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Prevalence And Patterns of Hepatitis B Virus Infection Serological Markers among HbsAg-Negative Patients Attending South Atlantic Petroleum Medical Centre, Keffi, Nasarawa State, Nigeria

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ABSTRACT

Routine screening for hepatitis B virus (HBV) infection in many healthcare settings relies primarily on the detection of hepatitis B surface antigen (HBsAg), which may fail to identify individuals with previous exposure, immunity, or possible occult HBV infection. This study determined the prevalence and pattern of HBV serological markers among HBsAg-negative patients attending South Atlantic Petroleum (SAPETRO) Medical Centre, Keffi, Nigeria. A cross-sectional study was conducted among 400 HBsAg-negative patients attending SAPETRO Medical Centre, Keffi. Socio-demographic information was obtained using a structured questionnaire, while clinical data were retrieved from hospital records. Approximately 3 mL of venous blood was collected from each participant, and serum samples were screened for HBV serological markers, including hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb), and hepatitis B core antibody (HBcAb), using the HBV-5 Rapid Panel Test Kit. Data were analysed using descriptive statistics and Chi-square tests, with statistical significance set at $P \leq 0.05$. Of the 400 HBsAg-negative participants, 52 (13.0%) were positive for HBsAb, 11 (2.8%) for HBeAb, and 21 (5.3%) for HBcAb, while none tested positive for HBeAg. Although the distribution of HBV serological markers varied across age groups and between genders, the differences were not statistically significant ($P > 0.05$). Based on serological patterns, 41 (10.3%) participants had vaccine-induced immunity, 11 (2.8%) had immunity due to previous natural infection, 10 (2.5%) exhibited serological evidence suggestive of possible occult HBV infection, and 338 (84.5%) were susceptible to HBV infection. A considerable proportion of HBsAg-negative individuals possessed HBV serological markers indicative of immunity, previous exposure, or possible occult infection, while most remained susceptible to HBV. These findings demonstrate the limitations of HBsAg-only screening and support the incorporation of comprehensive HBV serological profiling, alongside expanded hepatitis B vaccination and molecular testing where feasible, to improve HBV diagnosis and prevention.

Keywords: Hepatitis B virus, HBsAg-negative, serological markers, HBsAb, HBcAb, occult hepatitis B infection, Nigeria.

1.0 INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global public health challenge despite the availability of effective vaccines and antiviral therapies. According to the World Health Organization (WHO), an estimated 254 million people were living with chronic HBV infection in 2022, with approximately 1.2 million new infections occurring annually (WHO, 2024). HBV infection is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, accounting for substantial morbidity and mortality, particularly in low- and middle-income countries where the disease burden remains high (WHO, 2024; Adedewuyi et al., 2025). Sub-Saharan Africa, including Nigeria, continues to experience intermediate to high endemicity of HBV infection (Ajuwon et al., 2021; Musa et al., 2022 Adewuyi et al., 2025), making early and accurate diagnosis an essential component of disease control and prevention.

Routine laboratory diagnosis of HBV infection in many healthcare facilities relies primarily on the detection of hepatitis B surface antigen (HBsAg), which serves as the principal marker of active infection (Lok & McMahon, 2011). Although HBsAg screening is inexpensive, widely available, and suitable for large-scale screening programmes, it does not provide complete information regarding an individual's HBV infection status (Musa et al., 2022). Patients who test negative for HBsAg may still possess other HBV serological markers indicative of previous exposure, resolved infection, immunity, ongoing viral replication, or, in some cases, occult hepatitis B infection (OBI) (Raimondo et al., 2019; Mak et al., 2022). OBI is characterized by the presence of HBV DNA in individuals who are negative for HBsAg, with or without detectable antibodies to HBV, highlighting the limitations of relying solely on HBsAg for diagnosis (Mbaawuaga et al., 2014).

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A comprehensive HBV serological profile, including hepatitis B surface antibody (HBsAb), hepatitis B envelop antigen (HBeAg), hepatitis B envelop antibody (HBeAb), and hepatitis B core antibody (HBcAb), provides valuable information on an individual's infection history, infectivity, immune status, and stage of disease. Such serological profiling can identify individuals with resolved infection, vaccine-induced immunity, previous exposure, or possible occult infection that may warrant further molecular investigation (Mak et al., 2022; WHO, 2024).

