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Original Research Article

The value of serum C reactive protein in the assessment of severity of HIV infection among children in a resource limited setting

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ABSTRACT

Background: Human immuno-deficiency virus (HIV) infection has devastating impact especially on well-being of children. Management of HIV infected children in resource-limited countries poses significant difficulties. The role of C-reactive protein as a potentially useful/cost-effective tool for assessing severity of HIV infection is yet to be established. The relationship between serum C-reactive protein and severity of HIV infection among children aged 6 months to 12 years was evaluated.
Methods: Authors recruited 85 children each on combined antiretrovirals for ≥3 months, and apparently healthy HIV-negative controls. Severity of HIV infection was assessed by classification into immunologic categories 1, 2, 3 and clinical categories N, A, B, C according to the Centre for Disease Control revised classification system. Enzyme linked immunosorbent assay (ELISA) was used for serum CRP estimation. CD4 count was estimated by flow cytometry. Data analysis was with Statistical Package for Social Sciences version 20.
Results: Response rate was 160 (94.1%) with mean age of participants being 8.50±3.36 years. Male to female ratio was 1.35:1. Lymphadenopathy was the commonest clinical feature in 26 (32.50%) participants, 59 (73.75%) participants were asymptomatic, and 52 (65.00%) were immunologic stage 1. Median serum CRP of HIV infected group and controls were 4.2 (1-13.9) mg/l and 0.5 (0.2-1.9) mg/l respectively. There was an association between Clinical and immunolological stages of infection and levels of serum CRP, p values = 0.001 and 0.002 respectively.
Conclusions: The serum level of CRP may predict the severity of HIV infection among children.

Keywords: C- reactive protein, Cost-effective tool, Children, Severity of HIV infection

INTRODUCTION

Human immuno-deficiency virus infection/Acquired immune deficiency syndrome (HIV/AIDS) is an epidemic that has aroused global responses with significant accomplishments in the past decades.[1,2] Its associated morbidity and mortality remains a global concern with tremendous impact on children.

Though the Joint United Nations Program on HIV/AIDS (UNAIDS) in November 2016 noted a 50% global decline in new infections in children from 2010 figures, the incidence and prevalence rates of the infection still remained high.[1] In another publication by the organization in November 2018, an average of 1.8 million new infections occurred globally in the year 2017 and 180,000 of them were in children.[3]

HIV infection is a lifelong infection with progressive rise in viral RNA and a corresponding fall in CD_4 cell count.[4] Like other lifelong diseases, it requires reliable means of classification to guide in monitoring of disease

progression and response to treatment, prognostication and decision making on management options. The World Health Organization (WHO) staging of HIV infection is based on clinical assessment, while the Centre for Disease (CDC) classification is based on clinical and immunological derangements.[5,6] The two systems were developed to categorize the patient with respect to immunosuppression and disease progression. A number of tests are available for monitoring the progression of HIV infection and response to treatment, but the commonly used tests are viral load and CD_4 cell count. In resource-limited countries, these tests are too expensive for many patients and are not available at all in many secondary health care facilities, hence the need for less expensive modality of assessing severity of infection and response to treatment. It was found in Uyo, south-south Nigeria in 2015 that majority of HIV infected children belong to families with low socioeconomic status.[7] This negatively influences their access and affordability of health care services with attendant poor outcome.

C-reactive protein (CRP) is an acute phase protein produced by the liver during systemic inflammation and is assumed to play important role in the host defence against tissue damage and infection.[8] It was first discovered by Tillet and Francis in 1930, in patients infected with *Streptococcus Pneumonia*.[9] In the past, it was difficult to detect the protein in the sera of normal humans using the traditional methods such as precipitation with C-polysacharide (CPS), capillary immunoprecipitation and latex agglutination due to low sensitivity.[10] The protein can however be quantified more precisely with newer methods such as laser nephelometry, enzyme linked immunosorbent assay (ELISA) and Immunoturbidimetry.[10]

