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Molecular Prevalence and Liver Enzymes Profile in Occult Hepatitis B Infection among Blood Donors

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ABSTRACT

Background: Occult hepatitis B infection (OHBI), defined as positive seroreactivity for HBV DNA and negative seroreactivity for hepatitis B surface antigen (HBsAg), poses a challenge due to its hidden and asymptomatic nature. This study aimed to investigate the molecular rate of OHBI in blood donors and to assess the accompanying liver function profile.

Methods: Blood specimens from 200 HBsAg- negative blood donors were tested by a rapid chromatographic assay (Micro- point combo kit, USA) for HBV serological markers (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) and detection of HBV DNA by nested PCR.

Results: Of the 200 HBsAg-negative donors, 160 (80%) were seronegative for any hepatitis B serologic marker. Conversely, 5 (2.5%) HBsAb+, 4 (2%) HBcAb+, 11 (5.5%) HBsAb+HBcAb+, 20 (10%) HBcAb+HBeAb+ were positive. Of significance, 4 (2%) donors had evidence of HBV DNA, corresponding to a 2% OHBI prevalence in this population. Moreover, OHBI- positive donors presented higher levels of liver enzymes indicative of potential liver injury. OHBI was detected in 2% of blood donors, showing the risk of HBV transmissibility by blood transfusion from those who are seemingly negative for HBsAg. In addition, the high liver enzymes in OHBI-positive donors may signify a link with the outcome of chronic HBV infection.

Conclusions: Our data suggest the need to develop a sensitive screening strategy to identify OHBI among blood donors in order to reduce HBV transmission. It also highlights the importance of monitoring liver function tests in OHBI- positive individuals to prevent possible liver- related complications sequelae.

INTRODUCTION

Occult hepatitis B infection is reported to occur with varying prevalence in different countries and regions. In Africa, 11.2% of HIV-infected individuals have occult hepatitis B [1]. Africa South of the Sahara has a prevalence of 26.5% in the south, 11% in the north, 9.1% in the east, and 8.5% in the west [2]. Occult HBV infection is also an issue in Nigeria. Research in Ibadan reported a 0.5% frequency in blood samples [3]. Another Lagos blood donor study found 5.2% [4]. These findings emphasize the need for awareness, prevention, and screening, such as nucleic acid testing, to detect and prevent occult hepatitis B infection.

The safety of blood transfusion remains a medical concern due to the risk of transfusion-transmitted infections [5]. Among these infections is hepatitis B virus infection, caused by a DNA virus, a member of the hepadnaviridae family, which primarily attacks the liver, causing inflammation, fibrosis, and cancer [5, 6]. The primary diagnostic marker for hepatitis B infection and for blood donor screening is the hepatitis B surface antigen (HBsAg) [7]. Occult hepatitis B infections (OHBI) occur when HBsAg is negative, but serum or liver DNA is positive [8]. It is characterized by undetectable levels of surface antigen and detectable levels of viral DNA. OHBI is now a serious global threat and a major obstacle in the fight against hepatitis B infection, especially in low- and middle-income countries. [9-11]. HBsAg undergoes structural changes due to escape mutations in the S gene, which may cause OHBI [12]. As a result, the infection cannot be detected by the commonly used serological detection methods [13]. OHBI may also be linked to naturally occurring surface antigen mutations [12]. Therefore, the absence of HBsAg in the blood may not indicate the lack of circulating hepatitis B virus (HBV) in the presence of anti-HBc, which could be a potential source of new HBV infection in blood transfusion settings, especially where hepatitis B core antibody (HBcAb) or nucleic acid testing is not routine screening. Hence, the study was conducted to determine the molecular prevalence of occult hepatitis B infection among donors and its effect on the liver of healthy donors.

