Original Research Article

Coagulation parameters among individuals with Hepatitis B infections in Okada, Edo state , Nigeria

ABSTRACT

The liver plays a pivotal role in hemostasis and quantitative abnormalities of coagulation factors are known to occur in a diseased condition. The study was carried out to determine variations in platelet count, Activated Prothrombin time and prothrombin time.

Platelet count, APTT and PT were carried out on 60 known seropositive individuals for Hepatitis B virus using routine procedures for coagulation profile using the hematology laboratory laboratory, Igbinedion University Teaching Hospital, Okada within the period of July to September 2022.Seronegative individuals for Hepatitis B were used as controls. The tested individuals were in various disease progression and duration for the liver disease. There was marked thrombocytopenia in the infected individual with a mean platelet of 110.88 and statistically significant ($p < 0.001$), conversely, there was an increased prothrombin time as well as Activated partial prothrombin time with both having p- values < 0.001. The average PT and APTT were 25.53 and 48.89 respectively. There was marked changes in the coagulation cascade for individuals with Hepatitis B infection which could lead to bleeding or clotting disorders, an indication for loss of interity in the coagulation pathways. Individuals with Hepatitis B infection are thus liable to thrombocytopenia if not well managed.

Introduction

Hepatitis B virus (HBV) is one of the key etiological agents for liver diseases, including chronic hepatitis, liver cirrhosis and liver cancer [1]. It is the second commonest human carcinogen after tobacco [2], the virus is highly contagious and extremely resilience to environmental conditions[3]. The liver plays a pivotal role in the hemostatic system as it provides the framework for the coagulation factors and proteins involved in fibrinolysis[4]. Consequently, chronic or acute liver diseases frequently have a profound effect on the hemostatic system [5] Routine laboratory investigation for coagulation profile such as the platelet count, prothrombin time (PT), and the activated partial thromboplastin time (APTT) are frequently abnormal in patients with liver disease [6] .The combination of thrombocytopenia in association with prolonged PT and APTT is suggestive of a bleeding diathesis and is traditionally assumed that patients with liver disease are at a risk for bleeding as a results of these changes in the hemostatic framework.

In apparently normal healthy individuals, the hemostatic framework is in a fragile harmony between excessive bleeding state and clotting. Notwithstanding, irregularities in the framework cause either hemorrhagic or coagulating disorder [7]. There are various elements that influence the ordinary hemostatic framework, of which HBV disease is known to have been one of the primary drivers of hemostatic anomaly [8]. HBV contamination causes serious haemostatic difficulty particularly in the late phase of HBV , as resistant concealment, and the presence of simultaneous contamination or neoplastic sicknesses compounds the condition [9].

Coagulation irregularities in HBV patients can be credited from the impact of the infection which can cause various anomalies that incline the patients toward the events of coagulation issue. The

aim of this study was to evaluate the coagulation parameters such as Activated Partial Thromboplastin Time (APTT) and platelet count among individuals with Hepatitis B infection in Okada.

MATERIALS AND METHODS

Study area

The study was carried out at Igbinedion University Teaching Hospital, Okada town, Edo state, Nigeria to observe the changes in PT and APTT among people with hepatitis B infections.

Study subjects

The individuals being scrutinized are apparently healthy carriers and seropositive to Hepatitis B virus.

Study design

This is a cross-sectional intended to survey the quantitative values of APTT, PT and platelets using seropositive Hepatitis B and Hepatitis B seronegative as control

Sample size

A total of 60 subjects took part in this study utilizing the equation

N=(Z^2 PQ)/D^2

Where n is the base example size

Z standard deviation which is normally 1.96 which relates to 95% certainty level

D represents level of accuracy (taken as 0.05)

P commonness level of 0.5%

Q is elective extent (1-p) which is 1-0.5=0.5

Sample collection

8ml of venous blood test was collected and 5.0 ml of it was administered into a 0.5 ml of 3.2% trisodium citrate test bottle and spun to obtain plasma to be used for PT and APTT investigation, while the leftover 3.0 ml was apportioned into ethylenediaminetetraacetic for platelet count

Assay

Prothrombin Time Assay

PT was tested or analyzed involving the manual strategy as follows. The expected volume of PT reagent to be utilized was eliminated from the vial and brooded for 10 min at 37℃. Hundred microliters of the test plasma was added into a plastic corvette and incubated at 37℃ for 3 min. 200 microliters of the pre incubated PT reagent was quickly added and the clock was begun. The time taken for cluster to shape was recorded.

