ISSN - 2277 - 0593

Journal of Natural Sciences, Engineering and Technology

C-REACTIVE PROTEIN IN HOSPITAL PATIENTS IN A SOUTHWESTERN NIGERIAN COMMUNITY

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ABSTRACT

This study was conducted to evaluate c-reactive proteins on some inflammatory diseases among individuals aged 1 year and above attending out-patient clinic and emergency units of three hospitals in Abeokuta in southwestern Nigeria. Of the 266 blood samples examined overall, the CRP value was highest (16.7%) among age group 31-40 years and there was significant difference between CRP value and age groups (p=0.0060). More males (18%) had high CRP than female (16%), however there was no significant difference between the CRP values and sex. C-reactive protein was significantly high among the patients with anaemia (p<0.0001. r = -0.376). Of the patients with high CRP, all had significantly elevated erythrocyte sedimentation rate (p<0.0001). There was significant difference between CRP and both urea and creatinine (P<0.0001). There was association between CRP and cholesterol with X^2 value of 59.484 and significant at less than 1%.

INTRODUCTION

The C-reactive protein (CRP) was first recognized at Rockefeller University as a constituent in the serum of patients with acute pneumonia that formed a precipitation reaction with the C-mucopolysaccharide of certain groups of pneumococci and also gives the capsular swelling reaction when mixed with whole organisms (Tillet and Francis, 1930). In the serum it is present in the form of a glycoprotein complex which travels in the alpha-globulin region. Isolation and crystalized material had been injected into animals and an antibody raised against it which forms the basis of most laboratory methods of demonstrating its presence in patients (McCarty, 1947).

C-reactive protein has long been known as non-specific but constitutes optimal marker for identification and evaluation of acute-phase of infective and non-infective inflammation reactions (Jaye and Waites, 1997). In virtually all acute bacterial infections, respiratory diseases, some tumors and various types of tissue destruction, myocardiac infarction inclusive, the CRP rises (Roivainen *et al*., 2000). Another non-specific test to monitor inflammation is erythrocyte sedimentation rate (ESR). Both are elevated in the presence of inflammation, although, CRP appears and then disappears sooner than change in the ESR. The CRP level may fall to normal if treatment is successful but the ESR may still be abnormal for a longer period (Meier-Ewert *et al*., 2001).

C-reactive protein is an acute-phase plasma protein produced by the liver that rapidly change in concentration in the plasma in re-

sponse to a variety of stimuli most notably inflammation and tissue injury. When inflammation occurs there is a rapid rise in CRP levels, usually proportional to the degree of immunological stimulation and when inflammation resolves the CRP rapidly falls and therefore CRP level represents a good marker for disease activity and severity (Pepys and Hirschfield, 2003; Coventry *et al*., 2009). Reports have showed a very significant positive correlation between CRP levels and cardiovascular diseases (Gracia-Moll *et al*., 2000; Pasceri *et al*., 2000; Ridker *et al*., 2000) and between CRP levels and the extent of the arteroscIherotic diseases (Pepys and Hirschfield, 2003). Higher rate of cardiovascular diseases have been associated with human immunodeficiency virus due to inflammation activated by immune system and results in a proinflammatory state leading to elevated CRP level (Reingold *et al*., 2008).

CRP production is part of the specific and nonspecific acute phase response to most forms of inflammation, infection and tissue damage. The CRP blood level and other blood parameters would be useful tools in diagnosis and possibly to the physicians to monitor therapeutic response to treatment in some inflammatory diseases. This study therefore examines the CRP levels among patients routinely attending out-patient clinics.

MATERIALS AND METHODS

Study Area

The study was conducted in Abeokuta the capital city of Ogun State Southwestern Nigeria from October 2009 to April 2010 among patients at the out-patient and emergency unit at the Federal Medical Centre, Idi-Aba, State General Hospital, Ijaiye and Olikoye Ransome-Kuti Memorial Hospital at Asero, Abeokuta. The ethical approval was granted by the Ethical Committees of the hospitals.

