



Hepatitis B Virus Infection and Biomarkers Correlates of Liver Injury among Healthy Blood Donors in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIO conceived and designed the study. Authors NRA, MOI, INA and AIO were involved in samples collection and laboratory investigations. Authors AIO and IE analyzed the data obtained. Author AIO prepared the draft manuscript while authors NRA, IE and MOI provided ideas during draft writing. All authors read, reviewed and approved the final manuscript.

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ABSTRACT

Objectives: Destruction of blood cells and damage to hepatocytes are the hallmark of hepatitis B viral infection. This cross-sectional study was undertaken to evaluate some hematological parameters and biomarkers of liver injury in healthy blood donors infected with the occult and overt hepatitis B attending the University of Abuja Teaching Hospital (UATH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria.

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Materials and Methods: A total of 20 overt and 14 occult hepatitis B virus-infected blood donors were confirmed and enrolled using Roche® COBAS q-PCR from 212 consented pooled subjects. Hematological parameters and absolute CD4⁺ T-cell count were determined using the Sysmex hematology analyzer and Partec cyflow counter, respectively. Determination of serum Alanine Aminotransferase (ALT) and Alpha Feto-protein (AFP) levels were done spectrophotometrically and ELISA, respectively.

Results: The mean red blood cells, hematocrit, hemoglobin, white blood cells, CD4 and platelet count in the overt group were not statistically significantly different from the occult group $p > 0.05$. However, of the 34 infected subjects, 7 (20.6%), 6 (17.7%), 2 (5.9%), 13 (38.2%), 2 (5.9%) and 4 (11.8%) had abnormally low blood values for red blood cells, white blood cells, hematocrit, hemoglobin, CD4 and platelets respectively. The mean ALT and ALP values from the occult and overt groups were not also significantly different ($p > 0.05$).

Conclusion: This study found a decrease in the values of some blood cells. However, no significant changes in the liver enzymes were found. These infected individuals should be managed to prevent transmission of HBV infection to other people and subsequent destruction of blood cells and damage to liver cells. Future studies could focus on patients with chronic liver diseases/Hepatocellular carcinoma caused by HBV to determine the effects on the liver biomarkers and hematological parameters.

Keywords: Hematological parameters; liver biomarkers; occult hepatitis; blood donors; Nigeria.

1. INTRODUCTION

Hepatitis B virus (HBV) is one of the agents that cause viral hepatitis, a disease of major significance in term of both morbidity and mortality [1,2,3]. It is the major cause of inflammation of the liver and tends to be more serious than other hepatitis viruses [4,5]. Hepatitis B virus is also the most common cause of cirrhosis, liver failure and Hepatocellular carcinoma (HCC) as a result of its chronicity [6,7]. Hepatitis B virus infection can be occult or overt infection [5]. Occult hepatitis B virus infection (OBI) is characterized by the presence of HBV DNA in serum and/or in the liver of patients in the absence of detectable HBsAg with or without anti-HBV antibodies [8,9,10]. Whereas overt hepatitis B viral infection is simply defined as an infection in individuals who are positive for hepatitis B surface antigen (HBsAg) as well as HBV DNA. Overt and occult HBV infections have become a challenge to blood transfusion services and infected persons in Nigeria [11].

Hepatic pathology and extrahepatic abnormalities are common features in hepatitis B viral infection. Also, one of the frequently identified extrahepatic abnormalities often seen at the time of diagnosis of acute viral hepatitis is the hematological abnormality [12]. This is because every patient with acute viral hepatitis will require a full blood count to exclude bacterial sepsis as a cause of liver disease [12]. Hematological parameters such as red blood

