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Assessing the Impact of Hepatitis B Immunization among Children Aged 1-14 years in Ogbomoso, Oyo State, Nigeria

Busayo Kayode Akomolafe [1, 2, 3, *], Olawale Sunday Animasaun [2, 3], Etuvie Favour Akomolafe [5], Iyevhobu Kenneth Oshiokhayamhe [4], Olaniyan Oyejide Afolabi [6], Adebunmi Oluyemi Adigun [7], Adepoju Temitope Olufunmilayo [2], Adigun Prisca Funmito [2] and Rosemary Audu [3, 8]

[1] *Department of Medical Laboratory Sciences, Ajayi Crowther University, Oyo town, Oyo State, Nigeria.*
[2] *Department of Medical Laboratory Services, Oyo State Primary Healthcare Board, Ibadan, Oyo State, Nigeria.*
[3] *Department of Biological Sciences, Lead City University, Ibadan, Oyo State, Nigeria.*
[4] *Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.*
[5] *Department of Heamatology and Blood Transfusion Science, Bowen University Teaching Hospital, Ogbomoso, Oyo State, Nigeria.*
[6] *Department of Chemical pathology, Ladoke Akintola University Teaching hospital, Ogbomoso, Oyo State, Nigeria.*
[7] *Department of Medical Microbiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.*
[8] *Department of Microbiology, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria.*

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Abstract

Background: Hepatitis B Virus infection is a vaccine-preventable disease. The vaccine is recommended for all children at birth within the first 24 hours of life and during childhood to prevent perinatal and early childhood HBV transmission. Nigeria introduced HBV vaccines into the National Program on Immunization in 2004 and 2007 respectively.

Objective: This study aimed to assess the population impact made by hepatitis B vaccine after its integration into Nigeria's National Program on Immunization (NPI-NIG).

Materials and methods: A cross-sectional study was conducted from December, 2022 to October, 2023 in Ogbomoso, Oyo State, Nigeria. A simple random sampling technique was used to select 336 fully vaccinated children aged 1–14 years old with the presentation of their vaccination card. Blood samples were collected from each child and the plasma was used to determine status of hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), hepatitis B envelope antigen (HBeAg), hepatitis B surface antibody titer (anti-HBs) and HIV antibody using ELISA techniques.

Results: The seroprevalence of HBsAg, anti-HBc and HBeAg was found to be 4/336(1.2 %), 5/336(1.5 %) and 2/336(0.6 %) respectively. The positive cases were found among children aged 11-14 years. Of 336 fully vaccinated children, 43(12.8%) had anti-HBs titer > 10 IU/ml, while 293(87.2%) had anti-HBs titer <10IU/ml. All study participants tested negative for HIV antibody.

Conclusions: Though the prevalence of HBsAg (1.2%) in the study population was lower than the national prevalence of 8.1% for the country, the low proportion (12.8%) of the vaccinated children with protective anti-HBs titer value calls for further investigations to determine if booster doses of the vaccines may be required.

Keywords: Hepatitis B; Immunization; Children; Infection; Vaccine

* Corresponding author: Busayo Kayode Akomolafe

1. Introduction

According to the World Health Organization, 360 million people or more are chronic carriers of HBV, and one-third of the world's population has serological indicators of active HBV infection[1,2], with 1.2 million liver cancer-related deaths per year[3, 4]. Africa is regarded as an endemic region[5,6], and has been reported to have the second-highest population of persistent HBV carriers after Asia[6]. In Africa, about 60 million individuals have chronic hepatitis B infection through perinatal transmission[7,8]. In areas with high HBV endemicity, perinatal transmission is the main route of transmission. Among newborns who become infected with HBV, 90% remain chronically infected and carry into adulthood a one in four risk of premature mortality from HBV-related liver disease including liver cancer, and up to 25% of them may die from hepatocellular carcinoma during their lifetime[9,10.] Nigeria is one of the nations in the HBV hyper-endemic zone, where the degree of endemicity varies by target group and by region[5]. According to Nigeria's most recent nationwide sero-prevalence study, which was completed in 2018, the average prevalence of HBV in the nation is 8.5%[11]

A number of studies carried out in Nigeria have found that the prevalence is 14.0% among blood donors and 14.1% among expectant mothers who visit antenatal clinics[6]. Additionally, different rates have been reported among the pediatric population from different regions of the nation: 1.2% among adolescents attending secondary schools in Calabar, Nigeria; 13.9% in a hospital-based study of young people attending an outpatient clinic in Ilesha, South-West Nigeria; and 44.7% among primary school children in Borno, Nigeria [12–14].

