

# Studies on the Risk Factors of Hepatitis B Virus (HBV) Infection Among Students

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## ABSTRACT

**Background and Objective:** Globally, Hepatitis B Virus (HBV) infection is a major public health issue responsible for most cases of liver inflammation and complications worldwide. This study determines the prevalence of Hepatitis B Surface Antigenemia (HBsAg), its risk factors and the serological markers among the study subjects. **Materials and Methods:** A well-structured questionnaire was administered to consenting participants. Two hundred and ten volunteers were involved in the study. Blood samples were collected by venipuncture under aseptic conditions. Using the HBsAg test strip, the sera obtained were tested for the HBsAg (Acon Laboratory incorporated USA). Assay for qualitative assessment of Seromarkers was carried out using the HBV-5 panel test kit. **Results:** A prevalence of 22 (10.5%) among subjects screened. Considering gender, male subjects showed 5.7% positivity, while the female subjects recorded 4.8% [p-value 0.736 ( $p > 0.05$ )]. Considering age distribution subjects aged 16-25 years recorded a prevalence of 7.1%, [p-value 0.001 ( $p < 0.05$ )]. Clinical risk factors showed that 2.9% had a record of previous STD infection. Based on lifestyle, intravenous drug users and subjects that shared unsterilized sharp objects recorded a prevalence of 7.2%, [p-value of 0.027 ( $p < 0.05$ )]. Considering markers for HBV infection among seropositive subjects screened, the HBsAg marker showed positivity of 19 (100%) compared to the marker for HBeAg which recorded 4 (21.0%). Anti-HBs, showed 7 (37.0%) positivity while the anti-HBc marker showed a positive record of 14 (74.0%). **Conclusion:** This findings hence demands an urgent need for public health enlightenment among the population, while vaccination of individuals amongst the study group is strongly advocated.

## KEYWORDS

HBV, infection, seromarkers, students, risk factors, young adults, 5-panel test

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## INTRODUCTION

Hepatitis B Virus (HBV) infection is a global public health challenge, almost a third of the world's population are infected with HBV, While about 360 million of these populations are known to be chronic carriers. Furthermore, close to a million of these infected individuals die annually mainly from hepatitis B virus complications which includes liver cirrhosis and eventual liver cancer or hepatocellular carcinoma<sup>1</sup>.



However, the actual burden of HBV infection in Sub-Saharan Africa is underestimated, owing to inaccurate medical records keeping and under-reporting of cases, particularly from the rural communities. Estimates of hepatitis B antigenaemia seroprevalence of 6-20% have been reported, making Sub-Saharan Africa a hyper-endemic region. Nigeria is also a hyper-endemic country for HBV with various rates ranging from 0.5-44.7%<sup>2</sup>. Most infections worldwide are acquired through perinatal transmission at birth, through horizontal transmission between young children, through sexual contact and through Intravenous Drug Users (IVDU). Other routes of transmission, which have though declined in frequency with the implementation of strict control measures, include contaminated blood or blood products and unsafe medical practices, however, healthcare-associated infection remains a significant concern in both resource-poor settings<sup>3</sup>. However, the burden of HBV infection is marked with geographic disparity and dependent on the different modes of transmission predominant in the population and the resulting age at infection, which determines the probability of progression to chronic infection.

In addition, the epidemiology of HBV infection globally is changing because of the impact of universal infant vaccination programs<sup>4</sup>. The risk of liver-related complications is variable and influenced by a variety of host, viral and environmental factors determining the stage of liver diseases such that evidence of inflammation and fibrosis is such an important to guide towards therapeutic decisions and the need for Hepatocellular Carcinoma Screening (HCC). Although liver biopsy is recommended for assessing inflammatory activity and fibrosis<sup>5</sup>. Chronic hepatitis B accounts for approximately one-half of all HCC cases. Recent guidelines recommend screening for HCC every six months with abdominal ultrasonography and alpha-fetoprotein testing. If ultrasound findings are abnormal, then computed tomography or magnetic resonance imaging of the liver is recommended<sup>6</sup>.

The aim of this study, is to determine the rate of HBV infection among high-risk groups in our study population and to conduct a large-scale facility-based HBV prevalence study among the students' population screened.

## **MATERIALS AND METHODS**

**Study area:** This research was conducted among students of a tertiary institution in Oro, Kwara State, Nigeria, which serves as our study location. Assay of collected samples were done at the Department of Medical Laboratory, LMU Medical Center.

