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EVALUATION OF ACUTE PHASE PROTEINS (ALPHA-FETOPROTEIN, ERYTHROCYTE, SEDIMENTATION RATE AND LEUCOCYTES COUNTS IN VIRAL HEPATITIS PATIENTS IN ABA

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ABSTRACT

This research focuses on the acute phase proteins (alpha-fetoprotein, erythrocyte, sedimentation rate and leucocytes counts in viral hepatitis patients in Aba. 100 subjects (male and female) were used for the subjects HBV, HCV 70 hepatitis subjects and 30 non hepatitis subjects used as the control. The level of alpha fetoprotein was determined using microplate Enzymes Immunoassay method and some Hematological parameters; the Alpha-fetoprotein (6.42 ± 0.12), erythrocyte sedimentation rate (16.8 ± 0.88) and Leucocyte count (9.87 ± 0.33) were increased in viral hepatitis were significantly increased when compared with the control of (4.00 ± 0.15) respectively ($P < 0.05$). ESR and WBC count was increase in viral hepatitis patient when compared with the control the use of alpha-fetoprotein, Erythrocyte sedimentation rate and Leucocytes count could be an important diagnosis and evaluation, and therapeutics management of viral hepatitis patients.

Keywords: *alpha-fetoprotein, erythrocyte, sedimentation rate, leucocytes counts, viral hepatitis patients*

INTRODUCTION

Acute phase proteins have been well recognised for their application to human medicine and have been described to have value in the diagnosis and prognosis of various inflammatory and organ diseases, organ transplant, and cancer treatment. Erythrocytes sedimentation rate (ESR) which is one of the oldest laboratory test in clinical medicine is a broad-spectrum non-specific indicator of disease. Its usefulness is the monitor of disease progression (Isbister and Harmening 1988). It has been reported that ESR of 30mm per hour or more may be present over the age of 60years without any obvious cause (Miller et al., 1983). It is raised in a wide range of infections, inflammatory reactions, degenerative changes and malignant conditions associated with changes in plasma proteins particularly increases in fibrinogen, immunoglobulin's and C-reactive proteins (Obeagu *et al.*, 2017; Franscica *et al.*, 2017; Okara *et al.*, 2017; Obeagu, 2018)

On the other hand, the white blood cell count determines the number of each type of white blood cell, present in the blood. It can be expressed as a percentage (relative numbers of each type of WBC in relationship to the total WBC) or as an absolute value (percentage x total WBC) of these, the absolute value is much more important than the relative value.

The purpose of this study is to determine the effects of some acute phase proteins (alpha fetoprotein, ESR and leucocyte count in viral hepatitis patients.

MATERIALS AND METHODS

STUDY AREA

The study was done at Abia State University teaching hospital Aba.

STUDY POPULATION

A total of 100 subjects were used for this study. This comprised of 70 viral Hepatitis patients and 30 non hepatitis patients.

Ethical Approval/Informed Concept

Permissions were dully obtained from the ethical committee of Abia State University Teaching Hospital Aba.

Sample Size

100 samples of viral hepatitis patients were used for this study

Collection of Samples

5mls syringe filled with standard gauge disposable needle was used to withdraw 4mls of blood from the subjects. 2mls were dispensed into a dry plain tube and allowed to clot. 2mls were dispensed with an EDTA container. The clotted blood samples in the plain tubes were Centrifuged and preserved in Abia State University Teaching Hospital where the analysis was done.

Determination of Erythrocyte Sedimentation Rate (ESR) by Westergreen Method (Miller *et al.*, 200).

Procedure

Anticoagulated blood was placed in an upright tube and allowed to stand for one hour. After one hour, it was read macroscopically where the red cell meets the plasma and reported in mmlhr.

Determination of Leucocytes Count by Neubaur Counting Chamber Method (Bregman *et al.*, 2008)

Procedure

0.38mls of turks solution (diluent) was pipeted into a dry test tube

0.20mls of blood was added into the test tube, it was mixed properly, the neubaur chamber was charged by placing it into a moist petri dish and a drop of blood was added to the counting area. It was allowed to settle and counted schematically from the upper left small square of the square. The count was repeated in all the four comers of the chamber and the reading taken.

Determination of Alpha-Fetoprotein (Mizejewski *et al.*, 2001).**Procedure**

Format the microplates wells for each serum reference control, replace any unused micowell strip back into the aluminum bag, seal and store at 2-8°C

2. Pipette 0.025ml (25 μ l) of the appropriate serum reference control or specimen into the assigned well.
3. Add 0.100ml (100 μ l) of the anti AFP enzyme reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350 μ l) of wash buffer, decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used.
8. Add 0.100ml (100 μ l) of working substrate solution to all the wells
9. Incubate at room temperature for (15) minutes.
10. Add 0.05ml (50 μ l) f stop solution to each well mix gently for 15-20 seconds.
11. Read the absorbance in each well at 450nm.

Determination of PCV by Haematoant Method (Williams *et al*; 2009)**Procedure**

Plain capillary tube was properly filled with blood up to $\frac{3}{4}$ of the tube. Seated with plasticine and centrifuged for 5 minutes at 10,000vpm.

It was properly measured using the haematocut reader and results expressed as the percentage of the whole blood volume.

Determination of Differential Count by Longitudinal Method (Bregmanetal; 2008)

Procedure

This blood film was prepared using clean grease free slued.

