

Evaluation of Some Haemostatic Parameters as Co-Markers to Viral Load in the Management of HBV Infection Treatment Outcome in Delta State, Nigeria

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Abstract

Hepatitis B virus (HBV) infection is a viral infection that affects the liver causing acute as well as chronic disease worldwide. Poor diagnosis and prognostic factors remain one of crucial factors responsible for poor management of the disease despite progress in implementing vaccination programmes and development of new treatment perspectives in the management of hepatitis B virus (HBV) infections 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. Thus, this study was aimed at evaluating HBV infection treatment outcome using viral load and changes in haemostatic variables such as activated partial thromboplastin time, prothrombin time, fibrinogen and D-dimer 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. A total of one hundred and fifteen (115) adults aged 22 – 64 years were randomly enlisted in the study. The cross sectional study consist of fifty (50) confirmed hepatitis B negative subjects as negative controls whereas, the follow-up study consist 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 constrain. The blood samples collected in EDTA was used for platelet count using Sysmex® Automated Hematology Analyzer. Fibrinogen, viral load and D-dimer were analyzed using enzyme-linked immunosorbent assay method. Blood was also anticoagulated with 0.109 M trisodium citrate (9: 1 v/v) for the measurement of activated partial thromboplastin time and prothrombin time. The levels of platelet count did not differ significantly ($P > 0.05$) for the various groups whereas, the median levels of HBV viral load, fibrinogen, D-dimer, prothrombin time and APTT in the HBV naïve, three month post treatment and six month post treatment subjects where significantly higher when compared with the control group ($P < 0.05$). Thus, is possible that the distortions in synthesis of coagulation proteins in the liver which are reflected in prolongation of PT, APTT, increased D-dimer and low fibrinogen concentration plays a crucial role in pathogenesis HBV infection as such these parameters could be used as co – markers to viral load in monitoring the treatment outcome of HBV infection in Nigeria.

Keywords: HBV infection • D-dimer • Fibrinogen • Viral load • Treatment outcome

Introduction

Hepatitis B virus (HBV) infection is a viral infection that affects the liver causing acute as well as chronic disease [1]. The production of blood cells is under tight control by a group of haematopoietic cytokines, this cytokines can be affected by the HBV [2]. According to Ahmad EF, et al. [3] there is a significant increase in monocytes which are major players in innate and adaptive immune response such as antigen presentation and cytokine production, although lymphocytes have been shown to have reduced values in chronic stage of HBV infection. Alteration in haematological profile may predict those likely to have haematological complications even after recovery from the acute viral hepatitis [4]. 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 [5,6]. Another possible reason for deranged 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 [4].

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 [6-8]. Several research had showed a significantly raised haemoglobin concentration, absolute leukocytes, neutrophils, lymphocytes, eosinophils as well as monocytosis in HBV infection [6,9,10]. Thus, this study was aimed at evaluating HBV infection treatment outcome using viral load and changes in haemostatic variables such as activated partial thromboplastin time, prothrombin time, fibrinogen and D-dimer of HBV positive treatment naïve, on treatment at 3 months and treatment at 6 months subjects attending gastro-enterology clinic in Federal Medical Centre

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Received 15 January, 2022, Manuscript No. JBABM-22-51698; **Editor Assigned:** 17 January, 2022, PreQC No. P-51698; **Reviewed:** 19 January, 2022, QC No. Q-51698; **Revised:** 28 January, 2022, Manuscript No.R-51698; **Published:** 03 February, 2022, DOI: 10.4172/1948-593X.1000304

Asaba, Delta State, Nigeria. This will add to the existing level of information in Nigeria on HBV treatment outcome, which is beneficial.

