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Evaluation of Hepatitis B vaccine Immunogenicity in Relation to ABO and Rhesus Blood Group in vaccinated subjects in Bauchi State, Nigeria

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Abstract
Hepatitis B virus infection is a global public health issue. It can result in hepatocellular carcinoma (HCC), and liver cirrhosis when not properly managed. The discovery of Hepatitis B vaccine and its incorporation into immunization programmes has brought a drastic decline in the incidence of both chronic and acute hepatitis B. However, this achievement is being confronted by cases of vaccine sub-optimal immune response, non-responsiveness and breakthrough infection which may be associated with the host's genetic predisposition such as; ABO/Rhesus blood groups and hemoglobin genotype. The study aimed at investigating hepatitis B vaccines responsiveness (immunogenicity) and its association with the hosts' ABO and Rhesus blood group among vaccinated subjects in Bauchi State Nigeria. This was a cross-sectional investigation comprising of 352 subjects of both sexes from age 1 year to 60 years. Out of the 352 subjects, 196 were vaccinated while 156 were unvaccinated. 5mL of blood samples were collected and analyzed for determination of both ABO and Rhesus blood group while the plasma part of the samples was tested for anti-HBs antibodies by enzyme linked immunosorbent assay (ELISA). The results revealed that 96(49.0%) of the vaccinated subjects had anti-HBs level ≥ 10 IU/L, 65(33.2%) had ≤ 10 IU/L and 35(17.9%) were non-responders while 26(16.7%) of the unvaccinated subjects had anti-HBs level ≥ 10 IU/L (P = 0.003). On the relationship between the ABO/Rhesus group and vaccine immunogenicity, blood group AB had 100% sub-optimal response while group B had 34.2% suboptimal response and 21% non-responders. Blood group A and O had the highest ≥ 10 IU/L anti-HBs protective level of 52.8% and 50.0% respectively. However, there was no significant association between HBV vaccine responsiveness and hosts' ABO/Rhesus. The protective rate against HBV infection was moderate. Nevertheless, some blood types had higher responses than others. Therefore, a considerable proportion of vaccinated persons should be considered for either booster doses or revaccination.
Keywords: ABO; Hepatitis B Virus; Rhesus blood group; Vaccine Immunogenicity.

INTRODUCTION

Hepatitis B virus infection has been identified as one of the public health challenges that causes approximately 0.5 million to 1.2 million deaths annually following complications as a result of hepatocellular carcinoma, cirrhosis and chronic hepatitis (Zenebe *et al.*, 2014, Graber-Stiehi, 2018). According to the World Health Organization (2017) report, it was revealed that WHO Western pacific Region recorded the highest number of individuals living with active positive cases of hepatitis B virus. This case was estimated to be 6.2% which is approximately over 100 million cases (Magaji, *et al.*, 2021). Whereas in the African Region, there is an estimate of 60 million cases (6.1%). These two Regions together account for 68% of the world's hepatitis B cases (WHO 2017). The non-vaccinated group is considered one of the highest risk groups that are more susceptible to the disease. They are often vulnerable to hepatitis B infection on exposure to contaminated blood and or blood products (Fuad, *et al.*, 2017). In Nigeria, a national prevalent rate of 12.2% has been reported (Adebola, *et al.*, 2016).

Vaccination still remains the primary most effective baseline action in HBV infection prevention and control (Wiedermann, *et al.*, 2016). WHO has recommended that the HBV vaccine should be incorporated into the infant immunization program of every country (Gunasekaran and Sree, 2018). It has been reported that the vaccine has brought about a significant decrease in the rate of the infection. However, even with the introduction of the HBV vaccine in Nigeria since 2004, there seems to be cases of suboptimal and non-

response to the vaccine by the vaccinated individuals (Damulak, *et al.,* 2013). Different factors have been suggested to be responsible for the vaccine failure. These factors range from Human Leukocyte Antigen polymorphism (HLA), vaccine factor, dose schedule and sex of the host, (Sadoh, and Ofili, 2014). Other scholars have argued that S gene-related mutants may be responsible for the non-responsiveness (Chang, 2010, Qin, and Liao, 2018).