Nearly 200 nations have successfully incorporated the HBV vaccination program for infants into their regular immunization schedule, as of late 2022[15]. In Nigeria, the Expanded Program for Immunization (EPI) was incorporated with HBV vaccination in 2004. The diphtheria, tetanus, pertussis (DTP), hepatitis B (HepB) and Haemophilus Influenzae type b (Hib) vaccine was added as a pentavalent to the EPI schedules, and HBV vaccine is administered at 6-, 10-, and 14-weeks following delivery[15]. The hepatitis B birthdose was introduced in Nigeria in 2007.

In 2022, the national coverage of Hep B was 62% and much lower than the global coverage of 84%. Similarly, the coverage of HBBD was 52%. Both of these coverages are suboptimal and much lower than the 90% recommended by WHO[16]. Nigeria has reported 53% vaccination rates for pentavalent vaccination. Nevertheless, despite widespread vaccination, research continues to indicate vulnerability to HBV infection[17]. There is limited data on the effectiveness of the HBV vaccine among Nigerian children who have been fully vaccinated. This study therefore aimed to assess the impact of HBV vaccine, among fully vaccinated children in Ogbomoso, Southwest, Nigeria.

2. Materials and Methods

2.1. Study Design and Setting

This was a cross-sectional study conducted among hepatitis B vaccinated children in Ogbomoso, Southwest, Nigeria, from December 2022 to October 2023. Ogbomoso is a pre-colonial urban center and the second-biggest city in Oyo State, Nigeria[18]. Participants were recruited from schools, churches, mosques and their houses. Simple random sampling technique was used in recruiting study participants.

2.2. Participants Inclusion and Exclusion Criteria

Hepatitis B fully vaccinated children aged 1–14 years, who lived in Ogbomoso were included for the study. Also included were children whose parents or legal guardians consented and children 10-14yrs who gave their assent. Children who had not completed the full dose of the hepatitis B vaccine, children with known chronic ailment and those with present illness were excluded from this study.

2.3. Sample Size Estimation

The Fischer's formula for calculating the sample size for a population above 10,000 was used[2] in calculating the sample size for the study. $N=Z^2P(1-P)/d^2$. Where **N** is the sample size, **Z** is the statistic corresponding to level of confidence (95%), **P** is expected prevalence and d is precision (corresponding to effect size)[19].

$$N = \frac{1.96^2 \text{X } 0.085\ (1 - 0.085)}{0.05^2}$$

For this study, a prevalence of HBV infection in children (8.5%) previously reported in Nigeria was used[11]. The minimum sample size was thus calculated to be 220, However, a total of 336 samples was used for this study.

2.4. Data Collection Instrument and Method

A semi-structured interviewer administered questionnaire was developed using WHO recommended questionnaire used for data collection[20]. This was used to obtain relevant history and socio-demographic information from the parents of the subjects, history of previous hospital admission, blood transfusion, HBV vaccine completion and year of completion (verified from vaccination cards), parent educational level, occupation and location.

From each participant, 4ml of venous blood sample was collected into ethylenediaminetetraacetic acid (EDTA) container. This was centrifuged at 5000 rpm for 5 min to separate the plasma which was then transferred into cryovials and stored at −20°C until required for laboratory analysis.

2.5. Laboratory Analysis

All plasma samples were tested for the following serological markers: HBsAg, anti-HBc, HbeAg anti-HBs and HIV antibody. All serological tests were performed according to manufacturer's instructions on Perlong Medicals fully auto microplate reader medical ELISA reader (DNM-9602) (Nanjing Perlove Medical Equipment Co., Ltd.) using BIO-INTECO ELISA test kits (Bio-Inteco, INTECO DIAGNOSTICS (UK) LTD: Ken House,152-160 City road, London EC1V2NX, England, UK) . The ELISA Test kit has a sensitivity rate of 100% and specificity rate of 99.8% for HBsAg, HBsAb, HBeAg and anti-HBc, while for HIV antibody kit, sensitivity rate is 100% and specificity rate is 99.68% according to the manufacturer. {https://inteco-daemuk.com / Email: sales@inteco-daemuk.com}.

For the result interpretation, samples that test positive for either HBsAg, anti-HBc or HBeAg or both HBsAg and anti-HBc markers indicate exposure to HBV virus and if also positive to HIV was evidence of co-infection. Anti-HBs titer at >10 mIU/mL, indicates that anti-HBs has been detected at levels consistent with protective immunity against HBV infection.

