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Association of immune status with haematology and inflammatory markers in treatment naïve HIV positive patients from a tertiary health care institute

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High and low serum ferritin level in HIV patients is associated with the progression of disease with lowering of CD4 cell count in both adults and children. Elevated C-Reactive protein (CRP) has been a better predictor of child mortality and maternal disease progression to Acquired immuno deficiency syndrome (AIDS). Data related to ferritin, CRP together with anaemia and immune status in early stage of ART naïve HIV infection is scarce. Here, we evaluated haematological parameters, inflammatory markers like serum ferritin, CRP level and their association with immune status in Antiretroviral therapy (ART) naïve HIV positive cases. After obtaining written consent, 55 screening test (Meriscreen) positive & HIV-TRIDOT confirmed, antiretroviral treatment naïve patients above 18 years from both gender were included in the study. Serum ferritin, CRP and other haematological parameters were analysed along with CD4 and CD8 count. Anaemia found prominent in group having CD4 count less than 200 cells/µL with a significant fall in Hb%. Inflammatory parameters ferritin and CRP analyzed with respect to CD4 cells documented reverse phenomena in these cases. Significant inverse relation of ferritin with Hb%, total WBC and absolute lymphocyte count points towards concurrent presence of anaemia and leukopenia with inflammation in HIV cases. The results suggest that inflammatory markers like ferritin induced by cytokines may be considered as an auxiliary parameter for assessing disease severity in HIV positive cases at time of diagnosis for early therapeutic intervention.

Keywords: Acquired immuno deficiency syndrome (AIDS), Anaemia, Anti retroviral therapy, ART naïve, C-Reactive Protein, Ferritin, Human immunodeficiency virus (HIV)

Human Immunodeficiency virus (HIV) infection affecting both cellular and humoral immunity has become a global issue. An estimated 38.4 million people are living with HIV worldwide, the resource limited developing country being the most affected one as revealed by end of 2021[1]. Peripheral haematological manifestations are the most common observation being associated with progression of disease[2]. Anaemia of multifactorial cause is the most commonly found haematological manifestation being followed by leukopenia and thrombocytopenia[3]. Information related to haematological alteration and its relation to immune status at early stage, i.e., at time of detection and before ART initiation is scarce.

Contributory role of inflammatory markers like ferritin and C Reactive protein (CRP) by immunomodulation influencing immune status of the patient plays a critical role during infections[4,5]. Elevated ferritin as a marker of acute and chronic inflammation has shown its signature in a wide range of chronic diseases like in malignancy, infection and iron overload syndrome cases[6]. Raised plasma ferritin concentrations have been shown among HIV-infected patients in some studies while others have reported low ferritin concentrations[7]. Altered ferritin has also been associated with progression of disease with clinical worsening of HIV infection lowering CD4 cell count in both adults and children[7-9]. Immunomodulatory role of ferritin selectively inhibiting delayed type of hypersensitivity has been seen in in vitro studies[10]. Ferritin's correlation with Haemoglobin (Hb%) could not be shown significant in both anemic/nonanemic HIV Positive cases taking Antiretroviral therapy (ART)[11]. Ferritin level in adults before initiation of ART as well as its relation with Hb% at early stage of HIV infection is also less described[11].

Another inflammatory marker CRP, an acute-phase protein and pattern-recognition marker of systemic

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inflammation released from liver macrophages in response to IL-6 has revealed varied association with immune activation process in HIV infected cases in late stages and with opportunistic infections[12]. High concentration of CRP has been a better predictor of child mortality and maternal disease progression to AIDS[13]. Varied results of CRP with ART have been seen in different studies showing CRP being a predictor of disease progression[14]. Negative correlation of CRP with CD4 status was also observed in the HIV positive group with CD4 counts below 200 cells/μL with high CRP value[15]. Being independent of CD4 lymphocyte counts and HIV RNA levels, the levels of CRP were associated with HIV disease progression in other studies[16]. A significantly higher CRP levels (P <0.02) with lower CD4 counts (P <0.02) was observed in HIV with TB co-infection having no significant relationship between CRP levels with CD4 count in both the groups of HIV positive cases with or without tuberculosis[17]. However, blood CRP level has been shown to be related to anaemia in chronic inflammation cases like infection, cancer, auto-immune diseases[18]. Individuals with both anaemia and high CRP have an increased risk of disease progression in HIV cases, supporting the role of anaemia in the setting of inflammation[19].

