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**Haematological and Immunological Parameters in Patients with Chronic Hepatitis B Infection in Zaria, Nigeria**

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**Abstract**

Hepatitis B virus (HBV) is one of the major causes of morbidity and mortality worldwide. The aim of this study was to determine the haematological and immunological parameters in patients with chronic HBV infection in Zaria, Nigeria. Twenty individuals with confirmed chronic HBV (CHB) infection constituted the subjects while 20 non-HBV-infected individuals were monitored as controls. The subjects were enrolled purposively from the Gastroenterology Clinic of the Ahmadu Bello University Teaching Hospital Shika, Zaria Nigeria. Four millilitres of blood samples were collected from each study participants. Full blood count was conducted using the Swelab Alfa Haematology Analyzer, while CD4<sup>+</sup> T- Cell enumeration was performed using the Sysmex Partec CyFlow® Counter IVD flow cytometer according to the manufacturers' instruction. The mean (and standard deviation) age of the 20 participants with CHB was 32.7 ( $\pm 10.1$ ) years while that of the 20 HBV negative control participants was 30.0 ( $\pm 7.8$ ) years. Mann-Whitney test showed no significant difference between the two groups in their total WBC ( $p=0.6634$ ) and granulocytes ( $p=0.2386$ ). There was a significant increase in the monocytes count ( $p=0.0151$ ) and a significant decrease in the lymphocytes count ( $p=0.0006$ ) of patients with CHB compared to the healthy control. There was no significant difference in the mean CD4<sup>+</sup> T- lymphocytes count between subjects and controls ( $p=0.0633$ ). Unpaired Student *t*-test showed no significant difference between the two groups in the other haematological parameters. This study showed a significant increase in monocytes and decrease

in lymphocytes, a phenomenon that characterize the sustenance of infection by immune evasion mechanism.

**Keywords:** CHB, Haematological parameters, Immunocytes, Zaria

**Introduction**

Hepatitis B virus (HBV) is one of the major causes of morbidity and mortality worldwide (Caley *et al.*, 2012). About 2 billion people are thought to have evidence of past or present infection with HBV, with about 240 million chronic carriers of Hepatitis B surface antigen (HBsAg) (WHO, 2015). Worldwide, an estimated 650,000 people die each year from the complications of chronic hepatitis B (CHB) (Lozano *et al.*, 2012). In Nigeria, HBV infection is hyperendemic with the seroprevalence of HBsAg ranging from 10 – 40% (Ndako *et al.*, 2014; Bello *et al.*, 2011; Fasola *et al.*, 2008; Forbi *et al.*, 2008). Recent HBV seroprevalence studies in Nigeria indicate that HBV is hyperendemic. A prevalence rate of 13.6% was recorded by Musa *et al.* (2015) in their meta-analysis and systemic review, while Olayinka *et al.* (2016) recorded 12.2% in their national seroprevalence survey. The aim of this study was to investigate the haematological and immunological parameters in patients with chronic HBV infection in Zaria, Nigeria.

**Materials and Methods**

Study participants included 20 subjects with confirmed chronic HBV infection. They were enrolled purposively from the Gastroenterology Clinic of the Ahmadu Bello University Teaching

Hospital Shika, Zaria Nigeria. The choice of the clinic was informed by the availability of clinical history of participants, from which their respective hepatitis clinical staging was obtained. Chronic phase was defined by minimum of 6 months of hepatitis B surface antigen (HBsAg) seropositivity (WHO, 2018) and this was obtained from their clinical records in the gastroenterology clinic. Ethical approval (Reference Number: ABUTH/HREC/Y5/2016) was obtained from the Health Research Ethics Committee (HREC) of the Ahmadu Bello University Teaching Hospital, Zaria Nigeria before commencement of sample collection. Written informed consent was sought and obtained from each participant prior to enrollment into the study. Participants confirmed to be negative for both HIV and HCV, and positive for HBsAg for  $\geq 6$  months were recruited. Those confirmed negative for HIV, HCV, HBsAg and HBcAb were recruited as controls. Pregnant women were excluded. Patients with liver disease complications such as primary liver cell carcinoma (PLCC), chronic liver disease (CLD) and hepatocellular carcinoma (HCC) were also excluded. Those who failed to give consent were also excluded.

