



## ORIGINAL ARTICLE

# Association between ABO and RH blood groups and Hepatitis B virus infection among young Nigerian adults

BANKOLE HENRY OLADEINDE<sup>1</sup>, MATHEW FOLARANMI OLANIYAN<sup>1</sup>, MUSA ABIDEMI MUHIBI<sup>1</sup>,  
FERDINAND UWAIFO<sup>1</sup>, OMOREGIE RICHARD<sup>2,3</sup>, NWONU OKIKE OMABE<sup>1</sup>, AMINAT DAUD<sup>1</sup>,  
ONOLEN PHEBEAN OZOLUA<sup>4</sup>

<sup>1</sup>Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Medical Science, Edo University Iyamho, Edo State, Nigeria; <sup>2</sup>Medical Microbiology Division, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria; <sup>3</sup>School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria; <sup>4</sup>Department of Microbiology, Faculty of Science, Edo University Iyamho, Edo State, Nigeria

## Key words

Hepatitis B Virus • Blood groups • ABO • Rh antigen • Adolescents • Nigeria

## Summary

**Background.** Several diseases are reported to be associated with ABO/Rh blood groups. Data on the association between ABO and Rh D blood group antigens in the Nigerian population is sparse. This study aimed at determining the prevalence of Hepatitis B Virus (HBV) infection as well as its association with ABO and Rh D antigens among young Nigerian adults.

**Methods.** Whole blood was collected from 496 students and screened for the presence of HBsAg using an immuno-chromatographic technique. The ABO and Rh D antigen status of participants were also determined using standard techniques.

**Results.** In this study, the prevalence of HBV infection was 10/496 (2.10%). Of all factors assessed, only age of participants was identified as a risk factor ( $P < 0.05$ ) for HBV seropositivity. Over half

257/496 (51.5%) of subjects were of the blood group O type, while 18/496 (3.6%) were of the AB blood type which was the least in occurrence. Rh D negative blood group was observed among 24/496 (4.8%) subjects. Those with the B blood type were observed to have an insignificantly ( $P > 0.05$ ) higher prevalence of HBV infection. However, with respect to Rh D antigen alone, participants negative for the antigen were observed to have a five times higher risk of acquiring HBV infection than those positive for it ( $OR = 5.273$ ,  $95\% CI = 1.056, 26.321$ ,  $P > 0.05$ ). Combining the ABO and Rh blood group systems, an association ( $OR = 20.174$ ;  $P > 0.05$ ) was found to exist between B Rh D negative status and HBV infection.

**Conclusion.** Possession of B antigen without Rh D antigen is associated with increased risk of acquiring HBV infection.

## Introduction

Amongst several other blood group systems, the ABO and Rh blood group systems stand out as the most important in medicine today. The phenotypes of the ABO blood group system, A, B, AB and O, are determined by the presence or absence of one or both of A and B antigens on host red blood cell membrane. The Rh blood group system is highly pleomorphic containing about forty four different antigens, and is the second most important system to the ABO [1]. Of all the pleomorphic forms, the presence or absence of the Rh D antigen on red blood cell of the host is the most significant [1]. The presence or absence of ABO and Rh antigens on an individual's red blood cell is genetically mediated [2, 3]. HBV is the leading cause of liver disease worldwide, accounting for over 360 million cases of chronic hepatitis and 620,000 deaths per year [4]. HBV infection is hyper-endemic in Sub-Sahara Africa (SSA) and a major cause of chronic liver disease [5].

The distributions of ABO and Rh antigens have been extensively researched and their frequencies observed to vary considerably with respect to ethnic and geographical differences [1]. Apart from being resident on red blood cell membrane, studies have shown that the ABO

antigens are heavily distributed on the surfaces of human cells including the epithelium, sensory cells, platelets, leucocytes, saliva, seminal fluid, gastric secretion [6, 7]. Reports indicate that blood groups can serve as receptors for some microorganisms, facilitating the spread and establishment of infectious diseases [8]. This underlines the clinical relevance of blood group antigens in several other diseases besides transfusion and transplantation disorders. Studies have reported an association between ABO antigens and risk for the development of infectious diseases, [9] including hepatitis B virus associated hepatocellular carcinoma [10].