The associations between the ABO/Rhesus blood groups and vaccine failure have not been given much attention, unlike the association between ABO/Rhesus and diseases which has been studied since the early 1900s (Abegaz, 2021). However, because of the absence of some blood group antigens, there have been scientific arguments and counter arguments on the relationships between vaccine failure and ABO blood group (Fan, *et al.*, 2020). According to the theory of human genetic, infectious diseases, variation of inherence variants can influence any foreign body inoculated in the human body (Casanova, 2015). This implies that introduction of vaccine inoculum in the human body can be influenced. Cooling (2015), also came up with a suggestion and argued that; "Blood groups determine host immunogenicity to vaccine.'' Therefore, these two theories and other related theories that might possibly exist point to the possibility of genetic predisposition such as; ABO/Rhesus blood groups being capable of influencing Hepatitis B vaccine immunogenicity. The four major ABO blood groups include; type A, B, AB and O.

The antigens of the blood group are expression of inherited polymorphic traits in a population (Wang, *et al.,* 2012). Variation in hosts' blood group antigens has been associated with their susceptibility to antigens. Similarly, in terms of intracellular ingestion, ABO/Rhesus antigens ease the process of adherence via micro domains membrane (Gunasekaran and Sree, 2018). Respective types of blood can cause transformation in congenital immune response. There is an overrepresentation in the populace domiciled in malaria endemic region by respective phenotypes related to quantity of malaria resistance by host. Antibodies against ABO/Rhesus, blood types, Kell and T can be provoked by microorganisms, (Cooling, 2015).

An overview of some literature has lots of evidence that point to many factors that might influence vaccine immunogenicity. Some of these factors have been proven to have a relationship with the HBV vaccine response, while others have no validated evidence. Host genetic factors such as HLA have been investigated and found to influence HBV vaccine response. However, host genetic predispositions such as; ABO and Rhesus blood groups have not been given attention. There is therefore the need to evaluate hepatitis B vaccine immunogenicity in relation to ABO/Rhesus Blood Group in Bauchi State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out within the Bauchi metropolis which is the capital city of Bauchi State. Bauchi State is one of the 36 States in Nigeria and is located in the north eastern part of the country. The State is made up of twenty (20) Local Government Areas with a population estimate of 5.6 million. However, the estimated population of the metropolis is 1,500,000 (National Population Census, 2006). It has land mass of approximately 49,119km square area, representing about 5.3% of Nigeria's total land mass and is located between latitudes 9° 3' and 12° 3'N and is longitudes 8°, 50° and 11 East. The State is bordered by seven States, Kano and Jigawa to the North, Taraba and Plateau to the South, Gombe and Yobe to the East and Kaduna to the West. Bauchi State has a total of 55 tribal groups in which Hausa, Fulani, Sayawa, Gerawa, Jarawa, kirfawa and Karekare are the main tribes. Bauchi metropolis has a household population of 746, 735 (Bauchi Local Government house to house immunization record, 2018). There are eight (8) wards within the Bauchi metropolis.

Study Design

This was a cross-sectional investigation that was undertaken within the Bauchi State metropolis between January and November, 2021. The sample size was determined by using the rate of non-responsiveness as a baseline which is between 5-10% to the HBV vaccine. The sample size was found to be 239 but 352 was collected for convenience with 5% α level and 99% strength.The study subjects were from both sexes from age 1 year to 60 years. Out of the 352 subjects, 196 were vaccinated while 156 were unvaccinated. A random sampling technique was employed across all the twelve wards in the Bauchi metropolis. Human research ethical clearance was obtained from the Bauchi State ministry of health human's research ethics committee. A consent form and questionnaire were used to obtain the consent and data from the study subjects relevant to this study respectively.

