Research Article

Coagulation Parameters in Patients Positive for Hepatitis B Virus Infection

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Received: 05-Oct-2022,
Manuscript No. amhsr-22-71390;
Editor assigned: 07-Oct-2022,
Pre QC No. amhsr-22-71390(PQ);
Reviewed: 22-Oct-2022,
QC No. amhsr-22-71390;
Revised: 27-Oct-2022,
Manuscript No: amhsr-22-71390(R);
Published: 03-Nov-2022,
DOI: 10.54608.annalsmedical.2022.67

Abstract

Background: Hepatitis B is a liver infection caused by Hepatitis B Virus (HBV) that is transmissible from person to person through body fluids. The liver plays an important role in blood coagulation (homeostasis) being an important site of synthesis of all coagulation factors. **Objective:** This study was carried out to determine the effects of hepatitis B virus infection on coagulation parameters; Prothrombin Time (PT), International Normalised Ratio (INR), Activated Partial Thromboplastin Time (APTT), fibrinogen, platelet count and D-dimer in HBV positive subjects in Enugu. **Methods:** Subjects (n= 112)comprising 24 asymptomatic HBV positive subjects, 24chronic HBV positive subjects, 24 HBV induced liver disease subjects and 40 apparently healthy non-HBV controls subjects participated in this study. Coagulation parameters were analysed using standard haematological techniques. **Results:** The results revealed significant increase ($p<0.05$) in: PT of asymptomatic HBV positive subjects (12.5±1.7seconds), PT (16.8 ± 3.6 seconds), INR (1.4 ± 0.4), APTT (39.8 ± 6.9 seconds), fibrinogen (145.5 ± 31.1 g/dl), D-dimer (1537.9 ± 802.2 ng/ml)of HBV-induced liver disease subjects and platelets of chronic HBV(315 ± 85x10^9/L)compared to controls. **Conclusion:** The results revealed significant increase ($p<0.05$) in: PT of asymptomatic HBV positive subjects (12.5±1.7seconds), PT (16.8 ± 3.6 seconds), INR (1.4 ± 0.4), APTT (39.8 ± 6.9 seconds), fibrinogen (145.5 ± 31.1 g/dl), D-dimer (1537.9 ± 802.2 ng/ml)of HBV-induced liver disease subjects and platelets of chronic HBV(315 ± 85x10^9/L)compared to controls.

Keywords: Coagulation parameters; Hepatitis B virus; Liver; Liver damage; Homeostasis

Introduction

Hepatitis B Virus (HBV) causes inflammation of the liver which in most cases progresses to cirrhosis and hepatocellular carcinoma in chronic conditions. HBV is common in the underdeveloped world with no cure yet but supportive treatment to manage the conditions and improve prognosis. [1]However, this infection which has caused so many deaths world wide can be prevented through hepatitis B vaccine and the World Health Organization (WHO) hope to eradicate this infection by 2030. [1,2]Hepatitis B infection is endemic in Nigeria, common in the rural areas and can be contracted by contact with infected body fluids during sexual intercourse, contaminated syringes, contaminated sharp instruments and transmitted to babies during delivery[3] .

