

CD4+ T-Lymphocyte Counts, HBeAg Status, and Hepatic Transaminase Levels in Asymptomatic HBsAg-Positive Individuals in Sokoto, Nigeria

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ABSTRACT

Introduction: Hepatitis B virus (HBV) infection causes cirrhosis and hepatocellular carcinoma. Asymptomatic HBsAg-positive individuals often remain undetected, with disease progression depending on viral replication and host immunity. This study assessed CD4+ T-lymphocyte counts, HBeAg status, and hepatic transaminases in asymptomatic HBsAg-positive individuals in Sokoto, Nigeria. **Methods:** This cross-sectional study screened 430 individuals during World Hepatitis Day at Usmanu Danfodiyo University Teaching Hospital. HBsAg-positive individuals (confirmed by ELISA) were assessed for HBeAg, ALT, AST (spectrophotometry), and CD4+ counts (Partec CyFlow counter). Chi-square, Fisher's exact, and Student's t-tests were used for statistical analysis. **Results:** Of 430 individuals, 52 (12.1%) were HBsAg-positive with mean age 31 ± 7 years. Among these, 6 (11.5%) were HBeAg-positive, 7 (13.5%) had elevated ALT, and 4 (7.7%) had elevated AST. Mean CD4+ counts were significantly lower in HBeAg-positive individuals (366 ± 72 cells/mm³) compared to HBeAg-negative individuals (717 ± 234 cells/mm³; $P < 0.0001$). Ten participants (19.2%) had CD4+ counts below 500 cells/mm³, with 3 (5.8%) showing advanced immunosuppression (200–350 cells/mm³). No significant differences existed between sexes or age groups. HBeAg positivity was significantly higher among underweight individuals (42.9%) versus normal BMI (2.9%; $P = 0.007$). Predominant risk factors were tattooing (42.3%) and family history (36.5%). **Conclusion:** HBeAg positivity and elevated transaminases indicate active viral replication and hepatocellular injury among asymptomatic carriers. The significant association between HBeAg positivity and reduced CD4+ counts suggests impaired immune responses. Tattooing and family history as major risk factors highlight traditional practices and perinatal transmission routes. These findings emphasize the need for targeted screening programs and interventions to identify and manage asymptomatic HBV carriers in Nigeria.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major global public health concern [1]. HBV, a member of the *Hepadnaviridae* family, is carcinogenic and can integrate into the host genome during chronic infections [1]. HBV shares transmission routes with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), including blood-borne (*e.g.*, needle-stick injuries, contaminated blood products), sexual, and

perinatal pathways [2]. Consequently, co-infections with HIV and HCV are common in at-risk populations [3]. HBV infection can present as asymptomatic, acute, or chronic disease, or as fulminant hepatitis—a severe, rapidly progressive form of acute liver failure [1]. Asymptomatic individuals with HBV infection are a major reservoir for the virus, facilitating undetected transmission and complicating control efforts [2]. Early

identification of these asymptomatic individuals is critical for preventing disease progression and reducing transmission to the general population [2]. An effective recombinant three-dose vaccine is available that provides long-term protection against HBV infection, thereby reducing infection rates with widespread implementation [4, 5].

Globally, HBV infection is a leading cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC) [4, 6]. An estimated 2 billion people have been infected with hepatitis B virus (HBV) globally, and approximately 254 million individuals were living with chronic HBV infection as of 2022 [6]. In Nigeria, an estimated 8.1% of the population is currently infected with hepatitis B virus (HBV), with over 90% of cases undiagnosed, making the country one of the highest-burden regions globally [7, 8].

Chronic HBV infection, characterized by the persistence of HBsAg for more than six months, is a major public health concern [7]. Northwestern Nigeria, including Sokoto (the study area), continues to exhibit a high HBV burden, with recent studies reporting a regional prevalence of approximately 11.8%, which remains among the highest across Nigeria's six geopolitical zones [9]. The seroprevalence of HBV infection in Sokoto varies among different populations: 20.6% among individuals living with HIV infection [10], 11.7% among healthcare professionals [11], and 8.6% among blood donors [12]. Traditional practices such as group circumcision and tattooing, as well as polygamy, are common in the region and are recognized risk factors for HBV transmission [8]. As of 2023, an estimated 850,000 deaths worldwide were attributed to HBV infection, reflecting a continued global burden despite expanded prevention and treatment efforts [4].