In Nigeria, HBV screening in routine clinical practice is predominantly based on HBsAg testing alone, potentially overlooking patients with clinically significant HBV serological patterns. Recent Nigerian studies have continued to demonstrate the occurrence of additional HBV markers and even occult HBV infection among individuals who initially tested negative for HBsAg (Musa et al., 2022; Ugwu et al., 2025; Musa et al., 2026), underscoring the need for more comprehensive diagnostic approaches in high-endemic settings.

Therefore, this study aimed to determine the prevalence and pattern of hepatitis B virus infection serological markers among HBsAg-negative patients attending South Atlantic Petroleum (SAPETRO) Medical Centre, Keffi, Nigeria. The findings will provide insight into the spectrum of HBV serological profiles among individuals considered negative by routine screening and contribute evidence for improving HBV diagnostic strategies and patient management in resource-limited settings.

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was conducted at SAPETRO Medical Centre, Keffi, Nasarawa State, Nigeria. The medical centre is located within the premises of Nasarawa State University, Keffi, and serves as the university's primary healthcare facility. Established through a donation by SAPETRO Ltd, the centre provides both outpatient and inpatient healthcare services to students, university staff, their dependents, and members of the surrounding communities. In addition, the facility offers clinical consultations, laboratory diagnostic services, maternal and child healthcare, immunization, and other preventive and curative medical services.

2.2 Study Population

The study population comprised male and female patients of all age groups attending SAPETRO Medical Centre, Keffi, who voluntarily consented to participate and had tested negative for hepatitis B surface antigen (HBsAg) by the hospital laboratory. Participants included students, university staff, their dependents, and patients from the surrounding communities. Socio-demographic data were collected using a structured questionnaire, while relevant clinical information was obtained from participants' hospital records.

2.3 Ethical Approval and Consent

Ethical approval for this study was obtained from the Nasarawa State Ministry of Health Research Ethics Committee, Lafia, Nigeria (Approval No.: SMOH/LAF/HREC/4/26). Written informed consent was obtained from all participants before enrolment into the study and participation was voluntary. Confidentiality and anonymity of participants' information were maintained throughout the study.

2.4 Sample Size Determination

The minimum sample size for this study was determined using the formula described by Naing et al. (2006) for estimating a single population proportion at a 95% confidence level and a 5% margin of error:

$$n = \frac{Z^2 pq}{d^2}$$

where:

n = minimum required sample size;

Z = standard normal deviate corresponding to the desired confidence level (1.96 for 95% confidence interval);

P = estimated prevalence of hepatitis B virus (HBV) infection from a previous study (10.1% or 0.101) (Musa et al., 2022);

Q = 1 – p = 0.899

d = margin of error (0.05).

$$n = \frac{(1.96)^2(0.101)(0.899)}{(0.05)^2} = \frac{3.8416 \times 0.091}{0.0025}$$

$$= \frac{0.3496}{0.0025} = 139.8$$

$$n = 140$$

This was however rounded up to 400 samples to improve statistical power of the study.

2.5 Sample Collection and Processing

All samples were collected between November, 2025 and March, 2026. Approximately 3 mL of venous blood was aseptically collected from each participant by venepuncture at the Medical Laboratory of SAPETRO Medical Centre, Keffi, and dispensed into a labelled plain blood collection tube. The blood samples were allowed to clot at room temperature and centrifuged at 3,000 rpm for 5 minutes to separate the serum. The sera were carefully harvested into well-labelled cryovials and stored at −20°C in the Microbiology Laboratory, Department of Microbiology, Nasarawa State University, Keffi, until laboratory analysis.

2.6 Laboratory Analysis

2.6.1 Screening for Hepatitis B Virus Infection Serological Markers

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All serum samples were screened for HBV serological markers, including HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, using the HBV-5 Rapid Panel Test Kit (CTK Biotech Inc., San Diego, CA, USA). Briefly, the test cassette and serum samples were allowed to equilibrate to room temperature before testing. Using a disposable dropper, approximately 80–100 µL of serum was dispensed into the sample well of each test cassette. The test was allowed to run for 15–20 minutes, after which the results were interpreted according to the manufacturer's instructions. Positive and negative results were determined based on the appearance of coloured bands in the control and test regions of the cassette.

