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Role of Hematological and Alternate Markers in Human Immunodeficiency Virus Disease Progression

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Abstract: Problem statement: The knowledge of HIV infection has expanded tremendously which has led to significant improvement in the treatment, leading to decrease in morbidity and mortality. Considering the limitations in using CD4+T-cell counts and HIV RNA viral load as markers in the HIV disease progression, physicians treating the HIV patients now look for alternate markers that predict HIV disease progression and help in initiating and monitoring the Antiretroviral Treatment (ART). **Approach:** Blood samples were collected from 120 HIV sero-positive, antiretroviral therapy naïve patients attending the Integrated Counseling and Testing Centre (ICTC) of Kamineni Institute of Medical Sciences, Narketpally, Nalgonda. The study was carried out between January 2007 and June 2008. The CD4+T-cell counts, CD8+T-cell counts were estimated by Flow cytometry.TLC, AEC, HB%, ESR were performed by standard hematological methods. Latex agglutination test was used to measure CRP. **Results:** The mean CD4+T-cell counts, CD8+T-cell counts and CD4+:CD8+ratio were found to be 308±296.8 ($p<0.0001$), 976.20±548.61 ($p<0.0001$) and 0.314±0.198 ($p<0.0001$) respectively. The hematological parameters estimated included were Hemoglobin (10.7±204), AEC (391.8±180.1), TLC (8595.8±2146.9) and ESR (75.625±29) Significant positive correlation was observed between CD4+T-cell counts and AEC,TLC, HB% and CD4+:CD8+ratio. ESR and CRP was found to be negatively correlating with CD4+T-cell counts. **Conclusion:** The study findings reemphasize the importance of alternate markers to assess the stage of the disease, initiate antiretroviral therapy and monitor the response in disease progression. The study results also suggested that there is necessity to evaluate the hematological parameters before initiating antiretroviral therapy.

Key words: Hematological markers, TLC, CD4+, CRP

INTRODUCTION

Human immunodeficiency virus is the causative agent in AIDS. Since 1981, when the first AIDS cases were reported, more than 30 million people have been diagnosed as infected with HIV (UNAIDS, 2006). A large part of the infected individuals live in relatively poor and developing countries. Due to the large scale of morbidity and mortality it causes, HIV is fast becoming a major threat in developing/third world countries including the Indian sub-continent (Ramana and Mohanty, 2009). Infection with HIV is associated with prolonged latent period during which the virus continues to actively replicate, usually resulting in symptomatic illness (Miedema, 2006).

The HIV disease progression, which is highly variable in infected individuals, is characterized as rapid, typical or intermediate and late or non-progressors. The majority of infected individuals (70-80%) experience intermediate disease progression in which they show HIV RNA rise, CD4+T-cell decline and later development of AIDS related illness in 6-10 years. 10-15% of the infected patients whose CD4+T-cells rapidly decline and go into AIDS with in few years of infection are called rapid progressors. The late progressors (5%) can remain asymptomatic and healthy without showing significant changes in CD4+T-cell counts even for 10 years (Langford *et al*., 2007).

The biological basis of this variability in the disease progression is still unknown.

Many clinical and laboratory markers have been used to estimate disease progression in HIV1 infection. Markers of AIDS development include viral markers (plasma HIV RNA load, serum p24 Ag, serum anti p24 antibodies), Surrogate markers(antibodies against p17, gp 120, gp 41 and nef gene product) and nonspecific

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markers including CD4+T-cell counts, CD8+T-cell counts and Delayed Type Hypersensitivity test (DTH) (Kamat *et al*., 2008; Kannangai *et al*., 2008). Other Alternate markers include elevated serum β2microglobulin, neopterin (D-erythro-1',2',3'-trihydroprpylptrin), Dehydroepiandrosterone (DHEAS), serum cortisol and many others including CRP,ESR, serum albumin, Tumor Necrosis Factor (TNF), Interferon-γ, Interleukin-2(IL-2), IL-4 (Sevigny *et al*., 2007).

Though many tests are available, studies thus far showed that evaluation of CD4+T- cell counts and HIV RNA viral load can efficiently be used to monitor the HIV disease progression. However due to the limitations either in the scientific technology and infrastructure or in the finances many developing and poor countries cannot afford these tests. Studies now are concentrating on finding cheaper and useful alternate markers in monitoring HIV disease progression. Even with many advances in the availability and effectiveness of Highly Active Antiretroviral Therapy (HAART), still it is difficult to eradicate the infection. More so the patients receiving HAART will experience significant side effects (Berhane *et al*., 2004; Carr *et al*., 1998). Therefore it is suggested that before initiating HAART the patients hematological parameters are to be evaluated and regularly monitored during the therapy (Spacek *et al*., 2003). In the present study we evaluated some alternate markers and hematological parameters in HIV infected patients who are not on HAART.