Materials and Methods

A total of one hundred and fifteen (115) adults aged 22-64 years participated in this study. The cross sectional study consist of fifty (50) confirmed hepatitis B negative subjects as negative controls whereas, the follow-up study consist sixty-five (65) treatment naïve HBV positive subjects which were followed-up at three and six months on treatment with tenofovir respectively. Four of the participants (two in three months post treatment and two six months post treatment) dropped-out of the research due to time constrain. The blood samples collected in EDTA was used for platelet count using Sysmex® Automated Hematology Analyzer. Fibrinogen, D-dimer and viral load were analyzed using enzyme-linked immunosorbent assay method as previously been described by Ogawa S, et al. [11] and Riley RS et al. [12]. In briefly, fifty micro liters volume of standards or samples was added into their respective wells. A 100 µl volume of the enzyme conjugate was added into each well and mixed for 30 seconds. The wells were covered with the foil supplied with the kit and incubated at room temperature for 45 minutes. The wells were washed five times with a 300 µl volume of wash solution. A 100 µl volume of TMB reagents was added into each well and incubated at room temperature for 20 minutes in the dark with gentle shaking. The reaction was stopped by adding 100 µl volume of stop solution to each well. The intensity of the color produced was directly proportional to the amount of analyte present in the sample (s) and the intensity was measured at 450 nm. Blood was also anticoagulated with 0.109 M trisodium citrate (9:1 v/v) for the measurement of activated partial thromboplastin time and prothrombin time as previously been described by Fasola FA, et al. [4] whereas, the blood samples collected in anticoagulant free vacutainers, subsequently centrifuged at 750 x g for 15 minutes to obtain sera which was used for the evaluation of Hepatitis B surface antigen (HBsAg) using enzyme-linked immunosorbent assay as previously described by Shata MTM, et al. [13]. In briefly, fifty micro liters volume of standards or samples was added into their respective wells. A 100 µl volume of the enzyme conjugate was added into each well and mixed for 30 seconds. The wells were covered with the foil supplied with the kit and incubated at room temperature for 45 minutes. The wells were washed five times with a 300 µl volume of wash solution. A 100 µl volume of TMB reagents was added into each well and incubated at room temperature for 20 minutes in the dark with gentle shaking. The reaction was stopped by adding 100 µl volume of stop solution to each well. The intensity of the color produced was directly proportional to the amount of HBsAg present in the sample(s) and the intensity was measured at 450 nm.

Ethical consideration

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.

Informed consent

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

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.

Exclusion criteria

Subjects with other liver diseases e.g. 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 who tested negative to hepatitis B virus were also excluded.

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 ≤ 0.05 was considered as statistically significant.

Results

Table 1 shows comparison of median levels of platelet count, HBV viral load, fibrinogen, D-dimer, prothrombin time and APTT in the study population. The levels of platelet count did not differ significantly (P >0.05) for the various groups whereas, the median levels of HBV viral load, fibrinogen, D-dimer, prothrombin time and APTT in the HBV naïve, three month post treatment and six month post treatment subjects where significantly higher when compared with the control group (P <0.05). Table 2 shows the correlation of viral load with some clotting variables in treatment naïve HBV, the three month post HBV treatment and six month post HBV treatment. A weak positive correlation at three and six months post HBV treatment between HBV viral load and APTT (r = -0.570, P =0.002) and (r = 0.458, P =0.016) respectively.

Discussion

Despite progress in implementing vaccination programmes and in the

Table 1. Comparison of median levels of platelet count (cells/µl), HBV viral load (copies/mL), fibrinogen (ng/ml), D-dimer (ng/ml), prothrombin time (seconds) and activated partial thromboplastin time (seconds) in the study population.

Participants	Platelet	HBV Viral load	Fibrinogen	D- dimer	PT	APTT
HBV negative control (A) N= 50	234	0.01	66	145	12.5	24
HBV treatment naïve (B) N= 65	227.5	22840	85.5	242.5	20	54.5
Three months post treatment (C) N= 63	262	15720	57	252	19	53.5
Six months post treatment (D) N= 61	205	9800	55	218	17	55
Kriskal-wallis value	8.775	10.477	35.758	80.468	74.288	66.751
p-value	0.032	0.005	0	0	0	0
A vs. B	0.948	0	0.003	0	0	0
A vs. C	0.941	0	0.293	0	0	0
A vs. D	0.48	0	0.013	0	0	0
B vs. C	0.508	0.056	0	0.003	0.238	1
B vs. D	0.948	0.003	0	0	0.009	0.623
C vs. D	0.153	0.07	0.974	0	0.028	0.594

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

Table 2. Correlation of viral load with some clotting variables in treatment naïve HBV positive, 3 months on treatment HBV positive and 6 months on treatment HBV positive groups.

