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RESEARCH PAPER

HUMAN SERUM PROTEIN AND C-REACTIVE PROTEIN LEVELS AMONG HIV INFECTED SUBJECTS IN UROMI AND ITS ENVIRONS IN EDO, NIGERIA.

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ABSTRACT

Human serum protein and C-reactive protein levels were determined among HIV patients visiting St Camillus Hospital, Uromi, Edo State, Nigeria, between January to March, 2013. Fifty (50) HIV patients (20 males; 30 females) and 50 control subjects (24 males; 26 females) were enrolled for this study. The clinical status of the patients was obtained from their hospital records. Human serum protein and CRP levels were assayed spectrophotometrically, while CD4 count was done using a flow cytometry. Results showed that serum total protein (10.44±2.32 g/dL), globulin (7.06±2.37 g/dL) and C-reactive protein (22.64±12.45 mg/L) were significantly higher ($p<0.05$) in HIV patients than the controls. Albumin (ALB) levels (3.38±0.82 g/dL) and CD4 count of HIV patients (419.52±338.43 cells/µL) was significantly lower ($p<0.05$) than the control subjects. It was concluded that increased serum total protein, globulin, C-reactive protein and low levels of albumin and CD4 count, may be a valuable indices in the monitoring and management of HIV infections.

Key words: C-reactive protein, CD4, Total Protein, Albumin, Globulin, HIV

INTRODUCTION

Human immunodeficiency virus infection/Acquired immunodeficiency syndrome (HIV/AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV) (Sepkowitz, 2001). HIV is a retrovirus that primarily infects components of the human immune system such as $CD4^+$ T cells, macrophages and dendritic cells (Ochie and Kolhatkar, 2000; Ogeneh, 2002; Alimonti *et al*., 2003). It attacks the body's immune system (Siliciano, 2001; Kumar and Clark, 2002) resulting in the continuous depletion of CD4 T cells and progressively leads to immunodeficiency, opportunistic diseases and finally death (Grossman *et al*., 2002; Battegay *et al*., 2004**;** Mishra *et al*., 2009**)**.

Since HIV is characterized by inflammation, C-reactive protein (CRP), is currently the most widely used biomarker of inflammation used to monitor HIV infection (Pepys and Hirschfield, 2003; Baker *et al*., 2010; Funderburg *et al*., 2010; Neuhaus *et al*., 2010). CRP was so named because it was first identified as a substance in the serum of patients with acute inflammation that reacted with the C-polysaccharide of *Streptococcus pneumonia (*Pepys and Baltz, 1983; Ribeiro, 1997; Pepys et al., 2003) and discovered by Tillett and Francis in 1930 (Tillett and Francis, 1930). CRP is synthesized by the liver (Pepys *et al*., 2003), in response to factors released by macrophages and fat cells (adipocytes) (Lau *et al*., 2005).

This acute-phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP concentration can rise up to 300mg/L in 12-24 hours (Le Carrer *et al*., 1995; Vaishnavi, 1996; Hansson and Lindquist, 1997). Also total protein which measures the level of protein in the blood is increased in conditions that cause inflammation while plasma albumin is decreased in inflammatory condition (Fischbach, 2000; Sacher *et al*., 2000).

This study is therefore designed, to determine the levels of human serum protein and C-reactive protein among HIV infected subjects Uromi and environs in Edo State, Nigeria.

MATERIALS AND METHODS

Study design: The present study lasted for three months at St Camillus Hospital, Uromi, Edo State, Nigeria. The subjects were 50 HIV Positive patients (20 males and 30 females) (stage 1, stage 2 and stage 3) as described by CDC, (2011) aged 24 to 49 years, while 50 apparently healthy volunteer HIV Negative subjects (24 males and 26 females) aged 21 to 45 years were recruited as the control group.

Ethical approval: Ethical approval was obtained from the management St Camillus Hospital, Uromi, Edo State, for sample collection.

Inclusion and exclusion criteria: Already diagnosed HIV Positive patients from 24 years and above were recruited for the study. HIV Positive patients below 15 and above 50 years were also excluded from this study

Sample collection: Venous blood sample was collected from each subject using a 10mls sterile disposable syringe. This was dispensed into a 5mls plain sample containers and EDTA containers labelled with the subject's name, age and sex. The blood sample in the plain containers was spun for 5 minutes at 3000 rpm. The serum was separated from the red cells using a dry clean pasteur pipette into a dry clean plain specimen container. The serum was then stored at -20°C.

Analytical methods: Total protein was estimated by the Biuret method by Gomall *et al.* (1949), with kit assay system (Cromatest, Spain). Albumin was estimated by the Bromocresol green (BCG) method by Doumas *et al.* (1971), with kit assay system (Cromatest, Spain). Serum globulin was estimated indirectly by subtracting the albumin concentration from total protein concentration (Ochie and Kolhatkar, 2000).Serum globulin (g/dl) = Total protein (g/dl) - serum albumin (g/dl). C-reactive protein estimation was carried out by Latex enhanced immunoturbidimetry method; with kit assay system (Zeigenhagen and Drahovshy, 1983).CD4 count was done with Cy- Flow Counter which uses flurometric technique to detect CD4 cells (Westerman *et al*., 1994).

