Nig. J. Physiol. Sci. 26(June 2011) 109 – 112
www.njps.physocnigeria.org

Nig. J. Physiol. Sci.

Comparative study of C-Reactive Protein and other biochemical parameters in patients with hepatitis B and malaria in Calabar, Nigeria

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Summary: Serum levels of C-reactive proteins (CRP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total protein, albumin and globulins were investigated using high sensitivity Immunoturbidometric and colorimetric techniques in individuals with hepatitis (n=50), Malaria (n=50) and 40 control subjects in age range of 30 to 65 years. The hepatitis patients had a significantly higher ($P < 0.01$) level of aminotransferases when compared to malaria patients and control subjects. The mean value of ALT was 103.50 ± 71.4 IU/L and 46.72 ±17.48 IU/L for hepatitis and malaria respectively. The values for AST were 116.76 ± 63.27 IU/L and 57.74 IU/L ± 15.18 IU/L for hepatitis and malaria respectively while the values for control were 34.75 ± 14.64 and 35.25 ± 15.56 IU/L for AST and ALT respectively. The malaria patients showed a significantly higher level ($P < 0.01$) of aminotransferases when compared to the control. The mean serum CRP levels were 0.71 ± 0.11 mg/dL and 0.78 ± 0.13 mg/dL for hepatitis and malaria respectively. These values were significantly higher ($P < 0.01$) than those of the controls which was 0.32 ± 0.12 mg/dL. The values of CRP in malaria were significantly higher ($P< 0.05$) when compared with hepatitis. In malaria, AST correlated with CRP ($r = 0.58$). The mean serum proteins of hepatitis patients were significantly lower ($P < 0.05$) than those of the control and malaria while there were no significant differences between the total protein in malaria when compared with control. Albumin levels in both patients were significantly lower ($P > 0.05$) than those of the controls. The mean values were 33.40 ± 3.40g/L and 34.47 ± 3.56g/L for hepatitis and malaria respectively and 37.00 ± 3.43 g/L for the control. C-reactive protein correlated negatively with albumin in malaria ($r = -0.26$) while albumin had a negative correlation with globulin($r = -0.36$). Also albumin-globulin ratio were significantly ($P < 0.05$) decreased in both patients when compared with controls. This result suggests that a systemic acute phase response is present in hepatitis and malaria patients hence measurement of C-reactive proteins may be helpful in the diagnosis and management of hepatitis and malaria; especially in the malaria endemic region such as Nigeria.

Keywords*:* Hepatitis B, Malaria, C-reactive protein, Liver function tests.

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Manuscript Accepted: June, 2011

INTRODUCTION

C-reactive protein (CRP) is acute phase protein that is involved in the activation of complement, acceleration of phagocytosis and detoxification of substances released from damaged tissue. As such, C-reactive protein is considered to be one of the most sensitive indicators of inflammation (Schultz and Anorld, 1990). During bacterial and parasitic infections some plasma proteins increase many folds than their normal concentrations. Due to this increased response by those proteins they are often called “acute phase proteins”(Kushner,1993) . An acute phase proteins has been defined as one whose plasma concentration increases (positive acute phase proteins) or decreases (negative acute phase proteins) by at least 2 percent during inflammatory disorders (Morley and Kushner,1982; Cecilliani etal 2002).

C-reactive protein is synthesized and secreted mainly by the liver and generally measured by its capacity to precipitate C-substance and by Immunological techniques. Measurement of CRP is most frequently used for evaluation of injury in the body tissue or for the detection of inflammatory event somewhere in the body. In a study of CRP in liver diseases it was reported that the intensity of C-

reactive protein expression in hepatocytes is closely associated with the histology activity index score (Shima *et al*, 1996). Malaria parasite infection is another condition in which there is CRP response. This infection is caused mainly by plasmodium species and is accompanied by an increase in CRP (Hurt *et al*, 1994). The malaria episode is mainly caused by *Plasmodium falciparium*_It was proposed that levels of malaria – attributable CRP appear to track the acquisition of parasitological and clinical tolerance in this area with very high levels of P. falciparium transmission (Hurt *et al*, 1994; Ekvall *et al*, 2001). This would be an efficient approach to estimating malaria morbidity risks from small – scale serological surveys. This study aims to determine the levels of CRP; Albumin and Globulin; ALT and AST activities in hepatitis and malaria patients in endemic regions; and to determine the relationship between these parameters in both malaria and hepatitis B infections.