FBC was conducted in the Haematology Laboratory of the ABUTH Zaria, using Swelab Alfa Haematology Analyzer. CD4<sup>+</sup> T Cell enumeration was performed at ART LAB of Ahmadu Bello University Teaching Hospital, Shika Zaria using the Sysmex Partec CyFlow® Counter IVD flow cytometer according to the

manufacturer's instruction. The data were collated and validated using Microsoft Excel® spreadsheet. It was then analyzed using *GraphPad prism 6* statistical software package.

### Results

The mean (and standard deviation) age of the 20 participants with CHB was 32.7 ( $\pm 10.1$ ) years while that of the 20 control participants was 30.0 ( $\pm 7.8$ ) years. There were 11 male participants and 9 female participants with CHB, while the control group constituted of 10 male and 10 female participants.

Comparing the median and interquartile range (IQR) values of WBC, granulocyte, monocyte and lymphocyte counts in patients with CHB and healthy controls, Mann-Whitney test showed no significant difference between the two groups in their total WBC ( $p=0.6634$ ) and granulocytes ( $p=0.2386$ ), but there was a significant increase in the monocytes count ( $p=0.0151$ ) and a significant decrease in the lymphocytes count ( $p=0.0006$ ) of patients with CHB compared to the healthy control (Table 1). For haematological parameters in patients with CHB and healthy controls, Unpaired Student t-test showed no significant difference between the two groups in their red blood cells (RBC) count ( $p=0.1115$ ), packed cell volume (PCV) ( $p=0.8759$ ), haemoglobin concentration ( $p=0.2859$ ) and platelets count ( $p=0.0557$ ). Student t-test showed no significant difference in CD4 T-cells count between the two groups ( $p=0.0633$ )(Table2).

**Table 1: White Blood Cell (WBC) Counts in Patients with CHB and Healthy Controls in Zaria, Nigeria**

Parameter	Median (IQR)		p-value <sup>a</sup>
	CHB (n=20)	NHC (n=20)	
Total WBC ( $\times 10^9/L$ )	5.00 (4.65 – 6.28)	5.65 (4.60 – 6.90)	0.6634
Granulocytes ( $\times 10^9/L$ )	2.90 (2.30 – 3.08)	2.40 (1.88 – 3.20)	0.2386
Monocytes ( $\times 10^9/L$ )	0.40(0.33 – 0.50)	0.30 (0.23 – 0.40)	0.0151*
Lymphocytes ( $\times 10^9/L$ )	2.05 (1.70 – 2.50)	2.85 (2.23 – 3.38)	0.0006*

<sup>a</sup>Determined by Mann-Whitney U test; \*Significant difference

**Key:** CHB: Chronic Hepatitis B; NHC: Normal Healthy Controls; IQR: Interquartile Range

**Table 2: Some Haematological Parameters and CD4 Counts in Patients with CHB and Healthy Controls in Zaria, Nigeria**

Parameter	Value (mean ± SD)		p-value <sup>a</sup>
	CHB (n=20)	NHC (n=20)	
RBC (x10 <sup>12</sup> /L)	4.48 ± 0.53	4.19 ± 0.60	0.1115
PCV (%)	36.76 ± 4.48	36.54 ± 4.17	0.8759
Hb Conc. (g/L)	11.86 ± 1.62	11.35 ± 1.35	0.2859
Platelets (x10 <sup>9</sup> /L)	218.60 ± 45.22	190.30 ± 45.61	0.0557
CD4+ Count (cells/μL)	725 ± 248	869 ± 228	0.0633

