IMMUNIZATION (IMMUNE) STATUS OF A COHORT OF CHILDREN VACCINATED AGAINST HEPATITIS B VIRUS IN EKITI STATE OVER TEN YEARS AFTER INCORPORATION INTO NATIONAL PROGRAM ON IMMUNIZATION.

ABSTRACT

Hepatitis B vaccine has been introduced in Nigeria for over a decade now, yet, data on sero-conversion status of the immunized cohort in the population are scarce. Such data are important for objective evaluation of the impact and effectiveness of the HBV vaccination program. This study therefore aims at determining the sero-conversion status and the prevalence of HBV infection among the immunized cohort of children in Ekiti state, Nigeria. This cross-sectional study was conducted across all the three senatorial districts of Ekiti state, between October and December, 2017. A total of 441 children consisting of 226 males and 215 females (Male to female ratio= 1.1:1) between 5 to 10 years were recruited into the study. All subjects have had 3 full doses of hepatitis B vaccination before the age of 1 year. Multistage sampling technique was used. After caregiver's consent and assent from the children, 2 to 5mls of blood samples were collected from each subject and tested for the various hepatitis B viral markers (HBeAg, HBeAb, HBcAb, HBsAb and HBsAg) using Hepatitis B combo kit manufactured by Acumen. All subjects were negative for HBsAg, HBeAg, HBeAB and HBcAb. However, only 47 (10.7%) had detectable HBsAb. Among HBsAb positive patients 22 were males while 25 were females. Most were aged 5 years. Our findings showed zero prevalence of hepatitis B but minimal seroconversion rate among vaccinated children in Ekiti state, Nigeria. We conclude that majority of this children may be at risk of HBV at a later age. We recommend a booster dose of hepatitis

B vaccine at the school age of 6years to all children. However, since very low level antibody titres may not be detectable by quantitative detection methods as used in this study, further work using quantitative detection is required to overcome such major limitation.

Keywords: Immunization status, hepatitis B vaccine, sero-conversion, Ekiti State, Nigeria

Structure your Abstract: Introduction/Background, Aim, Methods, Results, Discussion, Conclusion. Similarly, your Manuscript-text with the same sub-headings.

Only use key-words found in #MESH.

Introduction:

Transmission and response to infection is dependent on age at infection, with young children infected commonly via contact with contaminated blood while the parenteral and sexually transmitted route is commoner in adolescents and adults.[1]About 80% of infections in childhood progresses to chronic infection while about 20% recovers fully without any sequel. 10-20% however becomes chronic carriers. The reverse is the case in those infected as adolescents or adults as the majority (up to 95%) of cases recovers fully and less than 1% becomes chronic carriers and 5-10% progressing to chronic infection, predisposing to hepatocellular carcinoma.

(The likelihood that hepatitis B will develop from an acute infection into a chronic infection depends on the age of the person infected. The younger a person is when infected with hepatitis B virus, the greater the chance of developing a chronic infection. Approximately 90% of infected infants will develop chronic infection. The risk goes down as a child gets older. Approximately 25%–50% of children infected between the ages of 1 and 5 years will develop chronic hepatitis B. By contrast, about 95% of adults recover completely and do not become chronically infected.)

(https://www.cdc.gov/hepatitis/hbv/bfaq.htm?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fhepatitis%2Fb%2Fbfaq.htm)

Worldwide about 1 million deaths annually are attributed to HBV-related liver disease and hepatocellular carcinoma.[1]It has been reported that approximately 30% of the world's population has serologic evidence of current or past HBV infection with a high proportion of chronic hepatitis B virus carriers worldwide currently estimated at 400 million individuals. (What is the "serologic evidence" implied here? An individual negative for HBsAg but positive for anti-HBs either has cleared an infection or has been vaccinated previously. Thus, anti-HBs antibody could be positive due to a past infection or past vaccination). this fact and the attendant complications notably liver cirrhosis and hepatocellular carcinoma makes HBV infection a disease of major public health importance worldwide.[2,3,4]

