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Case Report

DETERMINANTS OF RAISED SERUM C-REACTIVE PROTEIN IN HIV INFECTED CHILDREN ON HAART AT UNIVERSITY OF UYO TEACHING HOSPITAL, UYO

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ABSTRACT

Background: Paediatric HIV infection remains a major cause of morbidity and mortality among children in Sub-Saharan Africa. High levels of C-reactive protein (CRP) have been demonstrated in association with certain clinical manifestations of HIV infection among adults but such finding is yet to be documented among children. The relationship between clinical manifestations of HIV infection and serum level of C-reactive protein among children aged 6 months to 12 years was evaluated.
Method: Eighty HIV infected children receiving combined antiretrovirals for ≥3 months and age/gender matched apparently healthy HIV negative controls were recruited by the authors. The clinical manifestations of HIV infection were elicited through history taking, physical examination, review of case notes and laboratory investigations. Serum CRP was estimated using enzyme linked immunosorbent assay. Data was analyzed with statistical package for social sciences version 20.
Result: The mean age of the study participants was 8.50 ± 3.36 years. There were 46 (57.50%) males and 34 (42.50%) females, who participated in the study. The most common clinical manifestation was generalized lymphadenopathy, documented in 26 (32.50%) participants. The mean serum CRP of the study participants was significantly higher than that of the apparently healthy HIV negative control group (8.65 ± 10.89 mg/l vs. 0.89 ± 1.1mg/l), p = 0.04. Multiple regression analysis between serum CRP and clinical features demonstrated significant association between generalized lymphadenopathy and serum CRP (p = 0.04).
Conclusion: The presence of generalized lymphadenopathy may be the determinant of raised serum CRP among HIV infected children on HAART.

INTRODUCTION

Human immune deficiency virus infection is a chronic and progressive condition that continues to impact negatively on the well being of the child. As at 2019, about 13.8 million children under the age of 18 had lost one or both parents due to HIV, leading to increased risk of poverty, homelessness, school dropout and loss of opportunities.[1] Though the global response to HIV/AIDS has yielded significant progress towards curbing the infection, the epidemic continues to affect children globally.[1]

C-reactive protein is produced predominantly in the liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes during acute and chronic inflammatory conditions in response to cytokines.[2-5]It has been documented that the levels of CRP may increase up to 1000 fold during inflammatory response to certain infections.[5]Though the protein may play pathophysiologic role in certain diseases such as artheroslerosis, rheumatoid arthritis and idiopathic thrombocytopenic purpura, it also plays significant physiologic role in human immunity.[2-5]C-reactive protein is one of the parameters used commonly in clinical practice to diagnose and monitor inflammatory processes in some diseases.[3]

Higher levels of CRP have been demonstrated in children with HIV infection compared to apparently healthy uninfected group.[6-9] This may be attributed to immune activation as a result of the viral infection and presence of opportunistic infections.[10,11] Higher CRP levels and chances of progression to AIDS have also been demonstrated in HIV infected patients with opportunistic infections compared to those without opportunistic infections.[11,12]

Wagera *et al*[11] while investigating C-reactive protein as an early marker of opportunistic infection among 100 HIV infected patients attending antiretroviral therapy centre at Victoria Hospital, Bangalore in 2012 found that while the values of serum CRP in asymptomatic HIV infected patients were < 6mg/l, higher values were noted among those with opportunistic infections and other non-infectious diagnoses.

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The highest mean CRP was noticed among those who had coexisting cancer (160mg/l), followed by those who had both tuberculosis and diarrhoea (45mg/l). The mean CRP for those who had only tuberculosis was 41mg/l while the mean value for those who had fever without specific diagnosis was 24mg/l. For participants who had oral thrush, herpes infection and central nervous system infection, their mean CRP was 9mg/l. The study was conducted among adult participants and was able to demonstrate a relationship between levels of CRP and clinical manifestations in HIV infected individuals.

