Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 1194 - 1203

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

C-REACTIVE PROTEIN AS AN EARLY MARKER OF OPPORTUNISTIC INFECTIONS IN HIV

NAGESH.Y.WADGERA*[1], KALA YADHAV.M.L[2] AND NAGARAJA.B.S[3]

[1]3rd Year MBBS student, BMCRI [2]Associate professor of Microbiology, BMCRI
[3]Professor of Medicine, BMCRI

ABSTRACT

Opportunistic infections account for the majority of death in untreated patients with AIDS. CRP is a highly sensitive marker of infection & inflammation and its levels increase with infection. The present Study was undertaken among 1oo HIV+ patients, at ART center Victoria Hospital Bangalore. With the informed consent of the patient, a generalized proforma was filled up consisting of patient's clinical presentation and diagnosis. Their CRP level and CD4 count were measured. 56 HIV+ patients were asymptomatic and acted as control giving a negative test for CRP (<6mg/l), Showing no base line rise in CRP. Patients with infectious diagnosis showed a positive test for CRP, while patients on treatment were negative. CRP levels as high as 192mg/l were found in patients with endometrial cancer. Among the infectious cases, bacterial infection showed high level of CRP (mean 32mg/l) compared to viral/fungal infection (mean 9mg/l). Combinations of opportunistic infections produced a high level of CRP (mean 45mg/l). A graph of CRP along x-axis and CD4 count along Y-axis were plotted which showed a negative correlation ($r=-0.2324$, $p<0.01$ and $|z|=2.40$). From the graph, the CRP level at which ART can be started is >92.413mg/l [taking <200 (cells/μ l)as the CD4 count at which ART is started]. Patients showing negative test for CRP need not be started with ART, as their CD4 count is found to be approximately 329 cells/μl. CRP level in HIV patients has a prognostic significance and can be used as an early marker of Opportunistic infections.

KEYWORDS- C-reactive protein (CRP), Opportunistic Infections (OI), HIV+

NAGESH.Y.WADGERA
3rd Year MBBS student, BMCRI

**Corresponding author*

INTRODUCTION

Opportunistic infections account for the majority of deaths in untreated patients with AIDS. The actual frequency of opportunistic infections varies in different regions of the world. CRP, an acute phase protein mostly synthesized in the liver as a result of expression of cytokines such as IL-6, is an important component of the innate immune system. CRP is a highly sensitive marker of infection and inflammation and its levels increase with infection and inflammation. In healthy individuals, its concentration is less than 6mg/l in serum. However, during inflammatory response or infection, the levels may increase to 1000 fold[1]. Increased CRP levels may be detected as early as (5-10) hours after tissue damage. Elevated levels of CRP can usually be demonstrated in case of acute myocardial infarction, rheumatoid arthritis, bacterial and viral infections, acute rheumatic fever, with or without carditis, and in several types of malignancies, particularly those with metastasis. HIV is a progressive infection accompanied by destruction of the immune system largely through depletion of CD-4 cells. Fever, which is the result of cytokine mediated effects during acute phase response caused by tissue injury or inflammation leads to changes in serum protein levels. Fever and the rise in circulating concentrations of CRP are commonly used in clinical medicine for diagnosis of various infections and to monitor the response to Therapy[1]. Fever and other symptoms in an HIV infected individual are normally caused by opportunistic infections, which do cause a rise in CRP concentration[2&3]. However the relation between CRP concentration and Human Immunodeficiency Virus is still unclear. At present CD-4 count and HIV-RNA assay are potent markers of prognosis of HIV infection. But measurement of HIV-RNA level is highly expensive and not used in most of the hospitals. Low values of CRP have been shown to predict longer survival within HIV-infected individuals[4-7].It has been suggested that the measurement of CRP levels may be an inexpensive method for the study of prognosis of HIV infection and can be used as a vital tool to monitor the anti-retroviral therapy[4-7]. Infections in people living with HIV reflect the immune suppression of the host. Hence, CRP can be used as a marker of degree of immune suppression. In this study, we have tried to correlate CRP with other parameters of immune suppression in HIV patients in a tertiary care center. CRP assays being cost effective, labour intensive and takes much less time, is one of the best markers of opportunistic infections and can be used in all primary health setups.