**Ethical consideration:** After fulfilling all the ethical prerequisites for the use of humans as subjects of study, provisional ethical permit was obtained from LMU-Medical Centre Ethical Team. Informed consent from the recruited subjects was also obtained before the commencement of sampling protocols.

**Study population:** Two hundred (200) blood samples were collected from volunteers, which consist of both genders. Well-structured questionnaires were also used to secure relevant information and demographic data from the volunteer subjects.

**Inclusion and exclusion criteria:** The subjects recruited in the study were asymptomatic to HBV infection (apparently healthy) by routine screening and gave full informed consent. Subjects who failed to give consent or had been administered with the required doses of the vaccine, were excluded from the study. Subjects aged 16-55 years were recruited for the study.

**Collection and processing of specimens:** Under aseptic conditions, blood samples were obtained using standard ethical procedures. Thereafter, the samples were carefully labelled and transferred to a plastic microtitre tube containing Ethylene Diamine Tetra-Acetic Acid (EDTA). The obtained sera samples were then stored at -20°C prior use.

**Laboratory analysis:** To qualitatively detect the Hepatitis B Surface Antigen, (HBsAg) test strip (solid diagnostics) was employed for the test assay. The test kit is a rapid chromatographic immunoassay to detect hepatitis B surface antigen in plasma and serum. This kit has a record of 97.0% specificity with a sensitivity greater than 99%. The recorded results were interpreted further based on the manufacturer's specification.

**Screening using the 5-panel test kits for serological markers:** For assessing the hepatitis B (HBV) infection markers qualitatively in serum, whole blood and also plasma, the HBV-5 panel test was carried out. The panel test is a rapid test carried out to identify the major HBV infection markers which are mainly five, HBsAg, Anti-HBs (HBsAb), Anti-HBc (HBcAb), HBeAg and Anti-HBe (HBeAb).

**Statistical analysis:** The use of SPSS 17 (statistical package for social sciences version 17) was employed for statistical analysis. Obtained data from the questionnaire issued was further represented as figures, graphs and tables and in percentages. Pearson Chi-square test was also employed with a p-value of  $\leq 0.05$  as significant at a confidence interval of 95%.

## RESULTS

The age range was from 16-55, from the female subjects a total of 133 (63.3%) samples were obtained compared to 77 (36.7%) obtained from the males. The overall result showed that of the two-hundred and ten samples (210) assayed, for HBsAg, 188 (89.5%) tested negative while 22 tested positive, showing a prevalence of 10.5%. Twelve males tested positive for HBsAg (5.7%) while ten females representing (4.8%) were seropositive for HBsAg (Table 1).

Table 2 depicts the age ranges as it relates to the ratio of positive subjects examined for HBsAg to the number of students screened in total. Age ranges of 16-25 and 36-45 years, respectively had prevalence rates of 7.1 and 2%. A higher prevalence of 7.1% was observed among the age range of 16-25 and 2% seropositivity was observed in ages 36-45.

Table 3 shows the evaluation of the risk factors. Family history of HBV infection among males and females showed a prevalence of 1 and 0.5%, respectively. Also, previous records of sexually transmitted infections showed, a record of 1.9% among the male subjects compared to females with a record of 1%.

Table 4 showed risk factors as it relates to the lifestyle of the subjects screened. Individuals with a history of sharing sharp objects such as razors and clippers recorded a prevalence of 2.9 and 1.9% among males and females positive subjects screened, respectively.

Table 1: Sex distribution of HBsAg among subjects screened

Sex	No. of samples screened	No. of positive for HbsAg (%)	No. of negative for HbsAg (%)
Males	77	12 (5.7)	65 (30.9)
Females	133	10 (4.8)	123 (58.6)
Total	210	22 (10.5)	188 (89.5)

Table 5 showed the prevalence based demography of individuals screened. Students showed a higher prevalence of 7.1%, compared to teaching staff with a prevalence of 1.9%. To determine the level of possible liver derangement, Liver Function Tests (LFT) was carried out among the seropositive subjects, from this study, a total of 36.4% of the patients screened showed abnormality compared to the normal range, due to elevations in liver transaminases (Table 6).

Table 7 shows various serologic responses among the seropositive subjects screened. This includes the stage of HBV infection in chronic carriers and among individuals consistently affected by the virus. The well defined serologic markers among the positive subjects include the HBV Surface Antigen (HBsAg) and antibody, HBV e Antigen (HBeAg) and antibody t and the HBV core antigen.

