blood donor categories, interestingly, 19 (5.23%) of the VBD, and 3 (8.57%) of the PDBD showed evidenced $HBsAb^+$ seropositivity. That resulted in cummulative HBsAb seroprevalence of 7.71% among VBD, 2.78% among RBD and 11.43% among PDBD ($p < 0.05$). Study results showed that $HBsAb^+$ seropositivity remains the only evidence-based hepatitis B marker for confirming successful vaccination in vaccinated research participants or detecting individuals with passive or active immunization through blood transfusion or immune protected mother.

Table I: Mean Age and Gender Ratio, Marital and Educational Status of Vaccinated and Unvaccinated Research Participants

Research Participants' Demo- graphic Data	**VRP**	**UVRP**
n (%)	85 (18.1)	285 (81.9)
Mean Age ± SD (Years)	26 .5 ± 6.8	26.9 ±7.7
Sex		
Male	45 (52.9)	236 (61.3)
Female	40 (42.1)	149 (38.7)
Male: Female Ratio	1.2:1	1:6
Marital Status:		
Single	52 (61.2)	231 (61.0)
Married	32 (37.6)	143 (37.1)
Divorced/Separated	1 (1.2)	7 (1.8)
Educational Status:		
None	-	3 (0.8)
Primary	1(1.2)	4 (1.0)
Secondary	2 (2.4)	68 (17.7)
Tertiary	82 (96.4)	310 (80.5)

Key: **VRP** = Vaccinated research participants; UVRP=Unvaccinated research participants
n = Absolute number **%** = Percentage

Table II: The Patterns of HBsAb+ Seroprevalence in Research Participants Based on and Vaccination Index and Blood Donor Categories

Study Variables	Research Participants	VBD	RBD	PDBD	P-value
Overall No. Screened:					
n (%)	470 (100.0)	363 (77.23)	72 (15.32)	35 (7.2)	-
VRP:					
n (%)	85 (18.09)	78 (21.49)	6 (8.33)	1 (2.86)	-
UVRP					
n (%)	385 (81.91)	285 (78.51)	66 (91.67)	34 (97.14)	-
$HBsAb^+$ Seroprevalence in VRP:					
n (%)	12 (2.55)	9 (2.48)	2 (2.78)	1 (2.86)	> 0.05
$HBsAb^+$ Seroprevalence in UVRP:					
n (%)	22 (4.68)	19 (5.23)	0 (0.00)	3 (8.57)	< 0.05
Cum. $HBsAb^+$ Seroprevalence in UVRP:					
n (%)	34 (7.23)	28 (7.71)	2 (2.78)	4 (11.43)	< 0.05

* ***P-value was obtained by Student t-test***

Key: **SD**=Standard Deviation
RBD = Replacement blood donors
$HBsAb^+$ = Hepatitis B Surface Antibody-positive
Cum. = Cummulative
n (%) = Abs. number (percentage)
VBD = Voluntary blood donors
PDBD = Paid blood donors
VRP = Vaccinated Research Participant
UVRP = Unvaccinated Research Participants

3.3 Outcomes of Vaccinated Research Participants Tested at Different Hepatitis B Virus Post-Vaccination Intervals

Outcomes of interval between HBV vaccine reception and testing for HBV markers as observed in Figure 1 showed that 30 (6.38%) of VRP were tested within one month of post-vaccination. The percentage tested declined to 1.92% at 4-6 months interval, stabilized at 2.13% within 7-11 and 12-60 months of testing and rose to 2.98% at > 60 months.

3.4 Broad Spectrum HBV Markers Seroprevalence in Unvaccinated Research Participants Compared to Vaccinated Counterparts

Seroprevalence of each pattern of HBV markers of significance implicated in HBeAg+ and $HBeAg^-$ chronic infections and detected among VRP was 0.21% each. Hepatitis B core antibody-positive ($HBcAb^+$) seroprevalence was 0.43% among VRP. That yielded a total of 0.85% of HBV seropositive markers among VRP following exclusion of 2.55% of $HBsAb^+$ which is an evidence of successful vaccination. On the other hand, among the UVRP, a total of 11.05% of HBV markers seroprevalence implicated in acute, chronic

or occult hepatitis B was found. Only 22 (4.68%) and 1 (0.21%) had evidence of successful vaccination and natural immunity (i.e. $HBsAb^+$ and $HBsAb^+HBeAb^+HBcAb^+$ respectively).