Study population

This included individuals living in the Bauchi metropolis who were within the age of 1 year to 60 years. These were individuals, who

had been vaccinated with at least 2nd, 3rd doses or booster dose of hepatitis B vaccination as prescribed by the State health authority or those who had not been vaccinated and were apparently healthy. In this category of people, only those who consented to participate in this study were considered. However, Individuals who were within the age range of 1year to 60 years but did not consent and those who were not within the age bracket of 1 year to 60 years were excluded from the study.

Collection and Processing of Blood Samples

A volume of 5mL venous blood was aseptically collected in ethylene diamine tetra-acetic acid (EDTA) tubes from both the study subjects and the control subjects. The tubes were centrifuged at 2500 revolution per minute (RPM). The plasma was separated and stored at -21^{O}C at the virology laboratories of the infectious diseases Hospital Bayara (IDHB). The plasma was later retrieved to titration of anti-HBs levels. The packed red cells were re-suspended using 0.9% normal saline and used for ABO/Rhesus blood grouping.

Determination of ABO/Rhesus Blood Group

The ABO blood groups (A, B, O and AB) were determined using mouse-derived monoclonal antibodies produced by Ortho Bioclones Diagnostic Systems, Raritan, NJ. The ABO/Rhesus (Rh) grouping was performed based on conventional methods as described by Kim. *et al*, (2018). Subjects were classified as; ABO blood groups A, B, O or AB and Rhesus positive (Rh+) or Rh-negative (Rh-). Four separate drops of a 20% red blood cell suspension made with 0.9% normal saline were placed on a plastic tile with four predetermined spots. To 1st drop, 1 volume of anti-A serum was added, to second drop, 1volume of anti-B was added, while to the 3rd blood drop, 1 volume of anti-D was added and to the fourth blood spot normal saline was added to serve as control. Using a sterile applicator stick, one for each of the drops, the contents were mixed thoroughly. Then the tile was rocked gently for 2 minutes and the result was read by observing for agglutinations. Visible agglutinations in the mixture containing antisera A, B, or AB were considered blood group A, B or AB respectively. While visible agglutination in the mixture containing anti-D of any sample was considered Rhesus positive while absence of agglutination was considered Rhesus D negative.

Quantitative detection of Anti-HBs

The quantitation of the anti-HBs was carried out using a hepatitis B surface antigen Antibody ELISA Kit. The kit was obtained from Abnova Taipei City 114 Taiwan with a lot no: B21110PT. The Hepatitis B surface antigen Ab ELISA Kit is for both qualitative and quantitative detection of antibody to HBsAg in serum or plasma. The kit utilizes HBsAg on the wells as peroxidase-conjugate which is a solid-phase enzyme immunoassay that works based on the "sandwich principle." The assay was carried out according to the manufacturer's instructions.

Statistical analysis

The statistical analyses were carried out using the statistical package of social sciences (SPSS version 23.0). Computation was made using two-sided p values where p value <0.05 was regarded to have statistical significance. Comparisons of the baseline parameters were made using student's t-test and Pearson's v2 test.

RESULTS

The finding revealed 35(17.9%) of the vaccinated subjects to be non-responders to the vaccine with no detectable anti-HBs. However, 96(49.0%) of the vaccinated responded with ≥10 IU/L anti-HBs, 65(33.2%) had suboptimal anti-HBs response of <10 IU/L while among the unvaccinated, 26(16.7%) had suboptimal anti-HBs of ≤10 IU/L. The response of anti- HBs in the vaccinated group was significantly higher compared to the unvaccinated group (p= 0.001) as shown in table 1 below. There was no difference in anti-HBs protective level in both the participants who had taken two or three doses. On the contrary all the 16(8.2%) that had been administered booster dose showed anti-HBs titers of ≥100 IU/l. These are shown in table 1. Table 2 shows the association between vaccine immunogenicity and ABO blood group. Blood group A and O had the highest rate of anti-HBs protective level of 52.0% and 50.8% respectively. But blood group B had the lowest rate while type AB had 0.00% rate response to HBV vaccine. However, there was no statistical significant as regards the association as P= 0.33. Similarly, in the non-vaccinated subjects, blood group AB had 100% anti-HBs protective response followed by Blood group B and O with 27.8% and 15.2% respectively. There was equally no significant.