The relationship between the degree of immunosuppression and ability to mount CRP response in HIV infected individuals is another subject that is yet to be fully investigated. It was found earlier that the ability to mount a CRP response to Pneumocystis pneumonia infection is substantially small when CD_4 cell count is <50 cells/µl.[13]This finding suggests that a degree of immunocempetence is required for CRP response. An earlier report by Udoh *et al*[14] demonstrated an association between the immunologic/clinical stages of HIV infection and levels of serum CRP among HIV infected children on combined antiretroviral therapy. There is paucity of information on the relationship between the levels of serum CRP and specific clinical manifestations of HIV infection among children. It is also yet to be documented what clinical manifestations of the infection may determine the rise in serum CRP among children. This study sought to bridge the knowledge gap.

MATERIALS AND METHOD

The study was conducted between April and November, 2018 in the children out-patient clinic of the University of Uyo Teaching Hospital (UUTH), Uyo, Akwa Ibom State, Nigeria. It was a descriptive cross-sectional study, using data generated through clinical evaluation and review of case notes. The ethical clearance certificate was obtained from the University of Uyo Teaching Hospital Institutional Health Research Ethical Committee before commencement of the study. Eighty HIV infected children aged 6 months to 12 years who had been confirmed HIV positive and received HAART for minimum of 3 months were recruited consecutively after obtaining consent from their parents/guardian and assent from those 7 years and above. The exclusion criteria were the presence of symptoms and/or signs of illness not directly associated with HIV such UTI and URTI or the presence of chronic conditions not associated with HIV infection such as Sickle cell anaemia and congenital heart diseases. Newly diagnosed HIV infected children were excluded from the study to eliminate the bias of the effect of exposure/non-exposure to ARVs on the level of serum CRP. Equal number of apparently healthy children matched for age and gender served as controls. The inclusion criteria were parental consent/assent for those 7 years and above, HIV negative test result and absence of history and examination findings suggestive of any illness. Each prospective participant was clerked, examined and investigated. The case notes were also reviewed. Bedside urinalysis was performed using medi-test combi 9 urine dipstick (K39927, Macherey-Nagal) for each prospective participant and the result used to screen for UTI and metabolic abnormalities. The data generated was documented in case control form and used to screen, recruit and classify participants. The Centre for Disease (CDC) 1994 revised classification system for HIV infection in children less than 13 years of age was used to classify participants into immunologic categories 1, 2, 3 and clinical categories N, A, B and C.[15]

The diagnosis of HIV infection for children aged 6weeks to 18 months was done using deoxyribonucleic acid polymerase chain reaction (DNA PCR). Dried blood samples (DBS) using lasec DBS kit taken by a trained nurse at the children clinic were pooled and sent to the PCR laboratory where DNA PCR test was carried out. Confirmatory test was performed 4 to 6 weeks later.

Antibody test was used to make the diagnosis for children aged 18 months and above. About 0.5mls of venous sample was taken in an aseptic procedure and transferred to the HIV laboratory using a plain bottle. Antibody test was carried out using Alere determine kit (7D2343, Japan) and confirmed with Trinity Biotech Uni-gold kit (1206502, Ireland). Stat-Pak kit (44033015, New York, USA) was used as tie breaker.

The diagnoses of tuberculosis according to review of case notes were suspected in children with cough greater than 2 weeks with or without history of contact or clinical signs of tuberculosis. The confirmations of diagnoses were done through radiologic evidence of infection and positive acid fast bacilli or gene expert tests.

Enzyme linked immunosorbent assay (ELISA) colorimetry, using Calbiotech highly sensitive CRP kit (HS-CRP CR120C, California, USA) was used to test for serum level of CRP. It is a quantitative assay with sensitivity of 0.005mg/l. The principle of the test is based on quantitative sandwich enzyme immunoassay technique.[16] Two milliliters of venous blood samples were collected, pooled and transferred to the chemical pathology laboratory within an hour, where they were separated and stored at 2-8°C. The test procedure was carried out by an experienced medical laboratory scientist who closely followed the manufacturer's instructions. The concentration of serum CRP was expressed in mg/l.