cells (RBC), white blood cells (WBC), and platelets are blood cells in the complete blood count that have unique functions [13]. Studies have shown that these cells function and values are altered in disease conditions such as viral hepatitis [12,14]. Alanine Aminotransferase (ALT) is one of the enzymes released into plasma following cell death or injury. It serves as a biomarker, which its levels in serum could assist in the diagnosis and prognosis of diseases and injuries affecting the liver tissue caused by viral hepatitis [15]. The activities of serum ALT and aspartate transferase (AST) are significantly elevated several days before the onset of jaundice in acute viral hepatitis [15]. Alanine Aminotransferase is considered to be more specific for liver disease than AST because AST is elevated in cases of cardiac or skeletal muscle injury while ALT is not [3,15]. Many reports have shown that ALT is markedly elevated in acute HBV infection, moderately increased in chronic infection and remains normal in past and occult infection [11,16]. In addition, Alpha – Feto Protein (AFP) is a glycoprotein produced during fetal development of the hepatocytes and is the most useful marker for the diagnosis and management of HCC caused by viral hepatitis and HIV infection [2]. Elevated levels of AFP are found in patients with primary hepatoma and yolk sac-derived germ tumors [3].

Studies on healthy blood donors that tested positive for occult and overt HBV infections have been reported [4,5,10,17], but there is a paucity

of data on the hematological and biomarkers correlates of liver diseases. Infected blood donors may have high levels of ALT and AFP in their blood. This could result in liver cirrhosis and HCC if patients are not promptly and adequately managed. The current study is therefore intended to assess some hematological parameters and biomarkers of liver disease in apparently healthy blood donors infected with the occult and overt HBV infection at two teaching hospitals in Nigeria. Findings from this study will enable health providers make relevant recommendations for effective management of blood donors infected with the occult and overt HBV infections to prevent unforeseen public health conditions due to increased transmission and spread of HBV infection in the community.

2. MATERIALS AND METHODS

2.1 Study Areas, Design and Subjects Recruitment

This descriptive, cross-sectional study was carried out at the blood banks of the University of Abuja Teaching Hospital (UATH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi all in Nigeria. The subjects include 212 (108 from UATH and 104 from NAUTH), healthy blood donors. The selection of the subjects was done with the support of the staff of the hospitals' blood banks. Blood donors who have been found eligible for the donation were randomly selected and recruited for five months. Relevant Sociodemographic data and HBV associated risk factors of the participants were obtained using a well-structured questionnaire.

2.2 Specimens Collection and Laboratory Analysis

Ten (10) milliliters of venous blood was collected from each blood donor into K+ EDTA and plain bottles. All samples were analyzed for ALT, AFP, HBV serology, hematological parameters, and CD4+ T cell count. Samples that cannot be tested immediately were preserved at refrigeration temperatures. Also, samples for HBV DNA assays that cannot be processed immediately were preserved at -20°C until analyzed.

2.3 Detection of HBsAg by ELISA

The surface antigen for hepatitis B was tested by the ELISA technique on all specimens using the method of Burtis et al. [3]. Fortress Diagnostics

4th generation ELISA kit (Northern Ireland, UK) was used. The manufacturer's instructions on testing were strictly followed.

2.4 HBV DNA Studies

Hepatitis B virus DNA testing was performed on samples negative and positive for HBsAg ELISA to determine occult and overt hepatitis B virus infection using COBAS Roche Real-Time Polymerase Chain Reaction (PCR) as described by Osuji et al. [10]. The Standard Operating procedures were strictly followed to ensure accurate laboratory findings.

2.5 Determination of Alanine Aminotransferase (ALT) by Spectrophotometric Method

Alanine Aminotransferase (ALT) was determined by a standard method as described by Reitman and Frankel [18] using Randox reagents and Stax Fax 1904 Spectrophotometer. Alanine Aminotransferase (ALT) determination was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The kit manufacturer instructions and procedures were strictly followed.

2.6 Determination of Alpha Feto Protein by ELISA

Alpha-fetoprotein level was determined using the Perfemed Diagnostics ELISA Microwells Kit on all specimens that are positive for HBV DNA and negative samples using the method of Abelev [19]. Fourteen OBI samples, 20 overt HBV and 15 HBV negative samples making a total of 49 samples were assayed for AFP following manufacturer Standard Operating Procedures.

