

Evaluation of some Haematological parameters in the management of HBV infection treatment outcome in Delta State, Nigeria

ABSTRACT

Aims: To evaluate Hepatitis B virus (HBV) infection treatment outcome using the levels of white blood cells, haemoglobin, platelets counts and other red cell indices of HBV positive treatment naïve, on treatment at 3- and 6-months subjects attending gastro-enterology clinic in Federal Medical Centre Asaba, Delta State, Nigeria

Study design: Cross-Sectional and longitudinal study.

Place and Duration of Study: Federal Medical Centre Asaba (FMC) and Iykenon Medical and Diagnostic Co. Ltd, Awka. Federal Medical Centre, Asaba, between August 2019 and September 2020.

Methodology: A total of one hundred and fifteen (115) adults aged 22 – 64 years participated in this study. The study sites for this work comprised of Federal Medical Centre Asaba (FMC) and Iykenon Medical and Diagnostic Co. Ltd, Awka. The cross-sectional study consists of fifty (50) confirmed hepatitis B negative subjects as negative controls whereas, the follow-up study consists sixty-five (65) treatment naïve HBV positive subjects which were followed-up at three and six months on treatment with tenofovir respectively. Four (4) of the participants (two in three months post treatment and two six months post treatment) dropped-out of the research due to time constraint. Blood samples were collected from the subjects in EDTA bottles and were used for the analysis of white blood cell (WBC count), haemoglobin concentration, packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count (PLT). Statistical Package for social Science (SPSS) software version 26 was used in the analysis of data and $P < .05$ was considered statistically significant.

Results: There were no significant difference ($P > .05$) in neutrophil, lymphocyte, eosinophil and monocyte in the study population among the four groups whereas, the level of total white blood cell counts was significantly lower in the HBV naïve, one month post treatment and three-month post treatment when compared with the control group. The levels of haemoglobin, packed cell volume, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were significantly lower in the HBV naïve, three months on treatment and six-months on treatment when compared with the control group ($P < .05$).

Conclusion: In conclusion, it is possible that HBV infection has effect on iron metabolism and production of haematological precursor cells which are reflected in low levels of haemoglobin, PCV, MCV, MCH and MCHC as such these parameters could be used as co – markers to viral load in monitoring the treatment outcome of HBV infection in Nigeria.

Keywords: Hepatitis B Virus infection, red cell indices, platelets count, treatment outcome

1. INTRODUCTION

Hepatitis B virus is highly endemic in sub-Saharan Africa, China, South-East Asia and the Amazon Basin where around 8% of the population are chronic carriers [1]. The prevalence of chronic HBV infection is 2 – 4% in Japan, 5 – 18% in China and highest (15 – 20%) in Taiwan as well as several other countries in South-East Asia [1,2]. In Nigeria, a prevalence rates of 4.3% - 23.3% have been reported from different parts of the country [3]. A prevalence rate of 4.3% was reported from Port Harcourt [4], 5.7% from Ilorin [5], and 8.3%

from Zaria [6]. Several Nigerian researchers have reported that haematological parameters provide valuable information in HBV infection, since the condition may lead to changes in haematological values such as platelet, packed cell volume, haemoglobin and white blood cell in response to viral infection and inflammation [7,8,9,10,11]. It has been demonstrated that platelets play a major role in the pathogenesis of HBV infection [12,13,14]. Thus, this study was aimed at evaluating HBV infection treatment outcome using the levels of white blood cells, haemoglobin, PCV, MCV, MCH, MCHC and platelets counts of HBV positive treatment naïve, on treatment at 3 months and treatment at 6 months subjects attending gastro-enterology clinic in Federal Medical Centre Asaba, Delta State, Nigeria. This will add to the existing level of information in Nigeria on HBV treatment outcome, which is beneficial. The aim of this study was to evaluate Hepatitis B virus (HBV) infection treatment outcome using the levels of white blood cells, haemoglobin, platelets counts and other red cell indices of HBV positive treatment naïve, on treatment at 3- and 6-months subjects attending gastro-enterology clinic in Federal Medical Centre Asaba, Delta State, Nigeria