MATERIALS AND METHODS

This study was conducted on 50 patients suffering from hepatitis who tested positive for hepatitis B surface antigen, 50 malaria patients and 40 healthy control subjects. All subjects were between the ages of 30-65 years and the patients were attending the Medical Clinics of University of Calabar Teaching Hospital. Calabar, Cross River State.

Fresh blood sample drawn by venepuncture were collected from the subjects described above. The blood samples were allowed to clot and centrifuged at 3000rpm. The sera were obtained and used for the analysis of CRP using high sensitivity immunoturbidometric method. ALT, AST, total proteins, albumin were also estimated using colorimetric method (Reitman and Frankel, 1952; Kingsley, 1942; Douma and Peters, 1997). Malaria test was done using thin and thick blood films.

Method for CRP Assay

Test tubes were lined in a test tube rack and 0.4 ml of CRP latex suspension was pipetted into the test tubes. Then 25µl of test sample were added to the test tubes and the test tubes mixed well and incubated for 15 minutes. Thereafter 0.6ml of CRP buffer reagents

was added and left in water bath for another 5mins. The absorbance of the resulting milky solution formed was read at 570nm. A control serum was also treated as the test to monitor the accuracy. The CRP assay method used in this study has a sensitivity range of 0.005 to 1.00mg/dl using the manufacturers CRP multi-calibrator set.

Test for Malaria Parasite

The blood samples collected from the patients and controls were tested for the presence of malaria parasite. The blood sample was smeared on a clean grease free slide to make thin and thick films. The smear was allowed to dry in air and then stained with 2% Geimsa for 30 mins. These slides were washed with water and allowed to dry in air and the viewed under the microscope with ×100-lens using oil immersion. Presence of ring form of gametocytes is a positive result for malaria parasite.

Test for Hepatitis B Surface Antigen (HBsAg)

The test kit (Bioline Standard Diagnostics) was removed from the foil pouch and placed on a flat dry surface. 100µL of serum was added into the sample well. The test was timed for 20mins. The presence of colour in the test band was an indication of positive result.

Statistical analysis

Data obtained are expressed as the mean ± SEM. Student t-test and ANOVA were used to assess the level of statistical significance. The level of significant difference between the groups was evaluated at $P \leq 0.05$.

RESULTS

There were significant increase ($p< 0.01$) of CRP in both patient groups (hepatitis and malaria) when compared with controls. Also CRP levels were significantly higher in malaria patients ($p < 0.05$) than in those with hepatitis B. there were significantly higher ($p < 0.01$) levels of AST and ALT in the hepatitis B patients than in controls.

Table 1
The mean serum levels of CRP, AST and ALT in patients suffering from hepatitis B, malaria and normal subjects

Subjects	CRP (Mg/dI)	Enzymes (IU/L)	
		AST	ALT
Normal (n = 40)	0.32 ± 0.12	34.75 ± 14.64	35.25 ± 15.56
Hepatitis (n = 50)	0.71 ± 0.11	116.76 ± 63 .27	103.50 ± 71.40
Malaria (n = 50)	0.78 ± 0.13	57.74 ± 15.18	46.72 ± 17.48
t-test (Significant Value)	b vs a (p<0.01)	b vs a (p<0.01)	b vs a (p<0.01)
	c vs a (p<0.01)	c vs a (p<0.05)	c vs a (p<0.05)
	b vs c (p<0.05)	b vs c (p<0.01)	b vs c (p<0.01)

[a]Normal, [b]Hepatitis [c]Malaria [Vs] Versus

Table 2
The mean serum levels of total protein albumin and globulin in patients suffering from hepatitis, malaria and normal subjects.

Subject	Total Proteins	Albumin	Globulin	AlL / Glb ratio
Normal n = 40	65.19 ± 3.85	37.00 ± 3.34	27.93 ± 4.31	1.37 ± 0.29
Hepatitis n = 50	61.40 ± 4.11	33.40 ± 3.40	28.25 ± 5.27	1.24 ± 0.30
Malaria n = 50	64.37 ± 4.62	34.47 ± 3.56	29.92 ± 4.77	1.18 ± 0.25
t-test (Significant Value)	b vs a (p<0.05)	b vs a (p<0.05)	b vs a ns	b vs a (p<0.05)
	c vs a ns	c vs a (p<0.05)	c vs a (p<0.05)	c vs a (p<0.05)
	b vs c (p<0.05)	b vs c ns	b vs c ns	b vs c ns

[a]Normal, [b]Hepatitis [c]Malaria [Vs] Versus [ns]Non significance

Also there were significant increases ($p < 0.05$) in the level of AST and ALT in malaria patients when compared with controls as shown in table 1.