Most of these deaths were due to complications such as cirrhosis and HCC, although absolute mortality figures vary across regions depending on population size, access to healthcare, and prevalence rates [13]. The World Health Organization (WHO) African Region and Western Pacific Region have the highest HBV prevalence (6.1% and 6.2%, respectively), while the European Region and Region of the Americas have the lowest prevalence (<1%) [4], underscoring the disproportionate burden in resource-limited settings [4].

Regions with an HBsAg prevalence $\geq 8\%$ are considered hyperendemic for HBV; Nigeria, with a prevalence exceeding 8%, falls within this classification. In these regions, the predominant mode of transmission is perinatal (mother-to-child) transmission, which is associated with a high risk of chronic infection [2, 4]. HBV is also transmitted through percutaneous (e.g., needle-stick injuries, unsafe injections) and sexual contact [3].

Individuals with chronic HBV infection are at increased risk of developing cirrhosis and HCC [14].

Factors influencing progression to chronicity include age at acquisition, mode of transmission, and immune status [3, 5]; immunosuppression, in particular, favors viral persistence [1]. CD4⁺ T-lymphocytes are critical for coordinating adaptive immune responses and determining infection outcomes [15]. These cells facilitate B-cell activation and the production of anti-HBV antibodies, which are essential for viral clearance and long-term protection against reinfection [15, 16]. Thus, assessing immune status at HBV diagnosis may identify individuals at risk of disease progression, warranting closer monitoring [17]. Per WHO staging criteria, a CD4⁺ T-lymphocyte count <200 cells/mm³ indicates severe immunosuppression, while counts of 200–350 cells/mm³ indicate advanced immunosuppression [18]. Hepatic injury in HBV infection, which may progress through fibrosis and cirrhosis to HCC, is primarily immune-mediated rather than directly cytopathic, despite the hepatotropic nature of the virus [1, 3].

HBV infection is diagnosed by the detection of HBsAg in serum or plasma [4]. HBeAg is an important serological marker of active viral replication; its presence indicates high viral load and increased infectivity, thereby elevating the risk of progression to hepatic fibrosis, cirrhosis, and HCC in individuals with chronic HBV infection [14, 19]. Consequently, determining HBeAg status, especially in asymptomatic individuals, is crucial for preventing onward transmission and informing clinical management decisions [20].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serve as surrogate markers of hepatocellular injury [3]. Elevated levels in HBV infection indicate hepatocellular injury resulting from the host immune response to the virus [3]. Measuring serum transaminase levels can thus provide insight into the degree of hepatic inflammation or injury at the time of diagnosis [13]. Beyond individual patient assessment, global efforts are underway to address the HBV epidemic. Global initiatives, such as the WHO annual World Hepatitis Day, aim to raise public awareness of viral hepatitis and promote prevention and control efforts [21]. A key objective of these global initiatives is the elimination of HBV and HCV infections as public health threats by 2030 [22].

Data on immune status and markers of viral replication among asymptomatic HBV carriers in Northwestern Nigeria remain limited. This study aimed to assess CD4⁺ T-lymphocyte counts, HBeAg status, and hepatic transaminase levels in asymptomatic HBsAg-positive individuals who were identified during the 2015 World Hepatitis Day screening initiative at Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

MATERIAL AND METHODS

Study area and design. This hospital-based, cross-sectional descriptive study was conducted at Usmanu Danfodiyo University Teaching Hospital (UDUTH), a tertiary healthcare facility serving Sokoto State and neighboring states in Northwestern Nigeria. Participant recruitment occurred at a screening event during World Hepatitis Day 2015 (28 July), and data collection extended from 28 July through 18 August 2015.

Study population. The study population comprised 430 adults (aged ≥ 18 years) who voluntarily participated in the HBV screening initiative during the World Hepatitis Day event at the hospital.

Sampling technique. Non-probability convenience sampling was employed due to the voluntary nature of the screening event. No formal sample size calculation was performed, as all consenting eligible individuals presenting during the screening period were enrolled.