2.6. Data Analysis

Data was entered into Epidata software version 3.1, and then SPSS version 26 was used for analysis. Tables and figures were used to display the results. The relationship between the dependent and independent variables was demonstrated using binary logistic regression and Chi-square; a p-value of less than 0.05 was regarded as statistically significant.

2.6.1. Ethical Consideration and Consent to Participate

This study was ethically approved by the Research and Ethical Committee of the Bowen University Health Research Ethics Committee (HREC) with approval number BUTH/REC-852. Caregivers were appropriately educated on the significance of the research to get their optimal support. Written informed consent was obtained from the parents or guardian while assent was obtained from participants 10years and above.

3. Results

3.1. Socio-demographic Characteristics

In this study, 336 children between the ages of 1 and 14 years who had received full vaccinations for HBV were enrolled. Table 1 shows their socio–demographic characteristics and that of their parents. The children enrolled in the study consisted of 184 (54.8%) males and 152 (45.2%) females. The children were stratified into age strata of 1–5 years, 6–10 years, and 11–14 years. Majority of the children (61.6%) were aged 11 – 14 years, followed by those aged 6 – 10 years (20.2%).

3.2. Sero-positivity of HBV Markers and HIV

The overall positivity of HBsAg among all the children was 4/336 (1.2%) as shown in table 2. Three out of the four children who were positive for HBsAg, were males, and all the HBsAg positive children were aged between 11-14years. Furthermore, the overall prevalence of anti-HBc antibodies among the children was 5/336 (1.5%), HBeAg positivity among the children was 2/336 (0.6%) all were between the 11-14years as seen in table 2. Among fully vaccinated children, 43/332 (13.0%) of them had anti-HBs titer >10mIU/ml while 289/332(87.0%) had anti-HBs titer <10mIU/ml as shown in table 2. All participants were negative to HIV antibody.

3.3. Distribution of HBsAg and anti-HBc with Clinical and Socio-demographic Variables

Among the HBsAg and anti-HBc antibody seropositive children, one each had history of hospital admission and blood transfusion as shown in table 2. Three of the children positive to HBsAg were born to mothers who had secondary school education, while all four of the HBsAg positive children were born to parents who were artisans by occupation as seen in table 2.

Table 1 Socio–demographic Characteristics of Children and their Parents

Variables	Category	Frequency	Percentage (%)
Sex	Male	184	54.8
	Female	152	45.2
Age Group	1-5years	61	18.2
	6-10years	68	20.2
	11-14years	207	61.6
Academic Qualification of parents	Illiterate	94	28.0
	Primary school	89	26.5
	Secondary school	92	27.4
	Tertiary	61	18.2
Parents Age	21-30 years	61	18.2
	31-40years	244	72.6
	>40years	31	9.2
Occupation of parents	Artisan	245	72.9
	Banker	5	1.5
	Civil servant	29	8.6
	Farmer	31	9.2
	Teacher	21	6.3
	Student	4	1.2
	Lawyer	1	.3
Ethnicity	Yoruba	291	86.6
	Igbo	19	5.7
	Hausa	23	6.8
	Urhobo	3	.9
Religion	Christianity	256	76.2
	Muslim	80	23.8
TOTAL		336	100.0

Table 2 Seropositivity of HBV Markers with Socio–demographic and Clinical Variables