The hepatitis B vaccine was included in the immunization schedule in Nigeria in 1995 but the vaccine only became widely available in 2004, when the WHO policy of including HBV vaccination in the routine immunization schedule for children was implemented. The success of the immunization programme can be assessed by the timeliness of receipt of vaccines, the coverage of the vaccine and measurement of morbidity and mortality from the target disease.[5] In a study carried out at the Children Emergency Room of University of Benin Teaching Hospital, 83% of the children admitted within the study period were appropriately vaccinated but despite this high coverage rate in these age group, the study reported a high seroprevalence of HBV infection which was concluded to be due to lack of timeliness in administering the vaccine which hence rendered the vaccine ineffective.[5] (What was the serological test done? As I had said earlier, an individual negative for HBsAg but positive for anti-HBs either has cleared an

infection or has been vaccinated previously. Thus, anti-HBs antibody could be positive due to a past infection or past vaccination.) According to the WHO (year?), HBV vaccine has been introduced in 184 countries in the world with an average global vaccine coverage with 3 doses of hepatitis B vaccine estimated at 84%, as high as 92% in the Western Pacific.[6] In Nigeria however, few studies conducted on estimating global coverage is in a serogroup of individuals (this phrase not clear), the Health-care Workers (HCWs) and an average rate of 20% was reported in them. The risk of occupational exposure of these (this) group of individuals to HBV however remains high.[7]Mortality attributable to this preventable and curable infection is quite high, being a leading cause of death and disability worldwide. Unlike most communicable diseases (delete this phrase), the absolute burden and relative rank of viral hepatitis increased between 1990 and 2013.[8]The availability of effective vaccines and treatments suggests an important opportunity to improve public health – hence, the need to find out the actual burden of the infection, the susceptible proportion of the population with the aim of instituting a prompt intervention.

What is the Aim of your Study? From your Results and Discussion, it appears the Aim of your Study is to determine the immune-status of cohort of children vaccinated against hepatitis b virus in ekiti state (over ten years after incorporation into national program on immunization).

***Methodology*:** This cross-sectional study was conducted in Ekiti State, Southwest zone, Nigeria, between October and December, 2017.

Study area and study population: Ekiti state has 16 Local Government Areas (LGAs) within three senatorial districts. The current population of Ekiti State based on the projection from 2006 National Population Census and annual growth rate of 3.0% is 3,027,949. Subjects were children within the age of 5 to 10years (to accommodate children that have been captured

in the routine HB immunisation, since Hepatitis B vaccine was introduced into routine immunisation). (This Study should not have excluded those aged 1 – 5)

Sampling and Sample-size: Multistage sampling technique was used. At the first stage, two LGAs were selected by balloting from each of the three senatorial districts, making a total of 6 LGAs in all. Stage Two involved random selection by balloting for two health facilities from the selected LGAs. The third sampling stage was at the selected health facilities where subjects were selected based on equal allocation of the determined sample size (What was the sample-size determined? Show here the formula used and the calculation done. Was sample-size from previous similar studies used? If yes, what was that sample-size, and provide reference here in the text) across the total 12 facilities selected for the study, such that 30 to 42 subjects (children aged 5-10years) were recruited from each facility.

Questionnaire: A semi-structured, interviewer-administered questionnaire framed in English and back translated into Yoruba was used. Information was sought from the caregivers on the socio-demographic characteristics and immunisation history of the children using trained research assistants. Survey instrument was pretested in Efon LGA (an LGA outside the study LGA).

Ethical clearance: Prior to data collection, ethical clearance for the study was obtained from the Ethics and Research Committee of Ekiti State University Teaching Hospital, Ado-Ekiti and Ekiti State Ministry of Health. Written consent was obtained from the caregivers of the selected children. Permission to use the Health facilities was obtained from the State Ministry of Health and State Primary Health Care Development Authority. All data were handled in a confidential manner. Pre and post testing counselling was done.