Two hundred and sixty-six (266) samples were collected for this study. Venous blood samples were obtained from each patient into ethylediamine tetraacetate (EDTA) anticoagulated and non-anticoagulated tubes. The non-anticoagulated tubes were centrifuged at 2000 revolution per minute (rpm) for 5 minutes to obtain serum stored at 20^{o}C for serologic and biochemical analysis.

Haematocrit and Erythrocyte Sedimentation Rate Determination

The haematocrit (Hct) which is known as the packed cell volume (PCV) of each patient was determined by centrifugation of EDTA anti-coagulated blood in heparinized capillary tubes (with the end sealed) using Hawskley haematocrit centrifuge. The erythrocyte sedimentation rate (ESR) of each patient was determined by Westergreen-micro method using EDTA anticoagulated blood and the reading was taken at the end of one hour.

Serologic Analysis

The HIV-1 and HIV-2 screening kit used in this study was SD Bioline standard Diagnostic Inc. (Korea). This is immunochromatographic (rapid) method for quantitative detection of antibodies of all isotopes (IgG, IgM, IgA) specific to HIV-1 and HIV-2 simultaneously in serum, plasma or whole blood. This method used for each patient serum is highly sensitive and specific with very suitable control system incorporated.

The CRP reagent kit used was manufactured by Cypress Diagnostics, Belgium. The CRP reagent is a suspension of positive latex particle coated with the gamma globulin fraction of anti human CRP specific serum. When CRP is present in the sample, the resulting

turbidity was measured colorimetrically at 340nm. The CRP in each sample is then calculated using a standard CRP solution.

Biochemical Analysis

The cholesterol level was determined by spectrophotometric enzyme method using reagent kit of Randox Laboratories Ltd., Antrim, United Kingdom. Spectrophotometric analysis of urea and creatinine were carried out by the Oxime method and Jaffe reactions respectively (Bauer *et al*., 1974).

Data Analysis

The study was carried out and the proportion of individual CRP levels were calculated and level cross tabulated with age, sex, packed cell volume, erythrocyte sedimentation rate, human immunodeficiency virus infection, cholesterol, urea and creatinine using SPSS 11 windows packages. Relevant chi-square and correlation statistics were computed using Pearson correlation coefficient models.

RESULTS

Two hundred and sixty-six individuals attending out-patient clinics and emergency units were examined in this study as shown in Tables 1 and 2.

Of this, 154 (57.9% were male while 112 (42.1%) were female. There were more patients in the 31 – 40 year-age group 108 (40.6%) while fewer were seen in the 1 – 10 year-age group 6 (2.3%). Overall the CRP value was highest among the age group 31 – 40 years 18 (16.7%).

The chi-square statistics for the test of no association between age and C-reactive protein as shown in Table 1 had a value of 28.00% and statistically significant at less than 1% level and thus null hypothesis of no association is rejected. There was no significant difference between sex and c-reactive protein (p= 0.8080),), however, more males had higher CRP values, 28 (18.2%) than females 18 (16.1%).

In Table 3, of 104 patients with very high CRP values (>40mg/L), 36 (34.6%) had anaemia (Hct = 20 – 35%), 2 (1.9%) with moderately high CRP (11 – 40mg/L) and 66 (63.5%) normal CRP (0 – 10mg/L). The computed chi-square statistics for the test of no association between haematocrit and CRP levels had a value of 35.840 and is significant at less than 1% level. Thus the null hypothesis of no association is rejected.

The cross tabulation between the ESR and CRP in Table 4 shows that 2 (1.3%) of patients with high CPR of 11 – 40mg/L and >40mg/L respectively had normal ESR (3 – 8mm/h) while 44 (78.6%) patients with extremely elevated CRP (> 40mg/L) had ESR greater than 40mm/h. There is significant difference between the ESR and CRP (p< 0.0001).The cross-tabulation between serum urea/creatinine and CRP values of patients examined is presented in Table 5. It shows that 32 (13.6%) of the patient with normal blood urea (15 – 38mg/L) and creatinine (0.9 – 1.5mg/dL) had highly elevated CRP (> 40mg/L) while 2 (21%) with moderate and highly elevated values had CRP greater > 40 mg/L. The computed chi-square for the test of no association between blood urea/creatinine and CRP gave the same value of 43.816 and was statistically significant at less than 1% level. Thus the null hypothesis of no association is rejected in each case.