Ethical clearance. Ethical approval was obtained from the Research and Ethics Committee of UDUTH (approval number: UDUTH/HREC/2015/No. 898). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment, and participant confidentiality was maintained throughout the study by using anonymized identifiers for data management.

Inclusion and exclusion criteria. 1) Adults aged 18 years or older who provided written informed consent were eligible for the study. 2) Individuals with a known prior diagnosis of HBV infection were excluded.

Data collection. A structured, interviewer-administered questionnaire was used to collect data on socio-demographics, risk factors, and medical history from all participants. The questionnaire was developed in English with questions adapted from previously published studies on HBV risk factors; however, formal validation was not performed due to resource constraints, which is acknowledged as a limitation.

Sample collection and laboratory analysis. Venous blood samples (approximately 10 mL) were collected from all participants by trained phlebotomists using standard aseptic techniques. Serum, obtained by centrifugation of clotted blood samples, was used for the analysis of HBsAg, HBeAg, ALT, and AST, while whole blood collected in ethylenediaminetetraacetic acid (EDTA) tubes was used for CD4⁺ T-lymphocyte count determination. The upper limits of normal (ULN) for transaminases, according to manufacturer guidelines, were as follows: ALT, ≤ 49 IU/L for males and ≤ 34 IU/L for females; AST, ≤ 46 IU/L for males and ≤ 36 IU/L for females.

Participants were initially screened for HBsAg using Egens rapid immunochromatographic test strips (Reagent Technology, USA). Positive results were subsequently confirmed using an HBsAg ELISA kit (Fortress Diagnostics, UK) following the manufacturer's

instructions. The manufacturer-reported sensitivity and specificity of the ELISA kit were 99.75% and 99.87%, respectively. The sensitivity and specificity of the rapid test kit were not provided by the manufacturer.

Participants confirmed as HBsAg-positive underwent further testing. HBeAg status was determined using an HBeAg ELISA kit (DRG International Inc., USA), which had manufacturer-reported sensitivity and specificity of 100%. ALT and AST levels were measured using a kinetic UV assay with Agappe Diagnostics kits (Agappe Diagnostics Ltd., Switzerland). CD4⁺ T-lymphocyte counts were determined using a Partec CyFlow Counter II (Partec GmbH, Germany) following the Sysmex CD4 Easy Count Kit protocol. Briefly, an EDTA whole blood sample was incubated with a CD4-PE-conjugated monoclonal antibody in a 1:1 ratio for 15 min at room temperature before analysis. Quality control samples were included in each assay run according to manufacturer recommendations.

Statistical analysis. Data were analyzed using IBM SPSS Statistics, version 20 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), and categorical variables were presented as frequencies and percentages. Categorical variables were compared using the chi-square test or Fisher's exact test, with the latter applied when expected cell counts were < 5 . Continuous variables were compared using Student's t-test after confirming normality of distribution. A P-value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic characteristics of participants. Of the 430 individuals screened, 52 (12.1%) tested positive for HBsAg. Among the HBsAg-positive participants, 41 (78.8%) were males and 11 (21.2%) were females, with a male-to-female ratio of 3.7:1. The mean ($\pm$ SD) age was 31 ± 7 years. Regarding education level, most participants had attained tertiary education ($n = 39$, 75.0%), and the most common occupation was civil servant ($n = 24$, 46.2%) (Table 1).

Reported risk factors for HBV infection. The most frequently self-reported risk factors among the HBsAg-positive participants were tattooing ($n = 22$, 42.3%) and a family history of HBV infection ($n = 19$, 36.5%). Other reported factors included intravenous drug use ($n = 6$, 11.5%) and a history of blood transfusion ($n = 1$, 1.9%) (Table 2).

Laboratory findings in HBsAg-positive participants. Among the 52 HBsAg-positive participants, 6 (11.5%) were positive for HBeAg. Regarding liver enzymes, 7 (13.5%) participants had elevated ALT levels and 4 (7.7%) had elevated AST levels. The mean CD4⁺ T-lymphocyte count was 677 ± 249 cells/mm³. Overall, 10 (19.2%) participants had counts < 500 cells/mm³, while 3 (5.8%) had counts of 200–350 cells/mm³, a range indicating advanced immunosuppression (Table 3).