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study sites for this work comprised of Federal Medical Centre Asaba (FMC) and Iykenon Medical and Diagnostic Co. Ltd, Awka. Federal Medical Centre, Asaba is situated in the central Area of Asaba metropolis, the capital city of Delta State. The hospital is a tertiary health institution, a research and referral centre serving the entire state capital territory and neighboring towns like Ibusa, Iseleukwu, Ogwashiuku, Onicha – Ugbo, Onicha – Olona, Agbor etc. Federal Medical Centre Asaba is located in the South-South Geopolitical zone of Nigeria. The hospital was established on 12th August 1998 as a consequence of the Federal Government of Nigeria policy to setup a Federal Medical Centre in states where a Federal Teaching Hospital is non-existent.

Iykenon Medical and Diagnostic Co. Ltd is a private Medical Research and Diagnostic Centre situated close to the temporary site of Nnamdi Azikiwe University in the Central area of Awka the capital city of Anambra State.

2.2 Study Population

A total of one hundred and fifteen (115) adults aged 22 – 64 years participated in this study, who were chosen at convenience. This study consists of fifty (50) confirmed hepatitis B negative subjects as controls, whereas, the follow-up study consists of sixty-five (65) treatment naïve HBV positive subjects followed-up at three and six months on treatment with Tenofovir. Four (4) of the participants (two in three months on treatment and two six months on treatment) dropped-out of the research due to time constraint.

2.3 Selection Criteria

2.3.1 Inclusion Criteria

Male and female adult subjects aged between 18 - 65 years who tested positive or negative to Hepatitis B virus using One-Step Multi test strip, confirmed using both ELISA and PCR methods were included in the study. All confirmed negative and positive HBV subjects who gave informed consent by signing the consent form were included in the study.

2.3.2 Exclusion Criteria

Subjects with other liver diseases. For example, those who tested negative to hepatitis B virus using one-Step multi test strip, ELISA and PCR methods were excluded. Also, subjects below 18 years of age or above 65 years and those who withheld their consent before or in the course of the study were excluded from the study. Finally, individuals with haematological and/or haemostatic disorders also excluded.

2.4 Sample Collection and Analysis

2.4.1 Sample Collection

The blood samples were collected from the subjects in EDTA bottles and were used for the analysis of white blood cell (WBC count), haemoglobin concentration, packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count (PLT), using Sysmex® Automated Hematology Analyzer.

2.4.2 Sample Analysis

2.4.2.1 Estimation of Full Blood Count Red Cell Indices and Platelet Count using s auto haematology analyzer model manufactured by Shenzhen Mindray Bio – medical Electronics Co. Ltd

Full blood count of all participants in this study was carried out using the Mindray BC – 5000 5-part differential Auto haematology analyzer. The measurement methods used in this analyzer are; the Electrical Impedance method for determining the Red blood Cell (RBC) and Platelet (PLT) data, the colorimetric method for determining the haemoglobin, flow Cytometry by laser for determining the white blood cell data. Other parameter results including the Red blood Cell indices (MCV, MCH, MCHC, RDW) were obtained through automated calculation.

2.5 Statistical Analysis

Statistical Package for social Science (SPSS) software version 26 was used in the analysis of data. Comparison among groups was analyzed using analysis of variance (ANOVA) while comparison between groups was done using post Hoc analysis. Pearson's correlation was used to determine the relationship and association between parameters respectively. A value of $P < .05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Comparison of median of levels of total white blood cells, neutrophil, lymphocyte, eosinophil and monocyte in the study population