MATERIALS & METHODS

The present study was conducted for a period of two months at ART center, Victoria Hospital, Bangalore. 100 HIV+ patients were enrolled for the present study, among which 44% were symptomatic and remaining 56% were asymptomatic and acted as controls.

Inclusion criteria

All Patients above the age of 18 years seropositive for HIV-antibodies and attending ART center at Victoria hospital, Bangalore.

Exclusion criteria

(a) Patients below age of 18 years.
(b) Patients on ART therapy.
(c)Patients clinically diagnosed with AIDS.

The pre-ART patients were enrolled for the study with their informed consent. All the patients were tested for the HIV antibodies at the ICTC Infosys central laboratory Victoria hospital, Bangalore. Patients were explained about the study in detail in simplest manner possible as they could understand in their own mother tongue. Signature of the patient was taken on the consent form as a mark of their

approval in presence of the ART counselor. Permission to conduct the study was also taken from the KSAPS (Karnataka State AIDS Prevention Society). A generalized proforma was filled up with clinical history of the patient, presenting complaints, past clinical history and diagnosis. The study population consisted of varying age, all above 18 years. Most of the patients were of low socio-economic status. However age, sex, social background were not considered in the study.

Analytical methods Sample

The blood sample was collected under standard precautions at ART center for measurement of CD4 count in an EDTA vial and for CRP in a sterile plane vial without anticoagulant. The samples were immediately brought to the Microbiology lab for further processing. For CRP measurement, the serum sample was used and processed in the serology lab of the microbiology laboratory. CRP estimation was done by latex slide and tube test a diagnostic reagent kit for the in vitro detection of CRP in human serum by qualitative and semi quantitative rapid latex slider test. The principal behind the test is an immunological reaction between CRP as an antigen and latex particles that have been coated with mono specific anti human CRP sensitized to detect the level of greater than 6micrograms per ml[8]. CD4 COUNT- Peripheral blood samples were stained with monoclonal antibodies by means of whole lysing method and analyzed by means of 2 color flow cytometer FAC CALIBER and antibodies specific for CD3, CD4, CD8 lymphocytes.

RESULTS

Total of 100 HIV positive patients was enrolled for our study. Out of 100patients, 56% patients were asymptomatic and acted as control were CRP was negative (< 6mg/l). 44% patients were symptomatic patients with infectious /non infectious diagnosis (Figure no1). 56 patients were asymptomatic with generalized complaints such as anorexia, fatigue, and myalgia. The average CD4 count was found to be 421cells/µl and all showed negative test for CRP (<6mg/l). There was no base line rise in CRP among asymptomatic HIV patients. Out of 44 patients, 24 had infectious diagnosis, 12 had Non Infectious Diagnosis and 8 were on treatment for underlying opportunistic infection. Of 24 infectious cases bacterial were 18, viral 1, fungal 2, 2 were febrile cases and one case of unknown infection. 9 patients diagnosed TB with 8 being PTB and 1 extra pulmonary TB showed high level of serum CRP (41mg/l) and low CD4 count (130cells/µl). However CRP showed negative test with treatment to TB without much affecting the CD4count. CRP further decreased with the progression of the treatment. 4 patients with TB and Diarrhea showed a mean CRP of as high as 45mg/l and a low CD4 count of 72cells/µl. Mean CRP of 4 Diarrheic patients was also high (14.3mg/l) with low CD4 count. Treatment of diarrhea resulted in negative test for CRP. In case of fungal/viral infection like Oral thrush/herpes also showed mean CRP of 9mg/l. 2 febrile patients also showed high CRP of 24mg/l with low CD4 count of 34cells/µl. (figure no-2) Out of 12 non infectious cases, 1 was migraine, 2 cases were endometrial carcinoma, 1 of hypertensive-diabetic and 1 of allergic sinusitis. All these cases were found to be CRP positive. Other Non Infectious Diagnosis(ONID) 7 included cases of arachnoids cyst, congenital defective urethral valve, ANC, postpartum women, lipoma, schizophrenic which were CRP negative(<6mg/l)[figure no-3]. 2 patients with endometrial carcinoma, showed a very high level of CRP, as high as 192mg/l, with a good CD4 count of 697cells/µl. [figure no-3]. Patients with allergic sinusitis, migraine, hypertensive-diabetics also showed lower increase in CRP with good cd4 count. CRP was found to be high with opportunistic infection. CRP was much higher in bacterial infection than in the fungal or viral infection. Asymptomatic patients gave negative result for CRP test. There was clear cut direct correlation between CRP and opportunistic infection which did not vary with