Table 1. Socio-demographic characteristics of HBsAg-positive participants (N = 52)

Variable	Frequency (%), n = 52
Sex	
Male	41 (78.8)
Female	11 (21.2)
Age (years)	
Mean $\pm$ SD	31 $\pm$ 7
< 45	50 (96.2)
$\geq$ 45	2 (3.8)
Education Level	
None	2 (3.8)
Primary	1 (1.9)
Secondary	10 (19.2)
Tertiary	39 (75.0)
Occupation	
Civil servant	24 (46.2)
Student	17 (32.7)
Businessperson	4 (7.7)
Carpenter	2 (3.8)
Homemaker	2 (3.8)
Trader	2 (3.8)
Tailor	1 (1.9)

Table 2. Self-reported risk factors for HBV infection among HBsAg-positive participants (n = 52)^a

Risk factors	n (%)
Tattooing	22 (42.3)
Family history of HBV infection	19 (36.5)
Intravenous drug use	6 (11.5)
History of blood transfusion	1 (1.9)

^a Participants could report more than one risk factor; percentages are calculated based on the total number of HBsAg-positive participants.

Table 3. Clinical and immunological markers in HBsAg-positive participants (n = 52)

Parameter	Result
HBeAg Status	
HBeAg positive, n (%)	6 (11.5)
HBeAg negative, n (%)	46 (88.5)
Liver Transaminases^a	
ALT* (IU/L), mean $\pm$ SD	39.5 $\pm$ 40.1
Elevated ALT*, n (%)	7 (13.5)
AST** (IU/L), mean $\pm$ SD	27.8 $\pm$ 46.2
Elevated AST, n (%)	4 (7.7)
CD4+ T-lymphocyte Count^b	
CD4+ count (cells/mm ³), mean $\pm$ SD	677 $\pm$ 249
CD4+ count < 500 cells/mm ³ , n (%)	10 (19.2)
CD4+ count 200–350 cells/mm ³ (advanced immunosuppression), n (%)	3 (5.8)

^aElevated defined as ALT >49 IU/L; AST >46 IU/L.

^bNormal range: 500–1,500 cells/mm³. * ALT: Alanine aminotransferase; ** AST: Aspartate aminotransferase

Association of laboratory markers with sex and age. No significant associations were found between sex and the prevalence of HBeAg ($P = 0.626$), elevated ALT levels ($P = 0.767$), elevated AST levels ($P = 0.659$), or mean CD4+ T-lymphocyte counts ($P = 0.842$). Similarly, no significant differences were observed between age groups (<45 vs. $\geq$ 45

years) for HBeAg prevalence ($P = 1.000$), elevated ALT ($P = 0.828$), elevated AST ($P = 1.000$), or mean CD4+ counts ($P = 0.157$) (Table 4). Comparisons involving the $\geq$ 45 years age group should be interpreted with caution due to the small sample size ($n = 2$).

Table 4. Association of laboratory markers with sex and age in HBsAg-positive participants (n = 52)

Variable	Sex		P-value	Age group (years)		P-value
	Female (n=11)	Male (n=41)		< 45 (n=50)	$\geq$ 45 (n=2)	
HBeAg positive, n (%)	1 (9.1)	5 (12.2)	0.626	6 (12.0)	0 (0.0)	1.000
Elevated ALT levels, n (%)	1 (9.1)	6 (14.6)	0.767	7 (14.0)	0 (0.0)	0.828
Elevated AST levels, n (%)	0 (0.0)	4 (9.8)	0.659	4 (8.0)	0 (0.0)	1.000
Mean CD4+ count (cells/mm ³), Mean $\pm$ SD	665 $\pm$ 200	680 $\pm$ 262	0.842	687 $\pm$ 248	432 $\pm$ 65	0.157

Fisher's exact test was used for categorical variables due to small cell sizes; Student's t-test was used for continuous variables.

Association of HBeAg status with laboratory markers. The mean CD4⁺ T-lymphocyte count was significantly lower among HBeAg-positive participants than among HBeAg-negative participants (366 ± 72 vs.