HIV infection in the absence of other infections has been found to elicit acute phase proteins response characterized by higher concentrations of positive acute phase proteins.[11,12] Higher values of CRP have been demonstrated in HIV infected patients with or without inter-current infections.[12-15] The protein was identified as a potential prognostic marker that can be used to monitor HIV infected individuals.[16,17] Drain et al, demonstrated cost advantage with the use of c-reactive protein as a marker to monitor HIV infection when compared to viral load and Cd4 count. The usefulness of testing for serum CRP in HIV infected children has not been established and studies are scarce on this subject. It is necessary to conduct more studies on serum C - reactive protein among HIV infected children to discover useful knowledge that may contribute to patient care.

METHODS

Ethical clearance was obtained from the University of Uyo Teaching Hospital Institutional Health Research Ethical Committee before commencement of the study. The study was carried out from April to November 2018 in the children's out-patient clinic of the University of Uyo Teaching Hospital (UUTH), Uyo, Akwa Ibom State, Nigeria. It was a descriptive cross sectional study. Eighty-five HIV infected children aged 6 months to 12 years who had been on 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 acute symptoms of illness not directly associated with HIV such urinary tract infection and upper respiratory infection or the presence of chronic conditions not associated with HIV infection such as Sickle cell anaemia and congenital heart disease. Data generated through history taking, physical examination, investigations and review of case notes was documented in case control form and used to screen, recruit and classify respondents. Bedside urinalysis findings, using medi-test combi 9 urine dipstick (K39927, Macherey-Nagal) was used to screen for the presence of urinary tract infection and metabolic abnormalities. 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. The Centre for Disease (CDC) 1994 revised classification system for HIV infection in children less than 13 years of age was used.[18] The absolute CD_4 cell count and other laboratory results such as packed cell volume, chest X-ray, genexpert test for tuberculosis as well as clinical findings from history, physical examination and review of case notes were used to classify the respondents into immunologic categories 1, 2, 3 and clinical categories N, A, B and C. The anthropometric measurements were done using standard techniques. Weight was measured to the nearest 0.1kilograms using the solar seca bathroom scale and basinet scale for younger children. The standing height was measured to the nearest 0.1 centimetre using a stadiometer for children above 2 years of age while infantometer was used to measure the recumbent length of younger children.

The body mass index (BMI), BMI z-scores, weight for age z-scores, weight for height z-scores and height for age z-scores were determined using the SPSS script of the centre for disease control. The nutritional status of the respondents was determined from the findings. Deoxyribonucleic acid Polymerase chain reaction (DNA PCR) was used for diagnosis of HIV infection in children aged 6weeks to 18 months using lasec DBS (Dried blood sample) kit while antibody test was used to make the diagnosis for children aged 18 months and above using Alere determine kit (7D2343, Japan). Confirmation of the antibody test was done using Trinity Biotech Uni-gold kit (1206502, Ireland), while Stat-Pak kit (44033015, New York, USA) was used as tie breaker. Enzyme linked immunosorbent assay (ELISA) colorimetry, using Calbiotech highly sensitive CRP kit (HS-CRP CR120C, California, USA) was used to test for serum CRP. This method is a quantitative assay that can detect the level of

serum CRP in healthy individuals and has sensitivity of 0.005mg/l.

Serum CD_4 count was estimated by flow cytometry using Partec cyflow counter. The machine uses the principle of alignFree™ technology to perform true volumetric absolute counting (TVAC). The particle (CD_4 cells) concentration C is equal to the number (N) of counted particles in a given volume of blood V.[19]

$$C = N/V \text{ cells}/\mu l$$

The procedure was carried closely following the manufacturer's instructions. The absolute CD_4 cell count obtained was used to classify the respondent into the corresponding immunologic category. Data analysis was done using Statistical package for social sciences (SPSS) version 20. Categorical data were summarized using frequency and percentage. Quantitative data summarized using mean and standard deviation as well as median and interquartile range. ANOVA (f-test) was used to test for association between means of groups while Ranksum test was used to test for association between median of groups. The level of significance was taken as $p < 0.05$.