Activated Partial Thromboplastin Time (Modified with Kaolin) Assay

The anti- coagulated blood was centrifuged at 2500RPM for 15 minutes and the test procedure was done within two hours at room temperature. Plasma samples were stored frozen at -20℃ for up to a month, for tests not completed same day [10]

Procedure:

The sample mixture was brought to 37℃ and placed in test tubes. 100µl of plasma citrate was added, and 100µl of reagent added. Mixture was incubated for 3-5 minutes at 37℃ .

100µl of calcium chloride was added and the time for clot formation was recorded by the coaglometer

Platelet Count Assay

This was assayed using the Mythic 18 auto analyzer.

Data analysis

The data obtained from the research was entered into Microsoft succeed. Measurable investigation was conveyed with Chi-square utilizing SPSS (Statistical Package for Social Sciences), Frequency dispersion, Bivariate Correlation (Pearson and Spearman's rho Correlation Coefficients). Results were communicated as mean ± standard deviation, and correlations among gatherings and among bunches were broke down utilizing the autonomous t-test and the examination of difference, individually. The degree of importance was set at $P < 0.05$.

RESULTS AND DICSUSSIONS

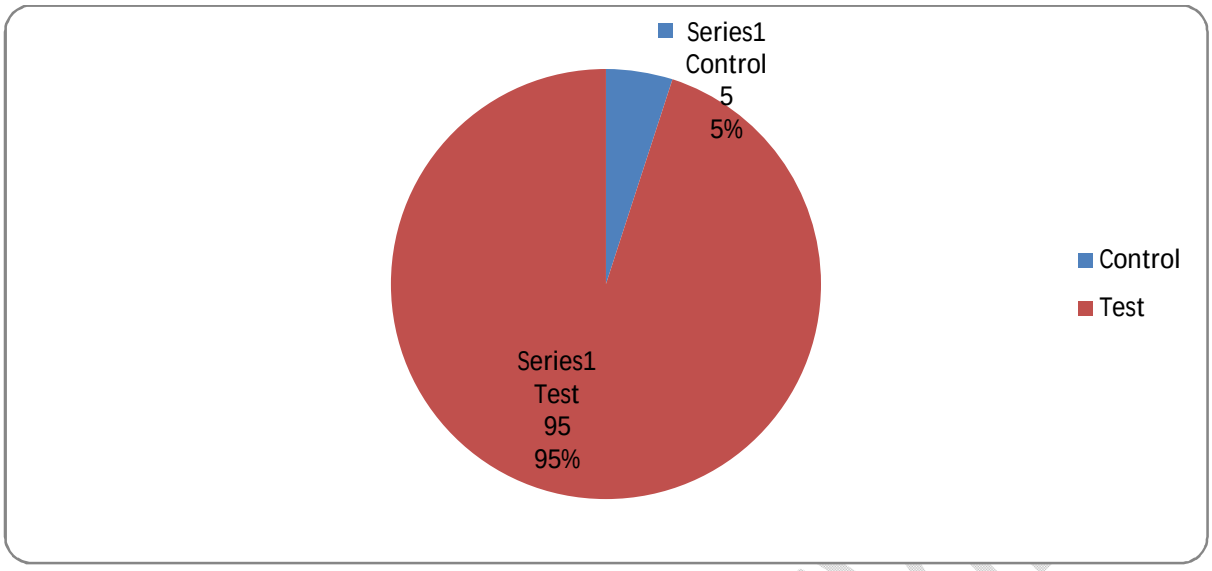

Figure 1: Percentage distribution of the subject

Table 1: Mean comparison of the variables between groups

Variables	Control	Test	t-test	p-value
PT	13±1.00	23.53±3.87	4.67	<0.001
PTTK	37.00±4.36	48.89±4.54	4.43	<0.001
INR	1.05±0.08	2.30±0.56	3.85	<0.001
PLT	282.33±33.01	110.88±26.53	10.81	<0.001