Blood-collection: After caregiver's consent and assent from the children, 2 to 5mls of blood samples were collected from each subject and tested for hepatitis B viral markers. Serologic testing for hepatitis B was done using rapid test kit searching for markers of HBV (HBeAg, HBeAb, HBcAb and HBsAb) (From what you list under first part of Results below and here, there should be five tests: HBsAg, HBsAb, HBeAg, HBeAb and HBcAb).with Hepatitis B combo kit manufactured by Innovita Biological Technology (lot:20170101). Manufacturer's instruction was carefully followed in testing procedures and interpretation of results for each subject. The results of the screening was later handed over to each participant's care-giver.

Statistical analysis: Briefly outline here all the statistical-tests done, any software used, and the p-value you set for significance-level

Results: A total of 441 children consisting of 226 (51.2%) males and 215 (48.8%) females between 5 to 10years were recruited into the study given a male to female ratio of 1.1:1. Majority (98.0%) belong to Yoruba ethnic group; 338 (76.6%) of them were in primary schools while only 5 were in secondary schools (Table 1). (The age distribution of subjects recruited ranges from 5 to 10 years. The highest age number of participants were age 5 years (27%) while the least was 10years (6.1%). (Include this in the Methodology under the subheading of Study-area and study-population)

All subjects had 3 full doses of hepatitis B vaccination before the age of 1 year. All subjects were negative for HBsAg, HBeAg, HBeAb and HBcAb. There was zero prevalence of hepatitis B viral infection among the study population. (From what you list under Methodology and here, there should be five tests: HBsAg, HBsAb, HBeAg, HBeAb and HBcAb).

(Avoid Font-change in the Manuscript-text)

Table 3 (Table 2, and not 3) shows the relationship between Respondents' Age and sex with the detection of Hepatitis B surface Antibodies. A total of 47 (10.7%) subjects had detectable HBsAb, though a greater proportion of the respondents with positive HBsAb were in the lower age group (12.7%) as against 7.0% in older age group, there was no significant difference in the detection of HBsAb across the various age of individuals in the study population. Furthermore, there was no significant gender difference between the proportion of those with positive Hepatitis B surface antibodies, 11.6% and 9.8% for females and male respectively.

Table 1: Socio-demographic characteristics of the Respondents

Variables		Frequency, n	Percent (%)
Sex	Female	215	48.8
	Male	226	51.2
Tribe	Yoruba	432	98.0
	Igbo	5	1.1
	Hausa	1	.2
	Others	3	.7
Child's Educational status	Pre-Primary	98	22.2
	Primary	338	76.6
	Secondary	5	1.1
	Total	441	100.0

Age in years	Frequency, n	Percent (%)
5	119	27.0
6	86	19.5
7	79	17.9
8	68	15.4
9	62	14.1
10	27	6.1
Total	441	100.0

Table 2: Relationship between Respondents' Age and sex with Hepatitis B surface Antibodies detection.

Age group of respondents	Hepatitis B surface Antibodies (HBsAb)			**Statistical test P=value**
	Neg	Pos	Total	
5 - 7.4yrs	248 (87.3%) (62.9%)	36 (17.1%) (76.6%)	284 (64.4%)	$X^2=3.413$ $P=0.065$
7.5 to 10yrs	146 (37.1%)	11 (22.4%)	157 (35.6%)	
Total	394 (100.0%)	47 (100.0%)	441 (100.0%)	
Sex	Hepatitis B surface Antibodies (HBsAb)			**Statistical test P=value**
	Negative	Positive	Total	
female	191 (88.4%)	25 (11.6%)	216 (100.0%)	$X^2=0.373$ $P=0.541$
male	203 (90.2%)	22 (9.8%)	225 (100.0%)	
Total	394 (89.3%)	47 (10.7%)	441 (100.0%)	

(Note the correction I have done on Age-group of Table2, and repeat similarly for gender. The cross-tabulation should total for Negative and Positive indicating 100% for the Total, and not totalled for the Age-groups as you have done.)