age, sex or other parameters. CRP lowered with treatment of underlying opportunistic infection. There was a negative correlation between CRP and CD4 count showing the prognostic significance of CRP in HIV, as shown in the graph (figure no- 4).

FIGURE 1

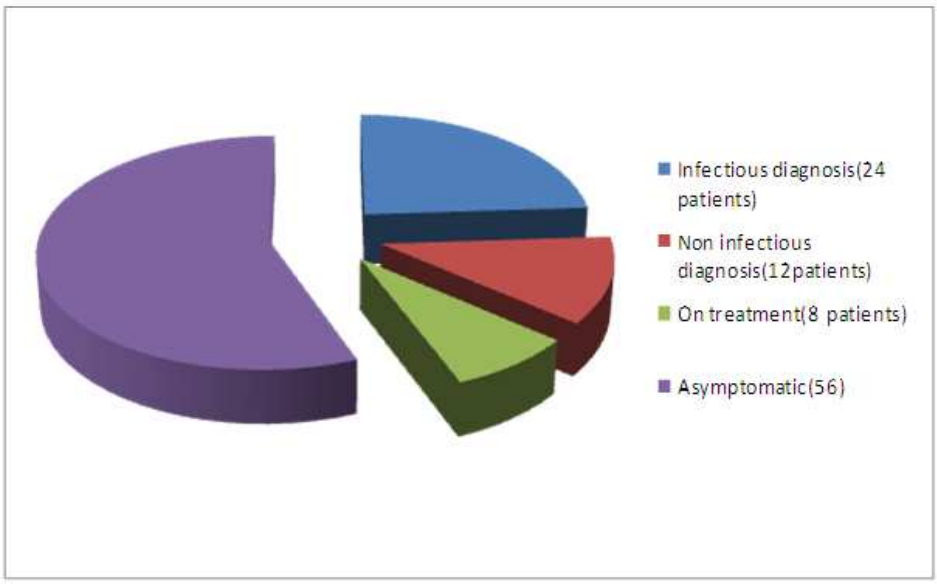
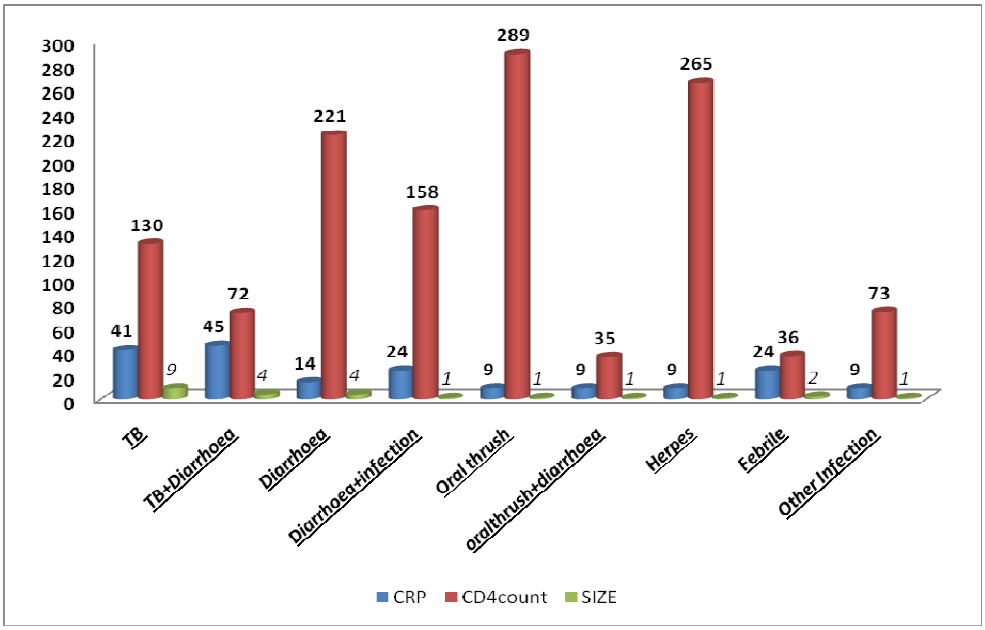