Table 1: C - reactive protein Distribution by Age of the Patients

Age (Years)	Number] Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
1 – 10	6	6 (100%)	0 (0%)	0 (0%)
11 – 20	26	24 (92.3%)	0 (0%)	2 (7.7%)
21 – 30	52	50 (96.2%)	0 (0%)	2 (3.8%)
31 – 40	108	86 (79.6%)	4 (3.7%)	18 (16.7%)
41 – 50	40	24 (60.0%)	2 (5.0%)	14 (35.0%)
51 – 60	26	18 (69.2%)	0 (0%)	8 (30.8%)
61 – 70	8	6 (75.0%)	0 (0%)	2 (25.0%)
Total	266	214 (80.5%)	6 (2.3%)	46 (13.3%)

Table 2: C-reactive Protein Distribution by Sex of the Patients

Sex	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
Male	154	122 (79.2%)	4 (2.6%)	28 (18.2%)
Female	112	92 (82.1%)	2 (1.8%)	18 (16.1%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

Table 3: Haematocrit Level in Relation to C - reactive protein

PCV (%)	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
35 – 54	162	148 (91.4%)	4 (2.5%)	10 (6.2%)
20 - 34	104	66 (68.5%)	2 (1.9%)	36 (34.6%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

Table 4: Frequency Distribution of Erythrocyte Sedimentation Rate in Relation to the C - reactive protein Level

ESR (mm/h)	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
3 – 8	152	148 (97.4%)	2 (1.3%)	2 (1.3%)
9 – 40	58	54 (93.1%)	4 (6.9%)	0 (0%)
> 40	56	12 (21.4%)	0 (0%)	44 (78.6%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

Table 5: Blood Urea and Creatinine of the patients in Relation to C-reactive Protein

Blood Urea Creatinine* (mg/dL)	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
15 – 38 (0.9 – 15)*	236	200 (84.7%)	4 (1.7%)	32 (13.6%)
29 – 60 (1.6 – 2.7)*	8	4 (50.0%)	2 (25.0%)	2 (25.0%)
61 – 120 (2.8 – 6.0)*	22	10 (45.5%)	0 (0%)	12 (54.5%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

The cross-tabulation between the blood cholesterol and CRP values of the patients examined is shown in Table 6. This table shows that 30 (12.2%) of the patient with normal blood cholesterol (140 – 200mg/dL) had highly elevated CRP values (> 40mg/L) while 16 (80.0%) with elevated cholesterol values also had high CRP values. There is significant difference between the blood cholesterol and CRP values ($P < 0.0001$).

The human immunodeficiency virus infection in relation to the CRP level is shown in Table 7. It shows that 16 (66.7%) of the patients with positive HIV infections had elevated CRP values (> 40mg/L) while 30 (12.4%) patients negative HIV infections had CRP values lesser than 40mg/L. The chi-square status computed for the test of no association between HIV infection and CRP level gave a value of 45.062 and significant at less than 1% level and thus the null hypothesis of no association is rejected.

Correlation analysis was carried out to examine the relationship between PCV, ESR,

Urea/Creatinine and cholesterol values with CRP values as shown in Table 8. The output from this analysis shows that there was positive, linear and significant relationship between each of these variables and CRP (<0.0001), the strength of association being 99%. However, a negative coefficient of -0.376 for the relationship with PCV suggesting that PCV was decreasing with a corresponding increasing CRP values.