2.7 Determination of ABO Blood Group

ABO Blood group/ type of all blood donors recruited was determined using cell and serum grouping methods as described by Contreras et al. [20]. Anti A, anti B and anti D reagents from Spectrum Biotech, USA was used and the manufacturer's instruction was strictly followed.

2.8 Determination of Some Hematological Parameters

A complete blood count of all blood donors enrolled in this study was done using Sysmex KN

21 auto hematology analyzer. The Sysmex KN 21 auto-hematology analyzer used was able to determine the hematological indices of the blood donors like HGB, RBC, WBC total and differential, HCT and Platelets. The Sysmex KN 21 Auto-hematology analyzer is a quantitative, automated hematology analyzer and leucocyte differential counter for *in-vitro* diagnostic use in clinical laboratories. The tests were done according to the instruction of the automatic multiparameter blood counter.

2.9 Determination of Absolute CD4 Cell Count

The CD4 cell count was performed on all blood donor participants following the method as described by the World Health Organization [21]. This was done using the Cyflow counter machine by Partec Germany. Flow cytometry is a method by which cell or microparticles in suspension is differentiated and counted according to the cell size, fluorescence emission, and internal structure.

2.10 Statistical Analysis

Data obtained from this study were entered on Microsoft excel and analyzed on SPSS version 20 for windows. Descriptive statistics were calculated and reported for Sociodemographic characteristics. Percentages were used to describe frequency analyses of categorical variables. A chi-square test was used to compare categorical variables. Analysis of variance (ANOVA) was used to compare the mean values of a continuous variable between three groups (occult, overt and HBV negative group). P-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 HBV DNA Assay

Out of 212 blood donors screened for occult and overt HBV infections by serological and molecular techniques, the result identified 14 and 20 persons as occult and overt HBV blood donors respectively. HBV DNA viral load among blood donors with occult HBV infection compared with individuals with overt HBV infection showed that the average HBV DNA for OBI blood donors (n=14) was 93±35 IU/mL with a range of 26-128 IU/mL. It was also observed that the average HBV DNA (viral load) for overt blood donors

(n=20) was 33176108±574579 IU/mL with a range of 924- 99520111 IU/mL.

3.2 Sociodemographic Characteristics and HBV Risk Factors

The significant findings from the table include; male subjects are more infected with the occult and overt HBV infection than the females between 18-40 years old more affected. In addition, students and artisans were more infected with hepatitis B virus infections than other occupational groups (Table 1).

Table 2 presents HBV risk factors among blood donors with overt and occult HBV infections in the study population. HBV risk factors assessed included: knowledge of HBV/ HBV infection, HBV vaccination status, occupation/domestic accidents, previous blood transfusion, multiple sexual partners, History of STDs to mention but a few. We observed no statistical significance difference between blood donors with the occult and overt HBV infection among the risk factors assessed. The principal observations indicated that most blood donors with occult and overt HBV infection lacked knowledge of HBV infection and have not received HBV vaccination.

3.3 Biochemical Markers of Liver Disease

Alanine Aminotransferase and Alpha Feto-protein levels among blood donors with the occult, overt and HBV negative individuals are presented in Table 3. All the OBI and overt blood donors have an insignificant level of ALT. In summary, the mean comparison using ANOVA showed no statistically significant difference between ALT and AFP comparing these 3 groups (OBI, Overt and HBV negative) p > 0.05.

