Original Article

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Microbiology Section

A Study of Alternate Biomarkers in HIV Disease and Evaluating their Efficacy in Predicting T CD4+ Cell Counts and Disease Progression in Resource Poor Settings in Highly Active Antiretroviral Therapy (HAART) Era

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ABSTRACT

Introduction: Human Immunodeficiency Virus (HIV), the causative agent of AIDS, has been a challenge to medical fraternity since it was first discovered in 1983. About 40 million people are living with HIV infection globally and 99% of the infected people are in south East Asia (SEA). Traditionally, HIV disease and progression, initiation of HAART and response to therapy is monitored by assessing in regular intervals, the T CD4+ cell counts and plasma HIV/RNA viral load. Resource poor, low and low – middle income group countries still have no finances to acquire infrastructure and scientific technology for performing such tests.

Objectives: Since very few studies are available, they have demonstrated the role of alternate biomarkers that can be used to predict CD4 cell counts and thereby, monitor HIV disease progression and HAART. We aimed to measure certain haematological parameters in HIV seropositive patients and to evaluate their efficacy in predicting TCD4+ cell counts.

Methods: The study group included 250 HIV seropositive patients with an age range of 18-65 years. 140(56%) males and 110(44%) females were included in the study. Absolute TCD4+cell counts and CD8+T cell counts were measured by using a flow cytometer. (MMWR Recommendations and Reports, 1992) TLC; HB%, AEC and ESR were estimated by using conventional haematological methods. CRP was evaluated by latex agglutination test (Immuno CRP Latex Agglutination Test).

Results: Among the tested haematological markers, a TLC of <1800 cells/mm^3 showed high specificity (100%) in predicting CD4 counts of < 200 cells/mm^3, with an accuracy of 61.46%. Haemoglobin and Absolute Eosinophilic counts showed high specificities of 84.09% and 94.32% respectively in predicting CD4 counts which were below 350 cells/mm^3. ESR with 98.98% sensitivity and AEC which had 83.67% sensitivity were able to predict CD4 counts of <200 cells/mm^3.

Conclusion: Among the tested biomarkers, it was seen that Absolute Eosinophilic counts of more than 550 cells/mm^3, Blood Haemoglobin which was less than 10 g%, ESR which measured more than 20 mm, CRP values of >1.2 and TLC of <1800 cells/mm^3 could be helpful in predicting CD4 cell counts of < 350 and <200 cells/mm^3.

Key words: Human Immunodeficiency Virus (HIV), HAART, Alternate biomarkers

INTRODUCTION

Human Immunodeficiency Virus (HIV), the causative agent of AIDS, has been a challenge to medical fraternity since it was first discovered in 1983 [1]. Laboratory diagnosis is affected by the window period in which patients show non – specific symptoms and the serum anti – HIV antibodies are present in undetectable amounts. Though the availability of advanced third and fourth generation tests have solved the problem, the cause of concern is the laboratory monitoring of the infected individuals. About 40 million people are living with HIV infection globally and 99% of the infected people are in south East Asia (SEA) [2]. With inception of Highly Active Antiretroviral Therapy (HAART), the quality of life of HIV infected individuals is gradually improving [3]. The number of people which contract new infections has been on decline globally and those who have access to HAART are increasing in number. Antiretroviral therapy was made available in India since April 2004 and currently, over three lakh people are on HAART [4]. The cause of concern now is the availability and efficacy of cost-effective disease monitoring strategies. T CD4+ cell counts and serum/plasma HIV/RNA viral load, which are traditional biomarkers for assessing the disease progression and for monitoring HAART, require sophisticated equipment and they cannot be affordable to financially constrained, low middle and low income group countries which actually carry a majority of burden of HIV seropositive individuals. We attempted to evaluate various haematological parameters in HIV seropositive patients and to evaluate their efficacy in predicting T CD4+ cell counts.

