IMMUNOLOGICAL RESPONSE TO RECOMBINANT HEPATITIS B VIRUS (HBV) VACCINE AMONG VACCINATED ADULTS IN KADUNA SOUTH, NIGERIA.

Salama K.Ibrahim[1*], Yetunde Faidero Isa[2], Hosea S. Hamafyelto[3] Lohya Nimzing[4], Zakka Sheyin[5]

[1]Hope for the Village Child Foundation, km 2 along Kachia Road, P.M.B 500, Kaduna State, Nigeria

[2]APIN/PEPFAR Laboratory, JUTH, P.M.B 2076, Jos Plateau State, Jos Nigeria

[3]Department of Medical Microbiology, University of Jos, Plateau State, Nigeria

[4.] Department of Microbiology, University of Maiduguri, P.M.B. 1069 Maiduguri, Borno State, Nigeria.

[5]Department of Medical Laboratory Science, University of Jos. Plateau State, Jos Nigeria.

Two and above authors with the same address then you give them same numbers with asterisk

Authors' contributions

This work was carried out in collaboration among all authors. Author SKI designed the study, wrote the protocol and the first draft of the manuscript, HSH performed the statistical analysis. Author YF managed the laboratory analyses of the study. Authors LN and ZS supervised and monitored the entire work. All authors read and approved the final manuscript.

ABSTRACT

Aim: The aim of this study was to determine the rate of immunological response to recombinant Hepatitis B Virus (HBV) vaccine among vaccinated residents of Kaduna South Senatorial district.

Materials and Methods: This was a cross-sectional study of 180 consented residents of Kaduna South Senatorial district, Kaduna State who have been vaccinated with HBV vaccines. Systematic sampling technique and written informed consent were used in recruiting subjects for this study. Five (5mls) of venous blood was collected from each subject after filling a structured questionnaire. Sera obtained from 180 subject were qualitatively assayed for HBV markers using SkyTech profile and quantitatively tested for Anti-HBs using ELISA KIT (Enzyme Linked Immunoassay for qualitative and quantitative determination of antibodies to Hepatitis B surface Antigen by DIA.PRO in Italy). The results from the laboratory analysis of the specimens were computed using SPSS version 21.

Results: The results represent 51.7% seropositive of HBsAb among subjects with male having 18.9% and female had 32.8% which statistically showed no significant difference between the groups (χ^2 =3.612,*P* = .43) see Table 1.In respect to age, 26 – 30years age grouped had the highest sero-conversion rate of 10.0%. This however, was not statistically significant (Table 2) (χ^2=5.604, *P* = .70). For the number of vaccine shots (Table 3) taken, 40.6% of those who completed their vaccination were sero-converted followed by those who took two shots with 4.4% while those who had one shot had 6.7% HBsAb (χ^2 =30.665, *P*< .001). Sero-conversion in relation to the quantity of HBV vaccine with titre values of ≥100IU/ml had 34.6%while ≤ 100IU/ml had 16.1% respectively. The result therefore showed statistically significant difference to the quantity of the vaccine administered at χ2 = 6.98, *P* = .08. In Table 4.

Conclusion: the findings in the research show that none of the subjects tested positive for Hepatitis B envelope Antigen or Hepatitis B envelop antibody. This could due to prior resolved HBV infection before the onset of vaccination or a resolved HBV infection mid-way into the vaccination process. Low sero-conversion rate was observed which could be due to the inclusion of subjects who failed to complete their vaccination.

Keywords*: Hepatitis B Virus, Sero-conversion, Sero-protection, Vaccination, Adults*

1. INTRODUCTION

Hepatitis is a general term meaning inflammation of the liver. This can be caused by Viruses, drugs, and poisons e.tc. Among the many known causes of hepatitis, viruses are the most important, with Hepatitis B virus being the most critical in terms of complications, morbidity and mortality. It is 50 to 100 times more infectious than HIV and can survive outside the body for at least 7 days[1].

Hepatitis B virus is a member of the Hepadnavirus family[2]. The virus particle called Dane particle / Virion[3] consist of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA (a DNA polymerase that has reverse transcriptase activity similar to retroviruses). The outer envelope contains embedded proteins which are involved in viral binding of, and entry into susceptible cells[4]. The Hepatitis B virus is known as a blood-borne virus because it is transmitted from one person to another via blood or fluids contaminated with blood. Another important route of transmission is from an infected mother to a newborn child, which occurs during or shortly after birth[5].

Currently, an estimated 2 billion people worldwide have had contact with the virus. 360 million of such infected persons progress to chronicity with about25% of chronic cases

developing cirrhosis and hepatocellular carcinoma[6]. HBV infection is of great concern in most developing countries. Sub-Saharan countries are reported to be hyper endemic (i.e.>8% of the population are infected) [7]. Among these Sub-Saharan countries, Nigeria has a pooled prevalence rate of 13.6%[8]. Though drugs are now available for treatment of HBV infection, present treatments are very costly and not readily available. However, vaccination is now the most effective and economical means for the prevention of this disease[9]. WHO in 1991 recommended the inclusion of Hepatitis B vaccines in the national vaccination program in all countries with the aim of reducing the global impact of HBV infections.