From table 2, the total protein level was significantly lower ($p < 0.05$) in hepatitis B infection (61.40 ± 4.11g/l) when compared with controls (65.19 ± 3.85g/l). There is a significant ($p < 0.05$) decrease in albumin levels in both patients when compared with controls. The difference between the globulin value in malaria patients and control was statistically significant ($p<0.5$).

DISCUSSION

The result obtained from this study showed that CRP a positive acute phase protein is highly elevated in hepatitis and malaria patients. These findings are in agreement with a previous study (Hurt *et al*, 1994) which reported such elevated levels in children under 6 years of age suffering from malaria and higher levels in children less than 1 year of age. Another study (Graminger *et al*, 1992) showed that serum CRP level is increased in liver diseases. It was proposed that C-reactive protein levels may probably be an indicator of recent clinical malaria episode in currently afebrile individual with high parasiteamia (Hurt *et al*, 1994, Ekvall *et al*, 2001).

This study also showed that albumin decreased significantly in both patients. It has been estimated that the normal liver makes 10g albumin per day but in the end stage liver diseases, production is reduced to 4g per day(James *et al*, 1992) and the level of this protein drops slowly in serum in acute liver diseases but permanently low in chronic end stage disease. Albumin Synthesis is immediately and severely depressed in inflammatory states such as burns, trauma, and sepsis (David, 1999). It is commonly depressed in patients with active rheumatic disorders or severe end stage malnutrition. Hence it was stated (Shoia *et al*, 1995) that serum levels of acute phase protein such as albumin are more closely associated with the degree of hepatic dysfunction than the hepatic growth factor (HGF) in which he established a negative correlation between HGF and albumin and also a positive correlation between HGF and CRP. The analysis of variance of this study showed that there is a significant variation in the levels of C-reactive protein in hepatitis, malaria and normal subjects.

The findings in this study also showed that the increase in the levels of CRP were significantly higher ($P<0.05$) in malaria than in the hepatitis patients. Inflammation is such a complex and highly orchestrated process involving many cell types and molecules some which attenuate it, and some of which cause it to resolve (Kushner, 1993). Hence this study suggests that inflammatory response is more intense in malaria than in hepatitis.

A major function of CRP a component of innate immunity is its ability to bind phosphocholin and thus recognize some foreign pathogens as well as phospholipids constituents of damaged cells (Volariakis, 1997). The changes in concentration of these acute phase proteins are due largely to changes in their production by hepatocytes (Cem and Irving, 1999, Bonnete etal 2003). This study also showed that serum total protein decreased in hepatitis patients. There was no difference in the level of total proteins in malaria patients when compared with controls. These findings did not agree with a previous study (Mishra et al, 1992) which showed that serum total proteins significantly decreased in malaria. Another study (Alumanah etal, 2000) however reported that serum protein did not vary significantly in malaria and this agrees with the findings from the current study.

The result showed an increase level of globulin in malaria. The globulin levels in malaria correlated significantly ($r=0.72$) with C-reactive protein. C-reactive protein correlated negatively with albumin ($r=-0.26$). Also albumin had a negative correlation with globulin in malaria ($r=-0.36$). This suggests that as albumin decreases globulin increases, and this finding supports the fact that albumin decreases in inflammatory conditions. There were increased levels of aminotransferases in both patients similar to other findings (Mazumder *et al*, 2002, Park *et al*, 2003).

There could be two to three times rise in levels of these enzymes in hepatitis patients because of

enzymes leakage is increased in any form of inflammation and this helps to test the functional integrity of the liver cells.

In conclusion, CRP is significantly increased in acute and chronic diseases such as malaria and hepatitis. The level of CRP in malaria patients was found to be significantly higher ($P<0.05$) than that of hepatitis. Also albumin was decreased in hepatitis and malaria. The aminotransferases were found to be significantly increased in both patients. The changes in these acute phase proteins enhances protection against invading organism, limit tissue damage and promote rapid return to homeostasis. Acute phase protein changes which reflect the presence and intensity of inflammation may be useful as clinical guide in the diagnosis of malaria especially in endemic regions such as Nigeria.

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