MATERIALS AND METHODS

One hundred and twenty HIV infected patients were enrolled from the Integrated Counseling and Testing Center (ICTC) of the Kamineni Institute of Medical Sciences hospital, Narketpally, Nalgonda. The study was carried out between Jan 2007 and June 2008. The protocol for the study was approved by the Institutional ethical committee and a signed consent from each subject was obtained. All the patients were antiretroviral therapy naive when the blood samples were taken. Blood was collected and processed following our established laboratory protocol. About 10 mL blood was collected in to sterile vacoutainers containing heparin/EDTA anticoagulant and without anticoagulant for blood and serum samples respectively. The daignosis of HIV infection was confirmed by three different ELISA's as recommended by National AIDS Control Society (NACO), India (NACO, 2006).

Table 1: The mean, standard deviation and p values of all the parameters in HIV patients

Parameter	Mean ± SD	p-value
CD4+T-cell counts	308.000±296.82	<0.0001
CD8+T-cell counts	976.208±548.618	<0.0001
CD4: CD8 ratio	0.314±0.198	<0.0001
TLC	8595.800±2146.9	<0.0001
HB (%)	10.742±2.464	<0.0001
AEC	391.800±180.18	<0.0001
ESR	75.625±29.03	<0.0001
CRP	1.725±1.267	<0.0001

Table 2: Correlation of markers with CD4+T-cell counts

	CD4+T-cell counts	
Marker	r	p
CD4+T-cell counts		
TLC	0.34	= 0.0059
HB (%)	0.33	= 0.0081
AEC	0.32	= 0.2270
ESR	-0.12	= 0.0004
CRP	-0.41	<0.0001

Absolute TCD4+cell counts, CD8+T-cell counts were measured using flow cytometry (MMWR Recommendations and Reports, 1992) TLC, HB%, AEC and ESR were estimated using conventional hematological methods. CRP was evaluated by latex agglutination test (Immuno CRP-Latex Agglutination Test).

RESULTS

The mean Hemoglobin, AEC,TC,CD4+T-cells, CD8+T-cells, ESR and CRP in the HIV seropositive individuals were found to be 10.742 g%, 391.8, 8595, 308 and 976.208 cells mm^{-3}, 75.625 mm and 1.725 mg dL^{-1} respectively. The mean, standard deviation and the p-value of all the parameters are shown in Table 1. The correlation of various markers against CD4+T-cell counts is shown in Table 2.

DISCUSSION

HIV infection is associated with extended clinical latent period in which the infected patient/person remains symptom less. During this period the virus actively replicates resulting in clinical illness. It is important for physicians concerned in treating HIV infected patients to understand the factors affecting disease progression which can facilitate clinicians to monitor and take treatment decisions. In developing and poor countries which usually have ill equipped laboratories to perform CD4+T-cell counts and HIV RNA viral load; it becomes imperative for the clinicians

to search for other available markers for disease progression. Our study results showed a significant positive correlation of CD8+T-cell counts, Hemoglobin, AEC and TLC against CD4+T-cell counts.ESR and CRP were found to be negatively correlating with CD4+T-cell counts The CD4+T-cell counts, CD8+T-cell counts and CD4:CD8 ratio among the study group were found comparatively lower to those observed by Ray *et al*. (2006) and similar to other studies by Sehgal *et al*. (2002); Chaudhary *et al*. (2008) and Walker *et al*. (2006) The Total Leucocyte counts positively correlated with CD4+Tcell counts as observed in other studies performed in India by Chaudhary *et al*. (2008). The study results showed a significant negative correlation of CD4+T-cell counts with ESR and CRP where as a positive correlation was observed against TLC, HB% and AEC.

CONCLUSION

The study results were found similar to previous studies and reaffirmed the importance of other available markers useful for monitoring the disease progression in HIV/AIDS patients.

We impress on the need for further studies on usefulness of alternate surrogate markers of HIV disease progression both before and during antiretroviral therapy.

The knowledge of HIV infection has expanded tremendously that led to significant improvement in the treatment, leading to decrease in morbidity and mortality. Unfortunately the progress achieved in the development of treatment and vaccine has been restricted or confined to western world. Disease progression, initiation and assessment of antiretroviral therapy in HIV infected individuals depend on CD4+T-cell counts and HIV RNA viral load. However due to the limitations either in the scientific technology and infrastructure or with the finances, many developing and poor countries cannot afford the cost of these tests. Therefore, research should concentrate on finding alternate and cost effective markers which can help physicians in deciding the start of antiretroviral therapy and predicting HIV disease progression.

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