MATERIAL AND METHODS

This study was carried out at Apollo Health City, a tertiary care hospital, between June 2011 to May 2012, which included 250 HIV sero-positive and antiretroviral therapy naive individuals. HIV sero –

positive patients who attended Integrated Counseling and Testing Centre (ICTC) which was situated at Area Hospital, Siddipet, India, were enrolled in the study. The study groups, at the time of their enrollment, were antiretroviral therapy naive, and those who were presently on HAART were excluded from the study. All the subjects who were included in the study were provided with a proforma with details of the study and informed and written consents were obtained from them. The study was approved by institutional ethical board. Blood samples were collected as per standard laboratory procedures. The HIV statuses of the subjects were confirmed as per NACO guidelines by using three different ELISA methods [5]. Absolute TCD4+cell counts and CD8+T cell counts were measured by using a flow cytometer. (MMWR Recommendations and Reports, 1992) TLC; HB%, AEC and ESR were estimated by using conventional haematological methods. CRP was evaluated by latex agglutination test (Immuno CRP Latex Agglutination Test).

Statistical software: Descriptive statistical analysis was carried out in the present study. The statistical softwares, namely, SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver. 2.11.1 were used for the analysis of the data (Spearman's rank order correlation, Sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) Accuracy and p – value). Microsoft Word and Excel were used to generate tables.

RESULTS

The median age of the subjects who were included in the study was 33 years, with an age range of 18-65 years. 140(56%) males and 110 (44%) females were included in the study. The cut – off for T CD4 + cell counts was taken as < 200 cell/mm^3 and < 350 cell/mm^3. The other variables with different cut-off values, which included ESR (>20 mm), AEC (>350 and > 550 cell/mm^3), TLC (< 2500 cell/mm^3 and <1800 cell/mm^3), HB (<10) and CRP (>1.2) were included, based on the WHO criteria and the published literature. The diagnostic performance of each variable in predicting T CD4 + cell counts, at a cut-off of < 200 cell/mm^3, has been shown in [Table/Fig-1]. Performance of each variable at its respective cut-off, in predicting T CD4 + cell counts of < 350 cell/mm^3, has been shown in [Table/Fig-2]. Spearman's rank order correlation and p-value of each variable at their cut-off values with respect to predicting T CD4 + cell counts (< 200, 200-350, > 350, 200-500 and > 500 cell/mm^3) have been shown in [Table/Fig-3].

DISCUSSION

Infection with HIV results in a variable disease progression, where many HIV infected individuals survive for more than 5 years even without undergoing an effective antiretroviral therapy [6]. A close monitoring of HIV infected patients is essential, for reducing their morbidity and mortality. Traditionally, HIV disease and progression,

	Sensitivity	Specificity	PPV	NPV	Accuracy	p value
ESR>20	98.98	15.56	43.56	96.15	49.00	<0.001*
AEC>350	83.67	58.94	56.94	84.76	68.67	<0.001*
AEC >550	30.61	90.07	66.67	66.67	66.67	<0.001*
TLC <2500	31.63	88.08	63.27	66.50	65.88	<0.001*
TLC <1800	2.00	100.00	100.00	61.13	61.46	<0.001*
Hb<10	37.76	77.48	52.11	65.73	61.85	0.009*
CRP>1.2	59.18	68.87	55.24	72.22	65.06	<0.001*

[Table/Fig-1]: Diagnostics performance of ESR, AEC, TLC, Hb and CRP for predicting the low CD4 count (<200)
*p Value statistically significant

	Sensitivity	Specificity	PPV	NPV	Accuracy	p value
ESR>20	94.41	19.32	68.16	65.38	67.87	<0.001*
AEC>350	70.19	64.77	78.47	54.28	68.27	<0.001*
AEC >550	24.84	94.32	88.89	40.69	49.40	<0.001*
TLC <2500	26.09	92.05	85.71	40.50	49.40	<0.001*
TLC <1800	1.24	100.00	100.00	35.63	36.14	0.541
Hb<10	35.40	84.09	80.28	41.57	52.61	0.001*
CRP>1.2	47.20	67.05	72.38	40.97	54.22	0.032*

