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Study on the Impacts of Haemoglobin Genotype on Hepatitis B vaccine Response among Vaccinated Individuals within Bauchi State, Nigeria

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Abstract

Hepatitis B virus (HBV) infection is one of the viral diseases of public health concern globally. It causes liver cirrhosis and hepatocellular carcinoma. Vaccine against hepatitis B has been incorporated into the routine infant immunization schedule of almost all the African Countries. However, reports on the vaccine failure, ranging from sub-optimal response, non-responsiveness, and vaccine breakthrough infection, threaten the vaccine efficacy globally. This study sought to assess the impacts of the Human hemoglobin genotype (HbG) on the vaccine response in Bauchi, north-eastern Nigeria. A cross-section of 196 vaccinated subjects of both sexes, aged 1 to 60 years, were recruited. The blood plasma was separated and used for the detection and titration of Hepatitis B surface antibodies by Enzyme-linked immunosorbent assay (ELISA). The red cell was used for determining Hemoglobin Genotype (HbG) of the subjects. The results indicated that genotypes SS (75%) and AS (61%) had the highest rate of optimal response to the hepatitis B vaccine, respectively, while genotype AC had the least vaccine response and the highest (50%) non-responders to the hepatitis B vaccine. Regarding genotype prevalence, genotypes AA and AS had the highest prevalence of 141 (71.9%) and 49 (25%), respectively. There was no significant relationship between various hemoglobin genotypes and Hepatitis B vaccine response. However, some genotypes have a greater affinity for the hepatitis B vaccine optimal response than others. There is a need for certain individuals to be re-vaccinated while others should go for a vaccine booster dose.

Key words: Hemoglobin Genotype, Hepatitis B, Immunogenicity, vaccine

INTRODUCTION

Hepatitis B infection (HBI) is caused by a virus from the class of hepadnaviridae. It has been globally recognized as one of the major viral diseases of public health burden (Graber-Stiehi, 2018). Hepatitis B virus (HBV) infection can culminate in chronic hepatitis and subsequently develop into life-threatening conditions such as liver cirrhosis and hepatocellular carcinoma (HCC) (Zenebe *et al.*, 2014; Graber-Stiehi, 2018) . The Knowledge of the incidence of HBV acute infection can provide vital data regarding the course of revealing outbreaks, transmission, and also the strength of prevention discourse. Hepatitis B is caused by a small circular DNA genome that is partially double-stranded (Fuad *et al.*, 2017).

Reports have shown that two billion people have been infected with HBV worldwide (Zhou & Terrault, 2019). About 400 million people worldwide are said to be chronically infected with HBV, which leads to approximately 1 million deaths annually due to complications as a result of liver cirrhosis and hepatocellular carcinoma (Fuad *et al.*, 2017). Hepatitis Bis the cause of up to 50% of hepatocellular carcinomas (HCC). Hepatitis B virus (HBV) infection remains a global challenge, with one-third of the world's population having serological evidence of current or previous infection (Mohammadali & Pourfathollah, 2018). In China, Southeast Asia, most of Africa, most Pacific Islands, parts of the Middle East, and the Amazon Basin, 8% to 15% of the population carries the virus, (Liu, 2018). In Nigeria, the Federal Ministry of Health (2016) reported HBsAg overall prevalence at 12.5%, and that 2-3 million persons are estimated to be carriers of chronic HBV. In Bauchi, a prevalence of 8% has been documented (Adebola *et al*, 2016).

According to the report of the World Health Organization (2017), it was revealed that the WHO Western Pacific Region has 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). In the African region, there are an estimated 60 million cases (6.1%) of prevalence. These two regions together account for about 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 and are often susceptible to hepatitis B infection on exposure to contaminated blood or blood products (Fuad *et al.*, 2017). In Nigeria, according to a 2016 HBV national survey, it was revealed that 12.2% of Nigerians are infected, which is approximately over 20 million who are acutely infected (Adebola *et al.*, 2016).