2.7 Data Analysis

Data were analysed using Smith's Statistical Package (SSP), Version 2.80 (Claremont, California, USA). Descriptive statistics were used to summarize the data and are presented in tables, and charts. The Chi-square (χ^2) test was used to assess the association between participants' age group and gender and the prevalence of hepatitis B virus (HBV) serological markers. A 95% confidence level was adopted, and a *P*-value of ≤ 0.05 was considered statistically significant.

3.0 RESULTS AND DISCUSSION

Among the 400 HBsAg-negative participants screened for HBV serological markers in this study, 52 (13.0%) tested positive for HBsAb, indicating evidence of immunity to HBV. None (0.0%) of the participants tested positive for HBeAg, while 11 (2.8%) were positive for HBeAb, suggesting previous viral replication with subsequent seroconversion. In addition, 21 (5.3%) participants tested positive for HBcAb, indicating previous exposure to HBV (Figure 1). These findings demonstrate that despite testing negative for HBsAg during routine screening, a proportion of the participants possessed other HBV serological markers suggestive of previous infection, possible occult infection, immune response, or resolved infection.

The 13.0% prevalence of HBsAb observed among the HBsAg-negative participants in this study indicates that only a small proportion had serological evidence of immunity against HBV, either through previous vaccination or recovery from natural infection (Mbaawuaga et al., 2014). This prevalence is lower than the 46.8% reported by Musa et al. (2022) among HIV-positive patients in Abuja, 28.3% reported by Egbu et al. (2020) among blood donors in Central Nigeria, 38.3% documented by Mohammed et al. (2019) among young people in Central Nigeria, and 31.6% reported by Musa et al. (2026) among blood donors in Abuja. The observed differences may be attributed to variations in study populations, HBV vaccination coverage, previous exposure to HBV, and geographical location. The relatively low HBsAb prevalence in the present study suggests that many HBsAg-negative individuals may remain susceptible to HBV infection, highlighting the need for improved hepatitis B vaccination and routine assessment of HBV immune status.

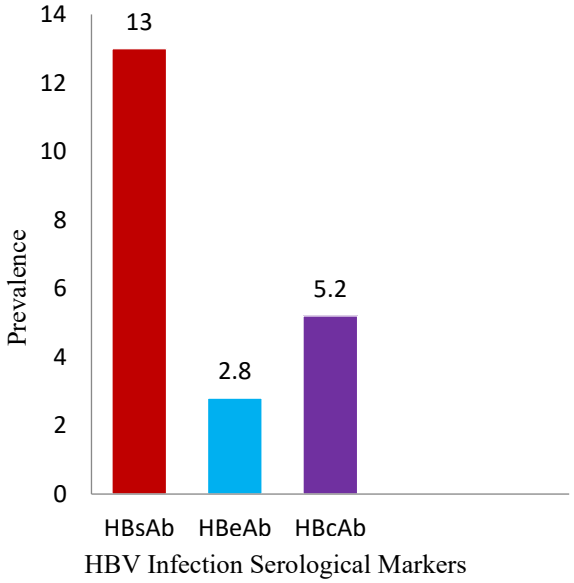

Figure 1: Prevalence of HBV infection serological markers among HBsAg-negative patients attending South Atlantic Petroleum medical centre, Keffi, Nasarawa State, Nigeria.

None (0.0%) of the participants in the present study tested positive for HBeAg, indicating the absence of active viral replication among the HBsAg-negative participants (Mbaawuaga et al., 2014). This finding was expected because HBeAg is generally associated with active HBV replication and is commonly detected in individuals with ongoing HBsAg-positive infection (Spearman et al., 2023). Similar findings have been reported in studies conducted among HBsAg-negative blood donors in Abuja, where no HBeAg-positive cases were identified (Musa et al., 2026). The absence of HBeAg in the present study suggests that none of the participants had highly replicative HBV infection at the time of sampling. However, this finding does not completely exclude the possibility of occult HBV infection, which can be confirmed by the presence of circulating HBcAb and viral DNA.

The HBeAb prevalence of 2.8% observed in this study suggests that a small proportion of participants had undergone seroconversion following previous HBV infection. HBeAb is generally regarded as a marker of reduced viral replication and lower infectivity after clearance of HBeAg (Chen et al., 2012). The prevalence recorded in this study is comparable to the 1.5% reported by Musa et al. (2022) among HIV-positive patients in Abuja but lower than the 12.8% documented by Musa et al. (2026) among blood donors in Abuja. The differences between studies may be

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explained by variations in participants' age distribution, previous HBV exposure, immune response, geographical location, and the endemicity of HBV in the respective study areas.