Variables		HBsAg		Anti-HBc status		Anti-HBsTitre mIUml		HBeAg	
		Negative (%)	Positive (%)	Negative (%)	Positive (%)	≥10	<10	Negative (%)	Positive (%)
Age group	1-5years	61(100.0)	0(0.0)	61(100.0)	0(0.0)	9(14.8)	52(85.2)	0(0.0)	0(0.0)
	6-10years	68(100.0)	0(0.0)	68(100.0)	0(0.0)	8(11.8)	60(88.2)	0(0.0)	0(0.0)
	>11years	203(98.1)	4(1.9)	202(97.6)	5(2.4)	26(12.8)	176(87.1)	2(50.0)	2(50.0)
Sex	Male	181(98.4)	3(1.6)	181(98.4)	3(1.6)	29(15.8)	155(84.2)	1(25.0)	2(75.0)
	Female	151(99.3)	1(0.7)	150(98.7)	2(1.3)	14(9.2)	138(90.8)	1(100.0)	0(0.0)
Educational Status of Mother	Illiterate	93(98.9)	1(1.1)	94(100.0)	0(0.0)	10(10.6)	84(89.4)	(0.0)	0(0.0)
	Primary school	89(100)	0(0.0)	89(100)	0(0.0)	8(9.0)	81(91.0)	(0.0)	0(0.0)
	Secondary school	89(96.7)	3(3.3)	87(94.6)	5(5.4)	13(14.1)	79(85.9)	90(97.8)	2(2.2)
	Tertiary	61(100.0)	0.00%	61(100.0)	0.00%	12(19.7)	49(80.3)	61(100.0)	0.00%
Hospital Admission	No	316(99.1)	3(0.9)	315(98.7)	4(1.3)	40(12.5)	279(87.5)	318(99.7)	1(0.3)
	Yes	16(94.1)	1(5.9)	16(94.1)	1(5.9)	3(17.6)	14(82.4)	16(94.1)	1(5.9)
Blood Transfusion	No	323(99.1)	3(0.9)	322(98.8)	4(1.2)	42(12.9)	284(87.1)	325(99.7)	1(0.3)
	Yes	9(90.0)	1(10.0)	9(90.0)	1(10.0)	1(10.0)	9(90.0)	9(90.0)	1(10.0)

3.4. Sero-protection Level of HBV Vaccine

Table 3 shows the interpretation of HBV serological markers by age strata among the children. Using CDC HBV serological result interpretation profile,[1] the children were grouped into Immunized (98.5%), Infection (1.2%) and Past exposure (0.3%).

3.5. Factors Associated with HBsAg and Anti-HBc Positivity

The bivariate and multivariable logistic regression of association of clinical factors (i.e., hospital admission and blood transfusion) and HBsAg is presented on table 4. One out of 17 children who had history of hospital admission and 1/10 children with history of blood transfusion were positive to HBsAg with p-value >0.05 which were not statistically significant. Also, the influence of the children's location and the occupation of their parents on their HBsAg positivity rate is presented on table 5 with p-value > 0.05 and not statistically significant.

Table 3 Interpretation of HBV Serological Markers by Age Strata (CDC HBV Serological Result Interpretation Profile)

					Total
Interpretation	Serological Markers	Age strata			
		1-5years	6-10years	≥11years	
Immunized	Anti-HBs positive alone	61(18.15%)	68(20.23%)	202(60.11%)	331(98.5%)
Infection	HBsAg positive alone	0 (0.0%)	0(0.0%)	2(0.6%)	2(0.6%)
	HBsAg, HBcAb & HBeAg positive	0(0.0%)	0(0%)	2(0.6%)	2(0.6%)
Past Exposure	Anti-HBc positive alone	0(0.0%)	0(0.0%)	1(0.3%)	1(0.3%)
Total		61(18.15%)	68(20.23%)	207(61.6%)	336(100%)

Table 4 Bivariate and Multivariate Logistic Regression of Associated Factors for HBsAg

Variable		HBsAg					
		Positive(%)	Negative(%)	COR(95%CI	P-value	AOR(95%CI)	P-value
Hospital Admission	Yes	1(5.9)	16(94.1)	01.67(0.03,94.90)	0.8	1.67(0.03,94.89)	0.80
	No	3(0.9)	316(99.1)	Ref		Ref	
Blood transfusion	Yes	1(10.0)	9(90.0)	0.13(0.00,7.22)	0.32	7.95(0.14,456.11)	0.32
	No	3(0.9)	323(99.1)	Ref		Ref	

NB: *: Candidate variable for multivariate analysis at P < 0.25 and **: significant variable by the multivariate analysis at P < 0.05; COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval; P–V: p–value; Ref: reference

Table 5 Comparison of Cultural Variables with the HBsAg Status in Children

Variable		HBSAg		Chi-square value	Df	AOR	P-value
		Positive(%)	Negative(%)				
Location	Rural	4(1.9)	212(98.1)	2.25[a]	1	3.56	0.130
	Urban	0(0.0)	120(100.0)				
Occupation of parents	Artisan	4(1.0)	241(98.4)	1.50[a]	6	2.55	0.959
	Banker	0(0.0)	5(100.0)				
	Civil servant	0(0.0)	29(100.0)				
	Farmer	0(0.0)	31(100.0)				
	Teacher	0(0.0)	21(100.0)				
	Student	0(0.0)	4(100.0)				
	Lawyer	0(0.0)	1(100.0)				