Literature is awash of studies exploring the relationship between ABO/Rh blood groups systems with infectious and non-infectious disease, but little is known about the correlation between these variables and HBV infection. Conflicting reports exists on the role of ABO and Rh antigens on susceptibility to HBV infection, with many of these reports showing a geographical and racial bias [11]. To the best of our knowledge, only one study [12], conducted in 1992 has evaluated the relationship between ABO blood group and HBV infection in Nigeria. This study [12], focused on blood donors only. Data on the relationship between Rh D antigen and HBV infection in Nigeria is missing. Knowledge of the association of various blood group types and diseases amongst

different groups in a population can give useful direction for the articulation and implementation of individualized approaches to management and prevention of diseases. Against this background, this study aimed at determining the prevalence as well as the association if any, between HBV infection and ABO and Rh blood group types among young Nigerian adolescents.

## Methods

### STUDY POPULATION

The study was conducted among undergraduate students of Edo University Iyamho, Edo State, Nigeria. Established in 2016, the University presently has student strength of about 3500 distributed across six different faculties. A total of 496 students were enrolled for this study consisting of 302 females and 194 males. The age range of the participants was 18-25 years. The students were drawn from all faculties/college in the University.

### SAMPLE SIZE DETERMINATION

The sample size was determined using the formula  $n = Z^2Pq/d^2$ , where:

$n$  = sample size

$Z$  = Standard normal deviate = 1.96 at 95% confidence limit

$p$  = prevalence of HBV in a previous Nigerian study = 12.5% = 0.125 [13].

$q$  = 1- $P$ ; = 1-0.125 = 0.875

$d$  = error margin = 0.05

Computing the value above will give

$N = (1.96)^2 \times 0.125 \times 0.875 / (0.05)^2 = 168$ .

Thus a sample size of 168 persons was obtained. However, to make room for non-responses and improperly filled and /or unreturned questionnaires, the sample size was increased to 496.

### ETHICAL CLEARANCE

Approval was sought and obtained from the Ethical Research Committee of Edo University Iyamho, Edo State, Nigeria. Informed consent was obtained from all consenting participants before commencement of collection of blood. Inclusion criteria was been registered as a student of Edo State University, Uzairue, Edo State, Nigeria.

### SAMPLE COLLECTION AND PROCESSING

Using a simple random sampling technique, a total of four hundred and ninety six (496) students were recruited for this study. Four milliliters of blood was collected from each consenting student and dispensed in an Ethylene-diamine Tetra acetic Acid (EDTA) container. Plasma obtained from the collected blood specimens were used for the serological detection of Hepatitis B surface antigen (HBsAg) using the immuno-chromatographic kits (Skytec Rapid Diagnostics USA), as previously described [14]. In brief, 20 ul of each participant's serum was placed on the adsorbent portion of the Skytec Rapid Test Strip that had been removed from its foil and placed

on a flat surface. This was allowed to stand for 5 minutes after which it was observed for the emergence of bands at strategic positions. The emergence of a single band at the control portion of strip indicated a negative result, while the appearance of a band on the control portion and another on the test region of strip was indicative of a positive result.

### ABO AND RH D BLOOD GROUP DETECTION

The slide agglutination technique as previously described was used to determine the ABO and Rh blood groups [15]. Briefly, a drop of each participant's blood was placed on three separate areas on a clean white tile. Each drop of blood was mixed with a drop of commercially prepared antisera A, B, and D, and observed for agglutination. Each mixture (blood plus antisera) was viewed microscopically to confirm agglutination.

### STATISTICAL ANALYSIS

The data obtained were analyzed using Chisquare ( $\chi^2$ ) or Fischer's exact test as appropriate and odd ratio analysis using the statistical software INSTAT®.