Cummulative HBV markers seroprevalence thus yielded a gross total of 15.94% among the UVRP. The data were presented in Table III. Subsequent interpretation of HBV markers seroprevalence results were made using flow-chart as shown in Figure 2.

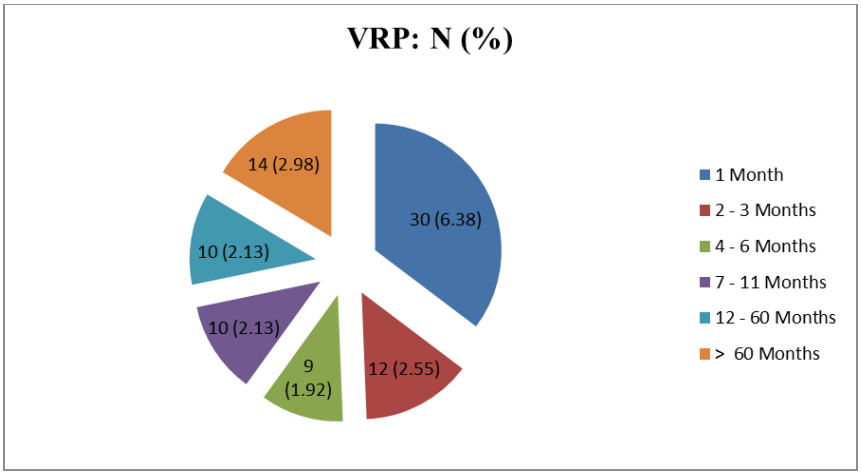

Figure 1: Absolute Numbers/Percentages of Vaccinated Research Participants Tested at Different Hepatitis B Virus Post-Vaccination Intervals

Key: VRP = Vaccinated Research Participants; n = Absolute number; % = Percentage
Overall number of VRP Tested: 85 (18.09%)

Table III: Broad Spectrum HBV Markers Seroprevalence in Unvaccinated Research Participants Compared to Vaccinated Counterparts

Patterns of HBV Markers Detected	**Vaccinated Research Participants: n (%)** 85 (18.09)	**Unvaccinated Research Participants: n (%)** 385 (81.91)
$HBsAb^+$	12 (2.55)	22 (4.68)
$HBsAg^+$ $HBeAb^+$ $HBcAb^+$	-	21 (4.47)
$HBsAg^+$ $HBeAg^-HBcAb^+$	1 (0.21)	3 (0.64)
$HBsAg^+$ $HBeAg^+$ $HBcAb^+$	1 (0.21)	1 (0.21)
$HBcAb^+$	2 (0.43)	16 (3.40)
$HBeAb^+$ $HBcAb^+$	-	4 (0.85)
$HBsAb^+$ $HBeAb^+$ $HBcAb^+$	-	1 (0.21)
$HBeAb^+$	-	1 (0.21)
$HBsAg^+$ $HBeAg^+$	-	2 (0.43)
$HBsAg^+$ $HBeAg^-$	-	1 (0.21)
$HBcAg^+$ $HBeAg^+$	-	1 (0.21)
$HBsAg^+$ $HBeAg^-$		
$HBeAg^+$	-	1 (0.21)
CHBVMSP: n (%)	16 (3.40)	75 (15.94)
ANHBVM: n (%)	69 (14.69)	310 (65.96)

KEY: Category 1: HBsAb+ Category 2: HBsAg+HBeAb+HBcAb+ Category 3: HBsAg+HBeAg- HBcAb+

Category 4: $HBsAg^{+}HBeAg^{+}HBcAb^{+}$ Category 5: $HBcAb^{+}$ Category 6: $HBcAb^{+}HBeAb^{+}$
Category7: $HBsAb^{+}HBeAb^{+}HBcAb^{+}$ Category 8: $HBeAb^{+}$ Category 9: $HBsAg^{+}HBeAg^{+}$
Category 10: $HBsAg^{+}HBeAg^{-}$ Category 12: $HBsAb^{+}HBeAg^{+}$ Category 11: $HBcAb^{+}HBeAg^{+}$
Category 13: $HBeAg^{+}$ HBVSPM = Hepatitis B virus seropositive markers % = Percentage
HBVMOP = Hepatitis B Viral Markers overall prevalence N = Absolute number + = Positive,
C.HBVSPM = Cummulative Hepatitis B Virus Seropositive markers
ANHBVM = All Negative Hepatitis B Virus Markers