Information relating both serum ferritin and CRP with haematological alteration and immune status in adult HIV cases at early stage i.e., at time of detection before initiation of ART is scarce. Hence, in the present study, we have tried to evaluate haematological parameters as well as inflammatory markers like serum ferritin and CRP level and their association with immune status in ART naïve HIV positive cases.

Material and Methods

This study was carried out in the Department of Biochemistry, at a tertiary care institute of eastern India with Institutional Ethics Committee clearance being obtained with reference no. IEC/AIIMS BBSR/PG Thesis/2018-19/14. After obtaining written consent, 55 screening test (Meriscreen) positive and HIV-TRIDOT confirmed, antiretroviral treatment naïve patients above 18 years of age from both sex, clinically symptomatic referred by the clinicians normal or high risk cases directly attending the clinic for HIV testing were included in the study. Pregnant women, seriously ill patients, patients taking vitamins supplements, and patients unable to answer the questions were excluded from the study[20].

After noting down history and clinical examination, 5 mL of fasting venous blood was collected and tested for haematological parameters as well as for serum ferritin and CRP immediately after collection of samples. Haematological parameters were analysed by automated Sysmex XT 4000-I analyzer.CD4 and CD8 count were estimated with the help of Beckman Coulter Navios Flow cytometer. Serum CRP was estimated with Beckman Coulter AU480 auto analyser and serum ferritin by Siemens Centaur XP Chemiluminescence Immunoassay analyser. 55 cases were divided into two groups as per their CD4 count <200/ μL of blood (n=25) and CD4 count ≥200/μL of blood, respectively[15,21].

Results of this study were analyzed by SPSS-21version. All data were expressed as Median with Inter Quartile Range (IQR). Correlation was studied using Spearman's correlation study. A value of P <0.05 was considered significant.

Results

Present study included 55 HIV positive cases. About 62.5% of cases were in age group of 30-50 yr, with male:female ratio being 4:1. Majority of cases were married as well as educated. Occasional alcohol intake was recorded in 87.5% cases. About 95% of cases had history of frequent intake of non-vegetarian diet. Mean BMI was found to be 18.22 kg/m^2. As per WHO Clinical staging, 85% cases were in early stage of the disease.

Twenty-five HIV positive cases were with CD4 count less than 200 cells/μL of blood and 30 cases were with higher than 200 cells/μL of blood. CD8 and CD4/CD8 ratio also registered parallel distribution in both these groups like CD4 cells, being significantly low (P <0.001)in cases having less CD4 cells/μL (Table 1).

Analysis of Haematological parameters revealed anaemia is more prominent in group having CD4 count <200 cells/μl with a significant fall in Hb%, showing its association with immunosuppression (P <0.001). Total WBC Count also revealed significant leukopenia in case group having CD4 count <200 cells/μL (<0.05), so also the absolute lymphocyte count showing marked fall (P <0.001) in this group of cases having CD4 count <200 cells/μL in comparison to CD4 count >200 cells/μL pointing towards effect of immunosuppression on bone marrow. Whereas, RDW CV% and total basophil count did not register any significant difference between these groups.

Table 1 — Haematological and immunological parameters with respect to CD4 count

Median (IQR)	CD4 < 200 cells/ µL (n = 25)	CD4 >= 200 cells/µL (n = 30)
CD8 Count (cells/µL)	351.17 (234.47 - 510.56)	996.07 (771.41 - 1261.43)*
CD4/CD8	0.18 (0.09 - 0.29)	0.38 (0.27 - 0.54)*
Ferritin(ng/ml)	691.4 (408.56 - 1789.2)	175.6 (65.78 - 396.38)*
CRP (mg/l)	12.45 (6.78 - 24.41)	10.09 (4.51 - 11.61)
Hb (g/dl)	8.9 (7.6 – 10.4)	12.3 (10.1 – 13.9)*
WBC (x1000 cells/cu.mm)	4.15 (3.31 – 6.22)	5.70 (5.19 – 7.47)*
RDW CV(%)	16.90 (14.90 – 18.60)	15.75 (14.20 – 17.90)
Absolute Lymphocytes/cu.mm	1145 (850 – 1190)	1930 (1320 – 2410)*
Absolute Basophils/cu.mm	10 (10 – 20)	10 (10 – 20)

[*P* value<0.05 compared to CD4 < 200 cells/µL]

Table 2 — Correlation of inflammatory parameters with haematological and immune status