717 ± 234 cells/mm³; $P < 0.0001$). However, no significant differences were observed in ALT and AST levels between these two groups ($P = 0.697$ and $P = 0.190$, respectively) (Table 5).

Table 5. Association of HBeAg status with laboratory markers in HBsAg-positive participants (n = 52)

Variable	HBeAg positive (n = 6)	HBeAg negative (n = 46)	P-value
CD4 ⁺ count (cells/mm ³), mean $\pm$ SD	366 ± 72	717 ± 234	< 0.0001
ALT (IU/L), mean $\pm$ SD	36.2 ± 16.7	40.0 ± 42.5	0.697
AST (IU/L), mean $\pm$ SD	17.0 ± 10.4	29.1 ± 48.9	0.190

Student's *t*-test was used for all comparisons.

Association between body mass index (BMI) and HBeAg status. Body mass index (BMI) was calculated and categorized according to WHO criteria. A significant association was found between BMI categories and HBeAg status using Fisher's exact test ($P = 0.007$). The

prevalence of HBeAg positivity was highest among underweight participants (42.9%) (Table 6). These results should be interpreted with caution due to small sample sizes in some BMI categories.

Table 6. Association between BMI and HBeAg status in HBsAg-positive participants (n = 52)

BMI category ^a (n)	HBeAg status	
	Positive, n (%)	Negative, n (%)
Underweight (7)	3 (42.9)	4 (57.1)
Normal (34)	1 (2.9)	33 (97.1)
Overweight (9)	1 (11.1)	8 (88.9)
Obese (2)	1 (50.0)	1 (50.0)
Total (52)	6 (11.5)	46 (88.5)

^aBMI categories: underweight (<18.5 kg/m²), normal (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), obese (≥ 30.0 kg/m²). Percentages are row percentages.

DISCUSSION

This study's finding of a 12.1% HBsAg prevalence in a cohort of asymptomatic individuals underscores the significant burden of undetected HBV infection in the region [7]. These data indicate that a large proportion of these individuals are chronic carriers of HBV, harboring the infection asymptotically, which poses a substantial challenge to HBV control efforts [4, 22]. This high prevalence is consistent with existing literature confirming that HBV infection often presents asymptotically and is endemic in West Africa [23, 24].

The prevalence of 12.1% is consistent with a national estimate of approximately 11% [7] and the 10.7% mean prevalence reported in a systematic review [25], though variations in study design, population, and testing methods limit direct comparisons [17]. Notably, this figure is identical to the reported prevalence for Northwestern Nigeria as a whole (11.8%) [9]. Similar high prevalence rates have been reported among pregnant women in other parts of Nigeria [26]. However, our finding is higher than prevalence rates reported in two studies conducted in the same area among blood donors (9.0% and 4.7%) [12, 24]. Ndakotsu and Musa [12] also reported a much lower prevalence of 1.9% among repeat blood donors, a group expected to have lower rates due to prior screening. Given that our study was conducted in 2015 and these comparison studies were conducted subsequently, this discrepancy may

suggest a decline in HBV prevalence in the study area over time, potentially attributable to improved public health measures.

The high frequency of self-reported risk factors, particularly tattooing (42.3%) and a family history of HBV infection (36.5%), implicates both vertical (perinatal) and intrafamilial transmission routes in the study population [8]. A family history of infection strongly suggests perinatal or early childhood transmission, which is known to substantially increase the risk of chronic HBV carriage [2]. These transmission dynamics likely contribute significantly to the high HBV prevalence in our region [7]. The 36.5% frequency of reported family history of HBV infection is similar to findings in China [27] but substantially higher than that reported in North-Central Nigeria (3.0%) [28]. The rate of intravenous drug use (11.5%) was comparable to that reported in Ekiti, Southwestern Nigeria (10.4%) [29], supporting percutaneous transmission as an important route [3].

The observed HBeAg prevalence of 11.5% is notably higher than rates reported in local studies among blood donors (0.6%) [24] and chronic HBV carriers in North-central Nigeria (4.5%) [30], and asymptomatic individuals in Jos (4.0%) [31]. This finding is clinically important, as HBeAg-positive individuals are highly infectious and face an increased risk of progression to cirrhosis and HCC [14, 19]. The higher prevalence in our

study may be attributable to the use of a more sensitive ELISA-based assay compared to rapid tests potentially used in other settings [17].