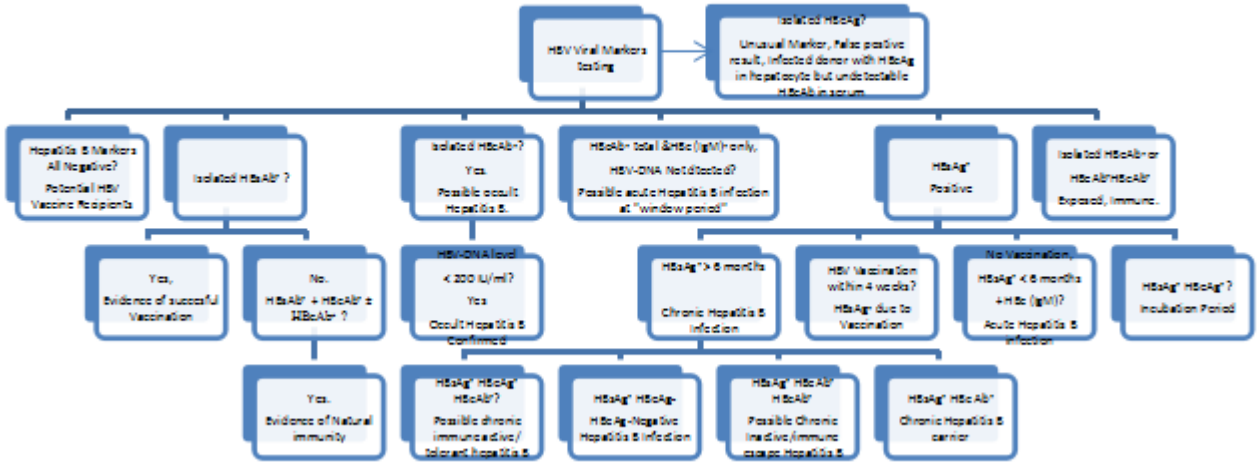

Figure 2: Flow-chart showing Hepatitis B Viral Markers Testing Outcomes and Interpretations in Vaccinated and Unvaccinated Research Participants

***Figure Legends*:**
HBsAg = Hepatitis B surface antigen HBsAb = Hepatitis B surface antibody
HBeAg = Hepatitis B envelope antigen HBeAb = Hepatitis B envelope antibody
HBcAb = Hepatitis B core antibody total + = Positive - =Negative

4. Discussion

Study finding in Table I which showed that only 85 (18.09%) of the research participants tested were VRP revealed low HBV vaccine preventable infectious disease coverage. The United Nations Children's Fund (UNICEF) and the World Health Organization estimated that only 41% of Nigerians were vaccinated against HBV in 2013 [26]. Inadequate attention and funding by the government, community rejection of immunization based on misconceptions, failure of the vaccinees to complete the three doses of the vaccine necessary for immune protection and HBV infection, religious, ethnic and cultural beliefs, fear and confusion, inadequate cold-chain management and lack of government commitment to fulfilling the Expanded Programme on Immunization (EPI) policy which targets at least 80% coverage rate have all been identified as the main obstacles to increasing HBV vaccine coverage rates in Nigeria [27-31]. Rural settings seem to be faced with pronounced challenge of limited coverage rate compared to the urban regions.

The mean age of both VRP and UVRP enrolled did not differ and that reflected the focus of the National Blood Transfusion Service (NBTS) on recruitment of targeted population of PBD for donation purpose. Study revealed increase in the number of male enrolled as VRP and UVRP compared to female counterparts as evidenced by 1.2:1 and 1.6:1 gender ratios for VRP and UVRP respectively. The fact that more than 60% of both VRP and UVRP respectively were singles while approximately 37% were married and at least 80.0% of both VRP and UVRP had tertiary education