[Table/Fig-2]: Diagnostics performance of ESR, AEC, TLC, Hb and CRP for predicting the low CD4 count (<350)
*p Value statistically significant

	CD4 count											
	<200		200-350		<350		200-500		>500		Total	
Pair	r	p	r	p	r	p	r	p	r	p	r	p
CD4 vs AEC	-0.115	0.26	-0.155	0.224	-0.084	0.439	-0.38	<0.001*	-0.341	0.065	-0.444	<0.001*
CD4 vs TLC	0.086	0.403	0.199	0.117	0.264	0.013*	0.304	<0.001*	0.14	0.462	0.381	<0.001*
CD4 vs Hb	0.377	<0.001*	-0.013	0.92	0.04	0.714	0.339	<0.001*	0.087	0.648	0.329	<0.001*
CD4 vs ESR	-0.108	0.291	-0.029	0.82	-0.1	0.359	-0.344	<0.001*	-0.2	0.289	-0.376	<0.001*
CD4 vs CRP	-0.299	0.003*	-0.222	0.080+	-0.138	0.198	-0.33	<0.001*	-0.204	0.279	-0.341	<0.001*

[Table/Fig-3]: Spearman Rank correlation CD4 with AEC, TLC, HB, ESR and CRP
Classification of Correlation Co-efficient (r)
Up to 0.1 : Trivial Correlation; 0.1-0.3 : Small Correlation; 0.3-0.5 : Moderate Correlation; 0.5-0.7 : Large Correlation
0.7-0.9 : V.Large Correlation; 0.9- 1.0 : Nearly Perfect correlation; 1 : Perfect correlation; *p Value Statistically significant