Statistical analysis: The results obtained in this study were analyzed statistically. The mean and Standard deviation values were calculated in each case. Student's t-test (independent t-test) and One Way Analysis of Variance (ANOVA, LSD) statistical method were employed for comparisons using a computer programme (SPSS) for "Windows Release 16.0". The comparison was done at 95% confidence level, a p-value equal to or less than 0.05 ($p \leq 0.05$) were considered statistically significant.

RESULTS

Table 1 shows that serum total protein (10.44±2.32 g/dL), globulin (7.06±2.37 g/dL) and C-reactive protein (22.64±12.45 mg/L), were significantly higher ($p<0.05$) in HIV patients than the controls. Albumin (ALB) levels (3.38±0.82 g/dL) and CD4 count of HIV patients (419.52±338.43 cells/µL) was significantly lower ($p<0.05$) than the control subjects. Table 2 shows the level of Serum CRP, CD4 count, Total protein, Albumin and Globulin in different clinical stages (Stages 1, 2 and 3) of HIV infected subjects (*see also fig. 1 and 2).*

Table 1: Levels of Serum CRP, Total Protein, Albumin, Globulin and CD4 count in HIV infected and control subjects

PARAMETERS	CONTROL (n=50)	TEST(n=50)	t-value	P- value
CRP mg/L	7.18 ± 3.99	22.64 ± 12.45	8.782	P<0.05 (S)
CD4 cells/µL	971.70 ± 213.16	419.52 ± 338.43	-11.537	P<0.05 (S)
Total Protein g/dL	7.12 ± 0.72	10.44 ± 2.32	10.126	P<0.05 (S)
Albumin g/dL	5.14 ± 0.63	3.38 ± 0.82	-15.30	P<0.05 (S)
Globulin g/dL	1.99 ± 0.78	7.06 ± 2.37	15.132	P<0.05 (S)

Key: n: Number of sample; CD4: Cluster of differentiation; CRP: C-reactive protein; S: Significant; df=49.

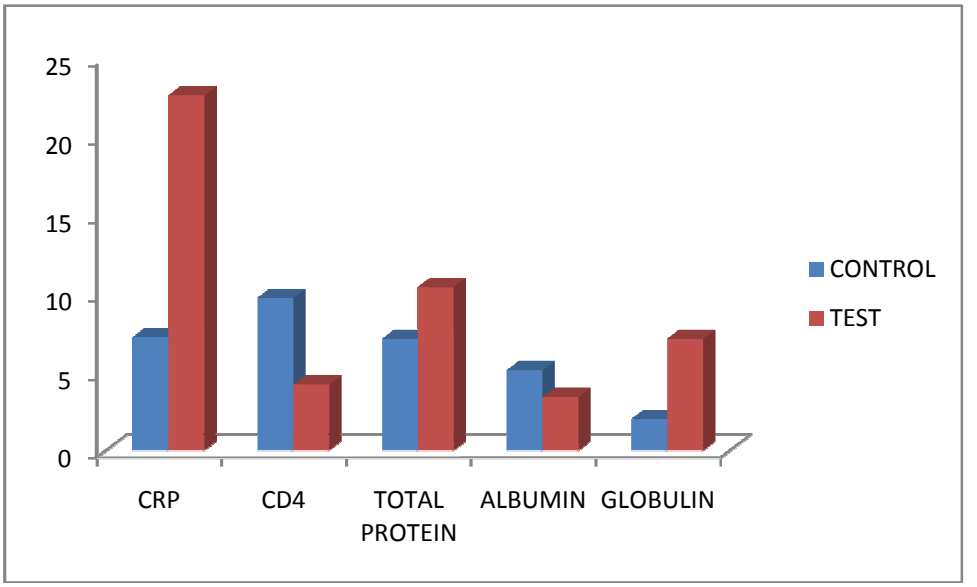

Figure I: Graph showing levels of Serum CRP, Total Protein, Albumin, Globulin and CD4 count in HIV infected and control subjects

Table 2: The levels of Serum CRP, CD4 count, Total Protein, Albumin and Globulin in different clinical stages of HIV infected subjects

PARAMETERS	CONTROL (n=50)	STAGE 1 (n=14)	STAGE 2 (n=20)	STAGE 3 (n=16)
CRP mg/L	7.18 ± 3.99^{a}	16.79 ± 9.05^{b}	17.70 ± 6.51^{b}	33.94 ± 13.45^{c}
CD4 cells/µL	971.70 ± 213.16^{a}	881.07 ± 256.39^{a}	337.35 ± 86.73^{b}	118.38 ± 55.47^{c}
Total Protein g/dL	7.12 ± 0.72^{a}	8.98 ± 1.62^{b}	9.61 ± 1.60^{b}	12.76 ± 1.83^{c}
Albumin g/dL	5.14 ± 0.63^{a}	3.23 ± 0.86^{b}	3.41 ± 0.73^{b}	3.47 ± 0.91^{b}
Globulin g/dL	1.99 ± 0.78^{a}	5.75 ± 1.97^{b}	6.20 ± 1.66^{b}	9.29 ± 1.91^{c}

Key: n: Number of sample; CD4: Cluster of differentiation; CRP: C-reactive protein; S: Significant; df=49; Values in a row with a different superscript are significantly different at P<0.05.