The occult hepatitis B infection (OHBI) is a public health problem worldwide, and prevalence can vary among populations and countries. The prevalence of OHBI among children and adolescents was 7.5% as reported by a systematic review and meta-analysis study, and the rates were higher among HIV-infected individuals (24.2%) and among children born to HBsAg-positive mothers (6.4%) [14]. The prevalence of OHBI differs by country, and the infection rate of OHBI is higher in low-income countries among blood donors and accounts for 0.2% in HBsAg-negative donors [15]. OHBI represents a significant risk for blood transfusion, since individuals with OHBI have the potential to be viremic with active HBV DNA in their blood but are asymptomatic (i.e., do not have detectable levels of circulating antibodies) and are hence at risk of transmitting their infection by blood transfusion or organ transplantation [16]. This further highlights the need for sensitive screening procedures, such as Nucleic Acid Testing (NAT), to detect OHBI and secure blood transfusion [9].

Hepatitis B virus (HBV) transmission in Nigeria occurs through unprotected sexual intercourse, traditional practices such as local circumcision, uvulectomy, scarification, tribal marks, and poor sterilization of surgical equipment. [1]. Homebirths that are not conducted in a proper medical environment with sterile conditions also pose risks [1]. Blood transfusions from donors carrying HBV or with occult HBV infection present a significant danger [3]. In Sokoto State, healthcare workers face a high risk of HBV infection through needle prick injuries and accidental contact with infected blood and body fluids, despite having adequate knowledge of transmission risks, due to low vaccine uptake [4]. Additionally, immunosuppressed individuals, including those co-infected with HIV, are at increased risk of HBV infection through standard transmission modes [5].

METHODS

Study design

It was a prospective cross-sectional study.

Study Area

The study was carried out at Murtala Muhammad Specialist Hospital in Kano State. Kano is a state among the 36 states of Nigeria, located in the North-Western part of the country, with a population of 11,058,300 (about twice the population of Arizona) and a total landmass of 20,130

km² (7,777 square miles) [17]. This hospital is the oldest medical facility in Kano city, serving people from within and outside the metropolis. It is a tertiary health institution established in 1926; it has an 826-bed capacity and up to 20-50 voluntary blood donors daily [18].

Sample Size

Sample size was calculated using OpenEpi (v2) with HBV prevalence of 14% [19], as follows:

where:

n = sample size

Z = 1.96 (95% CI)

p = 0.14

d = 0.05.

The calculated sample size was 187 + 9(5% error margin) = 196

However, a total of 200 donors were recruited for the study.

Inclusion and exclusion criteria

Inclusion: Healthy adults of 18-45 years, previously screened and found to be free from HBsAg, who provide either voluntary or replacement blood donations.

Exclusion: Respondents with other transmissible infections, those who have heard about the virus, those with a previous diagnosis of hepatitis, and those who refuse to participate.

Sampling Technique

Two hundred (200) blood donors were randomly recruited into this study, comprising both individuals who voluntarily come to the hospital to donate blood and those called to donate blood to patients (replacement donors).

Instrument of data collection

A self- developed questionnaire, with two sections, was used. The first section contains the relevant socio-demographic information, such as sex, age, and marital status. In contrast, the second section contains HBV risk factors, which include HBV vaccination and a history of blood transfusion. Participants were asked about sharing sharp objects, traditional marks, or tattoos.

Sample and data collection

Two hundred (200) negative HBsAg blood samples were collected from donors aseptically via venipuncture using a 2ml syringe, into a plain container at the Blood Transfusion Center. The questionnaires were completed by subjects, with some receiving assistance. A consent form was also given to the participants. Using a micropipette, a few drops of whole blood were immediately used for serological screening. The remaining blood was centrifuged at 3500 rpm for 10 minutes, and the sera were refrigerated and stored at -20 °C for further analysis.

Screening for Serological Markers of HBV

This was done using a one-step cassette-style HBV test (Micro point Diagnostic, Santa Clara, CA, USA) to detect the markers, including Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (HBAb), Hepatitis B e antigen (HBeAg), Hepatitis B e antibody (HBeAb), and Hepatitis B core antibody (HBcAb) in whole blood. Drops of blood were added to each well using

a pipette, followed by drops of buffer. The results were then read after 15 minutes, as per the manufacturer's guide. Only samples positive for both HBcAb and HBsAb were subjected to PCR for HBV DNA detection.