RESULTS

One hundred and seventy children were recruited; eighty-five into the study group and equal number as controls. Five participants were excluded from each of the groups due to sample spillage and voluntary withdrawal. One hundred and sixty (160) children completed the study, 80 in each group, giving a response rate of 94.1%.

Table 1: Demographic characteristics of the study participants.

Variable	Study group		Controls		Total	
	N	(%)	N	(%)	N	(%)
Age (Years)						
< 1	1	1.25	1	1.25	2	1.25
1-4	14	17.5	14	17.5	28	17.5
5-12	65	81.25	65	81.25	130	81.25
Total					160	100
Gender						
Male	46	57.5	46	57.5	92	57.5
Female	34	42.5	34	42.5	68	42.5
Total	80	100	80	100	160	100

The mean age of the participants was 8.50±3.36 years. Sixty-five (81.25%) children in each group were within the ages of 5 to 12 years, 14 (17.50%) participants in each group were within the ages of 1year to 4 years while one (1.25%) respondent in each group was less than 1 year of age. Ninety-two (57.50%) males and 68 (42.50%) females participated in the study with forty-six (57.50%) males and 34 (42.50%) females in each group. The male to female ratio was 1.35:1. The demographic characteristics of the participants are shown in Table 1 above. Table 2 demonstrates the distribution of clinical features derived from review of participants in the study group. The commonest clinical feature was lymphadenopathy, identified in 26 (32.50%) participants; while the least common clinical features were recurrent/chronic otitis media and splenomegaly each noted in one (1.25%) participant respectively.

Table 2: Clinical features among the study participants.

Variable	Frequency	Percent
Lymphadenopathy	26	32.5
Poor Weight gain/Weight loss	20	25.0
Dermatitis	7	8.8
Cough >1month	7	8.8
Pulmonary tuberculosis	5	6.3
Hepatomegaly	3	3.8
Anaemia	3	3.8
Recurrent/Chronic diarrhoea	2	2.5
Recurrent/Chronic otitis media	1	1.3
Splenomegaly	1	1.3

Table 3: CDC classification of the study 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 immune-suppression, 3 = Evidence of severe immune-suppression.

Fifty-nine (73.75%) and 52 (65.00%) HIV infected children were classified under the asymptomatic (clinical stage N) and no suppression (immunologic stage 1) stages of HIV infection respectively. None of the participants was severely symptomatic (clinical stage C), while 7 (8.75%) of them had severe immune suppression (immunologic stage 3). The classification of participants in the study group is as shown in Table 3 below. The median serum CRP of HIV infected and uninfected children as shown in table 4 were 4.2 (1-13.9) mg/l and 0.5 (0.2-1.9) mg/l respectively. The table also shows the median CRP for participants in the control group stratified according to age and gender. Wilcoxon rank-sum test for association showed no statistically significant association between median CRP and age ($p = 0.49$).

Similarly, no significant association was observed between median CRP and gender (p = 0.17). Fisher's exact test demonstrated significant association between the clinical and immunologic stages of HIV infection and mean serum CRP values (p = 0.001, 0.002) among participants in the study group as shown in Table 5. Pearson's correlation analysis found a weak negative statistically insignificant relationship between CD4 count and the CRP of respondents from 5 to 12 years (r = -0.08, p = 0.50).

Table 4: Median serum CRP of the participants and its association with socio-demographics among the control group.