initiation of HAART and response to therapy were monitored by assessing in regular intervals (6 months), the TCD4+ cell counts and plasma HIV/RNA viral load. Resource poor, low and low middle income group countries still have no finances to acquire infrastructure and scientific technology for performing such tests. Very few studies are available, that have demonstrated the role of alternate biomarkers that could be used to predict CD4 cell counts and thereby, monitor HIV disease progression and HAART. The present study used CD4 cell cutoffs of < 200 cells/mm^3 and < 350 cells/mm^3 and it compared the usefulness of alternate biomarkers. Among the tested alternative biological markers, a TLC of <1800 cells/mm^3 showed high specificity (100%) in predicting CD4 counts of < 200 cells/mm^3, with an accuracy of 61.46%. Haemoglobin and Absolute Eosinophilic counts showed high specificities of 84.09% and 94.32% respectively in predicting CD4 counts which were below 350 cells/mm^3. ESR with 98.98% sensitivity and AEC which had 83.67% sensitivity were able to predict CD4 counts of <200 cells/mm^3. Absolute Eosinophilic counts of more than 550 cells/mm^3 showed 90.07 specificity and a CRP of >1.2 mg/dL showed 68.87 specificity in predicting CD4 counts of <200 cells/mm^3. In agreement with other recent studies, our studies also suggested that TLCs of < 1800 and < 2000 cells/mm^3 may be more suitable for initiation of HAART. A moderate correlation was observed for Absolute Eosinophilic counts (r -0.444 and p <0.001) in predicting CD4 counts of <500 cells/mm^3. Haemoglobin (r 0.377 p <0.001) was a useful alternative for predicting CD4 counts of <200 cells/mm^3. ESR (r -0.376 p <0.001) and CRP (r -0.341 p <0.001) also correlated well with CD4 cell counts. As was observed by other studies, a TLC of <1200 cells/mm^3 was ineffective in predicting T CD4+ cell counts. We tried values of TLC at two different cutoffs of <1800 and <2500 cells/mm^3. A recent study tried to predict T CD4 cell counts of <200 cells/mm^3 by using the WHO HIV/AIDS clinical staging and found it to have a low sensitivity [7,8]. An Indian study evaluated haemoglobin, ESR and TLC and found that a TLC of < 1200 cells/mm^3 could predict CD4 counts of <200 cells/mm^3, with very low sensitivity [9]. Azzoni et al., recently used a prediction based classification(PBC) method by using alternate biomarkers like total lymphocyte count, in predicting CD4 cell counts, thereby reducing the cost of patient management [10]. Boulware et al., studied some biomarkers and found that a pre HAART evaluation of such markers could predict Immune Reconstitution Inflammatory Syndrome (IRIS) [11,12]. Another study which was done in a developing country, tried to evaluate non physician clinicians' roles in staging and monitoring HIV diastase [13]. Absolute Eosinophilic counts correlated well with CD4 cell counts in predicting the disease stage and thereby, they may prove to be a useful biomarker in assessing the disease progression, as was observed by Cohen et al., [14]. Assessment of utility of ESR in the current study revealed that a raised ESR correlated well with CD4 cell counts (Accuracy 67.87%) and that it could predict CD4 counts which were below 350 cells/mm^3. It can be used in decision making, before starting on HAART and thereby for monitoring antiretroviral therapy [15]. Few previous studies found no correlation between TCD4+ cell counts of less than 200 cells/mm^3 and ESR [16]. Only scanty data on the usefulness of ESR in predicting CD4 cell counts was found to be available on reviewing the literature [15, 17, 18]. Doing several studies on the utility of alternate biological markers in HIV could pave the way for a better understanding of immunopathogenesis of HIV disease and for improving laboratory testing strategies that can be used in predicting clinical stage, initiation of HAART and management of HIV disease [4]. Current study's results correlated well with those of a recent study, which found anaemia to be a useful predictor of CD4 cell counts of < 200 cells/mm^3 [19]. An Indonesian study showed good correlation of anaemia with reduced CD4 cell counts, as was seen in the current study, which observed that anaemia showed high specificity (77.48%) and accuracy in predicting CD4 cell counts of < 200 cells/mm^3. Elevated CRP was a good predictor of CD4 counts of <200 cells/mm^3, as was observed in the current study [20]. A moderate correlation was observed in our study between anaemia and CD4 cells of <200 cells/mm^3, as was observed in the study of Mata-Marin et al., [21]. Suresh Venkata Satya Attili et al., in a study which was done in northern India, studied the haematological profiles of 470 HIV seropositive patients and found that moderate to severe anaemia and leucopaenia were associated with CD4 cell counts of < 200 cells/mm^3 [22]. Haematological abnormalities are a cause of concern in HIV infected individuals and they could be useful in management of disease. Very few studies are available from India in this regards. This study also re emphasised the need of rethinking over a TLC of <1200 cells/mm^3 in predicting CD4 cell counts of <200 cell/mm^3 and initiation of HAART [7,9]. In another study which was done in Uganda, Miiro et al., evaluated low cost alternate blood markers in predicting CD4 counts of < 200 cell/mm^{3}and found that anaemia had a sensitivity (95% CI) of 47% and a specificity of 76% [23]. An Iranian study evaluated TLC counts of <1300 cells/mm^3 (which were slightly higher than the WHO recommended values) and found them to have optimum sensitivity and specificity in predicting CD4 counts of < 200 cell/mm^3 [24]. Marrigje A. de Jong et al., from Indonesia studied the utility of TLC among those who were on HAART and found that it was effective in predicting CD4 counts of <200 cells/mm^3 [25]. The study of Helena P W et al., revealed that a TLC of <1700 cells/mm^3 could predict CD4 counts of <350 cells/mm^3 [26].

CONCLUSION

The current study's results clearly indicated abnormal haematological parameters in HIV sero-positive patients. Among the tested biomarkers, it was seen that Absolute Eosinophilic counts of more than 550 cells/mm^3, Blood Haemoglobin of less than 10 g%, ESR which was more than 20 mm, a CRP value of >1.2 and a TLC of <1800 cells/mm^3 could be helpful in predicting CD4 cell counts of < 350 and <200 cells/mm^3. Such alternate biological blood parameters can be regularly measured in HIV sero-positive individuals, that are cost effective in making decisions on the start of HAART and they can be used further in the management of HIV disease and treatment response.

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