4. Discussion

The burden of hepatitis B has clearly decreased worldwide since universal hepatitis B vaccination programs were implemented[1]. Maintaining hepatitis B immunization programs is crucial for hepatitis B prevention and control, given its apparent effectiveness. Infant hepatitis B vaccine, was introduced into national immunisation schedules across sub-Saharan Africa between 1994 and 2014, Nigeria started in 2004. The World Health Organization (WHO) recommends commencing the first dose at birth but this has not been implemented in most sub-Saharan African countries. WHO has also suggested that the best way to measure the effectiveness of hepatitis B immunization programs and ensure that they are sustained and improved upon as needed is to use serosurveys[2]. Nigeria is yet to conduct a nationwide serosurvey to monitor the hepatitis B vaccine impact within the framework of Nigeria's National Program on Immunization (NPI-NG). In the absence of such nationwide surveys, a number of field and laboratory studies have been carried out in the nation; which only measure the prevalence of hepatitis B virus infection (HBsAg) among children not putting into consideration their vaccination status[5-7], thus, this is the only information currently available on the prevalence of HBV infection among fully vaccinated children. With the national hepatitis B vaccination program entering its second decade of implementation, the goal of this study was to assess the effectiveness of the vaccination in the nation. Therefore, this study concentrated on post-vaccination populations based on the year that the hepatitis B vaccine was introduced into NPI-NG, that is children aged 1 – 14years who had received the three doses of vaccine against hepatitis B virus. All research participants had their viral (HBsAg and HBeAg) and host (anti-HBc, anti-HBs, and anti-HBe) serological markers assessed in order to establish the impact of HBV vaccination among the study participants.

Findings from the study indicated that 1.2% (4/336) of vaccinated children in Ogbomoso, Southwest, Nigeria, aged 1 to 14 had HBsAg. The result is lower when compared to the national prevalence rate of 8.5%[11], but it is comparable to the 1.2% prevalence among adolescents aged 11 to 19 in the south-south region of the country[21]. In the emergency

department of a tertiary center in Benin, a different study also revealed a significantly greater prevalence (13.9%) of HBV infection in children between the ages of two months and fifteen years[22]. The prevalence rate in this study is much lower compared to another study carried out in Enugu which has a prevalence rate of 11.5%[23]. Another study reported 18.3% among children attending a tertiary health facility in North East Nigeria[24], 6.7% was also reported in a study carried out among children in Ekiti south west Nigeria[25]. The prevalence in this study was still lower than a low prevalence rate of 3.6% reported in Omuaran community south west Nigeria by another researcher[26], another study conducted within the same study region reported a 1.0% prevalence rate among children attending the children outpatient of the BUTH, Ogbomoso, Southwest Nigeria[27].

Introduction of the hepatitis B vaccine to the Nigerian NPI in 2004 precede the birth of the children recruited into this study, and the children were fully vaccinated which definitely is the reason for the low prevalence of HBV infection observed in this study. Several studies conducted outside Nigeria have also reported a definite decrease in the seroprevalence of HBV infection since the onset of the routine immunisation against hepatitis B[28,29]. As more children receive the HBV vaccine from birth, adhering to WHO recommendation, it will result in a decline in the national prevalence of HBV in the nation[28]. A previous study conducted in the same study region, Ibadan, Southwest, Nigeria on pregnant women found that majority (97.9%) of the women tested negative for HbeAg, suggesting that they were less likely to pass the virus on to their unborn children[30]. This may also be largely the reason for the low prevalence of HBV in the children in this study. Two of the study participants positive to HBsAg, Anti-HBc and HBeAg were siblings and had the same mother. The children, even though were fully immunized, never had hepatitis B immunoglobulin (HBIG) at birth. However, vaccine failure has been reported despite full vaccination. Mother-to-child transmission of HBV has been reported to be a major cause of chronic hepatitis B. WHO has advised giving hepatitis B vaccine to an infant whose mother has tested positive for the virus[2], within the first 12 hours of life. Use of HBIG although routinely available in high income countries, is usually unavailable in most LMIC. The World Health Organization recommends using antiviral prophylaxis in addition to infant vaccination to prevent mother-to-child transmission of hepatitis B[2]. It is possible that the mother of these infected children was not diagnosed for HBV during antenatal because patronage of traditional birth attendant is rampant in the community[31], hence, appropriate measures were not taken to stop mother-to-child transmission. The laboratory parameters in two of the HBsAg positive children, who were also positive for Anti- HBc, corroborates with the known fact that children are mostly infected from birth and develop chronic disease later in life and the likelihood of developing a chronic infection is more common in infants who acquire the virus perinatally from their mothers or before the age of 5 years[2,32]. These children may have acquired the infection from a presume HBV positive mother. Furthermore, the positive HBeAg status in these children makes them at increased risk to transmit the disease to others and develop complications from it[33].