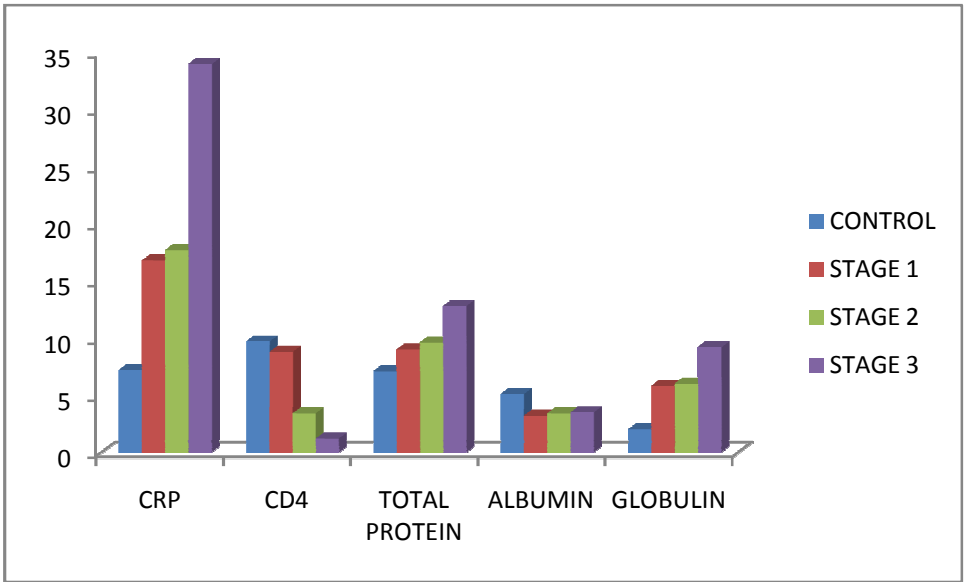

Figure 2: Graph showing the levels of Serum CRP, CD4 count, Total Protein, Albumin and Globulin in different clinical stages of HIV infected subjects .

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DISCUSSION

The hallmark of HIV disease is the continuous depletion of CD4 T cells, leading to progressive failure of the immune system, allowing life-threatening opportunistic infections, cancer and finally death (Saag *et al*., 1996). Since HIV is characterized by infection and inflammation, C-reactive protein (CRP) is the most widely used biomarker of inflammation (Pepys and Hirschfield, 2003).

The result of HIV infected subjects when compared with the control values, showed a statistical significant difference in the levels of total protein, albumin, globulin, C-reactive protein and CD4 Count ($P<0.05$). These results obtained in this study are in agreement with those reported by Doumas *et al.*, (1971); McGlynn *et al.*, (1995); Fischbach, (2000); Siliciano, (2001); Schleicher *et al*. (2005); Lau *et al*. (2006); Wadgera *et al.* (2012).

The level of serum Total protein, Globulin and CRP in HIV infected subjects were generally higher and low levels of Albumin and CD4 were observed when compared with the control subjects. These findings agree with the report of Wadgera *et al*. (2012); McGlynn *et al.* (1995); Hansson and Lindquist, (1997); Ribeiro (1997); Hattingh *et al.* (2009). The observed high level of total protein and globulin is as a result of acute-phase response that is rapidly harnessed to eliminate microbes, control further damage, clear infectious debris, and initiate repair processes and is in line with the reports of Yoshida *et al*. (1999); Hattingh *et al*. (2009).

The observed high level of CRP is due inflammatory response that is seen HIV patients and is in agreement with the report of Vaishnavi (1996); Schleicher *et al*. (2005); Wadgera *et al*. (2012). CRP was high because of opportunistic infections. Lawn S D *et al*., (2001) suggested that the serum CRP in HIV infected persons increase only in presence of opportunistic infections. The low CD4 count is due to the fact that HIV causes destruction of the immune system largely through depletion of CD4 cells which is in agreement with the report of Siliciano (2001); Grossman *et al.* (2002); Wadgera *et al*. (2012).

The results obtained from clinical staging indicated that as CD4 count declined, the CRP increased from stage 1 to stage 3. In stage 3, the CRP was significantly higher in stage 3 than stage 1 and 2, while CD4 count was significantly lower in stage

3. This is because as CD4 count declined, opportunistic infections set in and the patient developed AIDS at the later stage, which is in line with the report by Wadgera *et al*. (2012).

CONCLUSION

It can be concluded from this study that increased serum level of total protein, globulin, C-reactive protein and low levels of albumin and CD4 count were observed. These increased serum levels of total protein, globulin, C-reactive protein and low levels of albumin and CD4 count in HIV infected subjects, which were not gender or age related, reflected the damage to the immune cells as a result of the Human Immunodeficiency Virus (HIV). Therefore, CRP being less expensive and take much less time should be incorporated as a routine test in the monitoring and management of HIV infections.

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AUTHORS' CONTRIBUTIONS

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