## Result

The seroprevalence of HBV among study participants was 10/496 (2.01%). Age was identified as a risk factor ( $P < 0.05$ ) for HBV seropositivity, with participants within the age group of 24-26 years having the highest risk of infection. Although male participants had a higher prevalence of HBV than female, the difference failed to reach statistically significant proportion ( $P > 0.05$ ). With respect to marital status and religion, single students as well as those of the Moslem faith were observed to have a higher prevalence of HBV infection, albeit the difference was statistically insignificant ( $P > 0.05$ ). Similarly, student's faculty/college did not significantly affect the prevalence of HBV infection in this study ( $P > 0.05$ ) (Tab. I).

Over half 257/496 (51.5%) of the total number of study subjects were of the blood group O type. The distribution of A, B and AB blood types were 132/496 (26.6%), 89/496 (17.9%) and 18/496 (3.6%) respectively. Study participants with the B blood type had the highest prevalence 24/496 (4.5%) of HBV infection. No infection was recorded among participants of the AB blood type. Generally, the prevalence of HBV infection did not differ significantly ( $P > 0.05$ ) with respect to ABO blood type. The absence of the Rh D antigen on red blood cell of study subjects was found to be associated with HBV seropositivity (Rh Negative vs. Rh Positive: 2/24 (8.33%) vs. 8/472 (1.69%); OR = 5.273, 95% CI = 1.056, 26.321) albeit, it failed to reach statistical significance ( $P > 0.05$ ) (Tab. II).

A combination of ABO and Rh D blood groups revealed that only blood group B Rh D negative status was associated (OR = 20.714 95%CI = 0.800, 536.26;  $P > 0.05$ ) with higher sero-prevalence of HBV infection (Tab. III).

Tab. I. Prevalence of Hepatitis B virus infection among study participants.

Variables	N (%)	N. HBsAg positive (%)	OR	95% CI	P value
<b>Age (years)</b>					
15-17	67 (13.5)	0 (0.0)			0.039
18-20	190 (38.3)	3 (1.58)			
21-23	138 (27.8)	2 (1.45)			
24-26	101 (20.4)	5 (4.95)			
<b>Gender</b>					
Male	175 (35.3)	4 (2.28)	1.228	0.342, 4.413	0.7473
Female	321 (64.7)	6 (1.87)			
<b>Marital status</b>					
Single	483 (97.4)	10 (2.07)	0.5987	0.0333, 10.761	1.000
Married	13 (2.6)	0 (0.0)			
<b>Religion</b>					
Islam	92 (18.5)	2 (2.17)	1.100	0.229, 5/270	1.000
Christianity	404 (81.5)	8 (1.98)			
<b>Faculty/college</b>					
College of Medicine	228 (45.9)	5 (2.19)			0.5891
Faculty of Engineering	57 (11.5)	2 (3.51)			
Faculty of Arts Management and Social Sciences	58 (11.7)	0 (0.0)			
Faculty of Law	93 (18.8)	1 (1.08)			
Faculty of Science	60 (12.1)	2 (3.33)			

N: number of subjects; OR: odd ratio; CI: confidence interval; Test statistics used: Chisquare ( $\chi^2$ ) or Fischer's exact test.

Tab. II. Prevalence of Hepatitis B virus infection with respect to ABO and Rh blood type.

Variables	N. (%)	N. HBsAg positive (%)	OR	95% CI	P value
<b>Abo blood type</b>					
O	257 (51.8)	4 (0.78)			0.3105
A	132 (26.7)	2 (1.52)			
B	89 (17.9)	4 (4.50)			
AB	18 (3.6)	0 (0.00)			
<b>Rhesus blood type</b>					
Negative	24 (4.8)	2 (8.33)	5.273	1.056, 26.321	0.079
Positive	472 (95.2)	8 (1.69)			

N: number of subjects; OR: odd ratio; CI: confidence interval; Test statistics used: Chisquare ( $\chi^2$ ) or Fischer's exact test.