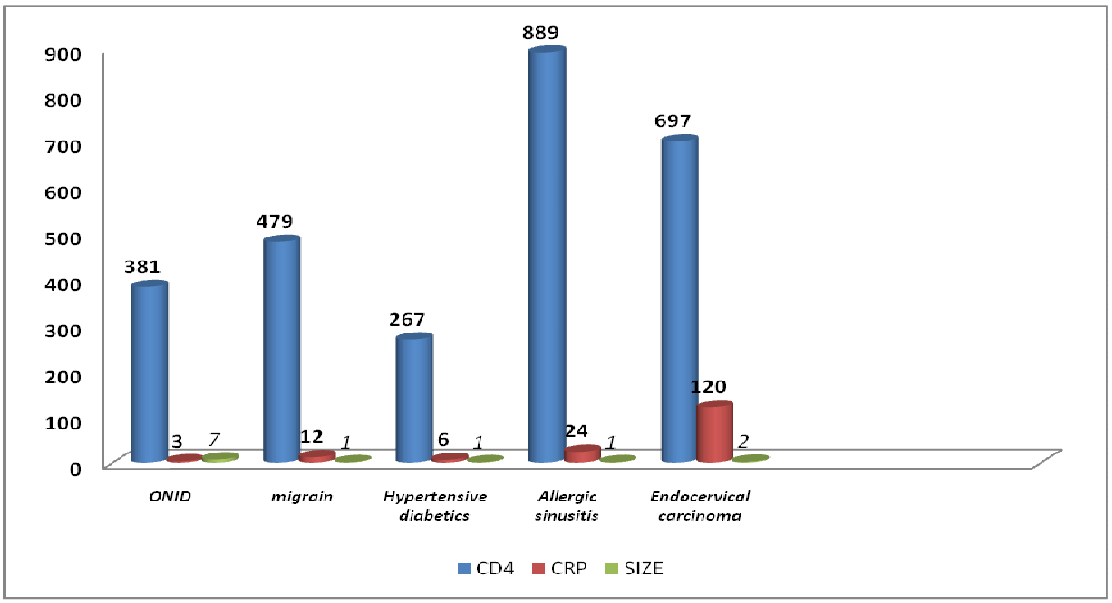
Figure showing, Case distribution with the sample size.

Figure 2



Graph showing rise in CRP level with Opportunistic infections, CD4 count and sample size. Greater the severity of opportunistic infection more is the rise in CRP. In cases of TB with diarrhea, CRP was found to be high. In cases of bacterial infection, CRP rise was high compared to viral or fungal. CD4 count was low in cases of bacterial infection compared to Viral/fungal.

Figure 3



Graph showing rise in CRP in case of non infectious diagnosis (NID). CRP was much higher in case of endometrial carcinoma with a good CD4count.Other Non Infectious Diagnosis (ONID) showed negative test for CRP.CD4 count was quite high compared to infectious patients.