Participants	Total white blood cells (cells/l)	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)
HBV negative control(A) N= 50	8100.00	46.00	45.00	2.00	6.00
HBV treatment naïve (B) N = 65	5250.00	47.50	46.00	2.00	5.00
Three months post treatment (C) N= 63	5900.00	49.00	44.00	2.00	4.50
Six months post treatment (D) N= 61	6700.00	47.00	44.00	2.00	5.00
Krskal-wallis value	0.000	2.096	0.087	1.981	10.251
p-value	0.000	0.553	0.993	0.576	0.017
A vs B	0.000	0.942	0.999	0.999	0.093
A vs C	0.000	0.832	1.000	1.000	0.005
A vs D	0.002	0.963	0.997	0.999	0.999
B vs C	0.009	1.000	1.000	1.000	0.987
B vs D	0.0420	1.000	1.000	0.936	0.582

C vs D	0.0001	1.000	1.000	1.000	0.204
--------	--------	-------	-------	-------	-------

α level set at 0.05
(P < 0.05) = Significant
(P > 0.05) = Not Significant

Table 2: Comparison of median levels of haemoglobin, packed cell volume, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration in the study population

Participants	Haemoglobin (g/dl)	Packed Cell Volume (%)	Mean Cell Volume (fL)	Mean Cell Haemoglobin (pg)	Mean Cell Haemoglobin Concentration (g/dl)
HBV negative control (A) N= 50	14.05	43.10	87.00	30.00	34.00
HBV treatment naïve (B) N= 65	10.06	36.00	79.00	26.00	30.00
3 months post treatment (C), N= 63	10.16	38.02	80.00	27.00	31.00
Six months post treatment (D) N= 61	12.10	40.00	82.00	28.00	32.00
Kruskal-wallis value	0.030	0.025	0.000	0.000	0.000
p-value	0.032	0.027	0.000	0.000	0.000
A vs B	0.000	0.005	0.003	0.000	0.000
A vs C	0.000	0.003	0.293	0.000	0.000
A vs D	0.000	0.002	0.013	0.000	0.000
B vs C	0.000	0.001	0.000	0.001	0.003
B vs D	0.000	0.007	0.000	0.000	0.004
C vs D	0.000	0.003	0.004	0.000	0.027

α level set at 0.05
(P < 0.05) = Significant
(P > 0.05) = Not Significant

Hepatitis B virus infection (HBV) is a major cause of concern worldwide causing significant morbidity and mortality [15]. Poor diagnosis and prognostic factors remain one of the crucial factors responsible for poor management of the disease. Development of new treatment perspectives in the management of hepatitis B virus (HBV) infection which still remain a major health problem worldwide, contributing considerably to cirrhosis- and hepatocellular carcinoma (HCC)-related mortality of 0.5 – 1 million per year [16,17].

Despite progress in implementing vaccination programmes and in the development of new treatment perspectives, hepatitis B virus (HBV) infections remain a major health problem worldwide [16]. Thus, it is postulated that changes in haematological parameters of HBV treatment might be related to various HBV treatment stages and can result in better prognosis and management of HBV patients. In this study we evaluated some haemostatic parameters as co – markers to viral load in monitoring the treatment outcome of HBV infection in Nigeria. The cross-sectional study consists of fifty (50) confirmed hepatitis B negative subjects as negative controls whereas, the follow-up study consists of sixty-five (65) treatment naïve HBV positive subjects which were followed-up at three and six months on treatment with tenofovir respectively. The levels of haemoglobin, PCV, MCV, MCH and MCHC were significantly lower in the HBV naïve, one month post treatment and three-month post treatment when compared with the control group ($P < .05$). This could be attributed to feeding style or a temporary bone marrow suppression and autoimmune haemolytic anaemia which may accompany viral hepatitis. Studies have shown that some abnormal haematological parameters in HBV infection include defect in levels of platelet numbers, packed cell volume (PCV), haemoglobin (Hb) and white blood cell (WBC) disorders which include absolute changes in Leukocyte numbers, involving neutrophils, lymphocytes and eosinophils in response to tissue injury, and inflammation [8,11,10]. Research has shown significantly

raised haemoglobin concentration, absolute leukocytes, neutrophils, lymphocytes, eosinophils as well as monocytosis in HBV infection [18,19,10]. Packed cell volume in most patients with acute viral hepatitis gradually decreases during the first three weeks of illness [20].