3.4 ABO Blood Group Distribution of Blood Donors with Occult and Overt HBV Infections

Fig. 1 presents the ABO Blood group distribution of blood donors, donation kind and repeatability of donation among blood donors with occult and overt HBV Infections. It is evident from the Fig. 1 that blood group O is the most prevalent among blood donors with occult and overt HBV Infections, with 12 (85.7%) out 14 OBI donors identified while 12 out 20 overt cases representing 60% prevalence belonged to blood group O. Classification of OBI blood donors in respect to their status is also presented in Fig. 1. The finding shows that 9 out of 14 blood donors with OBI belonged to family replacement donors

representing 64.3% while 5 donors with OBI were voluntary non-remunerated donors representing 35.7%. Fig. 1 also presents the prevalence of OBI among blood donors concerning the time of donation. The result shows that 9 out of 14 blood donors with OBI are Repeat (old) blood donors representing 64.3% prevalence while the remaining 5 donors are first-time donors representing 35.9% of the population.

Table 1. Sociodemographic characteristics of blood donors with occult and overt HBV infection

Blood donors demographics	Occult HBV blood donors n=14	Overt HBV blood donors n=20	Total (%) n = 34	Chi-square (P-value)
Gender				
Male	13	14	27 (79.4)	2.55 (0.110)
Female	01	06	07 (20.6)	
Marital status				
Married	08	07	15 (44.1)	1.59 (0.207)
Single	06	13	19 (55.9)	
Academic status				
Primary	02	01	03 (8.8)	0.90 (0.637)
Secondary	06	10	16 (47.1)	
Tertiary	06	09	15 (44.1)	
Age (Years)				
18-25	02	10	12 (35.3)	4.64 (0.09)
26-40	10	08	18 (52.9)	
41-60	02	02	04 (11.8)	
Occupation/Profession				
Applicants	01	00	01 (2.9)	5.55 (0.235)
Students	03	09	12 (35.3)	
Business/Trading	05	02	07 (20.6)	
Civil Servants	01	02	03 (8.8)	
Artisans/Farmers	04	07	11 (32.4)	

* $p < 0.05$ (statistically significant)

Table 2. Risk factors of blood donors with occult and overt HBV infection at UATH and NAUTH

HBV risk factors	No. (%) of OBI donors affected n =14	No. (%) of donors with overt HBV infection n = 20	Chi-square (p-value)
Lack of knowledge of HBV	10 (71.4)	10 (50)	1.5 (0.21)
No HBV Vaccination	12 (85.7)	20 (100)	2.9 (0.08)
Occupational/Domestic Accident (Needle prick/injuries)	8 (57.1)	17 (85)	3.1 (0.07)
Previous Blood Transfusion	2 (14.3)	0 (0)	2.9 (0.08)
Multiple sexual partner	1 (7.1)	0 (0)	1.4 (0.23)
History of Sexually Transmitted diseases	1 (7.1)	0 (0)	1.4 (0.23)
Alcohol/drug abuse	2 (14.2)	0 (0)	2.9 (0.08)
Previous Surgeries/Dialysis	1 (7.1)	1 (5)	0.06 (0.79)
Tribal marks/Tattoo	1 (7.1)	2 (10)	0.08 (0.77)
Visiting commercial Barber/Manicurist/Pedicurist	11 (78.6)	13 (65)	0.7 (0.39)
Organ Transplant	0 (0)	0 (0)	1.75 (0.18)

* $p < 0.05$ (Statistically significant)

Table 3. Alanine aminotransferase and alpha feto-protein levels among blood donors with occult, overt and HBV negative persons

Parameters (Significant value)	OBI n = 14 X±SD	OVERT n = 20 X±SD	HBV negative n = 15 X±SD	ANOVA (F) (p-value)
ALT (> 12 u/L)	4.9±3.3	5.8±2.4	3.5±0.87	1.622 (0.209)
AFP (> 8.5 ng/ml)	4.2±1.1	3.7±0.9	3.7±0.9	0.999 (0.376)

Statistically Significant ($\alpha < 0.05$); Key: ALT: Alanine Aminotransferase; AFP: Alpha Feto-protein; ANOVA: Analysis of variance