Figure 4

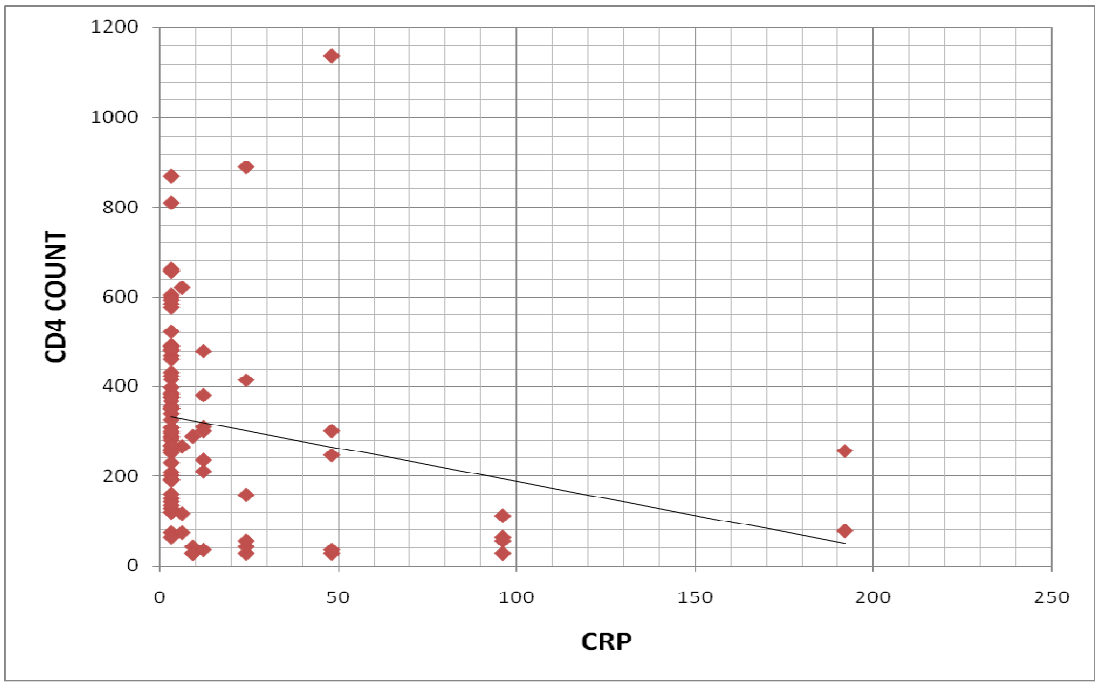


Figure showing relation between CD4 count and CRP.y=338.37-1.4973x is the equation of the line (r=-0.2324 and p<0.01(|z|=2.4).From the above graph CRP level at which ART can be started is >92.413mg/l(taking <200 cells/μ l as the CD4 count at which ART is started).Hence a symptomatic patient with CRP>92.413 should definitely be started on ART(ruling out any underlying inflammatory state). Asymptomatic patients without any underlying inflammatory conditions will have a minimum CD4 count of approximately 329 cells/μl (substituting x=5.9 in the above equation).

TABLE 1

DIAGNOSIS	*SIZE*	*Mean CRP (in mg/L)*	*Mean CD4 count (cells / µl)*
ASYMTOMATIC (CONTROLS)	***56***	***<6***	***421***
INFECTIOUS			
1)BACTERIAL			
TB	9	41	130
DIARHOEA	4	14.3	221
TB+DIARHOEA	4	45	72
DIARHOEA+OTHER INFECTION	1	24	158
	18	**31.1**	**145**
2)FUNGAL			
ORAL THRUSH	1	9	289
DIARHOEA+ORAL THRUSH	1	9	35
	2	**9**	**162**
3)VIRAL			
HERPES	1	9	265
	1	**9**	**265**
4)OTHER INFECTION			
FEBRILE	2	24	34.5
CNS INFECTIONS	1	9	73
	3	**16.5**	**53**
NON INFECTIOUS			
MIGRAIN	1	12	479
HYPERTENSION+DIABETIS	1	6	267
SINUSITIS	1	24	889
ENDOMETRIAL CARCINOMA	2	**120**	**697**
ONID	7	<6	381
	12		
ON TREATMENT			
TB	6	**<6**	159
DIARHOEA	2	**<6**	313
	8		
	44		

Distributions of cases, showing mean CRP (mCRP) and mean CD4count in different cases.