The transmission of the hepatitis B virus is primarily through blood and infected bodily fluids. The virus can spread through direct blood-to-blood contact, unprotected sex, unsafe injections, and blood transfusions, and from a woman infected with HBV to her newborn during delivery (WHO, 2018). The virus is believed to survive on moist surfaces for more than 7 days at room temperature (Patel *et al.*, 2019). In 2018, Mohaamadali & Pourfatholah indicated that the highest concentrations of virus are in the blood and serous fluids; lower titers were also found in other body fluids, such as saliva, tears, urine, and semen. Semen is a vehicle for sexual transmission, and saliva can be a vehicle of transmission through bites; other types of exposure, such as saliva through kissing, are unlikely modes of transmission (Liu et al., 2018). Transmission of HBV via tears, sweat, urine, stool, or droplet nuclei has not been documented (Kudo, 2017).

The hepatitis B vaccine is considered the primary and most effective means of HBV infection prevention and control. The incorporation of the Hepatitis B vaccine into the infant immunization program of every country has been recommended by the WHO (Gunasekaran and Sree, 2018). Nevertheless, even with the introduction of the HBV vaccine in Nigeria in 2004, there seem to be cases of vaccine failure, such as suboptimal and non-responsiveness among the vaccinated subjects (Adebola et al., 2016).

Despite the effectiveness and efficacy of the current HBV vaccine, there are still some groups of populations with suboptimal immunologic responses regarded as poor or non-responders to the HBV vaccine (WHO, 2018). Such suboptimal immunologic responses have been pointed toward the elderly, smokers, obese persons, and those with chronic liver or renal diseases, Diabetes mellitus (DM), or Human Immunodeficiency Virus (HIV) infection (Fuad *et al.*, 2017). Most diseases' virulence and vaccine immunogenicity have been associated with host genetic factors (Liu *et al.*, 2018). There are therefore, strong indications that HBV vaccine response may be influenced by human hemoglobin genotype as has been pointed out; "Blood genotype may influence host susceptibility to infection'' (Cooling 2015) and ''every body's genotypes have a greater influence on their susceptibility to transmissible diseases'' (Patel *et al.*, 2019).

The vaccine stimulates a varying degree of response that may have diverse protective efficacy. Some individuals, when vaccinated, do not often respond at all. These variations are determined partly by factors such as maternal antibodies and nutrition, including vitamin A, and partly by genetic predisposition of an individual, such as hemoglobin genotype. Since everybody's Genotype influences their susceptibility to transmissible diseases, there is a need to investigate the relationship between human hemoglobin genotype and hepatitis B vaccine response (Zhou & Terrault, 2019). This study aimed to assess whether hemoglobin genotype influences the optimal response to the hepatitis B vaccine. This may perhaps explain why some people respond optimally to the hepatitis B vaccine and why some do not.

MATERIALS AND METHODS

Study Design

A cross-sectional design was employed for the study as described by Tong & Revill (2016). The rate of hepatitis B vaccine non-responsiveness was used to determine the sample size, which is between 5-10%.

$$n = \frac{Z^2 Pq}{L^2}$$

The sample size was found to be 196. 5% α level and 99% strength were considered as confidence levels. The study population included both sexes within the ages of 1 year to 60 years who had been vaccinated with the hepatitis B vaccine. A stratified random sampling was used in the sample collection. Ethical clearance was sought from the Bauchi State Ministry of Health. A structured questionnaire, consent form, and assent form for minors were used as instruments of data collection.

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Study population

This consisted of men and women between the ages of 1 year and 60 years who resided in Bauchi city at the time of the research and had been duly vaccinated with 2 doses, 3 doses, or booster doses of hepatitis B vaccine.

Sample Collection and Processing

A venous blood sample of about 5 milliliters was collected from 196 vaccinated individuals who were selected by randomized sampling technique following the administration of a questionnaire, informed consent, and assent form for the minors to the study subjects. Only vaccinated adult subjects and minors who had their guardians' consent to participate in the study, were not sick, and were not under treatment with any corticosteroid drugs were recruited. The blood sample was transferred into a 10ml Ethylene Diamine Tetra acetic Acid (EDTA) capacity container and centrifuged for 5 minutes at 2500 revolutions per minute, and the plasma was separated into a 2ml capacity Sarstedt Screw Cap Macro Tube. The Plasma sample was used for the quantification of Hepatitis B surface Antibody titer (HBsAb) by Enzyme-Linked Immunosorbent Assay (ELISA) Kit procured from Abnova 108, St. Neihu District. Taipei City 114, Taiwan with lot no: B21110PT. Whereas the packed red cells were re-suspended with 0.9% normal saline and used for the determination of hemoglobin genotypes using a cellulose acetate paper electrophoresis machine (SearchTect Standard Instrument Model No: 300).