HBV DNA Extraction

A 200 µL serum sample was used for HBV DNA extraction using a QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Initially, the serum was added to a 1.5 ml tube, followed by the addition of 20 µL of protease and 200 µL of cell lysis solution. The mixture was vortexed thoroughly and incubated at 56 °C for 10 minutes. Subsequently, 50 µL of elution buffer was used to elute the extracted DNA, which was then stored at -20 °C for subsequent polymerase chain reaction (PCR) analysis [20].

Nested PCR

The hepatitis B S gene was amplified by PCR using two sets of primers, an inner and an outer primer, from the extracted DNA sample. The PCR consists of two rounds, with the first round using a 50 µl reaction mixture containing 1 µl of the DNA sample, 25 µl of pre-mixed PCR solution, 1.25 µl of forward and reverse primers (final concentration 0.5 µl), and 21.5 µl of nuclease-free water. The first round of amplification of the 916 bp segment, which included the S-gene, was conducted under the following conditions: 30 cycles of denaturation at 94 °C for 5 minutes, annealing at 63.8 °C, extension at 72 °C for 60 seconds, and a final extension of 10 minutes at 72 °C. The surface antigen's 656 bp S-gene amplicon was amplified by the inner primer during the second round under the following conditions: thirty (30) cycles of 94°C for five minutes, 94 °C for thirty seconds, 63.8 °C for thirty seconds, then 72 °C for sixty seconds, with a final extension at 72 °C for eight minutes [13]. The overall size of the Amplicons was assessed by comparing them to a DNA molecular marker after gel purification and separation on a 1.5% (w/v) agarose gel [9].

Liver Function Profiling

Serum samples that were positive after the molecular assay were screened for liver function tests, which were measured using a spectrophotometer according to the standard procedures described in the respective test kits (RANDOX Company, UK, and Human Germany). Alkaline phosphatase (ALP) was measured using the Wenger method, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using the Reitman and Frankel method, protein concentration using the Biuret method, and albumin (ALB) using the Bromocresol Green method.

Data Analysis

Microsoft Excel (2020) was used for data analysis. One-way analysis of variance (ANOVA) was used to analyze data on liver function parameters. In contrast, the chi-square test was used to determine significant differences in demographic characteristics and risk factors. All values were considered significant at $p < 0.05$.

Ethical Consideration

Ethical clearance was obtained from the Operational Research Advisory Council of the Kano State Ministry of Health (MOH/OFF/797/T.I./1812).

RESULTS

The prevalence of HBV markers among blood donors is detailed in Table 1. A total of 200 blood samples were screened for HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb. Among these samples, 40 tested positive for one or two markers in combination, while 160 were serologically negative for all markers. Notably, none of the samples tested positive for HBsAg, HBeAg, or HBeAb alone. HBsAb was detected in 5 (2.5%) samples, HBcAb in 4 (2%) samples, HBcAb&HBeAb in 20 (10 %) samples, and HBsAb&HBcAb in 11 (5.5%) samples. No sample tested positive for more than two markers. Table 1 also presents the demographic characteristics and HBV risk factors among blood donors with and without OHBI. Of the 200 blood donors screened, 4 (2%) had OHBI, with ages ranging from 18 to 31 and from 32 to 45 years. Among these OHBI donors, 3

(1.5%) were first-time donors, and only 1 was a repeated donor. Among the OHBI-negative donors, 20 (10%) were vaccinated, while 176 were unvaccinated, and 3 (1.5%) had a history of blood transfusion. Further demographic characteristics are delineated in Table 1. The liver function profile of infected OHBI donors is shown in Table 2, highlighting AST values ranging from 9.5 to 11.3 U/L, ALT from 10.8 to 12.8 U/L, ALP from 110.01 to 201 U/L, ALB from 3.0 to 5.0 g/dl, and Total protein from 6.2 to 8.0 g/dl. Moreover, Table 3 indicates that the combined prevalence of hepatitis B surface antibody (Anti-HBs) and hepatitis B core antibody (Anti-HBc) was 5.5%. PCR results in HBsAg-negative blood donors are based on HBV DNA detection, and only samples positive for both HBcAb and HBsAb were subjected to PCR analysis. Three of the nine samples tested amplified the HBV gene, producing a 656 bp DNA product, confirming the presence of HBV DNA (Figure 1).