Coagulation factors are one of the metabolites of the liver, an organ with high metabolic benefits to the body systems. The liver synthesizes, breaks down and detoxifies poisonous substances before they are cleared off from the system to avoid harming the body organs. A breakdown of this organ by infectious viruses like hepatitis B virus poses a life-threatening effect to the body. The synthesis of clotting factors is one of the functions of the liver. The liver releases the clotting factors into the circulation where they function to prevent bleeding from injured blood vessels. Any abnormality in coagulation factors will either cause bleeding disorder or thrombosis (abnormal clot formation). When the blood vessel is injured, the body develops a mechanism to prevent blood loss by forming platelet plug. This plug is stabilized by the clotting factors which become activated in a cascade of reactions to form the fibrin clot to stabilize the platelet plug in a physiologic condition. The body also form clots in pathologic condition which is life threatening. The chronic nature of HBV infection causes damage to the liver due to intra-hepatic inflammatory processes triggered by HBV specific CD8+ T-cells which affects synthesis of clotting factors, thus therapeutic target to limit the response of dysfunctional T cells will improve prognosis. [4]Chronic hepatitis B infection progresses to liver cirrhosis due to chronic inflammation and pro-inflammatory cytokines and IL-18 triggers apoptosis which results in liver damage. Acute liver failure due to increase apoptosis of the hepatocytes can be effectively treated with mesenchymal therapy. [5]Chronic hepatitis B even after treatment still develops hepatocellular carcinoma which is the most common type of primary liver cancer due to cell – free DNA. [6] Hepatitis B viral infection has been established by scientific research to affect treatment for other health conditions like hemophilia. Treatment for hepatitis B depends on the duration of infection. Acute hepatitis B infection does not usually need any specific treatment but may require treatment to relieve the symptoms while chronic hepatitis B infection can

How to Cite this Article: Onyekwelu K. A Case Report of Ds DNA Negative with Ana Positive SLE Induced Neuropsychosis Diagnosis Dilemma. Ann Med Health Sci Res. 2022;12: 336-343.

be treated with antiviral drugs. Prevention of Hepatitis B can be through immunization, bio-safety practice and living a healthy life style[1].

Hepatitis B virus like other viral hepatitis affects the liver, an organ with high metabolic activities and reduces the synthesis of coagulation factors resulting to prolonged clotting time [7,8], hypo fibrinogenemia. [9, 10] Hepatitis B viral infection has no permanent cure yet but relies on supportive care in the management of the disease. Understanding the effects of hepatitis B on coagulation can enhance supportive treatment and improve prognosis. This study aims at determining how HBV infection affects the coagulation system and to reconfirm results of previous research. The findings from this research will add to medical knowledge, enhance supportive treatment with respect to associated coagulopathies and improve prognosis among hepatitis B positive patients.

Methods

Area of study

The study was done at the University of Nigeria Teaching HospitalItuku/Ozalla, Enugu and Department of Medical Laboratory Sciences, College of Medicine, University of Nigeria Enugu Campus, Nigeria.

Subjects

The subjects comprise of HBV infected patients seeking medical attention at University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu. Informed consent of one hundred and twelve subjects was obtained which comprised of: 24 asymptomatic HBV positive subjects, 24chronic HBV positive subjects, 24 HBV positive subjects with HBV induced liver disease and 40 apparently healthy non-HBV control subjects.

Hepatitis B serologic test and recruitment of subjects

Hepatitis B serologic testing involves measurement of several Hepatitis B virus (HBV)-specific antigens and antibodies in which different serologic markers or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection. These antigens and antibodies include: Hepatitis B Surface Antigen (HBsAg), total Hepatitis B Core Antibody (anti-HBc) and IgM antibody to hepatitis B core antigen (IgM anti-HBc)

Hepatitis B Surface Antigen (HBsAg), a protein found on the surface of hepatitis B virus and total Hepatitis B Core Antibody (anti-HBc) are detected in high levels in serum during acute or chronic hepatitis B virus. Positivity of IgM antibody to Hepatitis B Core Antigen (IgM anti-HBc) indicates recent (acute) infection with hepatitis B virus while negativity of IgM antibody to Hepatitis B Core Antigen (IgM anti-HBc) indicates chronic infection with hepatitis B virus. Subjects that tested positive to HBsAg and anti-HBc; positive or negative to IgM anti-HBc were recruited for this study.

Inclusion criteria

Patients that tested positive to hepatitis B virus, not suffering from any other viral hepatitis or chronic disease like, diabetes, hypertension, or tuberculosis who gave their informed consent were recruited for this study.

Exclusion criteria

Pregnant women, HIV positive patients and other viral hepatitis patients or those with co-infection as well as those with other health conditions including, fatty-liver disease, diabetics, alcoholics, hypertension and those that did not give their informed consents were excluded from the study.