Serum CD_4 count was estimated by flow cytometry using Partec cyflow counter. The machine uses the principle of align Free™ technology to perform true volumetric absolute count (TVAC).[17]The test was carried out in HIV laboratory by an experienced laboratory scientist using two milliliters of venous blood sample collected from participants with plain bottle. The manufacturer's instructions were closely followed during the procedure.

The data obtained was analyzed using Statistical package for social sciences (SPSS) version 20. Categorical data were summarized using frequency and percentage while quantitative data were summarized using mean and standard deviation. Multivariate regression analysis was used to test for association between clinical findings/manifestations and serum CRP. The level of significance was taken as $p < 0.05$.

RESULTS

Eighty children aged between 6 months and 12 years participated in the study. The mean age of the study participants was 8.50 ± 3.36 years. Sixty-five (81.25%) participants were within the ages of 5 to 12 years. Fourteen (17.50%) study participants were within the ages below 5 years and 1year, while one (1.25%) participant was less than 1 year of age. There were 46 (57.50%) males and 34 (42.50%) females, who participated in the study. The male to female ratio was 1.35:1. The demographic characteristics of the study participants are demonstrated further in figure 1 below.

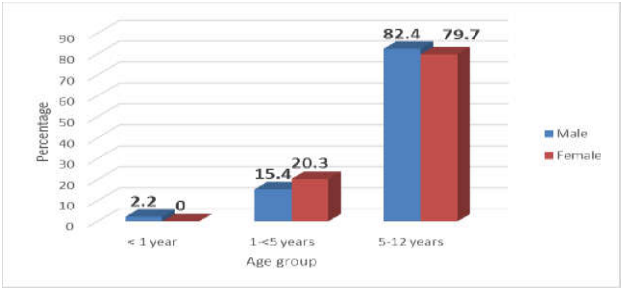

Figure 1 Demographic characteristics of the study participants

The most common clinical feature was generalized lymphadenopathy, identified in 26 (32.50%) participants, while the least common clinical features were recurrent/chronic otitis media and splenomegaly noted in one (1.25%) study participant respectively. The occurrence of other clinical findings is as demonstrated in table I. The staging of participants is demonstrated in table II. Majority of the participants 59 (73.75%) and 52 (65.00%) were classified under the asymptomatic (clinical stage N) and no suppression (immunologic stage 1) stages of HIV infection respectively. No participant was classified into severely symptomatic stage (clinical stage C), while 7 (8.75%) participants were classified as having severe immune suppression (immunologic stage C).

Table I. Clinical findings and mean CRP among the participants

Variable	Frequency	Percent	Mean CRP
Generalized lymphadenopathy	26	32.5	14.26± 12.24
Poor Weight gain/Weight loss	20	25.0	12.11 ± 13.03
Dermatitis	7	8.8	13.76 ± 10.78
Cough >1month	7	8.8	18.09±10.33
Pulmonary tuberculosis	5	6.3	18.52± 12.47
Hepatomegaly	3	3.8	21.7 ± 12.20
Anaemia	3	3.8	20.2 ± 13.27
Recurrent/Chronic diarrhea	2	2.5	18.9 ± 23.33
Recurrent/Chronic otitis media	1	1.3	14.0
Splenomegaly	1	1.3	12.0

Table II CDC Classification of participants

Variable	Frequency	Percent
Clinical Stage		
N	59	73.75
A	11	13.75
B	10	12.50
C	0	0.00
Total	80	100.00
Immunologic stage		
1	52	65.00
2	21	26.25
3	7	8.75
Total	80	100.00

N = Asymptomatic, A = mildly symptomatic, B = moderately symptomatic, C = severely symptomatic, 1 = No evidence of immuno-suppression, 2 = Evidence of moderate immunosuppression, 3 = Evidence of severe immunosuppression.

The highest mean CRP as shown in table I, was noted among participants with hepatomegaly (21.7 ± 12.20), and followed by those with anaemia (20.2 ± 13.27). Multiple regression analysis of the clinical features and serum CRP demonstrated significant association between generalized lymphadenopathy and serum CRP (p = 0.04). This is demonstrated in table III. The mean serum CRP of the study participants was 8.65 ± 10.89 mg/dl, while that of the apparently healthy HIV negative control group was 0.89 ± 1.1mg/l. Student t test demonstrated significant difference between the mean CRP in the two groups as shown in table IV.