Parameter	Hb%		TWBC (×1000 cells/cu.mm)		Absolute lymphocyte count/cu.mm		CD4 count cells/µL		CD8 count cells/µL		CD4/CD8	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Ferritin (ng/mL)	–0.590	<0.001	–0.332	0.013	–0.446	0.001	–0.295	0.029	–0.167	0.223	–0.334	0.013
CRP (mg/L)	–0.232	0.088	0.018	0.898	–0.055	0.691	–0.144	0.296	–0.138	0.314	–0.076	0.580

[CRP, C reactive protein; Hb, Hemoglobin; WBC, White Blood Count; RDW, Red Cell Distribution Width; CV, Coefficient of Variation]

Inflammatory parameters ferritin and CRP were also analysed with respect to CD4 cells. Both of them documented reverse phenomena in these cases. Ferritin revealed significant (*P* <0.001) rise in cases having CD4 Cells <200 cells/µL of blood. CRP though showed more rise in group having CD4 count <200 cells/µL of blood, yet the difference is not significant.

Spearman's correlation revealed significant negative correlation of ferritin with Hb%, suggesting for disproportionate iron distribution between ferritin synthesis and erythropoiesis during early stage of infection. Significant inverse correlation also could be observed between ferritin with total WBC and Absolute lymphocyte count. Similar negative correlation was also noted between raised ferritin with CD4 count as well as CD4/CD8 ratio pointing towards immunosuppression (Table 2).

CRP though found raised in group having CD4 <200 cells/µL of blood, yet it registered a negative association with Hb%, total WBC, Absolute lymphocyte count as well as with immune status, though not significant (Table 2).

A receiver operating characteristic (ROC) curve analysis for diagnostic sensitivity of these inflammatory parameters during early phase of HIV infection revealed area under curve for ferritin as well as ferritin with CRP a significant association (Fig. 1), whereas CRP alone did not reveal such relation (Table 3).

Table 3 — Area Under the ROC curve

Test result Variable(s)	Area	SE	p-value	Asymptotic 95% CI	
				LB	UB
Ferritin (ng/mL)	0.797	0.060	0.000	0.679	0.915
CRP (mg/L)	0.628	0.078	0.099	0.476	0.780
Ferritin (ng/mL) + CRP (mg/L)	0.777	0.064	0.000	0.652	0.902

[CI, Confidence Interval; CRP, C reactive protein; LB, Lower Bound; UB, Upper Bound]

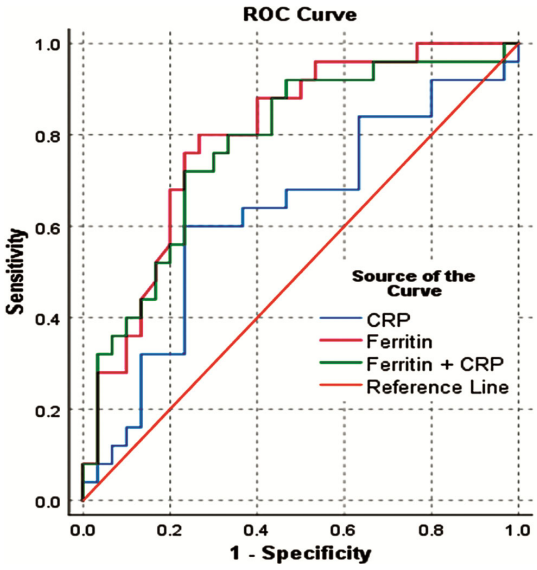

Fig. 1 — ROC curve analysis for diagnostic sensitivity of inflammatory parameters

Discussion

The present study aimed at analyzing alterations in haematological and inflammatory parameters in ART naïve HIV positive cases at the time of diagnosis, though the perturbations in these parameters have

been reported in prior studies in cases with progression of disease as well as during follow up with ART. In this study, 62.5% of the HIV positive cases were in the age group of 30-50 years with 80% male and 20% female, and young age group affected more being the moving population as suggested elsewhere[2]. Majority of cases were of low socioeconomic group, with more or less equal type of food habit. No one is a diabetic or with smoking habit. However 87.5% cases were chronic alcoholics. Preponderance of cases i.e 85% cases were in early stage of disease i.e., WHO clinical stage I & II (Table-1). Mean BMI was found to be 18.22kg/m^2, which may be attributed to their low socio-economic status, nutritional status and viral infection itself[20].