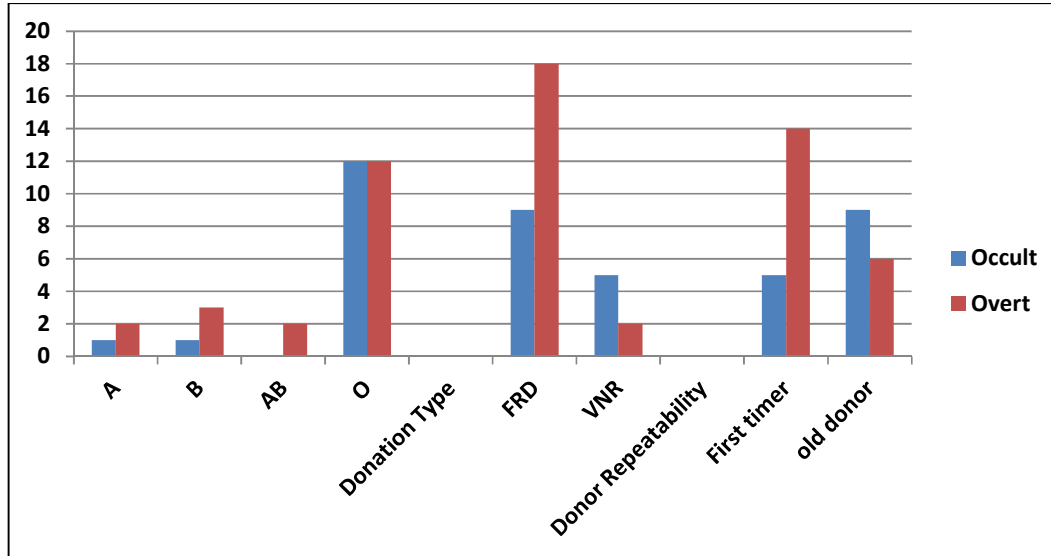


Fig. 1. ABO blood grouping and donation profile of blood donors with occult and overt HBV infection

Key: A: Blood group A, B: Blood group B, AB: Blood Group AB, O: Blood group O
FRD: Family Replacement Donor, VNR: Voluntary Non-remunerated Donor

3.5 Hematological Parameters and CD4 Count

Table 4 compared the mean count of red blood cells, hematocrit, hemoglobin, white blood cells, CD4 T-lymphocytes and platelets among occult and overt blood donors. There is no statistically significant difference across all blood cells examined between the two groups. The mean blood values of HCT, HGB, WBC, RBC, Platelets count and CD4 Cell count fall within the normal acceptable range.

Table 5 presents hematological parameters of occult and overt blood donors with low blood counts. Out of 34 subjects infected with occult and overt Hepatitis B, 2 (5.9%), 2 (5.9%), 13 (38.2%), 7 (20.6%), 6 (17.7%) and 4 (11.8%) had abnormally low blood values of CD4 cells, hematocrit, hemoglobin, red blood cells, white blood cells and platelets count respectively.

4. DISCUSSION

Occult and overt HBV infections could pose crucial challenge to blood transfusion safety in developing country where laboratory screening for blood donors enrollment are mainly by serological tests [17].

Sociodemographic data analysis from this study showed that most blood donors with occult and overt hepatitis B virus infections were within age 18-40 years old with median and mean age as 32 years and 31.4 years respectively. This finding correlates with the studies of Olotu et al. [22] and Oluyinka et al. [23] that reported that most OBI cases fall within persons between 31-39 years. It was also reported by Zheng et al. [24] that 28 years is median age of OBI blood donors in China. In a recent study by Omatola et al. [25], their finding showed that people of age bracket of 22-44 years were more infected with hepatitis B. This age bracket represents the most

sexual active period and most people may likely be infected with HBV and other transfusion transmissible viral agents (HIV and HCV). These viruses have common route of transmission among which is unprotected sexual intercourse [2]. However, the study did not find significant association between occult and overt blood donors, and the variables studied such as age, sex, educational status, marital status and occupation/profession (Table 1). This report is also similar to the finding of Olotu et al. [22]. All their OBI isolates were males and mostly below 36 years. This also conforms to the reports of Zheng et al. [24] which revealed the male to female ratio of OBI as 3.4:1 (17:5). Also, this study agrees with a recent study by Omatola et al. [25] that reported that male subjects were more infected with hepatitis B than the female counterpart.