showed the unreserved passion of younger blood donors in giving blood to save lives and the impacts of education on hepatitis B and blood donor recruitment [32]. Up to 77.23% and 21.49% of voluntary blood donors in the overall population of research participants and VRP respectively enrolled in the study in line with the advocacy of the World Health Organization on 100% voluntary non-remunerated blood donation [33-35] to minimize the risk of hepatitis B. Greater than 90% and 95% of RBD and PDBD were UVRP and susceptible to HBV infection. Irrespective of blood donor category, the number of VRP was low probably due to the fact that most research participants resided in rural settings [29]. The results of analyses showed that higher $HBsAb^+$ seroprevalence was observed among the unvaccinated group (4.68%) compared to the vaccinated group (2.55%). Studies have showed that the detection of HBsAb in VRPs' (also referred to as vaccinees) samples are evidence of successful vaccination [36-39]. In persons who recover from HBV infection, HBsAg is eliminated from the blood, usually within 3-4 months, and HBsAb develops during convalescence. The presence of HBsAb typically indicates immunity from HBV infection. However, such category of individuals who have natural recovery from HBV infection will also be $HBcAb^+$ thus distinguishing them from VRP. Previous infection or immunization with one genotype of HBV confers immunity to all genotypes. Based on blood donor categories, VRP had comparable $HBsAb^+$ seroprevalence (VBD, 2.48%; RBD, 2.71%, and PDBD, 2.86%) with no significant statistical difference ($p > 0.05$). Although we could not measure the concentrations of HBsAb in the blood donors, published data showed that HBsAb is the only easily measurable correlate of vaccine induced protection [40-41]. A previous study showed that persistence of detectable HBsAb after vaccination depends on the concentration of post-vaccination antibodies provided there is no exposure to HBV [42]. Furthermore, $HBsAb^+$ seroprevalence among unvaccinated research volunteers was higher especially among the PDBD (8.57%) and VBD (5.23%) compared to RBD (0.00%), $p < 0.05$. The blood donors might not actually know or have forgotten they had been vaccinated and had immune protection against HBV. Others might have passive immunization due to transfer of HBsAb from immune protected mother. Still, some of the blood donors might have been exposed to HBV and became immune due to development of HBsAb. Another possibility is that some of the blood donors might have passive immunization resulting from the transfer of HBsAb from transfused blood in the past [43]. Overall, the seroprevalence of HBsAb among research participants according to blood donor categories was highest among the paid blood donors (4 or 11.43%) followed by VBD (28 or 7.71%) and RBD had the least (2 or 2.78%). There is a statistical significant difference in the cummulative $HBsAb^+$ seroprevalence based on blood donor categories ($p < 0.05$). It has been shown that HBsAb seroprevalence is independent of age, sex or geographical location but rather the immune response of the vaccinees. For blood donor screening purpose, inclusion of HBsAb screening in conjunction with HBcAb screening in our routine testing protocol will help identify vaccinated blood donors and those who became immune by spontaneous natural mechanism following exposure to HBV. Through proper record-keeping, subsequent detection of HBsAb in recipients (who has never received HBV vaccines) of such transfused unit can help in surveillance study assessing passive immunization of recipients of blood transfusion through transfer of HBsAb from blood donors. Besides, introduction of HBV markers screening into public and private laboratories involved in blood donor recruitment through a well-organized and coordinated system will go a long way to capturing several Nigerians for HBV vaccination thus increasing vaccination coverage and progress towards 80.0% EPI policy.

Nearly two-third (11.71%) of VRP was tested at after ≥ 2 months of HBV vaccination, thus largely excluding the high tendency of detecting HBsAg due to HBV vaccine reception. One of the 6.38% VRP tested within one month of HBV vaccination had both HBsAg and HBsAb detected in her, showing evidence of humoral response to HBV vaccine and gradual disappearance of HBsAg. Different authors have discovered and published the possibility of mistaking VRP for confirmed HBsAg-positive blood donors especially in recently vaccinated subjects [37, 44-45]. The ideal time to perform HBsAb screening in VRP should be 2-3

months after receiving the last dose of the vaccine [39]. Three categories of vaccinees have been described by different authors based on at least one dose to complete 3 doses of HBV vaccine, namely, good responders (≥ 100 IU/L) and poor responders (< 100 IU/L) and non-responders or unimmunized (<10 IU/L). Majority of VRP were unaware of the need to have post-immunization testing done following HBV vaccination until enlightenment prior participation in this study. Outcomes of this study corroborated Tatsilong and his co-researchers' finding that up to 57% of vaccinated research participants who received three doses of HBV recombinant vaccine was unaware of the need for post-immunization testing. This was reflected by the number of VRP tested at different post-vaccination testing intervals including 12-60 months (10 or 2.13%) and > 60 months (14 or 2.98%) as shown in Figure 1. The study findings could have a notable impact on the testing outcomes should quantification of HBsAb titres be performed. Although Centre for Disease Control and Prevention gave contrary view on the need for post-vaccination testing generally, it was recommended that immunocompromised patients, those on haemodialysis, infected partners of those with HBV infection, co-infected patients with HCV or HIV, and healthcare personnel at high risk of contracting HBV through occupational exposures should have post-vaccination testing done to establish their need for possible revaccination or booster dose [39].