Alkaline phosphatase (ALP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), and occult hepatitis infection (OHBI).

The total number of samples =200, the number of positive samples = 40, the Number of negative samples 160.

Figures

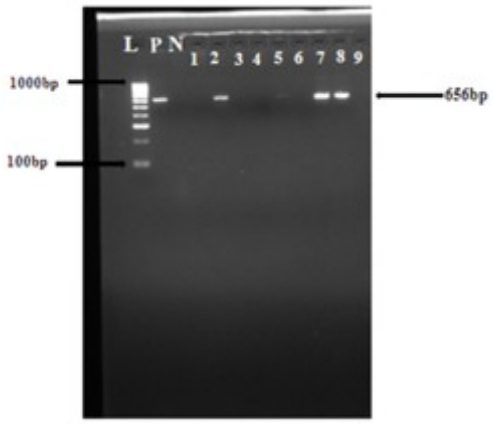

Figure 1: PCR result for the detection of HBV DNA in HBsAg-negative blood donors. Where both HBcAb and HBsAb were included in the PCR, three out of nine samples tested show successful amplification of HBV gene with 656bp amplicon DNA product, where L: ladder, P: positive control, N: negative control, bp: base pairs, all lanes show PCR products of the expected size, where Lanes P, 2, 7, and 8 show clear amplification bands corresponding to HBV DNA, molecular weight 656bp

Tables

Variables	OHBI (N=4)	HBsAg negative (N=196)	Chi-square (p-value)
Age in years			
18-31	1 (1%)	90 (45%)	0.69 (0.41)
32-45	3(1.5%)	106 (53%)	
Marital Status			
Single	3 (1.5%)	136 (68%)	0.58 (0.81)
Married	1 (1%)	60 (30%)	
HBV Vaccination			
Vaccinated	1 (1%)	20 (10%)	0.91 (0.33)
Not vaccinated	3 (1.5%)	176 (88%)	
Share a sharp/Barber			
Yes	3 (1.5%)	165 (82%)	0.24 (0.61)
No	1 (1%)	31 (15.5%)	
History of Blood Transfusion			
Yes	0 (0%)	3 (1.5%)	0.06 (0.8)
No	4 (2%)	193 (96.5%)	
First-time donors	3 (1.5%)	135 (67%)	0.068 (0.79)
Repeated donors	1 (1 %)	61 (30%)	
Had Traditional marks/ Tattoos			
Yes	0 (0 %)	7 (3.5%)	0.14 (0.7)
No	4 (2 %)	189 (94%)	

Table 1. Showing the prevalence of various hepatitis B virus (HBV) serological markers among blood donors and details the demographic characteristics and HBV risk factors among donors with and without occult hepatitis B infection (OHBI).

Variables	AST (U/L)	ALT (U/L)	ALP (U/L)	ALB (g/d)	TP (g/dl)
Positive control	13.01±0.000	15.21±0.000	285±0.000	2.8±0.000	4.7±0.000
Negative control	8.00±0.00	10.00±0.00	144.00±0.00	4.30±0.00	7.7±0.033
Sample 1	10.00±0.00	11.00±0.00	150.00±0.00	3.00±0.00	6.7±0.033
Sample 2	10.7±0.00	12.00±0.00	165.00±0.00	4.5±0.00	6.2±3.066
Sample 3	11.3±0.00	10.80±0.00	201.00±0.00	5.00±0.00	7.00±0.00
Sample 4	9.5±0.00	12.80±0.00	110.01±0.00	3.60±1.53	8.00±0.00
Reference value	0-12	0-12	98-279	3.5-5.5	6.0-8.0

Table 2: The Average Liver Function Profile of OHBI Blood Donors. It shows the liver enzyme levels of blood donors with occult hepatitis B infection (OHBI), including AST, ALT, ALP, ALB, and Total Protein. Elevated levels indicate potential liver damage in OHBI-positive donors. The positive control refers to confirmed HBV-positive serum used to validate the assay, while the negative control refers to pooled HBsAg-negative serum used to set reference values.