Hepatitis B virus risk factors assessment performed showed that most blood donors infected with occult and overt HBV infections have not been vaccinated with HBV vaccines. Twelve (85.7%) out of 14 occult blood donors have not been vaccinated with HBV vaccines while all the 20 overt HBV donors have not received vaccination. This conforms to a similar study by

Osuji et al. [26] on healthy blood donors in Nigeria that showed 86.6% of the population has not received HBV vaccination. Besides, many of these HBV infected blood donors recruited have at least one risk factor to HBV infection. Out of 14 OBI blood donors identified in this study, it was observed that 10 lacked knowledge of HBV, 8 had occupational/domestic accident and 11 blood donors with OBI had visited commercial barber/manicurist/pedicurist to mention but few among the risk factors. This represents 71.4%, 57.1% and 78.6% respectively. This study observed no statistical significant difference among blood donors with occult and overt HBV infections about HBV risk factors assessed. This means anyone can be infected with hepatitis B virus irrespective of risk factors. However, these findings collaborate with the study of Omatola et al. [25] that reported people that lacked knowledge of HBV infection are more infected with hepatitis B virus. In a study by Lavanya et al. [27] on prevalence of HBV markers among blood donors, they observed that the high prevalence of HBV markers among blood donors in India were found to be instigated with risk factors like alcoholism, smoking, tattooing, ear piercing, visiting barber's shop and family history of jaundice.

Table 4. Comparison of hematological parameters of occult and overt blood donors

Blood cells	Occult blood donors N=14 X±SD	Overt blood donors N=20 X±SD	p-value
RBC	4.8 ± 0.6	4.63±0.6	0.191
HCT	42.2 ± 5.0	38.7±3.8	0.401
HGB	12.7 ± 1.7	12.6±1.7	0.207
WBC	4.9 ± 1.1	5.0±1.1	0.841
CD4	773.6 ± 269.5	936.5±297.5	0.195
PLT	196.5 ± 50.6	215.2±35.2	0.26

Statistical significant (p<0.05)

Key: RBC-Red blood cells; HCT- Hematocrit; HGB- Haemoglobin; WBC - White blood cells; CD4 - Cluster of Differentiation type 4; PLT- Platelets

Table 5. Hematological parameters of occult and overt blood donors with abnormally low values

Hematological parameters (Reference values)	No. (%) of occult samples tested with low values n=14	No. (%) of overt samples with low values n=20	Total no. (%) of samples with low values n=34
CD4 (500-1500/ul)	1 (7.1)	1 (5)	2 (5.9)
HCT (35-54%)	0 (0)	2 (10)	2 (5.9)
HGB (12-18 g/dl)	6 (42.9)	7 (35)	13 (38.2)
RBC (4.3-6.0 × 10 ¹² /L)	2 (14.3)	5 (20)	7 (20.6)
WBC 4.0-10.0 × 10 ⁹ /L)	3 (21.4)	3 (15)	6 (17.7)
PLT (150-450 × 10 ⁹ /L)	1 (7.1)	3 (15)	4 (11.8)

The result of Alanine Aminotransferase (ALT) from this study showed that all OBI blood donors as well as HBV seronegative samples had normal serum ALT concentration. This result correlates with the finding of Lin et al. [28]. This corroborates with another report from Chinese blood donors with OBI, which reported normal ALT values from their subjects [23]. This result is not surprising because these occult and overt HBV infections were detected in apparently healthy blood donors. The hepatitis B virus has not attacked the liver cells to the level of liver damage with resultant elevated liver enzymes. The Alpha Feto- Protein (AFP) level among the 3 groups of blood donors showed no significant difference within the group.