Parameter		correlation	Fibrinogen	D-dimer	PT	PTTK
Treatment Naïve	Viral load	r	0.268	-0.009	0.122	-0.053
		p-value	0.152	0.962	0.52	0.78
3 months	Viral load	r	0.358	-0.041	-0.235	-0.570**
		p-value	0.061	0.834	0.229	0.002
6 months	Viral load	r	0.042	0.301	0.216	0.458*
		p-value	0.836	0.127	0.279	0.016

development of new treatment perspectives, hepatitis B virus (HBV) infections remain a major health problem worldwide, contributing considerably to cirrhosis- and hepatocellular carcinoma (HCC)-related mortality of 0.5–1 million per year [14]. Therefore, we can postulate changes in haemostatic parameters of HBV positive treatment might be related to various HBV infection 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 consist of fifty (50) confirmed hepatitis B negative subjects as negative controls whereas, the follow-up study consist 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 platelet count and red blood cell distribution width did not differ significantly ($P > 0.05$) for the various groups whereas, the median levels of fibrinogen, D-dimer in the HBV naïve, one month post treatment and six month post treatment subjects where significantly lower when compared with the control group ($P < 0.05$). Also, prothrombin time and APTT in the HBV naïve, one month post treatment and six month post treatment subjects where significantly prolonged when compared with the control group ($P < 0.05$). This could be attributed to selective derangement of haemostatic variable which had been associated with HBV infection. It has been reported that some degree of derangements in haemostatic indices occur in HBV infection due to associated liver damage resulting to disorders of the haemostatic mechanism [15]. It is well known that the liver plays a critical role in haemostasis as most of the coagulation factors, anticoagulant proteins and components of the fibrinolytic system are produced by the liver parenchymal cells. Therefore, these functions could be impaired in liver damage due to HBV infection. The onset of abnormal haemostatic mechanism resulting from aberrant liver function may most of the time be for different reasons including impaired coagulation factors synthesis, consumption of coagulation factors, altered clearance of activated coagulation factors as well as quantitative and qualitative platelet disorders [15]. Thus, it can be extrapolated that the distortions in synthesis of coagulation proteins in the liver which are reflected in prolongation of PT, APTT, increased D-dimer and low fibrinogen concentration plays a crucial role in pathogenesis HBV infection. However, the median levels of HBV viral load was significantly higher in the HBV naïve, one month post treatment and six month post treatment subjects when compared with the control group ($P < 0.05$). 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 [5,6]. Another possible reason for deranged 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 [4].

Conclusion

Thus, is possible that the distortions in synthesis of coagulation proteins in the liver which are reflected in prolongation of PT, APTT, increased D-dimer and low fibrinogen concentration plays a crucial role in pathogenesis HBV infection as such these parameters could be used as co-markers to viral load in monitoring the treatment outcome of HBV infection in Nigeria.

Authors' Contributions

SIO, GIA, NCI and AFE participated in the project design, data analysis and manuscript. SIO, AFE, JIE, RCC, OON, SO, AOU and URE performed major experiments. All authors read and approved the final manuscript.

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How to cite this article: Obu, Sylvia I, Grace Ifechukwudelu Amilo, Nancy Chiatogu Ibeh and Alfred Friday Ehiaghe, et al. "Evaluation of Some Haemostatic Parameters as Co-Markers to Viral Load in the Management of HBV Infection Treatment Outcome in Delta State, Nigeria." *J Bioanal Biomed* 14(2022): 304.