A higher prevalence of anaemia was reported previously in HIV seropositive women especially at lower CD4 levels[3,11,21]. In the present study 52.7% cases were having mild to moderate anaemia similar to another findings[3], median value of Hb% being significantly low in cases having CD4 <200 cells/μL of blood. Hb% and CD4 count did not reveal any marked association, though absolute lymphocyte count was shown to be in significant relation with CD4 count ($p = 0.006$, $r = 0.41$)in a study group of HIV positive patients stating that absolute lymphocyte count can sometimes be taken as a surrogate marker of assessing immunological status in ART naïve HIV patients, particularly in resource poor setting[22]. Absolute lymphocyte count was also reported to be a suitable cost-effective alternative for the monitoring of HIV positive patients showing a significant reduction in group having CD4 cell <200 cells/μL[23-25]. Individuals with raised viral load and lowered CD4 count had a higher prevalence of anaemia, but correlation of absolute neutrophil count and thrombocytopenia was not found statistically significant with absolute CD4 count and HIV viral load[26]. Present study also registered significant fall in total WBC and Absolute lymphocyte count in this group of HIV positive cases i.e., CD4 count <200/μL of blood. Both observation of anaemia and leucocyte count suggest towards impaired synthesis by bone marrow suppression resulting from nutritional deficit, socioeconomic factors or damage due to viral infection[26].

Raised serum ferritin level in HIV positive case has been shown previously with severity of disease irrespective of ART by different group of researchers correlating with immunosuppression defined as CD4 count <200 cells/μL, <350 cells/μL, respectively[7-9]. Contrary to this, serum ferritin along with serum folate had been documented to be significantly low in HIV positive females having low CD4 count[21].Present study revealed raised ferritin level in HIV positive cases, which is more marked in group having CD4 count <200 cell/μL at time of diagnosis, i.e., early stage of infection, which may be a reflection of inflammation due to viral infection rather than iron deficiency as suggested by others[11]. Ferritin probably induces immunosuppression by suppressing cell mediated immunity[8], but also induces IL-10 production in lymphocytes with inhibition of IL-2 production and lymphocyte proliferation[5,27]. Ferritin acts as a key player of inflammation through its signalling as part of the innate immune response as well as modulation of lymphocyte function. By binding to both T and B lymphocytes, it exhibits its immunosuppressive effects impairing T-cell proliferation, B-cell maturation and immunoglobulin production. Though these inhibitory effects are largely IL-10 and T Regs mediated, this ferritin-mediated lymphocyte suppression also includes downregulation of CD2[10].

Besides that, the present study also registered significant inverse relation of ferritin with Hb%, total WBC and absolute lymphocyte count pointing towards concurrent presence of anaemia and leukopenia with inflammation in HIV cases, which supports the explanation that uncontrolled inflammation is a contributor to these effects causing suppression of bone marrow rather than iron deficiency[22-25].

CRP plays a role in immunosuppression by binding to antigen specific T cells inhibiting both T cell signalling by suppressing immune synapse formation, early events in T Cell receptor engagement and its proliferation[28], with down-regulation of co-stimulatory molecule expression. In addition, classical complement pathway is also stimulated by binding of CRP to C1q bound to lyso-phosphatidyl choline on dead or apoptotic cells like an opsonin. It also suppresses expression of costimulatory molecules on mature dendritic cells[5].

Few researchers have documented rise of CRP and hsCRP with fall in CD4 cell in their study groups of HIV positive cases[8,13,14]. CRP was shown negatively correlated with CD4 counts, CRP value being highest in the group with CD4 counts below 200 cells/μL[15].

But no significant association was reported between CRP and CD4 cell count in the study group of HIV patients irrespective of TB co-infection[19]. Present study, though noted a rise in CRP in CD4 <200 cells/μL group in comparison to CD4 >200 cells/μL, the difference is not significant, which supports this earlier study[19].

Santos-Silva *et al*.[12] who studied a group of heterogenous patients admitted to the Emergency Department with an average prevalence of anaemic individuals of 44%, observed a significant negative relation between increased CRP and fall in Hb%, being more marked in aged population and in males revealing that fall in Hb% is seen in acute inflammation state also with opinion that monitoring of Hb% in patients with inflammatory conditions is of relevance for patients, as well as for treatment and follow-up[12].

The present study while looking for association between serum CRP level with haematological and immune parameters evaluated with Spearman's correlation did not register significant association with either Hb%, Absolute lymphocyte count, total WBC or with immune parameters, respectively.

Conclusion

Our observations in the above study reveal that though haematological alterations may be the cause or effect of the HIV disease process, inflammatory markers like serum ferritin may be considered as an auxiliary parameter for assessing disease severity at time of diagnosis of HIV positive cases. It may help in early therapeutic intervention and monitoring of patients in resource poor setting without facility for CD4, CD8 count evaluation.

Conflict of interest

Authors declare no competing interests.

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