STATISTICAL ANALYSIS

A graph of CRP along x-axis and CD4 count along the y-axis is plotted. CRP was the independent variable with CD4 count being dependent Variable. Z-test method of significance was employed for the analysis. No statistical software's were used for the analysis and was done manually with the help of an expert statistician. It showed a negative correlation($r=-0.2324$) between them, which is highly significant ($p<0.01$) at 1% level of significance. The linear regression line of CD4 count on CRP is drawn as shown in the Figure no-4. $y=338.37-1.4973x$ is the equation of the line .($r=-0.2324$ and $p<0.01(|z|=2.4)$.From the graph CRP level at which ART can be started is >92.413mg/l(taking <200 cells/µ l as the CD4 count at which ART is started).Hence a

symptomatic patient with CRP>92.413 should definitely be started on ART(ruling out any underlying inflammatory state).Asymptomatic patients without any underlying inflammatory conditions will have a minimum CD4 count of approximately 329 cells/µl (substituting x=5.9 in the above equation) .

DISCUSSION

The asymptomatic HIV patients showed negative test for CRP (<6mg/L), thus there was no baseline rise in CRP. Mahdad Noursadeghi and Robert F Miller (2005) UK[11] proposed that the base line CRP levels in the general population are <3mg/L. In HIV positive patients it was found to be higher than the general population and it was 5.9mg/L and possibly reflects sustained acute phase response as a consequence of HIV infection. Bryan lau et al(2006)[14] showed in their study, lower level of CRP concentration predicts longer survival within HIV infected population .For a population with on-going HIV infection ,level of CRP was shown to be relatively low(<4mg/l),which indicates HIV is not a highly inflammatory state. Baseline of 3.52mg/l was suggested by a Vellore based study[16]. In our present study all asymptomatic patients with No clinical symptoms gave negative results for CRP done by latex agglutination method, which detects CRP greater than or equal to 6mg/l concentration. As suggested by Mahdad Noursadeghi and Robert F Miller[11] there might be slight/no elevation of CRP in HIV patients, but is insignificant.

We had three basic observations, firstly the infectious patients having some opportunistic infection and presenting with clinical symptoms, Secondly, patients with non infectious diagnosis and thirdly patients with the treatment history of underlying opportunistic infection. Infectious cases were all positive for CRP. The rise in CRP was more with bacterial infection. The viral and fungal did produce increase in the CRP level, but was not as high as that of the bacterial. CRP was very high in case of combination of opportunistic infections. Lawn S D et al[12] suggested that the serum CRP in HIV infected persons increase only in presence of opportunistic infections. Chalmer J D et al[13] showed CRP as an independent predictor of severity in community acquired pneumonia. CRP was elevated in patients with Pneumocystis Carini Pneumonia (120mg/l) and TB showed as high as 44mg/l. In our study too, patients with TB had an average CRP of 42mg/l. Grutzmeier s et al[7] says patients with the opportunistic infection of bacterial origin have higher rise in CRP compared to patients with other opportunistic infection. Mahdad Noursadeghi and Robert F Miller[11] show CRP to be elevated in HIV patients and were much higher in bacterial infections. Febrile cases were CRP positive. Mahdad Noursadeghi and Robert F Miller[11] found CRP to be positive in febrile patients and showed decrease in its level with treatment. Pepys Mark B et al[1] says CRP is elevated in Cases of fever, since fever being a cytokine mediated process. In the present study two cases with fever showed an elevated CRP of about 24 mg/l. Patients diagnosed with non infectious diagnosis like endometrial carcinoma, sinusitis, migraine, and hypertensive diabetics showed positivity for CRP with a good CD4 count. A study conducted by Dimitrios Trichopoulos et al[15] shows that the increased plasma CRP level is a potential marker of increased cancer risk. This correlates with our study were a case endometrial carcinoma showed a very high CRP level of 120mg/l with good CD4 count.