HBV Serological Marker	Positive	Percentage
HBsAg	0	0
HBsAb	5	2.5
HBcAb	4	2
HBeAg	0	0
HBeAb	0	0
HBsAb +HBcAb	11	5.5
HBcAb +HBeAb	20	10

Table 3: Prevalence of HBV markers among HBsAg-negative Blood donors (N=200). Showing the prevalence of various hepatitis B virus (HBV) serological markers among 200 HBsAg-negative blood donors, including HBsAb, HBcAb, HBeAg, and HBeAb, highlighting the percentage of donors testing positive for each marker.

DISCUSSION

The prevalence of occult hepatitis B infection (OHBI) in this study, estimated at 2% among 200 HBsAg- negative blood donors, falls within the range reported in studies conducted across different regions. For instance, a study at Lagos Teaching Hospital reported a similar prevalence of 3%, while a survey at Benue Teaching Hospital documented a lower prevalence of 0.7% among blood donors [21]. Conversely, a higher prevalence of 5.4% was reported in Ile- Ife, indicating regional variability within Nigeria itself [22]. Prevalence rates in other countries exhibit further diversity. For example, Egypt, Taiwan, and Saudi Arabia (Riyadh) reported lower prevalence rates of 0.5%, 0.11%, and 0.2%, respectively. Conversely, Malaysia, Burkina Faso, and Northeast China documented higher prevalence rates of 5.5%, 7.3%, and 33.3%, respectively [13, 20, 23]. Such disparities underscore the influence of factors such as HBV endemicity, liver disease prevalence, and screening methodologies on OHBI prevalence rates across different geographical locations. On the other hand, the prevalence of HBV markers, such as Hepatitis B surface antibodies (Anti-HBs) and Hepatitis B core antibodies (Anti-HBc), varied across studies, indicating differences in immunity due to natural exposure and past infections. Isolated anti-HBc, indicating the risk of ongoing silent hepatitis B infection, was observed in some cases [24, 25]. Additionally, the presence of Hepatitis B envelope antigen (HBeAg) and its antibody (HBeAb) indicated the replicative phase of the virus and reduced infectivity, respectively [26].

The assessment of liver function parameters among occult hepatitis B-infected donors revealed elevated levels of liver enzymes, including Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Albumin, and Total protein [27]. This observation aligns with findings from southeast Nigeria, where OHBI- infected blood donors exhibited similar elevations in liver enzymes, contrasting with reports from Nigeria's capital city and Egypt, where donors showed normal liver enzyme levels [28, 29]. This study suggests that a combination of antibodies indicates immunity acquired through natural exposure. This prevalence rate (2%) is higher than the 1.7% reported in Abuja and Anambra, but lower than the 4.6% documented in other Nigerian studies [30,31]. Contrasts starkly with the notably higher rate of 50% reported in Ile-Ife, Nigeria [32]. The presence of antibodies against HBV core antigen (anti-HBc) and surface antigen (anti- HBs) alongside hepatitis B envelope antigen (HBeAg) and its antibody (HBeAb)

plays a crucial role in determining the infectious state and transmission risk of the virus [26]. However, our study recorded zero prevalence of HBeAg and HBeAb individually, consistent with previous findings [33] that reported a similar absence of these markers alone. This contrasts with the prevalence rates of 1.8% for HBeAg and 22.8% for HBeAb observed in Burkina Faso [23], indicating regional differences in HBV infectivity and transmission dynamics. The presence of anti-HBe in combination with anti-HBc, in the absence of HBsAg, anti-HBs, and a core HBV mutant, indicates a state of reduced contagiousness and convalescence.