All the OBI blood donors as well as overt and negative samples had insignificant levels of AFP in the blood. Few studies have been done on the relationship between AFP and transfusion transmissible infectious viral agents in Nigeria. The result obtained from the present study indicated normal values of AFP among healthy blood donors infected with occult and overt HBV. In this study both ALT and AFP levels were normal in healthy blood donors with occult and overt HBV infections. A possible explanation for this is the viral load in OBI is low and the viral particles are not multiplying in these healthy blood donors.

The ABO blood group among blood donors with occult HBV infection showed that blood group O is the most prevalent among blood donors with occult HBV infection with 12 out of 14 OBI identified representing 85.7% belonged to blood group O while blood group A and B have one OBI blood donor each. This represents 7.1% prevalence. There is no OBI associated with blood donors with AB Blood group. In a similar study by Lavanya et al. [27] they found a contrasting result showing that blood group B is more prevalent followed by O but it is not statistically significant. Also, 9 out of 14 blood donors with OBI in this study were family replacement donors representing 64.3% while 5 blood donors with OBI are voluntary non-remunerated donors representing 35.7%. No blood donor with OBI was identified as commercial paid donor. Repeat (old) blood donors have more prevalent rate of occult HBV infection than first time blood donors. Findings from this study showed that 9 out of 14 blood donors with OBI are Repeat (old) blood donors representing 64.3% while the remaining 5 donors are first time donors representing 35.7% of the population. This could be that any blood

donor could be infected with HBV infection irrespective of blood donor status they belong. It also depends on exposure to risk factors and the immune status of the individual [27]. Whosoever is recruited for blood donation should be adequately screened for these transfusion transmissible viral agents using highly sensitive and specific test kits to detect and prevent transmission of hepatitis B virus to blood recipients and its attendant consequences.

Hematological parameters and absolute CD4 Cell count analysis showed that blood samples of some blood donors infected with occult and overt HBV infection have low values of some hematological indices. Findings from our hematological and CD4⁺ T cell analysis corroborates with the study of Ajugwo et al. [14] in Nigeria, which also found no significant difference in hematological indices (HCT, HGB, WBC Total and ESR) between symptomatic and asymptomatic patients infected with HBV. They concluded that patients with HBV infection have the tendency to develop anaemia if not adequately and properly managed. A comparative analysis of some hematological parameters and CD4⁺ T cell count of blood donors with OBI and overt samples in this study showed no significant difference between these 2 groups of blood donors. In addition, the low values of some hematological parameters observed among blood donors with occult and overt HBV infections, showed alteration and destruction of blood cells. This finding is in concordance with the report of Fasola et al. [12].

5. CONCLUSION/RECOMMENDATIONS

No significant changes were observed in the hematological parameters and biomarkers of liver disease in healthy blood donors infected with occult and overt hepatitis B. However, we found a decrease in some blood cells of these individuals. There is, therefore, a need to manage these subjects to prevent transmission of HBV infection to other people and subsequent destruction or alteration of blood cells, damage to liver cells resulting to liver cirrhosis or Hepatocellular carcinoma. Finally, blood donors in particular and the population in general should be protected from hepatitis B virus infection with administration of potent HBV vaccines.

6. LIMITATIONS OF THE STUDY

The small number of occult and overt samples of blood donors used in this study could be the

reason of no significant changes in biomarkers of liver markers and hematological parameters observed in this study. Secondly, there are limited funds as there is no grant and sponsorship from donor agencies.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Health research ethics committee (HREC) of both hospitals and informed consent was obtained from the subjects before sample collection. Subjects that declined to participate were excluded from the study. The approval letters from Health Research Ethics Committees with reference numbers for University of Abuja Teaching Hospital Abuja is FCT/UATH/HREC/PR/514 and that of Nnamdi Azikiwe University Teaching Hospital Nnewi is NAUTH/CS/66/VOL.9/40.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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