Hepatitis B vaccine is a genetically engineered (man-made in the laboratory) piece of the virus. It does not contain live virus, thus cannot cause hepatitis due to reversion as in some live attenuated vaccines. This vaccine works by helping the body produce immunity (through antibody production) that prevents infection from Hepatitis B virus. It is pertinent to note Hepatitis B vaccine does not protect from other viral infections[1]. There are two types of products available for immunization against Hepatitis B: a vaccine that confers active immunity and a specific immunoglobulin that provides passive and temporary immunity while awaiting response to vaccine. Hepatitis B vaccines are routinely given intramuscularly in the upper arm or anterolateral thigh. The buttock must not be used because vaccine efficacy may be reduced[10].

Protection by HBV vaccine is based on the production of immunity or antibodies to the surface protein or outer coat of the virus. This outer coat is called Hepatitis B surface antigen or HBsAg[11]. Successfully vaccinated individuals will be positive for only anti- HBs while persons who got immunity as a result of HBV infection will be positive for both anti-HBc and anti- HBs[12].

However, around 10% to 15% of adults fail to respond to three shots of vaccine or respond poorly. Poor responses are mostly associated with age (over 40 years), obesity and smoking[13]. Lower sero-conversion rates have also been reported in alcoholics, particularly those with advanced liver disease[14]. Thus the need for post - vaccination testing for HBsAg and anti-HBs following HBV vaccination.

Though this vaccine is commonly used in Nigeria, there is no sufficient data on Nigerian subjects regarding immunologic response or lack thereof in terms of its immunogenicity[15]. And many among those who start to receive the vaccine fail to complete the 3 recommended doses of the vaccine.Thus the need to study the immunological response to HBV vaccine, among vaccinated persons, in Kaduna south Senatorial District.

2. MATERIALS AND METHODS

2.1 Study Area

Blood random samples were collected from residents of Kaduna South senatorial district in Kaduna State. Kaduna State is located at $10^0$31"N7^0 26'25"E in north-western Nigeria. The population of Kaduna as at the 2006 Nigerian census was 760,084 but this is believed to have grown to over 1.8 million as of 2013. Collected samples will be appropriately.

2.1.1 Study population

Persons with any history of Hepatitis B vaccination who had no knowledge of being HBsAg positive and consented to be part of the study were included and also excluded subjects who had knowledge of being HBsAg-positive, above the age of 60, non HBV vaccinated, between July and November 2016 from Kaduna South Senatorial district**.**

2.1.2 Study design

The study was a hospital based descriptive, cross-sectional study that recruited 180 consenting adults in Kaduna South Senatorial district, Kaduna State**.**

2.2 Ethical Consideration

Before the commencement of the project, relevant ethical clearance/approval was sought for and obtained from the Kaduna State Ministry of Health.

2.3 Sample Collection

Five (5) ml of venous blood was collected from the forearm of one hundred and eighty residents of Kaduna South Senatorial district, Kaduna State after the signing of a consent form and the administration of questionnaire. Collected samples were placed in sterile plain tubes and labeled appropriately. The blood samples were centrifuged at 2000 rpm for five minutes. Sera were separated using automated pipette and stored in the freezer at -20^0C prior to usage.

All samples were qualitatively tested for five Hepatitis B Markers HBsAg, HBsAb, HBeAg, HBeAb and HBcAb using HBV 5-Panel test kit (SkyTech, USA). Quantity of HBsAb was also tested using ELISA KIT (Enzyme Linked Immunoassay for qualitative and quantitative determination of antibodies to Hepatitis B surface Antigen in human serum and plasma by DIA.PRO in Italy).

2.4 QUALITATIVE ANALYSIS USING HBV PROFILE TEST KIT

2.4.1 Principle of HBV Detection with HBV Profile Test Kit

The product uses the colloidal gold and membrane chromatography technology, measures HBsAg, HBeAg in whole blood, serum and plasma with dual-antibody sandwich method, measures HBsAb with dual – Antigen sandwich method and measures HBeAb and HBcAb with neutralization competitive inhibition method.

2.4.2 Assay procedure of HBV Profile Test

The right side of the HBV profile test card were placed horizontally from the original package, from left to right, respectively corresponding to HBsAg, HBsAb, HBeAg, HBeAb, HBcAb. Serum was dropped into each of the five sample wells of the test board using micropipettes. A drop of buffer was added to each of the five wells on the test card. The results were observed and recorded within fifteen minutes.

2.4.3 Results Determination

Negative results for HBsAg, HBsAb, HBeAg (sandwich method), were represented with only one purple bar at the control line in the control (C) zone. While positive results were by detecting two purple bars, one in the test (T) zone and the other in the control (C) zone. Negative results for HBeAb and HBcAb (competition method) was done by detecting two purple bars in T and C zone and Positive results were by detecting only one purple bar (control line) in the control zone.

2.5 Data Analysis

The data obtained from the study were analysed using Statistical Package for Social Sciences (SPSS) version 21 (IBM SPSS Inc, USA).Proportions were compared using Chi-square with confidence limit (p-value) of < 0.05considered significant.