The average PT, PTTK INR and PLT of the HBV subject (Test) were 23.53, 48.89, 2.30, and 110.88 respectively with exception of the PLT, the values of theses variable were lesser in the control subject. The mean comparison by t-test showed that PT, PTTK and INR level of the HBV patient was statistically significantly higher than that of the control group ($p<0.05$). The mean PLT was significantly higher ($p<0.05$) in the control group than the test.

Table 2: Logistics regression analysis

Variables	B	S.E.	p-value	Odd Ratio
PT	3.43	1007.94	0.99	31.006
PTTK	0.53	968.04	1	1.693
PLT	-0.13	87.42	0.99	0.881
Constant	-50.36	46245.429	0.99	0

Multivariate regression analysis was done to know the predictor that significantly associated with HBV status while other variables are held constant. All the independent variables were enter into the model except INR because it has nearly a perfect correlation with PT and PTTK to avoid multicolinearity. The model significantly predict HBV status $\chi^2(3) = 23.8$, $p<.001$. The model account for 32.8% (cox&snell R^2) of the variance in the HBV status. No variable was significantly independently associated with HBV status with other variable held constant.

Table 3: The Correlation of the variables in the control and the Test

Status	Variables	PT	PTTK	INR	PLT
Control	PT	1			
	PTTK	-0.803	1		
	INR	0.866	-0.993	1	
	PLT	-0.515	0.924	-0.875	
Test	PT	1			
	PTTK	.418**	1		
	INR	.969**	.375**	1	
	PLT	-0.07	-.397**	-0.075	1

The table showed the output of the Pearson correlation coefficient (r). The association of the variables with each other in the control group was not significant. PT showed a very strong correlation with PTTK and INR. PTTK showed very strong to near perfect correlation with INR and PLT but not significant. This outcome may have resulted from a very small sample size. In the Test group PT showed statistically significant positive moderate and very strong correlation with PTTK and INR respectively. PTTK showed statistically significant positive and negative moderate correlation with INR and PLT respectively.

Hepatitis B virus remains a major health problem worldwide, contributing considerably to cirrhosis and hepatocellular carcinoma- related mortality of 0.5 -1million per year [(11)]. It is well known that the liver plays a critical role in hemostasis as most of the coagulation factors;

anticoagulant and components of the fibrinolytic system are synthesized by the liver parenchymal cells. Therefore, these liver functions can be impaired as a result of HBV infection.

The research findings showed that there is a marked thrombocytopenia (p- value < 0.001). This was in line with work done by [12,13,14,15], this reduced platelets count could be as a result of the disease progression, the individuals in this case are chronic hepatitis B seropositive carriers and also as a result of impaired hepatic synthesis of thrombopoietin which is the principal physiological regulator of platelet production [16]. Nevertheless, the INR (International normalized ratio) value obtained were high, p- value < 0.001, an indication that the subjects are at risk for bleeding or clotting disorder.

Also there exists a statistical change in prothrombin test and Activated partial thromboplastin (APTT using modified Kaolin). According to Yang-Mei et al., 2008, infection of the liver by virus especially those not self- limiting such as Hepatitis B causes virus induced tumor necrosis factor production which mediates a significant liver pathology. These changes can therefore be explained on the basis of the state of the diseased liver which is saddled with the responsibility of clotting factor synthesis [18] and most likely loss of hepatic function following HBV infection creating hepatic inflammation as a result of hepatitis B virus X protein which is pro-inflammatory cytokine mediating a Fas- mediated cell apoptosis [19].

Conclusion

Viral hepatitis B can be deduced to cause alterations in the coagulation factors as seen in this study. Hence, coagulation process backing, checking and evaluation ought to be important for routine operation in the management of patients with Hepatitis B infection.

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