Determination of Hepatitis B surface Antibody (HBsAb) using ELISA

The microtiter plate is made up of 100 wells. Well, one (1) of the microtiter plate was reserved for blank control, well two (2) for positive control, and well three (3) for negative control as instructed by the manufacturer. 50*uL* of each control and/or specimen was added to the appropriate wells of the reaction using a clean pipette tip for each sample. Additionally, 50uL of HBsAg Peroxidase was added to each well, except for the blank. The plate was then tapped gently for 3 minutes for homogeneity. The plate was incubated in a water bath for 1 hour at 37 °C, and then washed 3 times. After this, a 100 µL mixture of Buffered A and B was added to each well, including the blank, and the plate was then covered with a black cover and incubated for 30 minutes at 25 °C. After this, 100 μL of $2NH_2SO_4$ was added to each well, including the blank. The absorbance of both the controls and the test specimens was determined within 30 minutes with a photometer at 450 nm wavelength with 620-690nm reference wavelength using the blank well sample to blank the photometer. The result for each well was calculated following the manufacturer's instructions. The result was interpreted based on the World Health Organization (WHO) standard, which recommended that Hepatitis B Surface Antibody (HBsAb) ≥10mIU/mL is considered as optimal response to the Hepatitis B vaccine, and HBsAb <10mIU/mL is a suboptimal response, while <0mIU/mL is considered non-responsiveness.

Determination of Hemoglobin Genotyping by Cellulose Acetate Paper Electrophoresis

The red blood cell (RBC) separated from the plasma was used to determine the various genotypes of the study subjects. The red cell was re-suspended using 0.9% normal saline and washed three times the supernatant was discarded, and the red cells were lysed by adding an equal volume of distilled water, one quarter (1/4) volume of toluene, and one drop of 3% potassium cyanide and was properly homogenized. A prepared buffer with PH 8.4 solutions was transferred into the chamber of the electrophoresis. Wicks that were already soaked in the buffered solution were placed such that they had contact with the buffer solution. A cellulose acetate paper was re-soaked in the buffered solution and allowed to soak for 25 minutes. The excess buffered solution was drained by placing the plate between the absorbent papers. 0.2ml of the hemolysate samples of both the test and control (AS Genotype) were applied approximately 3cm away from the cathode using an applicator stick.

The cellulose acetate membrane plate containing the samples was placed immediately in the electrophoresis tank. The tank (chamber) was then connected to a power supply and the plate was electrophoresed for 20 minutes at approximately 220mAh. After 20 minutes, the power supply was put off and the result was read.

Result: haemoglobin genotypes run based on the mobility distance in the electrophoresis tank. Genotype 'S' and 'C' gene move a longer distance than 'A' gene as can be seen on Plate 1 below (Alvaro *et al.*, 2024).

RESULTS

The findings indicated that genotype AA had the highest prevalence of 141 (71.9%) in the hepatitis B vaccinated subjects. On the other hand, Genotype AS had the second highest prevalence of 49 (25%) in the vaccinated group. Genotypes SS and AC had the least prevalence rates of 4(2.0%) and AC 2(1%) in the vaccinated subjects. Nevertheless, there was a significant difference in the distribution pattern of hemoglobin genotypes, as indicated by a P value ≤ 0.05, as shown in Table 1 below.

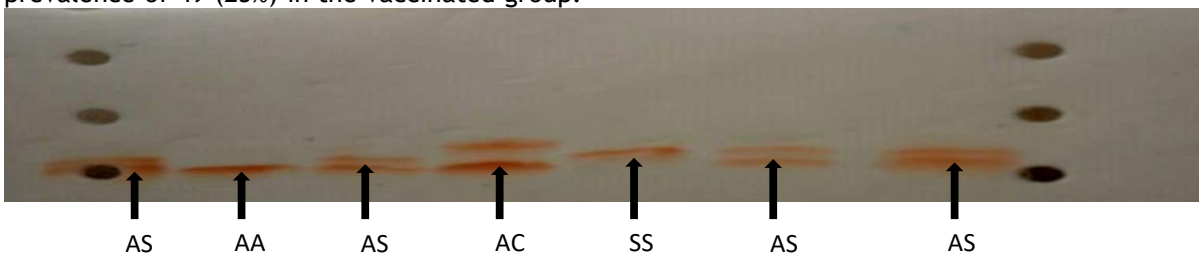

Plate 1: Hemoglobin genotype electrophoresis acetate cellulose paper
Key: Control-known genotype AS used as a standard, SS-Sickle cell trait genotype, AA-Adult gene AC-adult gene with C variant gene.