Table 1: Level and distribution of anti-HBs in the vaccinated and non-vaccinated subjects

Anti-HBs	Vaccinated (%)	Non-vaccinated (%)
Protected (≥ 10)	96(49.0)	26(16.7)
Not-protected (≤10)	65(33.2)	
Non-responders	35(17.9)	
Total	196(100.0)	156(100.0)

Table 2: Anti-HBs immunogenicity among the different blood groups

Vaccinated		**Anti-HBs**		
ABO blood groups	No. examined	≥10 UI/L (%)	≤10UI/L (%)	No response (%)
A	25	13 (52.0)	7 (28.0)	5 (20.0)
B	38	17 (44.7)	13 (34.2)	8 (21.1)
O	130	66 (50.8)	42 (32.3)	22 (16.9)
AB	3	0 (0.0)	3 (100.0)	0 (0.0)
Total	196	96 (49.0)	65(33.2)	35 (17.9)
Statistical analysis: $\chi 2 = 6.905$; df = 3; p-value = 0.33 (p < 0.05) – not statistically significant				
Non-Vaccinated ABO blood groups				
A	12	1(8.3)	0(0.0)	
B	18	5(27.8)	0(0.0)	
O	125	19(15.2	0(0.0)	
AB	1	1(100.0)	0(0.0)	
Total	156	26(16.7)	0(0.0)	
Statistical analysis: $\chi 2 = 7.394$; df = 3; p-value = 0.06 (p < 0.05) – not stat. significant				

The table 3 below presents the relationships between vaccine response and Rhesus blood group in both the vaccinated and non-vaccinated subjects.Rhesus negative blood group had the highest anti-HBs ≥10UI/L of 56.3% while Rhesus positive blood group had 48.3% of anti-HBs ≥10UI/L. In the non-vaccinate, 16.9% of the Rhesus positive had anti-HBs ≥10UI/L while only 12.5 of Rhesus positive group responded with anti-HBs ≥10UI/L.

Table 3: Distribution of Anti-HBs in the study subjects in relation to Rhesus blood group

Vaccinated		**Anti-HBs**		
Rhesus blood groups	No.examined	Anti-HBs ≥10UI/L	Anti-HBs< 10 UI/L	No. response (%)
Positive	180	87 (48.3)	61(33.9)	32 (17.8)
Negative	16	9 (56.3)	4 (25.0)	3 (18.8)
Total	196	96 (49.0)	65 (33.2)	35 (17.9)
Statistical result: $\chi 2 = 0.546$; df = 2; p-value = 0.76 (p < 0.05) – not statistically significant				
Non-Vaccinated Rhesus blood groups				
Positive	148	25(16.9)	0 (0.0)	
Negative	8	1(12.5)	0 (0.0)	
Total	156	26(16.7)	0 (0.0)	
Statistical result: $\chi 2 = 0.105$; df = 1; p-value = 0.75 (p < 0.05) – not statistically significant				

Key: **Anti-HBs;** antibody to Hepatitis B surface Antigen

DISCUSSION

This is probably the first study in Bauchi to investigate the association between hepatitis B vaccine immunogenicity with ABO/Rhesus blood group. Our findings indicated a moderate hepatitis B vaccine response of 49% among the vaccinated study subjects in Bauchi. Hepatitis B vaccine rate of non-responsiveness has been estimated to be 5%-15% globally, (Wiedermann, *et al.*, 2016). This study has shown that 17.9%) of the vaccinated subjects to be non-responders which is evidently higher than the estimated level. The finding on the rate of non-responders did not agree with the global estimated levels as it was 3% higher than the maximum level.