Tab. III. Prevalence of Hepatitis B virus infection with respect to ABO and Rh Blood grouping.

Blood group	N	No. HBsAg positive (%)	OR	95% CI	P value
A +	127	2 (1.57)	0.578	0.026, 12.638	1.000
A -	5	0(0.0)	ND	ND	ND
B +	84	2 (2.38)	0.879	0.040, 19.275	1.000
B-	5	2(40.0)	20.714	0.800, 536.26	0.059
AB +	18	0(0.0)	ND	ND	ND
O +	243	4 (1.65)	0.545	0.028, 10.623	1.000
O -	14	0 (0.0)	1	1	

N: number of subjects; ND: not done; Test statistics used: Fischer's exact test.

## Discussion

There is paucity of data on the prevalence and associated risk factors for HBV infection among young Nigerians. Although reports abound on the association of ABO and Rh blood group systems with disease, none have specifically focused on the relations between the blood group systems and HBV infection among young adults

in Nigeria. Against this background this study was conducted.

The finding of a HBV prevalence of 2.1% is consistent with a value of 3/300 (1.5%) recorded in an earlier Nigerian study [16]. Our finding is however at sharp variance with 37/800 (4.6%) and 47/407 (11.5%) reported in other African studies respectively [17, 18]. The observed variation in result may be due to

differences in geographical location of studies as the studies by Ekouevi *et al.*, 2015 [17], and Tesfa *et al.*, 2021 [18], were conducted in Togo and Ethiopia respectively. Students within the age group of 24-26 years had a significantly higher risk of being infected by HBV while younger participants in the age group of 15-17 years recorded no HBV infection. This observation is at variance with findings from a previous Nigerian study where undergraduate students less than 18 years were reported to have a significantly higher risk of HBV seropositivity than older ones [13], It is however in agreement with a Togolese one [17]. Nigeria commenced her universal HBV immunization program in 2004 [19]. Although, the HBV vaccination history of our study participants were not noted at time of research, it is possible that students in the age category of 15-17 years are better beneficiaries of the young Nigerian HBV vaccination program in Nigeria. This may have accounted for the zero prevalence of HBV infection observed among them. In this study, gender, religion and faculty/college of participants were not found to significantly affect the prevalence of HBV infection. Findings from other studies [13, 16], have supported our observations.

The distribution of ABO blood types among study participants was O 257/496 (51.8%), A 132/496 (26.6%), B 89/496 (17.9%) and AB 18/496 (3.6%). A similar pattern has been reported by other African studies [1, 20]. One Asian study [21] however, documented blood group B as the most predominant blood type followed by O. while others [22, 23, 24] reported blood group A as the most common among their study populations. Of all participants examined in this study, only 4.8 percent were found to lack the rhesus D antigen. A Rh D negative prevalence of 6%, 7.2% have been reported by studies from Nigeria [25] and Ethiopia [26] respectively. Asia and Africa are generally known to have a low number of Rh D negative individuals [27]. Blood group types are inherited and its frequencies vary from one population to another [25]. This may explain the observed variation in blood types in the aforementioned studies.

Studies have reported an association between certain diseases and the ABO/Rh blood group systems. In this study, the prevalence of HBV was observed to be highest among participants with B blood type, followed by blood type A. Participants with blood type AB had no incidence of HBV infection. A similar trend had been reported by an earlier study [28]. However, findings from a meta-analysis study of thirty-eight articles showed the contrary with blood group B individuals reported to have the lowest risk for HBV [11]. The variation in result could be due to several reasons. Firstly, the study by Jing and his colleagues [11], focused on a disproportionately higher number of non-African studies, with only seven of them being from the African continent, out of which just two were from Nigerian. Again all thirty articles analyzed by Jing and his colleagues [11] focused on either blood donors or patients from hospital settings in contrast to our study population which comprised of young undergraduate students. Other studies [29, 30]