Table 1: Distribution pattern of Hemoglobin genotype in the vaccinated subjects in Bauchi

Hb Genotype	Frequency (n)	Percentage (%)
AA	141	71.9
AS	49	25
AC	2	1
SS	4	2
Total	**196**	**100**
p-value		**0.01**

Key: Hb=Hemoglobin, AS=Sickle cell carrier, AC=Adults hemoglobin with C trait, SS=Sickle cell trait, P value <0.05 means significant relationship.

Relationship between Haemoglobin Genotype and Hepatitis B vaccine Response

In the area of impact and relationship between Hemoglobin genotypes (HbG) and Hepatitis B vaccine response, WHO standard units were employed to determine whether a vaccinated person is protected following hepatitis B vaccination or not. The protective antibody response level is determined by titrating the volume of antibody (HBsAb) produced after at least six months and above following vaccination. The WHO recommends that; an HBsAb level of ≥ m10 IU/L is considered to be a protective response level to the HBV vaccine, while a level <10m IU/L is considered (suboptimal) to have no protective response. However, the absence of either any detectable antibody response of ≥10mIU/L or <10mIU/L is considered as no response (non-responder). Therefore, subjects found to have ≥10mIU/L were considered protected against HBV infection, while those with <10mIU/L were considered not protected and those without any detectable level of the antibody response were considered non-responders (Negative).

The results indicated that genotypes SS and AS had the highest vaccine optimal response of 75% and 61.2% respectively. Genotype AA had the lowest (44.0%) optimal response and the highest (39.0%) suboptimal response. On the other hand, genotype AC had 50% on both optimal and non-response respectively. There was an overall non-responder of 17.9%. This rate of non-response to the hepatitis B vaccine was relatively higher compared to the global rate which is said to be between 5-10%. There was a significant difference regarding the association between Hepatitis B vaccine response and hemoglobin genotype, as evidenced by a P-value of < 0.05. Nevertheless, the findings indicated that some hemoglobin genotypes have an affinity to respond optimally to the Hepatitis B vaccine than other genotypes as indicated in Table 2 below.

DISCUSSION

Among the (196) vaccinated group subjects, 96(49%) responded optimally (≥10mIU/mL) with a protective level to HepB vaccine. The 49% optimal response was similar with to 55% reported in an earlier study carried out in Taiwan and Niger State, Nigeria. However, this 49% did not agree with the report of Fuad, et al. (2017), who documented 72.2% to have responded to the HBV vaccine with an optimal response and 27.8% having no protective response against HBV infection in Yemen. In another similar report among the subjects within

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the age bracket of 20–55 years, it has been found that 96.5% had protective immunity to hepatitis B, and the HB antibody response was similar in both males and females. In India, 86% were reported to have shown good seroconversion in response to the Hepatitis B vaccine, while 10% responded suboptimally (Yang et al., 2016).

Table 2: Relationship between Hepatitis B vaccine response and haemoglobin genotypes (HbG) among the study groups in Bauchi State

Hb Genotype	No. Examined	≥10 mIU/mL (Optimal)	<10 mIU/mL (Sub-optimal)	<0 mIU/mL (Non-detectable)
AA	141	62 (44.0%)	55 (39.0%)	24 (17.0%)
AS	49	30 (61.2%)	10 (20.4%)	9 (18.4%)
AC	2	1 (50.0%)	0 (0.0%)	1 (50.0%)
SS	4	3 (75.0%)	0 (0.0%)	1 (25.0%)
Total	**196**	**96 (49.0%)**	**65 (33.2%)**	**35 (17.9%)**
p-value		**0.13**	**0.03**	**0.11**

Key: HBV VS =Hepatitis B vaccinated subjects, HBsAb=Hepatitis B surface antibody, Hb=Hemoglobin, Calculated P Value <0.05 means significant relationship