With the treatment of underlying opportunistic infection the CRP level decreased progressively and showed a negative test. E k sage et al[9] showed in those with CRP value that remain elevated after > 4days of treatment there was a greater risk of treatment failure and increased mortality. A study conducted by R Kannangai[16] shows, compared to healthy individuals & asymptomatic, HIV-1 infected individuals not on ART: the mean CRP level was high in

symptomatic HIV-1 infected individuals who underwent ART, similarly after ART there was a drop in the mean CRP level. However there was no much significant difference. In the present study there was no base line rise in the CRP. The HIV patients with no opportunistic infection showed a negative test. This clearly shows CRP is independent of HIV viral infection and is due to the opportunistic infection. We would suspect a transient rise in CRP during an initial few days of contracting HIV infection. Such cases are impossible to study as are rare. .Opportunistic infections showed positive for CRP test and further the CRP was found to be high with the bacterial infection & least in viral/fungal infection. However CRP in itself is a nonspecific marker and could not show a clear cut level for particular opportunistic infections. CRP might be organism sensitive as it showed a very high level in bacterial infection than the other infections. With treatment of the underlying opportunistic infection, the CRP level slowly decreased to negative. CRP levels were thus a reflector of opportunistic infection and thus are a marker of opportunistic infection. Relation of CRP with cd4 count showed a negative correlation. The graph of CRP against CD4 count clearly shows that with increase in CRP, CD4 count decreases.

CONCLUSION

There is no base line rise in CRP due to HIV infection. The acute phase response produced by the HIV is highly insignificant.CRP increases with the Opportunistic infection, more being in the bacterial infection & combination of infections. Hence CRP is a marker of Opportunistic infection. Certain Non –infectious condition involving underlying inflammatory process like cancer, migraine, hypertension, diabetics also cause rise in CRP, but the rise is much higher in Cancer.CRP level decrease with treatment of underlying opportunistic infection. By our study, Asymptomatic patients without any underlying inflammatory conditions would have a minimum CD4 count of approximately 329 cells/µl. Negative test for CRP implies that patient does not have an Opportunistic infection & is with good CD4 count (>200 cells/µl) and ther is no need to be started with the anti-retroviral therapy without even doing CD4assay.CRP above 92mg/l indicates CD4 count less than 200. Based on our findings, we suggest immediately starting ART in patients with CRP levels of more than 92mg/dl**.**

SUGGESTIONS

All the HIV patients at their nearest primary health care center should undergo periodic CRP assay at least once in a month to keep in check their opportunistic infections. For an HIV positive asymptomatic patients it is generally advised to have CD4 count done, every three months. The yearly expenditure ratio for CRP assay and CD4 count would be 1:4, which shows cost effectiveness of CRP assay over CD4 count. CD4 count and HIV RNA assay are advised rottenly depending on the clinical scenario of the patients. Positive test for CRP implies some underling opportunistic infections, which should be evaluated further by doing CD4 count and other relevant tests to manage the patients. An asymptomatic HIV positive patient with Negative test for CRP would suggest that the patient is with good CD4 count and is probably free of Opportunistic infections. It can be easily done in a primary health setup where as CD4 count is done at few tertiary care centers. Hence CRP is a very significant marker of opportunistic infections in developing nation having large rural background like India. CRP assay can also be used as a guide to start ART therapy where in situation CD4 count could not be done.