The finding that 13.5% of participants had elevated ALT, a relatively liver-specific marker of hepatocellular injury, suggests that a substantial minority of asymptomatic carriers have active liver inflammation [6]. The concurrent elevation of AST in 7.7% of participants, though less specific, further supports the presence of hepatocellular injury [19]. Although not statistically significant, there was a trend toward higher prevalence of HBeAg and elevated transaminases in males. This aligns with established evidence that males may have a higher risk of persistent HBV and adverse outcomes such as HCC, though our study was likely underpowered to detect this difference statistically [5, 13].

Although HBeAg positivity is typically associated with active viral replication and hepatocellular injury, HBeAg-positive individuals in this cohort did not have significantly higher transaminase levels. This could be explained by the small number of HBeAg-positive individuals ($n = 6$), which limited the statistical power to detect a difference, or it could reflect that some participants were in the immune-tolerant phase of chronic infection [17].

The most significant immunological finding was the strong association between HBeAg positivity and lower CD4+ T-lymphocyte counts, which is consistent with previous studies [15]. This finding suggests that HBeAg-positive individuals may have impaired T-cell-mediated immune clearance, potentially increasing their risk of chronic HBV disease progression [31]. Furthermore, 5.8% of participants had CD4+ counts indicative of advanced immunosuppression (200–350 cells/mm³), and 19.2% had counts below normal. These findings highlight a subset of asymptomatic carriers who may be particularly vulnerable to persistent infection and opportunistic infections [16].

Strengths of this study include the use of confirmed ELISA testing for both HBsAg and HBeAg, and the comprehensive assessment of immunological parameters alongside serological markers. However, this study has some limitations. First, serological confirmatory testing for chronic HBV infection was not performed. Second, HBV DNA was not measured, precluding the identification of occult HBV infection and quantification of viral load, a key predictor of disease progression [31]. Third, co-infections with hepatitis D virus (HDV), HIV, and HCV were not assessed; these co-infections are known to impact HBV natural history [31, 32]. Fourth, the questionnaire used to collect risk factor data was not formally validated. Fifth, the use of convenience sampling limits the generalizability of the findings. Finally, the exclusion of individuals with a known prior diagnosis of HBV may have introduced selection bias,

potentially underestimating the true burden of markers such as HBeAg in chronic carriers.

In conclusion, this study highlights a high prevalence of asymptomatic HBsAg carriers in Sokoto, with a subset exhibiting markers of high infectivity (HBeAg positivity) and active liver disease (elevated transaminases). Key findings include the strong association of HBeAg positivity with lower CD4+ T-lymphocyte counts and the novel link between underweight BMI and HBeAg status. Together, these results underscore the potential for undetected transmission and disease progression within this population, highlighting that individuals with HBV may also be at risk for co-infection with other blood-borne viruses, such as HIV and HCV [32]. Based on these findings, several public health recommendations can be made. These include: (1) integrating HBV screening and counseling into routine clinical services [18, 21]; (2) strengthening programs for antiviral prophylaxis and vaccination of infants born to HBV-infected mothers [2]; and (3) enhancing public education on preventive measures [5]. Moreover, our results suggest that routine assessment of BMI and CD4+ T-lymphocyte counts may be valuable additions to the initial evaluation and ongoing management of individuals with HBV infection.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AI DISCLOSURE

No artificial intelligence (AI) tools, machine learning systems, or automated data analysis software were used in the design of the study, data collection, data analysis, interpretation of results, or preparation of this manuscript. All aspects of the work were carried out solely by the authors.

DATA AVAILABILITY

The data supporting the findings of this study are available within the article. Additional data related to this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

BH conceived the study, performed laboratory and statistical analyses, and wrote the manuscript. AY

provided clinical expertise and assisted with data interpretation. HYR performed sample collection and laboratory analyses. UMA contributed to study design and statistical analysis. All authors reviewed and approved the final manuscript.

ETHICS STATEMENT

This study was approved by the Research and Ethics Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria (approval number: UDUTH/HREC/2015/No. 898). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

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