DISCUSSION

This study showed that the CRP is highest in the age group 31 – 40 years among patient in Abeokuta metropolis. This report corresponds with previous report studies (Pepys and Hirschfield, 2003; Zacho *et al*., 2008; Cushman *et al*., 2009). This may be attributed to coronary artery risk development in young adults (Cushman *et al*., 2005; Reingold *et al*., 2008 and Cushman *et al*., 2009). There was no significant difference between sex and CRP, although more men have increased CRP than women in this investigation which is in contrast to the previous reports (Rider and Cook, 2004; Cushman *et al*., 2005; Cook *et al*., 2006; Ridker *et al*., 2007; Cushman *et al*., 2009). The difference may be due to the various socio-economic, geographical and ethnic groups in the study areas.

In the report of Kanfer and Nicol (1997) which is similar to the finding in this study, increased CRP was significantly observed in patients that were anaemic (PCV = 20 – 35%). This observation also supports the reports of others (Pepys and Hirschfield, 2003; John *et al*., 2004; Clyne and Jonathan, 2009; Reingold *et al*., 2008). In this study, increased CRP is observed in patients with extremely elevated erythrocyte sedimentation rate (ESR). Previous reports (Brigden, 1998; John *et al*., 2004; Cook *et al*., 2004) are in support of this finding that the levels of ESR and CRP are useful indicators for inflammatory reactions. This shows that the level of inflammatory reaction is significantly high with increased CRP as excellent predictive value than ESR. Generally, elevated blood urea and creatinine are indicative of renal malfunction (Davidsohn and Henry, 1974 and Brooks *et al*., 1998). In this study, both blood urea and creatinine were elevated in patients with increased CRP which is indication of renal malfunction. Also, this report showed that there were associations between the CRP and cholesterol which is a heart index (arteriosclerosis). This may be attributed to the effect of cholesterol and the lipoproteins involvement in immune response and tissue repair as recorded by previous observations (Ridker *et al*., 2002; Ridker, 2003; Cushman *et al*., 2009).

CONCLUSION

The result of this study showed significant increase in the CRP of the HIV positive patients which shows the level of inflammatory reaction. This is in agreement with previous investigations (Lau *et al*., 2006 and Reingold *et al*., 2008). This may have implications for cardiovascular diseases among HIV-infected patients (John *et al*., 2004; Lloyd-Jones *et al*., 2006; Lau *et al*., 2006; Reingold *et al*., 2008). Future studies should evaluate carefully the epidemiology of CRP with cardiovascular diseases in HIV-infected persons. Also, several articles in other parts of the world have been published on relationship between CRP and cardiovascular diseases especially coronary artery disease and therefore, future studies would have an important role in assessing and treatment of these patients in this study area where most physicians are not aware of the importance of CRP as diagnostic tool in inflammatory diseases.

Table 6: Total Blood Cholesterol and C - reactive protein

Blood Cholesterol (mg/dL)	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 n (%)	11 – 40 n (%)	> 40 n (%)
140 – 250	246	210 (85.4%)	6 (2.4%)	30 (12.2%)
> 250	20	4 (20.0%)	0 (0%)	16 (80.0%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

Table 7: Human Immunodeficiency virus Infection in the Patients and C-reactive Protein

HIV Infection	Number Examined (n)	C-Reactive Protein (mg/L)		
		0 – 10 N (%)	11 – 40 n (%)	> 40 n (%)
Positive	24	8 (33.3%)	0 (0%)	16 (66.7%)
Negative	242	206 (85.1%)	6 (2.5%)	30 (12.4%)
Total	266	214 (80.5%)	6 (2.3%)	46 (17.3%)

Table 8: Correlation Coefficients between CRP with PCV, ESR, Urea/ Creatinine and Cholesterol

Variables	Correlation Coefficient (R)	R Square	Std. Error	P Value
PCV/CRP	-0.376	0.141	4.7973	<0.0001
ESR/CRP	0.723	0.522	21.774	<0.0001
Urea and Creatinine/CRP	0.283	0.080	15.3437	<0.0001
Cholesterol/CRP	0.375	0.140	32.5014	<0.0001

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(Manuscript received: 17th May, 2010; accepted:, 14th July, 2010).