The prevalence of HBcAb (5.3%) among the HBsAg-negative participants indicates previous exposure to HBV and suggests that some individuals had resolved infection or may possibly be carriers of occult infection (Chen et al., 2012; Egbu et al., 2020). This finding is consistent with reports from several Nigerian studies demonstrating that HBcAb may be detected even in individuals who test negative for HBsAg (Ugwu et a., 2025; Musa et al., 2026). Even though, the prevalence observed in the present study is lower than most previous reported studies in Nigeria and Africa (Mohammed et a., 2019; Adjei et al., 2021; Musa et al., 2022; Ofori-Asenso & Agyeman, 2023), Nevertheless, this calls for concerns because some of these HBsAg-negative patients may have occult HBV infection or may probably be in the window period of the infection. It also further demonstrates the limitation of relying solely on HBsAg screening for HBV diagnosis, as previous exposure to the virus may remain undetected without comprehensive serological testing.

The distribution of HBV serological markers according to age showed that HBsAb positivity was highest among participants aged 21–30 years (14.2%), followed by those aged 10–20 years (13.8%), while a lower prevalence was observed among participants aged 31–40 years (5.9%). No participant aged 41 years and above tested positive for HBsAb. In contrast, HBeAb and HBcAb were more frequently detected among the older age groups, with the highest prevalence recorded among participants aged 31–40 years (8.8%) and those aged ≥41 years (8.3%), while HBcAb was highest among participants aged 31–40 years (11.8%). These differences were not statistically significant (p>0.05) (Table 1). However, the higher HBsAb prevalence among younger participants may reflect better hepatitis B vaccination uptake or acquired immunity following previous exposure (Breakwell et al., 2023). Conversely, the increased occurrence of HBeAb and HBcAb among older participants may be attributed to cumulative lifetime exposure to HBV and a greater likelihood of previous infection and subsequent seroconversion. Similar age-related patterns have been reported in previous Nigerian studies (Alaku et al., 202; Musa et al., 2022), although some authors observed higher or lower frequencies of specific HBV serological markers across different age groups (Egbu et al., 2020; Musa et al., 2022). These findings suggest that age influences the distribution of HBV serological markers and should be considered when implementing hepatitis B screening, vaccination, and prevention strategies.

In relation to gender, HBsAb positivity was slightly higher among females (13.6%) than males (12.5%). Similarly, HBeAb was higher among females (3.4%) compared with males (2.2%), whereas HBcAb was more prevalent among males (6.7%) than females (3.4%). However, the observed differences in the distribution of all HBV serological markers between male and female participants were not statistically significant ($P > 0.05$) (Table 1). The slightly higher HBsAb prevalence among females may reflect better health-seeking behaviour and higher uptake of hepatitis B vaccination, while the higher HBcAb prevalence among males may be associated with greater exposure to behavioural and occupational risk factors for HBV infection. Similar gender-related patterns have been reported in previous African and Nigerian studies (Mohammed et al., 2019; Alaku et al., 2020; Bayo et al., 2021).

Table 1: Prevalence and distribution of HBV infection serologic markers among HBsAg-negative patients attending South Atlantic Petroleum medical centre Keffi in relation to socio-demographics.

Socio-demographic	No. Screened (N=400)	No. Positive (%)		
		HBsAb	HBeAb	HBcAb
Age (Years)				
10-20	94	13(13.8)	2(2.1)	8(8.5)
21-30	260	37(14.2)	5(1.9)	8(3.1)
31-40	34	2(5.9)	3(8.8)	4(11.8)
41≥	12	0.(0.0)	1(8.3)	1(8.3)
P-Value		**0.5223**	**0.2311**	**0.4080**
Gender				
Male	224	28(12.5)	5(2.2)	15(6.7)
Female	176	24(13.6)	6(3.4)	6(3.4)
P-Value		**0.1320**	**0.2111**	**0.8164**

Analysis of the patterns of HBV serological markers showed that 41 (10.3%) participants had serological evidence of immunity due to hepatitis B vaccination, while 11 (2.8%) were immune due to previous natural HBV infection (Table 2). The higher proportion of vaccine-induced immunity compared with naturally acquired immunity suggests that hepatitis B vaccination has contributed to protective immunity among a subset of the study population. However, the relatively low prevalence of both immune profiles

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indicates that a considerable proportion of the HBsAg-negative participants lacked serological evidence of protection against HBV and may therefore remain susceptible to infection. The prevalence of vaccine-induced immunity and immunity due to natural previous exposure observed in the present study were both lower than that reported among young people in Central Nigeria (Mohammed et al., 2019) which were 16.0% and 22.3% respectively. These differences may be attributed to variations in study populations, age distribution, hepatitis B vaccination coverage and previous exposure to HBV.