Also, another study has shown that some individuals do not show a protective anti-HBs antibody response even after a complete course of primary vaccination (Magaji, *et al.*, 2021). Surprisingly, studies from Rajasthan and Bulgaria showed a higher non-response rate of 30% and 20%, respectively (Weler and Zeuzem, 2016, Huang, *et al*, 2013). The rate of non-responders in this study still falls within the average level of the highest rate of non-responsiveness reported. Similarly, 5-10% in a given population has been approximately estimated to be suboptimal or poor responders to HBV vaccine (Wiedermann, *et al*, 2016). This category is believed to produce non-protective anti-HBs (< 10IU/L) levels (Szmuness, *et al*,

2018). In this study, 33 % vaccinated subjects had suboptimal or non-protective antibody levels (< 10IU/L) against HBV. This finding does not agree with the global estimate of poor response to HBV vaccine. However, it agrees with the report of Fuad, *et al,* (2017) who documented 27.8% having no protective level against HBV infection in Yemen following vaccination.

The non-vaccinated subjects were incorporated to serve as a control cohort. Ordinarily, anti-HBs should not be found among the unvaccinated, for it is usually produced following inoculation during the vaccine (World Health Organization, 2017). However, it has been established that the same hepatitis B surface antigen (HBsAg) used as active ingredient in the hepatitis B vaccine has the same infective particle in the hepatitis B components (Mei, et al, 2018). Therefore anti-HBs can be present among the non-vaccinated subjects who have been exposed to the HBV. This has been considered to be a result of natural clearance of the viral agents from the host following strong immune response (Olumuyiwa, *et al,* 2011). This typical scenario was evident as can be seen in table 2 above and 3 below. A total of 26 (16.7%) of the non-vaccinated had ≥10 UI/L anti-HBs level. However, the remaining 130(83.3%) were considered negative for HBsAg unlike the vaccinated group that were considered non-responders.

On the other hand, the rate of protective level of anti-HBs response revealed 49.0%) had an HBV protective level of antibody (≥ 10 IU/L). However, Olumuyiwa, (2011) had earlier in a similar study in Niger State, reported 55% hepatitis B vaccine response in Niger State Nigeria. This has a wide margin with our present findings. It still did not agree with the finding of Fuad, *et al,* (2017) who reported 72.2% to have responded to HBV vaccine with ≥ 10 IU/L and 27.8% having no protective antibody against HBV infection in Yemen. In another similar study in subjects within the age bracket of 20–55 years, its findings revealed 96.5% had protective immunity to hepatitis B, and the anti-HBs response was similar in both male and female, (Abegaz *et al.,* 2021). In another recent study in India, 86% was reported to have shown good seroconversion in response to the Hepatitis B vaccine, while 10% were suboptimal responders, (Yang, 2019). It seems geographical locations and ABO/Rhesus factors may partly account for the heterogeneity of the immune response, as the highest rate of response seems to come from the same region. On the association of the responsiveness with the ABO/Rhesus type, type A and O had higher levels of anti-HBs protective level and compared to other blood types (Berinyuy *et al.*, 2019). However, there was no statistical significance.

CONCLUSION

This study indicated blood group O and Rhesus positive blood group had the highest rate of hepatitis B vaccine failure, compared with other blood groups and Rhesus factor. This is an indication that some blood group and Rhesus factors do not have affinity to hepatitis B vaccine response. In addition, 16% of the unvaccinated were found to have ≥10 UI/L anti-HBs levels of which most of them were of blood group O and Rhesus Positive blood. However, there was no statistical significance. Therefore, it seems blood group O and Rhesus Positive subjects have the ability to produce protective anti-HBs following exposure to hepatitis B virus. There is a need for more investigation on the association between ABO/Rhesus and HBV vaccine failure, especially the chemistry of the blood.

Contribution to Knowledge: the study revealed that some blood groups and Rhesus factors respond to hepatitis B vaccine more than others.

Conflict of interest: None declared

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