4.1 Evidence of Immune Protection

Furthermore, study showed that majority of VRP had evidence-based picture of immune protection despite low HBsAb seroprevalence of 2.55%. This might not be unconnected with loss of detectable HBsAb as at post-vaccination testing interval, number of doses received and immune response of VRP as well as the diagnostic device detection limit [46-47]. It has been established that immunocompetent individuals who achieve HBsAb concentrations of >10 IU/L after pre-exposure vaccination have nearly complete protection against both acute and chronic hepatitis B infection, even if HBsAb concentrations decline subsequently to < 10IU/L [47]. At first sight, the 2.55% seroprevalence of HBsAb among VRP here might imply low empirical evidence of immune protection among the vaccinated blood donors. However, there was a greater possibility of loss of HBsAb after a prolonged period. It has been reported that after primary immunization with hepatitis B vaccine, HBsAb levels decline rapidly within the first year and more slowly thereafter [47]. Another report showed that among young adults who responded to a primary vaccine series with antibody concentrations of >10 IU/L, 17%–50% have low or undetectable concentrations of HBsAb (reflecting HBsAb loss) 10 – 15 years after vaccination [48]. Regarding study results however, a more critical evaluation based on the aforementioned reports might provide reasons for low HBsAb seroprevalence in spite of possible immune protection. The diagnostic device used in this study had a detection limit of ≥ 30 IU/L and so might not detect those with lower HBsAb concentration. In other words, the commercially available serologic tests for confirming vaccination status should be improved to detect lower but protective levels of antibody produced by vaccination.

4.2 Hepatitis B vaccination Outcomes

Only 1 (0.21%) each of chronic carrier of hepatitis B (evidenced by $HBsAg^{+}HBeAg^{-}HBcAb^{+}$ detection) and chronic immune tolerant hepatitis B (as evidenced by $HBsAg^{+}HBeAg^{+}HBcAb^{+}$ detection) respectively was found among VRP in this study. Serum HBV-DNA and alanine aminotransferase levels of these two infected VRP were respectively 707 IU/mL and 15.2 IU/L; and > 27 million IU/mL and 16.9 IU/L (not shown in this study results). Also, HBcAb (total) was detected in two. Detection of HBcAb has been connected with occult hepatitis B [49]. Overall HBV markers (besides HBsAb) seroprevalence of 0.85% among VRP (and non-detection of HBV markers in 17.24% that made up the 18.09% VRP) gave an evidence of 95.3% immune protection. That was higher than 80.0% vaccine efficacy reported by Odusanya and co-researchers in their study [30].

On the other hand, UVRP showed evidence of broad spectrum of HBV markers seroprevalence reflecting the different phases of HBV infection. Based on the array of combination of HBV markers