<sup>a</sup>Determined by Student t-test

**Key:** CHB: chronic hepatitis B; NHC: Normal healthy controls; SD: standard deviation

### Discussion

Findings from this study indicated a significant increase in monocyte counts and decrease in lymphocyte counts in patients with CHB as compared with healthy controls. There was also an insignificant decrease in CD4+ T cell counts among the CHB subjects compared to the healthy controls. Moreover, there were no significant changes in the total WBC, granulocytes, RBC counts, PCV, haemoglobin concentration and platelet counts. Our finding is in agreement with the findings of Francisca *et al.* (2017). Our finding is however at variance with the findings of Fasola *et al.* (2009) where the focus of their findings was strictly on patients with acute hepatitis B (AHB) viral infection. This study focused only on participants with CHB. This could be the reason for the discrepant results. During the acute phase of HBV infection in immunocompetent individuals, innate immunity generally plays a central role to limit the success of the virus while initiating development of an adaptive immune response. Being central innate effector cells in viral infections, natural killer (NK) cell, monocyte and its derivatives, and other non-cellular components respond appropriately to eliminate viral infections by detecting the viral infection, hence the observable increase in all immunocytes during the acute phase (Fiscaro *et al.*, 2009). While phagocytes engulf to destroy the detected 'foreign' viral particles by different mechanisms such as release of antimicrobial proteins and peptides, release of proinflammatory cytokines and employment of reactive oxygen species, antigen presenting cells

carry on with the function of activating the adaptive immune response (Owen *et al.*, 2013). Innate cellular mechanisms that detect the viral particle can be triggered by viral replication to stimulate the production of type 1 interferons (IFNs) which in turn activate fully the innate response as well as trigger adaptive response stimulation. NK cells can also be activated by recognition of stress-induced molecules and the modulation of the quantity of major histocompatibility complex (MHC)-class 1 molecules on the surface of infected cells (Moretta *et al.*, 2005; Heil *et al.*, 2004). As complication due to HBV infection are mostly immune mediated, it is important to note that HBV does not stimulate strong innate response during the first weeks of acute infection, but that does not affect the recovery rate (Rehermann and Nascimbeni, 2005).

During the chronic phase of HBV infection, patients tend to have late, transient or narrowly focused T-cell responses, suggestive of the decrease in the lymphocyte counts observed in this study (Rehermann and Nascimbeni, 2005). Additionally, the sustained increase in monocyte counts in the chronic phase of HBV infection was supported by the findings of Zhang *et al.* (2011) where, in both immune tolerant (high HBV DNA and normal liver enzymes) and immune active (detectable HBV DNA and high liver enzymes) groups of patients there was a significant increase. This is due to the strong association with liver inflammation in those with CHB as CD16+ monocytes are known to preferentially release inflammatory cytokines

which is likely involved in expansion of Th17 cells and induction of liver injury (Zhang *et al.*, 2011). It is noteworthy that monocytes play both pro and anti-inflammatory roles in HBV-infected liver due to their plasticity that allows for their differentiation into M1 inflammatory macrophages or M2 anti-inflammatory macrophages, while in the periphery they serve as depot of HBsAg that could be exploited for presentation to T-cells (Maini and Gehring, 2016; Ginhoux and Jung, 2014).

### Conclusion and Recommendation

In conclusion, this study highlighted a significant increase in monocytes which are the major players in innate and adaptive immune response such as antigen presentation and cytokines productions. Significant decrease in lymphocytes was also observed in this study, a finding which suggests dampening of the immune response due to chronic phase of HBV infection, a phenomenon that characterize the sustenance of infection by immune evasion mechanism.

It is therefore, recommended that the mechanisms causing decrease in lymphocytes in CHB should be elucidated further. This could lead to immunotherapeutic approach in augmenting vaccine and treatment modalities available today.

### Conflict of Interest Declaration

Authors declare no conflict of interest.

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