The overall seroprevalence of anti-HBc positivity in this study was 1.5% which is lower when compared with studies reported from Addis Ababa (5.6%)[34] and Gondar (6.3%)[35]. On the other hand, the current result of anti-HBc seroprevalence is similar to finding from Niakhar-Senegal (1.5%)[36] but relatively higher than that report from Dakahleya-Egypt (0.11%)[37]. In contrast, higher prevalence was reported from Hawassa city (19.5%)[38] and Gambian Villages (10.2%)[39]. These variations may be due to difference in geographical area, age range of study participants, HBV vaccination status, income level, socio cultural practices, and inclusion of birth dose of HBV vaccine within 24 hours. In this study, children who had history of hospital admission and blood transfusion were likely to be anti-HBc positive although not statistically significant. This might be due to the horizontal transmission of the virus from the hospital environment or at childhood.

Quantifying anti-HBs in the absence of any other serological marker allowed researchers to assess the effectiveness of the hepatitis B vaccine, which is defined as enhanced immunity to HBV infection. In this investigation, the lowest detectable titre of anti-HBs was 5IU/ml. But, all subjects with detectable anti-HBs titres (≥10 IU/mL) were considered protected against HBV infection[40] for the purposes of this study. The study participants had an overall immunity rate to HBV infection of 98.5%. The sero-protection level of hepatitis B vaccine among children in this study is considered highly effective since safe and effective HBV vaccine has a protection level of 98 to100% according to WHO report[2]. The immunity to HBV infection recorded in the study population is much higher than the report from Ethiopia which ranged from 32.2–58.4%[41], and higher than the two reports 86.8% and 87.0% within vaccinated cohorts below five years in South Africa[42,43]. The 98.5% immunity level observed in this study is higher than 96% reported in Malawi in 2021[44]. In a health facility-based study conducted in Tanzania five years post vaccination, 69.3% vaccinated children below 5 years of age were said to have developed immunity to HBV infection[45]. While Egypt, reported a 54% immunity rate 20 years post vaccination era in the country[46]. higher immunity to HBV infection rate has also been reported elsewhere[47,48]. Although, 98 % of the children had evidence of anti-HBS which is evidence that the vaccine was effective, it is however noted that only 12.8% had levels exceeding 10 IU/ml. This suggests a wanning of immunity and has implications for re-infections. The role of booster dose therefore should be considered in these populations. The disparity recorded might be due to; differences in cold chain system of the vaccine, differences in nutritional status, age groups, vaccine

seroconversion rate and different extent of exposure to natural boosters. HBV vaccine protective ability against hepatitis B virus has recorded low success in some individuals due to a number of factors, including the virus, host immune system, genetics, sociodemographic, cultural, and programmatic factors, even though the WHO declared that the vaccine would confer 98–100% protection against HBV[49,28]. Owing to those causes, after five to fifteen years of vaccination, 15–50% of the children may have undetectable concentrations of anti-hepatitis B surface antibody, and 10% may still be prone to contracting HBV [50,51].

Nevertheless, several studies have established that anti-HBs titres below the stipulated 10 IU/mL are still protective due to primed immune memory cells which effect an anamnestic response upon subsequent challenge with viral antigen[52,53]. Larger studies may be essential to confirm the level of immunity in HBV vaccinated children in Nigeria. However, there is a need to consider booster dose of HBV vaccine in Nigeria. Studies have shown that individuals with anti-HBs levels above 2 IU/L continue to respond rapidly to a booster dose and maintain a post-booster level that represents an excellent protective level[54].

5. Conclusion

Introduction of the hepatitis B vaccine into NPI-NG has shown remarkable success in the studied children significantly reducing the prevalence of HBV infections while increasing immunity to HBV infection. However, further studies are required to evaluate the wanning of immunity and implications re-infections among children age 1-14 years in the country using a larger sample size and there may be a need to consider booster dose of HBV vaccination in the country.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

This study was ethically approved by the Research and Ethical Committee of the Bowen University Health Research Ethics Committee (HREC) with approval number BUTH/REC-852. Caregivers were appropriately educated on the significance of the research to get their optimal support.

Statement of informed consent

Written informed consent was obtained from the parents or guardian while assent was obtained from participants 10 years and above.

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