also had a different finding, with blood group A donors reported to have the highest prevalence of HBV infection. Reports have shown that blood antigens may serve as receptors for bacteria, parasites and viruses, leading to colonization and invasion of host or evasion of its immune system [31]. It is interesting to note that the preferences for histo blood group antigens by some microorganisms including *norovirus* and *Helicobacter pylori* are influenced by specific genotypes and subtypes [31, 32] As the genotypes of HBV are geographically diverse [33]. It is possible that the variation in these reports could be attributed to differences in preferences of histo-blood group antigens by predominant HBV genotypes in these regions. Further investigations are however, needed to verify this.

Participants with blood type AB had the least prevalence of HBV infection in this study. Some studies [12, 28] have reported similar findings. The reason for this is unclear. Perhaps, the small size of samples of blood group AB tested may be responsible for this finding, or the co-existence of histo -antigens A and B on host cells may represent a resistance factor for HBV infection. This will definitely require further studies to verify. Generally, the prevalence of HBV was not significantly affected by ABO blood group system. This is contrasting to an earlier report [15].

The finding of a higher prevalence of HBV among Rh D negative subjects in this study has been previously reported by an Indian study [28]. Indeed, subjects that tested negative to the Rh D antigen in our study were observed to have a five times higher risk of acquiring HBV than their Rh positive counterparts in this study. It is however at variance a report elsewhere [15]. Basically, three molecular mechanisms have been reported for development of Rh D negativity namely, total deletion of the Rh D gene, (RHD), the presence of pseudo Rh D gene (RHD<sub>s</sub>) (inactive) in association with ce allele in the RHCE, and the presence of hybrid gene, with the latter two mechanisms leading to the production of a non-functional Rh protein [3]. Interestingly, the expression of these forms has been reported to vary vastly with respect to race, location and tribe [3]. Genetic variations in human populations plus environmental factors contribute to susceptibility to infectious diseases [34]. Perhaps this could explain the variation in observations earlier reported. Generally however, the prevalence of HBV was not significantly affected by Rh status. This is in line with findings from other studies [28, 30].

Combination of the ABO and Rh D blood group systems, revealed an association between HBV sero-positivity and B Rh D antigen negative blood group. Indeed, a twenty times higher risk (OR = 20.714) for HBV sero-positivity was observed among participants with B Rh D negative blood group status. Interestingly, no such association (OR = 0.879) was found amongst subjects with B and Rh antigens. Findings from some studies points to the protective effect of Rh D antigen against Hepatitis B virus infection [20, 35]. Also, studies have reported a generally lower health status among persons who lack the Rh D antigen [36, 37]. Differences in blood

group antigen expression can increase or decrease host susceptibility to many infections [31]. Thus the observed pattern of result in this study may be as result of the concomitant effect of the expression of B antigen and the lack of Rh D antigen on their red blood cell of host. This definitely requires further study to substantiate. The gold standard for diagnosis of hepatitis B virus infection is the detection of HBV nucleic acid in blood or liver [38]. This study focused on the detection of HBsAg in blood serologically. This is a limitation in this study.

## Conclusion

Generally, the prevalence of HBV infection was 2.01% among study subjects. Age was identified as a risk factor for HBV seropositivity. Subjects with Blood group antigen B but lacking the Rh D antigen were more at risk of acquiring HBV infection. These findings may prove valuable to health managers and planners in articulating and implementing HBV infection control strategies.

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## Conflict of interest statement

Authors have no conflict of interest to declare.

## Disclosure statement

The authors report no conflict of interest.

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## Author's contribution

All authors took part in the study design, generated and analyzed data and substantively took part in the drafting of manuscript.

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**Correspondence:** Bankole Henry Oladeinde - Tel: +2348053096120 - E-mail: oladeinde.bankole@edouniversity.edu.ng - bamenzy@yahoo.com

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