Table 2: Age distribution of subjects screened

Age group	No. of sample screened			No. of positive for HbsAg (%)			No. of negative for HbsAg (%)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
16-25	48	112	160	7 (14.6)	8 (7.1)	15 (7.1)	41 (85.4)	104 (92.9)	145 (69.0)
26-35	6	4	10	1 (16.7)	1 (25.0)	2 (1.0)	5 (83.3)	3 (75.0)	8 (3.8)
36-45	15	12	27	3 (20.0)	1 (8.3)	4 (2.0)	12 (80.0)	11 (91.7)	23 (11.0)
46-55	8	5	13	1 (14.3)	0 (0.0)	1 (0.5)	7 (87.5)	5 (100.0)	12 (5.7)

Table 3: Risk factors of individuals based on clinical history

Risk factors	No. of positive patients for various risk factors (%)		No. of patients negative for various risk factors (%)	
	Males	Females	Males	Females
<b>Clinical history</b>				
Blood transfusion	5 (2.4)	3 (1.4)	7 (3.3)	7 (3.3)
Surgery	1 (0.5)	5 (2.4)	11 (5.2)	5 (2.4)
Sexually	4 (1.9)	2 (1)	8 (3.8)	8 (3.8)
<b>Transmitted disease</b>				
Infection in family	2 (1)	1 (0.5)	10 (4.8)	9 (4.5)

Table 4: Risk factors based on lifestyle of subjects screened

Risk factors	No. of positive for various risk factors (%)		No. of negative for various risk factors (%)	
	Males	Females	Males	Females
<b>Life style</b>				
Several sexual partners	6 (2.9)	1 (0.5)	6 (2.9)	9 (4.3)
<b>Share sharp</b>				
Unsterilized object	6 (2.9)	4 (1.9)	6 (2.9)	6 (60)
Intravenous drug users	3 (1.4)	1 (0.5)	9 (4.3)	9 (4.3)

Table 5: Distribution of HBsAg based on demographic factors among subjects screened

Occupation	Number tested	Sex		No. of positive (%)		No. of negative (%)	
		Male	Female	Male	Female	Male	Female
Student	160	48	112	7 (3.3)	8 (3.8)	41 (19.5)	104 (49.5)
Teaching staff	30	12	18	3 (1.4)	1 (0.5)	9 (4.3)	17 (8.1)
Attendants	10	6	4	1 (0.5)	0 (0.0)	5 (2.4)	4 (1.9)
Security	10	4	6	1 (0.5)	1 (0.5)	3 (1.4)	5 (2.4)

Table 6: Overall determination of serum alanine aminotransferase (ALT) levels on positive subjects screened (36.4% of the seropositive subjects screened showed abnormality to ALT-Assay)

Sex	No. of screened (%)	Normal ALT (%)	Abnormal ALT (%)
Male	12 (54.5)	7 (31.8)	5 (21.7)
Female	10 (45.5)	7 (31.8)	3 (13.6)

Table 7: Overall prevalence of HBV markers among subjects screened

Age	Sex	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb
40	Male	Positive	Negative	Negative	Negative	Positive
19	Male	Positive	Negative	Negative	Positive	Positive
18	Male	Positive	Negative	Negative	Positive	Positive
18	Female	Positive	Negative	Negative	Positive	Positive
33	Male	Positive	Negative	Negative	Positive	Positive
22	Male	Positive	Negative	Negative	Positive	Positive
19	Male	Positive	Negative	Negative	Positive	Positive
21	Female	Positive	Negative	Negative	Positive	Positive
27	Female	Positive	Negative	Positive	Negative	Positive
41	Male	Positive	Negative	Negative	Positive	Positive
40	Female	Positive	Negative	Negative	Positive	Positive
20	Male	Positive	Negative	Negative	Positive	Positive
20	Female	Positive	Negative	Negative	Positive	Positive
21	Male	Positive	Negative	Negative	Negative	Positive
19	Male	Positive	Negative	Positive	Negative	Positive
20	Male	Positive	Negative	Positive	Negative	Positive
18	Female	Positive	Negative	Negative	Negative	Negative
22	Male	Positive	Negative	Negative	Positive	Positive
21	Male	Positive	Negative	Negative	Positive	Positive