seroprevalence among the UVRP, a cummulative 5.96% hepatitis B surface antigenaemia was observed compared to 0.42% among the vaccinated counterparts (i.e. a 14-fold HBsAg seroprevalence or evident overt hepatitis B). This was more than two times the 6-fold hepatitis surface antigenaemia among unvaccinated children compared to the vaccinated group reported by Odusanya et al (2005) [30]. Among the UVRP, other eleven patterns of HBV markers seroprevalence (totalling 11.05%) were detected through this study besides the specific patterns found in successfully vaccinated participants (evidenced by $HBsAb^+$) and another with natural immunity ($HBsAb^+HBeAb^+HBcAb^+$) which constituted the remaining 4.89%. The eleven patterns of HBV markers seroprevalence in UVRP included 4.47% of $HBsAg^+HBeAb^+HBcAb^+$ (possible chronic inactive/immune escape hepatitis B depending on whether HBV-DNA level is < 2000IU/mL or > 2000IU/mL and the serum alanine aminotransferase (ALT) is normal or elevated) [50]; 0.64% of $HBsAg^+HBeAg^-HBcAb^+$ (HBeAg-negative chronic carriers); 0.21% of $HBsAg^+HBeAg^+HBcAb^+$ (evidence of either chronic immune active/tolerant hepatitis B depending on whether HBV-DNA level > 200,000IU/mL and serum ALT is persistently elevated or normal) [16, 50]; 3.4% of $HBcAb^+$ (associated with occult hepatitis B if HBV-DNA level < 200IU/mL is found without HBcIgM, or acute hepatitis B during window period if HBcAb total differentiation reveals seropositive HBc(IgM) alone; 0.85% of $HBcAb^+HBeAb^+$ and 0.21% isolated $HBeAb^+$ (HBV markers showing exposed immune UVRP with no evident cure); 0.43% of $HBsAg^+HBeAg^+$ (hepatitis B infection at incubation phase); 0.21% of $HBsAg^+HbeAg^-$ (chronic hepatitis B infection), 0.21% of $HBcAb^+HBeAg^+$ (acute or chronic hepatitis B infection depending on whether HBc(IgM) or HBc(IgG) is detected following HBcAb total differentiation); 0.21% of $HBsAb^+HBeAg^+$ (unusual markers but might be connected with acute hepatitis B at clearing phase), and 0.21% of $HBeAg^+$ (unusual marker, false positive HBeAg or infected donor with HBcAg in hepatocyte but undetectable HBcAb in serum). Overall, based on study findings of HBV markers seroprevalence among UVRV, Ekiti state, Nigeria can be classified as a high endemic region for hepatitis B [13].

4.3 Hepatitis B Viral Markers Testing Interpretation Guidelines

Finally, this study has used flowchart (Figure 2) to elucidate the interpretations of hepatitis B viral markers testing outcomes in vaccinated and unvaccinated research participants. Hepatitis B viral markers screening rather than HBsAg is a good entry point of testing in early diagnosis of HBV infection [51]. Besides giving the broad revelations on different phases of HBV infection and showing evidence of immune protection in vaccinated research participants, it ensures relative ease in the monitoring of chronically infected individuals by circumventing the associated delays in follow-up. Weighing the clinical consequences of HBV infection with the cost-effectiveness of diagnostic device (0.7-2.9 USD/kit), adopting this entry point procedure will save a lot of cost and psychological trauma associated with HBV infection. Several authors have described the various phases of hepatitis B infection [37, 39, 50]. Different authorities in hepatology have used different guidelines to interpret hepatitis B viral markers testing results and the differences in their interpretations could be traced to advances of testing procedure beyond qualitative serologic screening such as inclusion of quantification serologic assay, differentiation assay-HBcAb total differentiation into hepatitis B core IgM (HBcIgM) and hepatitis B core IgG (HBcIgG), molecular diagnosis with advanced techniques including quantification of HBV-DNA level with real-time PCR,HBV genotyping and sequencing, performance of biochemical assessments and liver scan [13, 37, 50, 52-55]. This study interpretive algorithm is fundamental and compares with several others and can be adopted in clinical settings to accurately diagnose HBV infections and assess HBV vaccination outcomes.

5. Conclusion

Low evident vaccine coverage showed the need for a more aggressive and strategic campaign on ‘Know your HBV status’ among Nigerian citizens especially at the grassroots to get more people vaccinated against Hepatitis B. Low HBV

markers seroprevalence among the VRP compared to evident broad spectrum of various HBV markers seroprevalence patterns implicated in different phases of HBV infection among UVRP a working preventive programme. Improved testing technique with lower detection limit of 10IU/L will enhance a better picture of successfully vaccinated research participants. Post-vaccination testing 2-3 months after the last dose of HBV vaccine is recommended for eligible individuals. Accurate interpretations of HBV markers testing results should follow recommended guidelines and algorithm to aid early diagnosis and facilitate prompt treatment of infected donors where necessary.

Limitation of Study

Since this was a descriptive cross-sectional study, the number of vaccinated research participants among the overall population studied was limited and outcomes of research might be better pictured with an experimental study involving larger population of vaccinated research participants comparable to the unvaccinated counterparts. Future studies can look in this direction.

Conflict of Interest

Authors declared there was no conflict of interest of any kind with respect to this study.

Authors' contributions

KAF designed the study, searched for literatures and performed the diagnostic and statistical analyses. AOD performed some serologic assays, proof-read the manuscript and made contributions to its content. Both authors read and approved the study.

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