In the present survey, the prevalence of anti-HBe/anti-HBc was 10% compared to 39.6% in Golestan province, Iran [34]. These regional differences in CHB prevalence further highlight the complex interplay of factors that shape HBV epidemiology, including health practices, immunization, and population susceptibility. Regarding HBV immunization, a significant proportion of both OHBI and HBsAg-negative donors were not vaccinated against HBV. Notably, 3 (1.5%) OHBI donors and 176 (88%) HBsAg-negative donors were not vaccinated, a finding in line with Osuji et al. (2021) [35], who reported a high non-vaccination rate (86.6%) among healthy donors in Nigeria. Interestingly, none of the OHBI donors reported a history of blood transfusion, whereas only 1.5% of HBsAg-negative donors did. This is in line with earlier studies by Salawu et al. (2011) [32], who observed a high percentage of donors without a transfusion history (98%) and first-time donors (70%), a trend also observed in the present study.

The demographic characteristics of blood donors in this study show interesting trends in age distribution and in vaccination status for occult HBV infection (OHBVI) and HBsAg-negative status. The majority of OHBI donors were aged 18-45 years, a finding in keeping with previous studies [37], which reported a peak prevalence of OHBI cases amongst individuals aged 31-39 years. The majority of OHBI donors (75%) were aged 32– 45 years, consistent with previous reports showing higher occurrence among middle-aged adults [37]. Marital status among donors revealed that the majority were not married, with prevalence rates of 3 (1.5%) among OHBI donors and 136 (68%) among HBsAg-negative donors. This aligns with the findings of Salawu et al. (2011) [32], who reported a similar trend among unmarried donors in Ile-Ife (55.9%). However, this contrasts with the findings of Busari et al. (2018) [37], who observed that more than half of their donors were married. Despite these demographic and risk factor differences, no significant distinction was observed between donors with OHBI and those without it in terms of age, transfusion history, vaccination status, marital status, or other factors. This lack of a significant difference may be attributed to the small number of individuals with OHBI in the study, consistent with previous findings [22, 33].

In conclusion, 2% of blood donors in this study were found to have occult hepatitis B infections. Despite no significant variation in demographics or HBV risk factors between donors with and without OHBI, differences in liver parameters were observed. This underscores the challenge posed by hidden hepatitis B infections to blood transfusion safety. Therefore, it is suggested that blood transfusion centers consider implementing HBV profiling or semi-automated molecular screening to better detect occult hepatitis B infections in donors, rather than relying solely on HBsAg testing. Such approaches could enhance blood transfusion safety and reduce the risk of HBV transmission. The introduction of NAT in blood centers in low-resource settings will markedly improve blood safety by detecting OHBI and other transfusion-transmitted infections. Low-cost technologies, including Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA), as well as simplified point-of-care NAT kits that are easy to use and require limited expertise, can be applied. Training of health workers and strengthening of laboratories are critical. Health organizations and NGOs with a global remit can provide funds and technical assistance. Strong quality control and community involvement, as well as efficient data management systems, are also essential for success.

Limitations of the study

Several limitations in this study should be acknowledged. A convenience sample of 200 blood donors may not accurately reflect the broader population, potentially limiting the generalizability of the findings. Additionally, the study was conducted in Kano state, and the situation there may not precisely reflect the pattern of OHBI prevalence and risk factors in other regions of Nigeria or internationally. Therefore, larger studies from different areas could be valuable in providing a more comprehensive understanding of OHBI prevalence and its impact on blood safety.

CONFLICT OF INTEREST

None to declare

AUTHOR CONTRIBUTIONS

Conceptualization was carried out by Kauthar Mohammad and Shuaibu Abdullahi Hudu; methodology by Usman Aliyu Dutsinma; formal analysis by Ahmed Subeh Alshrari; investigation by Kauthar Mohammad and Usman Aliyu Dutsinma; resources by Ahmed Subeh Alshrari and Shuaibu Abdullahi Hudu; and writing — original draft preparation by Kauthar Mohammad and Shuaibu Abdullahi Hudu. All authors reviewed and approved the final version of the manuscript.

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