Table III Association of CRP with clinical features among the study participants

Clinical feature	B	S.E.	Wald	Df	P value	Odds	95% C.I.	
							Lower	Upper
Generalized lymphadenopathy	-1.56	0.75	4.32	1.00	0.04*	0.21	0.05	0.92
Dermatitis	-0.40	1.32	0.09	1.00	0.77	0.67	0.05	0.92
Cough>1month	-20.43	1.22	0.00	1.00	1.00	0.00	0.00	0.11
Chronic/Recurrent otitis media	-19.88	4.05	0.00	1.00	1.00	0.00	0.00	0.01
Poor weight gain/Weight loss	-0.36	0.66	0.31	1.00	0.58	0.70	0.19	2.52
Hepatomegaly	-20.11	9.70	0.00	1.00	1.00	0.00	0.02	0.13
Splenomegaly	20.62	6.30	0.00	1.00	1.00	0.92	0.04	0.08
Constant	3.60	1.80	0.00	1.00	1.00	0.12		

B = coefficient of regression, SE = standard error, df = degree of freedom, CI = confidence interval, * = statistically significant.

Table IV Comparison of serum CRP between the Study group and controls

	CRP				
	N	Mean	SD	t test	P value
Study group	80	8.65	10.89	6.10	0-001
Control	80	0.89	1.10		

DISCUSSION

The clinical manifestations of HIV infection in children are similar globally with slight age and regional differences.[18]Most clinical manifestations of HIV infection occur as a result of inflammatory response to the virus and the presence of other infections that capitalizes on the immunodeficiency state occasioned by the viral infection. Common clinical findings among infants include lymphadenopathy, failure to thrive, hepatosplenomegaly, oral thrash and interstitial pneumonia while persistent fever, progressive weight loss, chronic/ recurrent diarrhoea, skin manifestations, lymphadenopathy and hepatosplenomegaly occur commonly in older children.[18,19] Systemic and pulmonary findings predominate the presentations in USA and Europe while children with the infectionin Africa present more commonly with chronic diarrhoea and malnutrition.[18,19]The prevalence of each of the clinical manifestations of HIV infection documented in this study was low compared to other studies because the participants had received care and HAART for minimum of three months prior to recruitment. Some of the clinical features at presentation may have resolved in the course of treatment prior to recruitment. Generalized lymphadenopathy was the commonest clinical finding in this study, its prevalence being comparable to 33.3% and 35.5% reported by Brown *et al*[20] in Ibadan, Nigeria and Sehgal *et al*[21] in Delhi, India respectively. The high prevalence of lymphadenopathy compared to other clinical findings in this study may be explained by the fact that this clinical finding tends to remain persistent in HIV infected individuals irrespective of commencement of treatment.[18,22-25]The occurrence of generalized persistent lymphadenpathy in HIV infected individuals have been associated with lymphoid hyperplasia as a result of the viral infection and the presence of opportunistic infections such as toxoplasmosis, disseminated fungal infection, atypical mycobacterial infection and cytomegalovirus.[22-25] Malignances such as non-hodgkins lymphoma may also be responsible for persistent generalized lymphadenopathy. The finding of significant association between the level of serum CRP and the presence of generalized lymphadenopathy in this study is novel. It may suggest a relationship between lymphoid hyperplasia and ongoing immune activation and inflammation among HIV infected individuals. The mean serum CRP was significantly

higher among HIV infected children compared to apparently healthy HIV-uninfected control group. This finding corresponds to earlier reports in many studies among children,[9, 26-28]Higher CRP in HIV infected children may be as a result of sustained acute phase response due to ongoing immune activation by HIV and opportunistic infections.

CONCLUSION

There was an association between the level of serum CRP and the presence of generalized lymphadenopathy among HIV infected children. This finding may suggest that the presence of generalized lymphadenopathy may be the determinant of raised serum CRP among HIV infected children.

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