The HBV vaccine is one of the only two available vaccines that can prevent cancer (Yang et al., 2016). The World Health Organization recommends either a 3 or 4-dose schedule of the HB vaccine. In the case of a 3-dose regimen, the first dose is given at 0 age (at birth), whereas the second and third doses are given at an interval of four weeks. Similarly, for a 4-dose schedule, a monovalent at zero age is normally followed by 3 monovalent or combined vaccine doses. This is always administered together with other routine infant vaccines. The complete and appropriately administered vaccine is believed to stimulate the production of protective HBsAb levels in over 90% of young adults, children, and infants. For this reason, WHO does not recommend booster doses for persons who have received the complete 3-dose vaccination schedule (WHO, 2018).

The Centers for Disease Control recommends that all children and adolescents less than eighteen years who live in countries with low or intermediate endemicity should be vaccinated (CDC, 2020). In such areas, there may be more people who fall into high-risk groups. These groups should also be vaccinated, they may include; Individuals who always need blood or blood products, patients on dialysis and recipients of solid organ transplantations; those in prisons, persons who inject drugs, sexual contacts of people with chronic HBV infection, people with multiple sexual partners, healthcare workers, travelers to endemic areas should receive the vaccine before leaving for endemic areas. The practice of safe injection can also reduce the transmission of HBV.

Despite the availability of the vaccine, HBV infection persists as a global issue (Yang et al., 2019). However, HBV vaccine non-response has been described in the general health population as well as in patients with chronic disease. About 5%-15% of the general population has been estimated to produce no protective level of antibody response, and this is referred to as non-responders (Casanova, 2015). Some studies have shown that a response rate as low as 20% is observed in inflammatory bowel disease and diabetes (Gunasekaran & Sree, 2018). Anti-HB levels have been reported to decrease after 10-31 years and fall below a level considered protective in approximately 25% of cases (Graber-Stiehi, 2018).

Some studies have reported that vaccines stimulate a variable production of the hepatitis B antibody in different individuals which leads to inconsistent efficacy (Kimman & Vandabriel, 2017). The findings corroborate these reports, for we noticed drift with some hemoglobin genotypes. The hepatitis B surface antibody (HBsAb) titer was found at an optimal level of response (>10 mIU/mL) as recommended by the WHO, while some had suboptimal (<10 mIU/mL) and others had no detectable (< 0m IU/mL) response level at all (WHO, 2018). Our study indicated that individuals with Genotype SS had the highest (75%) rate of optimal response to the hepatitis B vaccine, which is the protective level of HBs antibody. Genotype AS followed this with 61.2%. This finding contradicts the reports of Han et al. (2023), which documented that individuals with sickle cell traits or diseases do not respond optimally to vaccines. However, genotypes AC and SS indicated the highest rate of 50% and 25% non-responders to the hepatitis B vaccine, respectively. This non-response corroborates the postulation of Abegaz (2021). Although there was no significant relationship between hemoglobin genotypes and hepatitis B vaccine optimal responses, it was obvious that

some genotypes have an affinity for optimal response to hepatitis B vaccine than others. Similarly, there were high rates of vaccine suboptimal response in genotypes AA and AS. This might be a result of the fact that genotype AA is the most dominant Genotype in Bauchi, and genotype AS is next, and the first variant trait genotype.

CONCLUSION

This study provides valuable insight into the impacts of haemoglobin genotypes on hepatitis B vaccine response among vaccinated individuals in Bauchi. The findings suggest that individuals with different haemoglobin genotypes may exhibit varying levels of immune response to the hepatitis B vaccine. The knowledge of these variations can inform the development of personalized vaccination strategies and improve vaccine efficacy in diverse populations. Further study is needed to explore the underlying mechanisms and the clinical implications of these findings.

RECOMMENDATION

There is a need for a deeper investigation into the relationships between hemoglobin genotype and HBV vaccine response among vaccinated individuals. These investigations may help unveil the role of different hemoglobin genotypes in response to hepatitis vaccines and other vaccine-preventable diseases globally.

CONFLICT OF INTEREST

None declared

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AUTHORS' CONTRIBUTIONS

Thomas Kyauta Mallam and Mispha Titus assisted in reviewing the manuscript, study design, and data analysis.

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