It has been well established that many haematological abnormalities occur in HBV infection possibly due to cell distortions that occur following inflammation caused by the infection which may likely result to alterations in iron metabolism, aberrant production of haematological precursor cells as well as defect in red blood cell morphology [7,10]. Another possible reason for the changes in haematological indices in HBV infection is disruption of liver functions due to liver damage since the liver has indisputable influence on several essential functions of many organs in the body, the haematopoietic system inclusive. Outside its role as an extravascular haematopoietic organ in early foetal life and bone marrow infiltrative disease, the liver synthesizes and stores many of the elements and proteins necessary in blood production. It also plays a crucial role in the haemostasis [20].

Some abnormal haematological parameters in HBV infection include levels of platelet numbers, packed cell volume (PCV), haemoglobin (Hb) and white blood cell (WBC) disorders which include absolute changes in Leukocyte numbers, involving neutrophils lymphocytes and eosinophils in response to tissue injury, and inflammation [8,11,10]. Several researchers have reported that haematological parameters provide valuable information as regards manifestation of HBV infection since the condition may lead to changes in haematological values such as platelet, Packed Cell Volume (PCV), Haemoglobin (Hb) and White Blood Cell (WBC) disorders which include; absolute changes in Leukocyte numbers, involving Neutrophil, Lymphocyte and Eosinophil in response to viral infection and inflammation [7,8,9,10,11]. It is possible that inflammation caused by HBV infection has effect on iron metabolism and production of haematological precursor cells. Research has shown significantly raised opposite haemoglobin concentration, absolute leukocytes, neutrophils, lymphocytes, eosinophils as well as monocytosis in HBV infection [18,19,10].

CONSENT

Both oral and written consent of each HBV positive and control subjects were obtained before recruitment into the study.

ETHICAL APPROVAL

Ethical approval was sort and obtained from the Research and Ethics Committee of Federal Medical Centre (FMC) Asaba, Delta State where the participants were recruited from. The approval letter from this committee with reference number FMC/ASB/A81 VOL. XII/119.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE

RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

1. MacLachlan, J.H. and Cowie, B.C. Hepatitis B virus epidemiology. *Cold Spring Harb Perspect Med*, 2015; 1;5(5): 21410-4.
2. Chien-Jen, C., Li-Yu, W. and Ming-Whei Y. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *Wiley Online Library*, 2002; 15: 3-36.
3. Berinyuy, B. E., Alawode, R. A., Mohammed, A. B. and Babalola B. S. Prevalence of Hepatitis B virus in Nigeria: Review Update. *Annal of Public Health and Epidemiology*, 2019; 1(1): 1 – 7.
4. Akaani, C. I., Ojule, A. C., Oporum, H. C. and Ejilemele, A. A. Seroprevalence of Hepatitis B surface antigen in pregnant women in Port Harcourt, Nigeria. *Niger Postgraduate Medical Journal*, 2005; 12(4): 266 – 70.
5. Agbede, O. O., Iseniyi, J. O., Kolawole, M. O. and Ojuowa, A. Risk factors and seroprevalence of Hepatitis B surface antigenemia in mothers and their preschool age children in Ilorin, Nigeria. *Therapy*, 2007; 4(1): 67–72.
6. Jatau, E. D. and Yahaya, A. Seroprevalence of hepatitis B virus in pregnant women attending a clinic in Zaria, Nigeria. *Science World Journal*, 2009; 4: 7–9.
7. Eze, M. E. and Buseri, F. I. Effects of hepatitis B infection on haematological parameters in Pregnancy in Port Harcourt, Nigeria. *Research Journal of Medical Sciences*, 2009; 3(6): 194–7.
8. Pan, C. Q. and Zhang J. X. Natural history and clinical consequences of hepatitis B virus infection. *International Journal of Medical Sciences*, 2005; 2: 36 - 40.
9. Park, K. S., Lee, Y. S. and Lee, S. K. A study of markers of viral hepatitis in adults living in Daegu and Gyungbuk area, Korea. *Journal of Gastroenterology*, 2003; 41: 473–9.
10. Ali, S. J. A correlative study between haematological and biochemical parameters in hepatitis B. *Ibn Al Haitham Journal for Pure and Applied Science*, 2019; 32 (2): 21-9.
11. Nwokediuko, S.C. and Ibegbulam, O. Quantitative platelet, abnormalities in patients with hepatitis, B virus-related, liver disease. *Gastroenterology Research*, 2009; 2(6): 344-9.
12. Claushuis, T. A. M., de Stoppelaar, S. F., Stroo, I., Roelofs, J. J. T. H., Ottenhoff, R., Van der Poll, T. and Van't Veer, C. Thrombin contributes to protective immunity in pneumonia-derived sepsis via fibrin polymerization and platelet-neutrophil interactions. *Journal of Thrombosis and Haemostasis*, 2017; 15: 744–57.
13. Chen, J., Li, X., Li, L., Zhang, T., Zhang Q., Wu, F., Wang, D., Hu, H., Tian, C., Liao, D., Zhao, L., Song, D., Zhao, Y., Wu, C., and Song, X. Coagulation factors VII, IX