## DISCUSSION

This study determined the prevalence of HBV infection and its associated risk factors. The seroprevalence of HBV recorded in this study was 10.5%. These findings were however higher than a prevalence of 5.5% recorded in a study conducted by Ndako *et al.*<sup>7</sup> among sexually active young adult in a rural community of South West-Nigeria. Comparatively, a prevalence rate of 29.8% was reported in Central Nigeria which is reportedly higher compared to the result obtained in this study<sup>8</sup>. In a similar work carried out by Zakka *et al.*<sup>9</sup>, a lower prevalence rate of 8.9% was recorded among blood donors in Gombe- Nigeria<sup>9</sup>. In another study conducted among Makere Medical students, an overall prevalence of 11.0% was recorded for HBsAg, which was slightly higher compared to the 10.5% prevalence recorded in this study, which is also higher than the result of a study obtained in Port Sudan Ahlia College among student as reported by Osman *et al.*<sup>10</sup> According to Omatola *et al.*<sup>11</sup>, WHO has established low prevalence as <2% and moderate prevalence as 2-8% and high incidence rate of >8% HBsAg positivity. It can then be deduced that the population screened in this study is highly prevalent with 10.5% reported. Globally, liver disease is majorly caused by the hepatitis virus. In most developed countries HBV occurrence is around 2% which is low as compared to 8% of HBV occurrence in developing countries were the endemicity of the infection is mostly caused by age difference, socio-economic and constant sexual activities.

Based on gender, female subjects had lower seropositivity of 4.8% for HBsAg compared to their male counterparts which recorded 5.7% prevalence, which was however not statistically significant, this might be as a result of the lesser number of males recruited for the research. Uneke *et al.*<sup>12</sup> proposed that both males and females are uniformly prone to HBV infection while gender may not be a determining factor for the epidemiology of HBV infection. Nonetheless, result obtained in this study is similar to the findings made by Ugwuja and Ugwu<sup>13</sup>, who stated that HBsAg seropositivity does not differ based on gender.

With regards to age, a prevalence of 7.1% was recorded among subjects aged 16-25 years, which is similar to the results of a study conducted among attendees of the Association for Reproductive Family and Health (AFRH) centre in Ibadan, southwestern Nigeria, where the age specific distribution of HBsAg among subjects in the study showed that subjects in the age group of 16-29 years had a higher prevalence rate of 7(7.1%)<sup>14</sup>. Moreover, the prevalence of 10.5% recorded in this study, is relatively higher than the 6.0% recorded among apparently healthy urban Nigerians in a study considering data from pre-vaccination tests in Nassarawa, Adoga *et al.*<sup>15</sup> and also higher than 9.0% prevalence among seemingly healthy students in the University of Ilorin-Nigeria in the work of Udeze *et al.*<sup>16</sup>.

In a similar study conducted, an increased prevalence was seen among students in the age range of 16-25 years compared to individuals of other age brackets while the rate of infection was found to be higher in females compared to the male subjects, this finding is however in contrast with the report of Udeze *et al.*<sup>16</sup>, where a higher prevalence of HBsAg was recorded among males subjects compared to the females. However, in Osun State, South-West, Nigeria, similar tests were conducted which revealed an increased prevalence in female children as compared to males although the difference in statistics failed to show any level of significance<sup>17</sup>.

This study has shown a gradual upsurge of Hepatitis B Virus (HBV) infection in the study group at such an alarming rate. The high prevalence observed among the age group of 16-25 calls for concern as they constitute the socially active group. A very high number of persons at our study location showed a lack of knowledge of the Hepatitis B virus, which could lead to further spread of the infectious agent in the community.

In the accurate diagnosis of acute HBV infection, an identifying serologic profile is established. In the occurrence of an acute infection, virologic markers and host antibody responses are presented in a characteristic pattern based on the findings of Ikogba *et al.*<sup>18</sup>. The identification of Hepatitis B Surface Antigen (HBsAg) can take place averagely between 30-60 days (1-12 weeks) in the sera of most patients, after the occurrence of the disease and is usually the first serologic marker to appear. A serologic response occurs in the acute stage of HBV infection in chronic carriers similar to a response which occurs in individuals consistently affected by the virus. HBsAg and anti-HBc (IgG antibodies) continue to exist lastingly in cases of chronic HBV infection and nucleic acid amplification methods can be used to detect HBV DNA<sup>19</sup>.