Discussion:

The coverage rate of HBV vaccine among children in this study was 100%, this is similar to the findings of Patel MK, et al in 2014 where 98% coverage was found following routine infant immunization schedule in French Polynesia[9], and in China where a coverage of 94 percent was found by Xiaofeng Liang et al (2009) when it was found that Hepatitis B vaccine coverage (3 doses) increased from 30.0% for children born in 1992 to 93.4% for children born in 2005[10]. However coverage rate seen in this study is higher than the coverage rate seen in Yemen (2017) by Fuad A. A. Alssamei et al where a rate of 87.3% was

found among children from 6 to 59 months [11.] This Study is comparable with the findings of Bekondi et al. (2015) where overall HBV immunization coverage based on immunization cards was 99 %, 49 % and 100 % in Cameroon, CAR (Write out in full) and Senegal, respectively and that based on maternal recall was 91 %, 17 % and 88 % in Cameroon, CAR and Senegal, respectively. (annotate) In this study, our assessment of coverage was based on immunization cards. (Describe this under Methodology also) The coverage rate shows that hepatitis B vaccination has been successfully integrated into routine infant immunization program in most parts of Ekiti state.

There was zero prevalence of hepatitis B among vaccinated children in this study, this is in contrast with the findings of Bekondi et al. (2015) where a HBsAg positivity prevalence of 0.7 %, 5.1 %, and 0.2 % were seen among children in Cameroon, Central Africa Republic (CAR) and Senegal respectively.[12] However, our findings (Tabulate this, and present under Results) is similar to that of Patel MK, et al in 2014 among French Polynesia where none of the children were positive for hepatitis B infection.

This study showed that only 10.7% of children vaccinated against hepatitis B virus in infancy had detectable antibodies 5 to 10 years after the vaccination. The level of seroconversion recorded in the index study is very low when compared to studies from other countries within and outside Africa. Dassah S et al.[13] and Chakraborty et al.[14] reported 100% seroconversion in Ghana and Bangladesh respectively while Freitas da Motta et al.[15] reported 98% seroconversion among vaccinated children in Brazil. Our findings is however show similarity to other findings which showed a decline of HBV vaccine protective level with age in Yemen [16, 17]. Saudi Arabia [18], Europe [19], and China [20, 21]. (List the rates experienced in these countries, along with the relevant studies, and not just provide the references here. Discuss in greater detail. Is there not any other similar studies done in Nigeria? If such is the vaccine-failure rate, what is the incidence of HBV in this age-group in your State, and how does this incidence compare with

various Nigerian-states, besides various countries and regions? You state in your Abstract “All subjects were negative for HBsAg, HBeAg, HBeAB and HBcAb”. Don’t you find your results very unusual that only 10.7% of your subjects were HBsAb positive yet none were HBsAg positive till the age 10?) (Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 mIU/ml in the recipient's serum. The vaccine is more effective in children and 95 percent of those vaccinated have protective levels of antibody. This drops to around 90% at 40 years of age and to around 75 percent in those over 60 years. The protection afforded by vaccination is long lasting even after antibody levels fall below 10 mIU/ml. Lee, Chuanfang; Gong, Yan; Brok, Jesper; Boxall, Elizabeth H; Gluud, Christian (19 April 2006). "Hepatitis B immunisation for newborn infants of hepatitis B surface antigen-positive mothers". Cochrane Database of Systematic Reviews (2): CD004790. doi:10.1002/14651858.CD004790.pub2. PMID 16625613.)