and X are effective antibacterial proteins against drug-resistant Gram-negative bacteria. *Cell Research*, 2019; 29: 711-24.

14. Burzynski, L. C., Humphry, M., Pyriou, K., Wiggins. K. A., Chan, J. N. E., Figg, N., Kitt, L. L., Summers, C., Tatham, K. C., Martin, P. B., Bennett, M. R. and Clarke, M. C. H. The coagulation and immune systems are directly linked through the activation of interleukin-1 α by thrombin. *Immunity*, 2019; 50: 1033-42.
15. Abdulrazzaq, N. Z., Nihad, K. T. and Ahmed A. M. Expression of IL-1b, IL-12, IL-17a, TNF-a and Fox-P3 in Patients with Low, Medium and High-Hepatitis Viral Load. *Annals of the Romanian Society for Cell Biology*, 2021; 25 (4): 9239–50.
16. Hu, P. and Ren, H. Interpretations of EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *Zhonghua Gan Zang Bing Za Zhi* 2017;25(6)415–8. [Accessed 10th December 2021]. 2017. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28763857>.
17. Rybicka, M., Woziwodzka, A., Sznarkowska, A., Romanowski, T., Stalke, P., Dręczewski, M., Verrier, E. R., Baumert, T. F. and Bielawski, K. P. Genetic variation in IL-10 influences the progression of hepatitis B infection. *International Journal of Infectious Diseases*, 2020; 96: 260-5.
18. Das, S.K., Mukherjee, S., Vasudevan, D. M. and Balakrishnan, V. Comparison of haematological parameters in patients with non-alcoholic fatty liver disease and alcoholic liver disease. *Singapore Medical Journal*, 2011; 52(3): 175-80.
19. Onwuasoanya, U. F., Ihongbe, J.C., Obeagu, E., Ifeanyichukwu, M., Nwachukwu P. E. and Ochiabuto O. Haematological indices of hepatitis, B infected subjects, in Nnamdi Azikiwe, University Teaching Hospital, Nnewi, Anambra State, Nigeria. *Journal of Biomedical Sciences*, 2017; 6(3): 17-24.
20. Fasola F. A., Otegbayo, J. A., Abjah U. M. A. and Ola S.O. Haematological parameters in Nigerians with acute viral hepatitis. *Nigerian Journal of Gastroenterology and Hepatology*, 2009; 1(1): 27 - 31.