3. RESULTS

Of the 180 respondents, 61 were male while 119 were female. The overall sero-conversion rate of HBsAb 51.7% out of which male had 18.9% representing 34 positive rate while female with 32.8% had 59 tested positive. The results there showed no statistically significant difference between male and female (χ^2 =3.612, *P* = .43) see Table 1. The study revealed the highest HBsAb sero-conversion rate of 10.0% among the age group 26 – 30 years with the lowest rate of 3.3% among 16 – 20 years age group. This however, was not statistically significant (Table 2) (χ^2=5.604, *P* = .70).

For the number of vaccine shots, 40.6% of those who completed their vaccination were sero-converted followed by those who took two shots with 4.4% while those who had one shot had 6.7% HBsAb at χ^2 =30.665, *P*< .001. This showed a significant difference between the vaccine shots (Table 3). Protective sero-protection in relation to the quantity of HBV vaccine with titre values of ≥100IU/ml had 34.6% while ≤ 100IU/ml had 16.1% respectively. The result therefore showed statistically significant difference to the quantity of the vaccine administered at χ2 = 6.98, *P* = .08. In Table 4. This study did not recruit equal number of respondents in the one shot, two shots and three shots category and did not take into consideration other the cellular and genetic factors that influence sero-conversion

Table 1. Sero-conversion of adult subjects to HBV vaccine in relation to gender.

Gender	No. tested for HBsAb	No Positive for HBsAb	%Positive
Male	61	34	18.9
Female	119	59	32.8
Total	**180**	**93**	**51.7**

(χ^2=3.612, *P*= .43).

Table 2. Sero-conversion of adult subjects to HBV vaccine in relation to age.

Age group	No. tested for HBsAb	No Positive for HBsAb	%Positive
16-20	14	6	3.3
21-25	20	11	6.1
26-30	34	18	10
31-35	11	8	4.4
36-40	20	12	6.7
41-45	30	16	8.9
46-50	22	8	4.4
50-55	14	6	3.3
≥55	15	8	4.4
Total	**180**	**103**	**57.2**

Chi-Square (χ^2) =5.604, *P*=.70.

Table 3. Sero-conversion of adult subjects to HBV vaccine in association with the number vaccine shots received.

No of shots Received	No. tested for HBsAb	No Positive for HBsAb	%Positive
One	47	12	6.7
Two	27	8	4.4
Three	106	73	40.6
Total	**180**	**93**	**51.7**

(χ^2=30.665, df = 2, *P*< .001).

Table 4. Protective sero-conversion in association with quantity of HBsAb produced after vaccination.

Quantity of HBsAb	No. tested for HBsAb	No Positive for HBsAb	%Positive
<100	94	29	16.1
>100	86	64	34.6
Total	**180**	**93**	**51.7**

χ^2 =13.98, *P* = .08.

4. DISCUSSION

The rate of sero-conversion for all subjects observed by this study was 51.7% (93/180) regardless of the number of vaccine shots received. None of the subjects tested was positive for Hepatitis B envelope Antigen (HBeAg) or Hepatitis B envelop antibody. This showed that none of the subjects was at the infectivity phase or has recovered from the phase. This could be as a result of prior resolved HBV infection before the onset of vaccination or a resolved HBV infection mid-way into the vaccination process.

In this study, the 18.9% rate of sero – conversion among male subjects lower than 32.8% sero – conversion rates recorded in female subjects. This was in agreement with findings of [16]who suggested that the high level of testosterone in men represses transcription factors implicated in immune activation and with findings by [17]that found higher rates of sero-conversion in women than men. However, this study agrees with that by [15]who found that men were more likely to sero-convert to HBsAb than women.

The highest rate of sero –conversion of 10.0% in age group 26 – 30 years had no relationship with sero – conversion after HBV vaccination as was found by[18, 19]. This finding may be as a result of active immunity as a result of physical exercise possessed by the older subjects in this study.

The number of HBV vaccine shots received by subjects was analyzed in this study. Result of the analysis showed that there is a relationship between the rate of sero-conversion and the number of vaccine shots received. [7]This buttresses the importance of completing the course of vaccination as 40.6%of the subjects who completed their vaccination sero-converted and just 6.5% of those who received just one shot of the vaccine sero-converted.

In this study, sero-conversion of 34.6% (64/180) with titre values of ≥100IU/ml of HBsAb (level deemed protective by the European Health Sector) was lower than the 85-90% of full response of vaccinated individuals as reported [20]and the 82.9%[21]. This low sero-

conversion rate reported by this study may be as a result of the inclusion of subjects who received one or two shots of the vaccine.

5. CONCLUSION

It was concluded based on the findings that none of the subjects tested were positive for Hepatitis B envelope Antigen (HBeAg) or Hepatitis B envelop antibody. This could be as a result of prior resolved HBV infection before the onset of vaccination or a resolved HBV infection mid-way into the vaccination process. Low sero-conversion rate was observed which could be as a result of the inclusion of subjects who received one or two shots of the vaccine.

CONSENT

All the authors reviewed and gave their consent for this article to be submitted for publication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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