A well-described age-related modulation of the immune system is the decline of de novo generation of T and B cells. In addition, the accumulation of memory cells and loss of diversity in antigen specificities caused by a lifetime of exposure to pathogens have also been described [22].This is a call for great concern and a recommendation for booster doses of Hepatitis B vaccine for school age children to prevent hepatitis B infection. Electric power supply is very poor in Ekiti State and many other states in Nigeria, this may cause broken cold chain of stored vaccine and result in poor sero-conversion in ‘vaccinated’ children. Other possible causes of low sero-conversion include poorly manufactured vaccine, expired vaccine, improper administration and incomplete dosages among others. However among our study population, these are not likely because of the usual steps taken to ensure potency of vaccines used in the NPI programs. The need for booster doses can be assessed by

annual HBsAb testing, and it has been proposed that a booster dose should be given when the HBsAb concentration is less than 10 mIU/mL. Therefore measuring titers is being considered. Furthermore “For other immunocompromised people (eg, HIV-infected people, hematopoietic stem cell transplant recipients, and people receiving chemotherapy), the need for booster doses has not been determined” (Who are you quoting? Besides, the sentence is not relevant to your Study, since you do not state in your Methods whether you either include or exclude these group of children), so likely it has not been determined that the need for booster doses in healthy patients has been determined.(Discuss this part, from Line 199, alongside References).

Conclusion and Recommendation: Hepatitis B vaccination protects children against HBV in the study population. However, the seroconversion rate seen in this study showed that majority of the children may be at risk of HBV infection at a later age. To avert a setback in the goal of hepatitis B viral eradication by year 2030, we recommend booster dose of hepatitis b vaccine at the school age of 6 years to all children in our environment. (Discuss alongside that the WHO till date does not recommend routine booster dose. See below.).

***Study Limitation*:** However, since very low level antibody titres may not be detectable by qualitative detection methods as used in this study, further work using quantitative detection methods is required to confirm total lack of immunoglobulin or otherwise among vaccinated children. (Study Limitations are conventionally discussed under Method, and not at the end of the Manuscript).

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Conflicts of interest: Authors declared no conflicts of interest in this work

WHO (2018): https://www.who.int/news-room/fact-sheets/detail/hepatitis-b The hepatitis B vaccine is the mainstay of hepatitis B prevention. WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 hours. The low incidence of chronic HBV infection in children under 5 years of age at present can be attributed to the widespread use of hepatitis B vaccine. Worldwide, in 2015, the estimated prevalence of HBV infection in this age group was about 1.3%, compared with about 4.7% in the pre-vaccination era. The birth dose should be followed by 2 or 3 doses to complete the primary series. In most cases, 1 of the following 2 options is considered appropriate:

a 3-dose schedule of hepatitis B vaccine, with the first dose (monovalent) being given at birth and the second and third (monovalent or combined vaccine) given at the same time as the first and third doses of diphtheria, pertussis (whooping cough), and tetanus – (DTP) vaccine; or

a 4-dose schedule, where a monovalent birth dose is followed by three monovalent or combined vaccine doses, usually given with other routine infant vaccines.

The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is probably lifelong. Thus, WHO does not recommend booster vaccination for persons who have completed the 3 dose vaccination schedule.

All children and adolescents younger than 18 years-old and not previously vaccinated should receive the vaccine if they live in countries where there is low or intermediate endemicity. In

those settings it is possible that more people in high-risk groups may acquire the infection and they should also be vaccinated. They include:

- people who frequently require blood or blood products, dialysis patients, recipients of solid organ transplantations;
- people interned in prisons;
- persons who inject drugs;
- household and sexual contacts of people with chronic HBV infection;
- people with multiple sexual partners;
- healthcare workers and others who may be exposed to blood and blood products through their work; and
- travellers who have not completed their hepatitis B vaccination series, who should be offered the vaccine before leaving for endemic areas.

The vaccine has an excellent record of safety and effectiveness. Since 1982, over 1 billion doses of hepatitis B vaccine have been used worldwide. In many countries where between 8–15% of children used to become chronically infected with the hepatitis B virus, vaccination has reduced the rate of chronic infection to less than 1% among immunized children.

In 2015, global coverage with the third dose of hepatitis B vaccine reached 84%, and global coverage with the birth dose of hepatitis B vaccine was 39%.

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