About 15.8% (3/19) of students that reacted to HBsAg also had HBeAg which implies that the replication of HBV is taking place following an increase in the levels of HBV in the infected person. Increased infectivity and greater levels of viral replication are largely associated with the manifestation of HBeAg in a patient's serum<sup>20</sup>. Therefore, such individuals are capable of transmitting the virus using the exchange of body fluids or blood. Chances of spreading the infection to others are increased due to the presence of HBeAg if there be any form of contact with the vody fluid. Hepatitis Be Antigen (HBeAg) becomes evident for a while after the appearance of the HBsAg.

However, tests for HBV DNA in serum will indicate the occurrence of HBV DNA before HBsAg or HBeAg appears, at levels of HBV DNA. Previous studies observed the appearance of HBeAg in serum, this implied that the viral cells were actively replicating in the host body. A higher rate of infectivity and greater HBV DNA levels is denoted by the recurring appearance of HBeAg. Absence or relatively low levels of HBV and normal rate of hepatic aminotransferase is largely observed in chronic carriers of HBV infection who usually have an appearance of anti-HBe alongside resolutions of their HBeAg. Due to spontaneous seroconversion to the antibody against HBeAg, a decrease in the rate of seropositivity with age was recorded and 1.6% HBeAg (Anti-HBe) positivity among youths.

Further analysis indicated that 13% of the HBsAg reactive individuals had developed the (anti-HBe) antibody. The production of this antibody occurs temporarily in the immune system when the HBV infection is active, which connotes that the infection is resolved or a lower level of the virus. A noticeable reduction in the viral load occurs after the production of antibodies against the foreign agent which leads to clearing of the HBe during the natural events of the infection. This implies that the patients do not have the virus and are incapable of transmitting the virus denoting successful recovery from the infection<sup>20</sup>.

The first to appear which is the Hepatitis B core antibody had been developed in 93.3% of the individuals. Within six months, during the stage of acute infection, the IgM anti-HBc is usually found in a high titre and eventually wares off, but it remains persistent in some cases of chronic hepatitis. It is harder to ascertain the stage at which the subjects got infected with the HBV as the presence of anti-HBc in the host's serum denotes the occurrence of a past or recent hepatitis B virus infection<sup>19</sup>.

Efforts at initiating therapy to chronic Hepatitis B virus shows the the for accurate determination of alanine aminotransferase levels (ALT) of a patient, which is highly important due to the immune-mediated inflammation caused by the elevation in ALT levels for clearing of HBV-infected hepatocytes (in the Liver cells) and an increased rate of hepatitis B virus e antigen (HBeAg) seroconversion<sup>14</sup>.

Liver inflammation is usually positively associated with most positive subjects. Furthermore, it has been observed that at normal ALT levels, liver damage of infected patients is at a lower rate compared to infected patients observed with irregular or a near normal/elevated ALT levels. From this study, 35.3% of seropositive subjects recorded a higher level of aminotransferases (ALT) i.e., above the normal range of 12 IU L<sup>-1</sup>. Therefore, the commonest and easiest method of detecting inflammation of the liver in individuals, especially with chronic HBV infection is by measuring the level of aminotransferase by sequential analysis and close observations of variances. Once an individual has been diagnosed with chronic HBV infection, follow-up testing must be performed for alanine aminotransferase (ALT), a marker of liver cell inflammation<sup>19</sup>.

## **CONCLUSION**

This study revealed a high prevalence of hepatitis B virus infection among the student population screened at our study location. This could be attributed to lack of awareness of the universal precautionary guidelines, lack of knowledge on sharing of sharp objects and possible needle stick injury and lack of access or outright ignorance on the need for immunization among the infected subjects. This calls for the need to provide training on universal precautionary guidelines for health caregivers. Safety procedures coupled with enlightenment and prompt vaccination among the study population is strongly advocated.

## **SIGNIFICANCE STATEMENT**

This study will assist researchers to uncover critical needs for accurate screening and prompt diagnosis which may have not been explored. Thus, a new and prompt approach to assaying for HBV may be arrived at using the 5-Panel test kit which has been able to assess past HBV infection and future immunity. This method of tests for Hepatitis B virus infection, as shown in this study would indicate whether a person is immune either due to HBV vaccination or due to having recovered from a past infection. This screening method may also be used to assess whether vaccination successfully generated immunity and to identify patients who are at an increased risk of HBV reactivation.

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