	Age group (in years)	Frequency	Percent	Median CRP (IQR)mg/l	Rank-sum test p-value
Study group		80	100	4.2 (1-13.9)	
Control		80	100	0.5 (0.2-1.9)	
	Less than 1	1	1.3	0.1 (0.1-0.1)	p=0.49
	1-4	10	12.5	0.4 (0.2-1.3)	
	5-12	69	86.2	0.5 (0.2-2.0)	
Gender					
	Male	45	56.25	0.4 (0.1 -1.0)	p=0.17
	Female	35	43.75	0.7 (0.2-3.3)	

Table 5: Association of mean serum CRP levels with stages of HIV infection.

Variables	Mean	SD	F test	p value
Clinical stage				
N	5.59	8.68	11.14	0.001
A	17.18	12.94		
B	17.33	11.68		
Immunological Stage				
1	8.39	10.88	6.51	0.002
2	5.13	6.58		
3	21.15	13.61		

DISCUSSION

Mobilization of human and material resources in addition to focused research and development of novel interventions and practices remain the panacea to cubing the impact of HIV infection in low income countries. The finding that majority of the HIV infected children who participated in this study were within the ages of 5 to 12 years may be a reflection of declining incidence of Paediatric HIV infection due to the gains of prevention of mother to child transmission and improved survival rate among HIV infected children in the study area. It is comparable to other reports from studies in Cameroun and Senegal, where majority of the participants were aged 5 to 15 years and 5 to 10 years respectively.[20,21]

Male preponderance observed in this study may be related to the practice of male child preference in the region, which may influence the health seeking behaviour of parents and caregivers for male children compared to females, thereby affecting gender proportions.[22] Many studies in Nigeria on Paediatric HIV infection reported similar finding.[23-25]

The median serum CRP of apparently healthy control group in this study falls within normal serum CRP of 1mg/l documented for healthy individuals.[26] It is comparable to the earlier finding of 0.45mg/l, 0.5mg/l and 0.43 mg/l in Brazil, Italy, and Chile respectively.[10,27,28] Present study found no association between median serum CRP and age among apparently healthy children contrary to an earlier observation by Hokama and Nakamura, where CRP appeared to progressively increase with age.[29] Schlench et al, on the other hand noted a slight negative age trend in median CRP concentration among children in Europe.[27] The absence of significant association between median CRP and gender among apparently healthy children in the present study corroborates with an earlier documentation by Monica Acevedo et al, where no significant difference was noted between median serum CRP in male children compared to female. Schlench et al, on the other hand found a slightly higher serum CRP in girls than in boys of the same age while Ford et al, and Shanahan et al, noted significant increase in serum CRP in females from the age of 15 years.[27,28,30,31] The increment was attributed to inflammatory challenge among females occasioned by physical and behavioural changes of adolescence as against anti-inflammatory effect of male sex hormones.[31] Mean serum CRP was found to be significantly associated with CDC clinical/immunologic categories of HIV infection in this study. HIV infected children in clinical category B and immunologic category C had the highest levels of serum CRP. The reason for this finding may be explained by the fact that HIV infection causes progressive decline in CD_4 count and predisposition to opportunistic infections which are responsible for

deteriorating clinical condition of the patient and corresponding rise in serum CRP.[30,32] Similar findings were reported earlier by Wadgera et al, and Ugwu et al, among adult populations. A baseline serum CRP and follow-up monitoring of serum levels of the protein may be useful in assessing the severity and progression of HIV infection.[32,33]

A weak negative and statistically insignificant correlation was noted between serum CRP and absolute CD_4 count among HIV infected children. This finding is consistent with previous report from a study in 2008 involving children, adolescents and young adults, where CRP did not vary significantly with CD_4 %.[34] Other investigators in adult populations however documented strong negative correlation between serum CRP and CD_4 count.[14,16] The reason for the observed difference in findings is yet to be identified.

CONCLUSION

There was an association between the levels of serum CRP and the stages of HIV infection in children, indicating that serum CRP may be valuable in evaluating HIV infected children. There is need for longitudinal studies that demonstrates the changes of CRP over time following the commencement of HAART to establish the usefulness of estimating serum CRP in the management of HIV infection.

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