Ethics

The procedures followed in this study were in accordance with the ethical standards of the ethics committee of University of Nigeria teaching hospital, Ituku/Ozalla, Enugu on human experimentation (approval number: NHREC/05/01/2008B-FWA00002458-IRB00002323) and also in accordance with the Helsinki declaration of 1975, as revised in 2013. Oral or written informed consent of the subjects was obtained before the administration of questionnaires and collection of blood sample.

Sample collection

Blood sample (6.0 ml) was collected from each subject under aseptic conditions and 4.0 ml was transferred into tri-sodium citrate container for coagulation studies, 2.0 ml into ethylene -diamine-tetra-acetic acid container for platelet enumeration and D-dimer estimation.

Sample assay

Quantitative in-vitro analysis of fibrinogen level and qualitative analysis for PT and APTT was done using semi- auto coagulation analyser. Platelet was estimated using five part haematology analyser, Mindray BC5000. A predetermined volume of blood was aspirated, diluted at specific ratios with ammonium oxalate solution inside the machine. The diluted blood cells are channelled through traducers with small holes. The suspended blood cells pass through apertures, causing direct current resistance to change between the electrodes. Blood cell sizes are detected as electric pulses and blood cells are counted by counting the pulses and the count of each cell type displayed.

D-dimer was measured using Selex on Multiple Bio-Marker analyzer which uses test cassettes. Monoclonal antibodies which bind to a specific epitope on the D-dimer are designed and coats the test region. Antigen specific to the D-dimer present in the sample binds to the corresponding antibody on the test cassette. The labelled antigen-antibody complex is detected as red on the detection zone. 100 ul of blood was applied on the test region and allowed for 10 minutes, red cells were removed, the plasma passes through the detection zone with the appearance of a red line, and its intensity is proportional to the concentration of D-dimer.

Statistical analysis

Data were subjected to descriptive and inferential statistics and analyzed using student's t-test and one-way analysis of variance at 95% confidence interval.Probability value less than 0.05 was considered statistically significant.

Results

Characteristics of study population

Out of 112 subjects who completed the questionnaires and participated in the study, males were 85 (75.9%) and females were 27 (24.1%). The mean age for males was 37 ± 10 years while the mean age for females was 27 ± 7 years.

Coagulation parameters among asymptomatic Hepatitis B positive subjects

The result of asymptomatic hepatitis B virus positive subjects is shown in Table 1.There was a statistically significant ($p<0.05$) increase in prothrombin time (12.5 ± 1.7 sec) when compared to the control (10.9 ± 0.8 sec).

Coagulation parameters among chronic Hepatitis B positive subjects

The result of subjects with chronic hepatitis B virus infection showed a statistically significant ($p<0.05$) increase in platelet count (315 ± 85) when compared to control (247 ± 53)(Table 2).

Coagulation parameters among subjects with Hepatitis B virus induced liver damage

In table 3, the result of subjects with hepatitis B virus induced liver damage showed a significant ($p<0.05$) increase in prothrombin time (13.5 ± 3.9sec), INR (1.1 ± 0.3), APTT (31.3 ± 8.4secs), Fibrinogen (213.5 ± 59.6 g/dl) and D-dimer (607.1 ± 811.7(ng/ml) when compared to the control (10.9 ±0.8 sec), (0.8 ± 0.1), (27.6 ± 4.4sec), (278.4 ± 55.4g/dl) and (94 ± 4.4ng/ml) respectively.

Discussion

The liver parenchyma cells play an important role in the synthesis of proteins including proteins of the coagulation system (coagulation factors), fibrinolytic systems (plasminogen) as well as anticoagulants, however, once the liver is diseased, its synthetic function is impaired which also causes some changes in hemostatic function. This study on the effects of hepatitis B virus infection on coagulation system was designed to assess changes associated with some coagulation parameters following hepatitis B virus infection.