Table 2: Patterns of HBV infection serologic markers among HBsAg-negative patients attending South Atlantic Petroleum medical centre Keffi, Nigeria.

Pattern of HBV Serologic Markers	Interpretation	No. of Patients	Prevalence (%)
$HBsAb^+$, $HBcAb^-$, $HBeAg^-$, $HBeAb^-$	Immune due to vaccination	41	10.3
$HBsAb^+$, $HBcAb^+$, $HBeAg^-$, $HBeAb^+$	Immune due to natural previous exposure	11	2.8
$HBsAb^-$, $HBcAb^+$, $HBeAg^-$, $HBeAb^-$	Possible HBV occult infection	10	2.5
$HBsAb^-$, $HBcAb^-$, $HBeAg^-$, $HBeAb^-$	Unexposed (Susceptible)	338	84.5
Total		**400**	**100**

Among the HBsAg-negative participants, 10 (2.5%) exhibited a serological profile suggestive of possible occult HBV infection (Table 2), characterized by the presence of HBcAb in the absence of HBsAb and HBeAb (Mbaawuaga et al., 2014). Although occult HBV infection is definitively diagnosed by the detection of HBV DNA in individuals who are HBsAg-negative (Chen et al., 2012), the serological pattern observed in this study indicates previous exposure to HBV and raises suspicion of occult infection. Similar findings have been reported in Nigeria and other HBV-endemic regions, where HBsAg-negative individuals with isolated HBcAb positivity were found to harbour occult HBV infection following molecular testing (Raimondo et a., 2019; Candotti et al., 2022; Musa et al., 2026). The occurrence of this serological profile highlights the limitation of relying solely on HBsAg for routine HBV screening, as individuals with possible occult infection may remain undetected and could potentially transmit the virus through blood transfusion, organ transplantation, or other routes. However, since HBV DNA testing was not performed, the cases identified in this study should be regarded as possible rather than confirmed occult HBV infections. Nevertheless, these findings underscore the need to incorporate molecular assays, particularly for high-risk individuals and blood donors, to improve the detection of occult HBV infection and enhance transfusion and public health safety.

Furthermore, 338 (84.5%) of the participants were unexposed and susceptible to HBV infection (Table 2), indicating the absence of detectable HBV serological markers and, consequently, no evidence of previous exposure or protective immunity (Akinyemi et al., 2022). This finding demonstrates that the majority of the HBsAg-negative patients remained vulnerable to HBV infection despite testing negative by routine HBsAg screening. The predominance of susceptible individuals in the present study is consistent with findings from previous Nigerian studies, which likewise reported that most individuals lacked protective HBV serological markers and therefore remained susceptible to infection (Mohammed et al., 2019; Musa et al., 2022; Musa et al., 2026). The consistently high proportion of susceptible individuals across these studies may reflect suboptimal hepatitis B vaccination coverage among adults, missed opportunities for catch-up vaccination, limited awareness of HBV prevention, and inadequate post-vaccination serological testing. It may also indicate that many individuals have neither been naturally exposed to HBV nor received complete vaccination. These findings underscore the need to strengthen hepatitis B vaccination programmes, particularly among adolescents and adults, and support the incorporation of comprehensive HBV serological screening into routine clinical practice to identify susceptible individuals who would benefit from vaccination and other preventive interventions.

4.0 CONCLUSIONS

This study demonstrated that although all participants were negative for HBsAg by routine screening, a proportion possessed other HBV serological markers indicative of vaccine-induced immunity, previous natural infection, or possible occult HBV infection. More importantly, the majority of the participants remained serologically susceptible to HBV infection, highlighting gaps in protective immunity within the study population. These findings underscore the limitations of relying solely on HBsAg screening and emphasize the importance of comprehensive HBV serological profiling, expanded hepatitis B vaccination programmes, and, where feasible, molecular testing to improve HBV diagnosis, prevention, and control.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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