Slides were labeled with the patient name with grease pencil. It was allowed to air dry adequately and placed on a staining rack. It was flooded with leishman stain and allowed to act for 2 minutes. Double diluted with buffer distil water PH 6.8 and allow to act for 8 minutes. The slide was washed off. The back of the slide cleaned and allowed to air dry. It was viewed under the microscope using low power for overall impression and using general appearance of blood cells. Then with high power tens with a drop of immersion oil and with x100 objective tens. Differential white blood cell was counted and noted.

A total of 100 white were counted and reported.

Statistical Analysis

Data were entered into Microsoft Excel 2010 (Microsoft corporations Inc. USA) and transported to the software package SPSS for window version 20.0 for analysis. Differences between the group means were compared using the student's t-test as well as standard error of the mean for analysis of variances (ANOV A) Statistical significant was set at $P < 0.05$.

RESULTS

Table 1: The mean value of Alpha-Fetoprotein level in viral hepatitis patients

Parameter	Reference range	Control (n=30)	Hepatitis patients n=70	P value
Alpha-Feto Protein	<8.5ng/ml	4.00 ± 0.12	6.42 ± 0.15	P<0.05

The result showed that there is significant difference in Alpha-Fetoprotein (6.42 ± 0.15) compared to the non-patients (4.00 ± 0.12) in the viral hepatitis patients.

Table 2; comparison of mean value of Erythrocytes sediments rate and leucocytes count

Parameters	Control N=30	Hepatitis patients N=70
ESR mm/hr	5.70 ± 0.19	16.8 ± 0.88
WBC x $10^9/1$	4.81 ± 0.29	9.87 ± 0.33
Neu x $10^9/1$	45.72 ± 0.64	35.01 ± 0.12
Lymp x $10^9/1$	41.94 ± 0.73	47.63 ± 0.15
Eosino x $10^9/1$	1.94 ± 0.65	2.55 ± 0.25
Monox $10^9/1$	1.80 ± 0.03	2.76 ± 0.50

Key:

- WBC - White Blood Cell
- PCW - Packed Cell Volume
- ESR - Erythrocytes Sedimentation rate
- NED - Neutrophil
- LMY - Lymphocyte
- EOSIN- Eosinophil
- MONO- Monocyte

Table 2 showed a comparison of ESR, PCV and Leucocytes count of viral hepatitis patients with the non-hepatitis patients. The result showed that there is significant difference in ESR (16.8 ± 0.88) and (5.70 ± 0.19) compared to nonhepatitis patients. Leucocytes count (9.87 ± 0.33) and (4.81 ± 0.29) and were (2.86 ± 3.41) and (3.51 ± 0.33) high compared to the non-hepatitis patients. Similarly, the lymphocytes, monocytes and eosinophils level (47.63 ± 0.15), (2.55 ± 0.25) and (2.76 ± 0.50) were higher in viral hepatitis patients compared to the non-

hepatitis patients (41.94 ± 0.73), (1.80 ± 0.03) and (2.55 ± 0.25) respectively. Neutrophils which is lower in viral hepatitis patients compared to the non-viral hepatitis patients (35.01 ± 0.12) and (45.72 ± 0.64).

DISCUSSION

The study exposes the analysis of some acute phase proteins (Alpha-Fetoprotein, ESR and Leucocytes count) in viral hepatitis patients. Several studies have been conducted to demonstrate the elevation of serum AFP level in a patient with hepatitis B (Mansi, 2006) one of the study showed that the major cause of AFP elevation in patients with Chronic hepatitis B is an exacerbation of disease. AFP elevation occurred shortly after the onset of an exacerbation in the underlying chronic hepatitis.

Chronic inflammation is found in the liver related, disorder such as neonatal hepatitis (NH), hepatitis C. virus (HCV) and Hepatitis B virus (HBV) often associated with hepatic Cirrhosis. AFP has been implicated in all forms of chronic hepatitis. HCV is major cause of acute and chronic liver disease. Several studies reported the prevalence of viral chronic liver disease (CLD). Erythrocytes sedimentation rate (ESR) is the rate which red blood cell sediment in a period of one hour and is a non-specific measure of inflammation in the body. However, another study was done to find the relationship between ESR and all types of hepatitis and the finding shows that the high ESR were more frequent in HBV than in other types of hepatitis (Kassa, 1997). Erythrocyte Sedimentation Rate red to the control. Viral hepatitis joins the conditions such as liver Cirrhosis, inflammatory CA and conditions in which leucocytes count appears to be high. These results are consistent with the theory that inflammation has a role in etiology of viral hepatitis. However, the erythrocyte sedimentation rate was significantly associated with viral hepatitis (Adler and Green, 2005). Our study also showed that the peripheral total WBC, monocyte and lymphocytes count were high in viral hepatitis count were stronger predictors of incident control. Similar finding was reported that the peripheral blood monocytes in chronic HBV infection proliferate and increase in number (*Macaulay et al.*, 2011). It is now clear that there is considerable and complex cross talk and collaboration between cells of both these systems, both in acute and chronic disease both these systems are implicated in the chronic inflammatory syndrome in viral hepatitis (*Akarsu et al.*, 2008) Since inflammation

plays a role in development of viral hepatitis, understanding its role is important in the development of prevention strategies for viral hepatitis.

CONCLUSION

In conclusion this study suggests that using micro-plate enzyme immunoassay method in monitoring the AFP in patient with HBV and Haematological parameters e.g ESR and Leucocytes count is also necessary because it can help in predict potential transition of viral hepatitis, administering anti-inflammatory medication in addition to lifestyle, changes may bolster efforts in preventing viral hepatitis disease.

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