REFERENCES

1. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003 Jun;111(12):1805-12. Review. Erratum in: J Clin Invest. 2003 Jul;112(2):299.
2. Florence E, Bottieau E, Lynen L, Colebunders R. Patients with HIV infection and fever: a diagnostic approach. Acta Clin Belg. 2002 Jul-Aug;57(4):184-90.
3. Sullivan M, Feinberg J, Bartlett JG. Fever in patients with HIV infection. Infect Dis Clin North Am. 1996 Mar;10(1):149-65.
4. Feldman JG, Goldwasser P, Holman S, DeHovitz J, Minkoff H. C-reactive protein is an independent predictor of mortality in women with HIV-1 infection. J Acquir Immune Defic Syndr. 2003 Feb 1;32(2):210-4.
5. Melchior JC, Niyongabo T, Henzel D, Durack-Bown I, Henri SC, Boulier A. Malnutrition and wasting, immunodepression, and chronic inflammation as independent predictors of survival in HIV-infected patients. Nutrition. 1999 Nov-Dec;15(11-12):865-9.
6. Süttmann U, Ockenga J, Selberg O, Hoogestraat L, Deicher H, Müller MJ. Incidence and prognostic value of malnutrition and wasting in human immunodeficiency virus-infected outpatients. J Acquir Immune Defic Syndr Hum Retrovirol. 1995 Mar 1;8(3):239-46.
7. Grützmeier S, Sandström E. C-reactive protein levels in HIV complicated by opportunistic infections and infections with common bacterial pathogens. Scand J Infect Dis. 1999;31(3):229-34.
8. Tillett WS, Francis T. SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS. J Exp Med. 1930 Sep 30;52(4):561-71.
9. Sage EK, Noursadeghi M, Evans HE, Parker SJ, Copas AJ, Edwards SG, Miller RF. Prognostic value of C-reactive protein in HIV-infected patients with Pneumocystis jirovecii pneumonia. Int J STD AIDS. 2010 Apr;21(4):288-92.
10. Fei MW, Kim EJ, Sant CA, Jarlsberg LG, Davis JL, Swartzman A, Huang L. Predicting mortality from HIV-associated Pneumocystis pneumonia at illness presentation: an observational cohort study. Thorax. 2009 Dec;64(12):1070-6. Epub 2009 Oct 12. PubMed PMID: 19825785;
11. Noursadeghi M, Miller RF. Clinical value of C-reactive protein measurements in HIV-positive patients. Int J STD AIDS. 2005 Jun;16(6):438-41.
12. Lawn SD, Wiktor S, Coulibaly D, Ackah AN, Lal RB. Serum C-reactive protein and detection of tuberculosis in persons co-infected with the human immunodeficiency virus. Trans R Soc Trop Med Hyg. 2001 Jan-Feb;95(1):41-2.
13. Chalmers JD, Singanayagam A, Hill AT. C-reactive protein is an independent predictor of severity in community-acquired pneumonia. Am J Med. 2008 Mar;121(3):219-25.
14. Lau B, Sharrett AR, Kingsley LA, Post W, Palella FJ, Visscher B, Gange SJ. C-reactive protein is a marker for human immunodeficiency virus disease progression. Arch Intern Med. 2006 Jan 9;166(1):64-70.
15. Trichopoulos D, Psaltopoulou T, Orfanos P, Trichopoulou A, Boffetta P. Plasma C-reactive protein and risk of cancer: a prospective study from Greece. Cancer Epidemiol Biomarkers Prev. 2006 Feb;15(2):381-4.
16. Kannangai R, Kandathil AJ, Ebenezer DL, Mathai E, Prakash AJ, Abraham OC, Sudarsanam TD, Pulimood SA, Selvakumar R, Job V, Sridharan G. Usefulness of alternate prognostic serum and plasma markers for antiretroviral

therapy for human immunodeficiency virus type 1 infection. Clin Vaccine Immunol. 2008 Jan;15(1):154-8. Epub 2007 Nov 14.
17. Walzer PD, Evans HE, Copas AJ, Edwards SG, Grant AD, Miller RF. Early predictors of mortality from Pneumocystis jirovecii pneumonia in HIV-infected patients: 1985-2006. Clin Infect Dis. 2008 Feb 15;46(4):625-33.
18. Chaudhary M, Kashyap B, Gautam H, Saini S, Bhalla P. Role of C-reactive protein in HIV infection: a pilot study. Viral Immunol. 2008 Jun;21(2):263-6.