From the results of this study, it was observed that hepatitis B virus infection caused significant changes in coagulation parameters (platelets, PT, APTT, Fibrinogen and D-dimer) and these tally with the claims of other researchers that hepatitis B infection has a statistically significant change in APTT, prothrombin time, fibrinogen and platelets[7].

The significant increase in PT in the asymptomatic subjects could be explained as increased expression of tissue factor on immune cells (monocytes and neutrophils) and platelets following hepatitis B virus infection. Tissue factor activates factor VII, initiating extrinsic coagulation cascade resulting in fibrin clot formation to protect the body with subsequent decrease in factor VII. [10] There was also a significant increase in platelets in chronic hepatitis B positive subjects. This increase could be attributed to the fact that platelets are acute-phase reactants which increase in response to various stimuli including chronic inflammation due to infection of the liver by HBV. [11] In the hepatitis B induced liver disease subjects, there was a statistically significant increase in prothrombin time, INR, APTT, D-dimer and a significant decrease in fibrinogen. The increase in prothrombin time could be as a result of decreased synthesis of prothrombin due to decrease in the functionality of the hepatic cells as a result of cirrhosis. In cirrhosis, the liver cells are replaced by scar tissue which block the flow of nutrients and hormones within the liver cell resulting in cell death and organ failure. [12] Research also reports that the increase expression of tissue factors on monocytes, neutrophils, platelets and endothelial cells during infection triggering coagulation, consumes the clotting factors resulting in prolonged prothrombin time[13].

There was a statistically significant decrease in fibrinogen and this could be attributed to liver damage. Viral infections trigger the production of tumor necrosis factor which causes a significant damage to the liver. [14]The diseased liver remains unable to synthesis clotting. D-dimer is shown to be significantly increased and could be attributed to increase intravascular coagulation as a result of chronic inflammation by Hepatitis B virus infection. High levels of D-dimer are suggestive of increase thrombolytic activity. [15] High D-dimer levels have been registered as poor prognosis in cancer patients including hepato cellular carcinoma and D-dimer levels of >1500ng/ml is associated with increased mortality in COVID 19 patients[16, 17].

Table 1: Coagulation parameters among chronic HBV positive and control subjects, *p < 0.05 (Significant).

	Mean ± SD of control subjects	Mean ± SD of asymptomatic HBV positive subjects
Platelets (X 109/L)	247 ± 53	232 ± 67
Prothrombin (seconds)	10.9 ± 0.8	12.5 ± 1.7*
INR	0.8 ± 0.1	0.9 ± 0.1
APTT (seconds)	27.6 ± 4.4	28.4 ± 5.7
Fibrinogen (g/dl)	278.4 ± 55.4	246.1 ± 46.5
D-dimer (ng/ml)	94 ± 4.4	110.8 ± 101.2

Table 2: Coagulation parameters among chronic HBV positive and control subjects, *p < 0.05 (Significant).

	Mean ± SD of control subjects	Mean ± SD of chronic HBV positive subjects
Platelets(X 109/L)	247 ± 53	315 ± 85*
Prothrombin (seconds)	10.9 ± 0.8	11.2 ± 1.8
INR	0.8 ± 0.1	0.9 ± 0.2
APTT (seconds)	27.6 ± 4.4	25.8 ± 5.0
Fibrinogen (g/dl)	278.4 ± 55.4	248.9 ± 24.7
D-dimer (ng/ml)	94 ± 4.4	172.6 ± 149

Conclusion

This study concludes that hepatitis B virus causes significant changes in coagulation parameters (platelets, PT, APTT, Fibrinogen, and D-dimer) of hepatitis B virus infected patents. The coagulation alterations are pronounced in hepatitis B virus induced liver damage and the severity corresponds to the degree of liver damage. Therefore, therapeutic measures geared towards maintaining the functionality of the coagulation system will enhance supportive care for Hepatitis B virus patients with chronic infection.

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