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RESEARCH ARTICLE

CAN HAEMOGLOBIN ESTIMATION BE USED INSTEAD OF CD4 COUNTS TO MONITOR HIV DISEASE PROGRESSION IN RESOURCE LIMITED SETTING

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ABSTRACT

Background: At the end of 2008, 3 people out of 1000 between 15 to 49 years of age were affected with HIV in India. Commonly used prognostic markers like CD4 count and viral loads for HIV monitoring are expensive and sophisticated. Anemia is a frequent complication that occurs in 20-80% of HIV infected persons and is associated with faster disease progression and mortality. Therefore we aimed to determine the changes in Hb concentration and its correlation with CD4 counts and also to determine whether Hb alone can be used as an indicator for disease progression.

Materials and Methods: 2 ml of venous blood was drawn for CD4 cell count and Hb% once monthly for a period of 3 months from 100 patients between the age of 18 to 60 years attending the Antiretroviral therapy (ART) Centre of our hospital. The results were compiled and descriptive and inferential statistical analyses done

Results: Of the 100 patients majority of patients had CD4 counts above 200 and most had hemoglobin more than 12g/dl i.e. 63% while anemia was found in 37%. Over 3 months, out of the patients who had anemia, majority had CD4 counts below 500 and also, CD4 count was found to be less in people having Hemoglobin less than 12g/dl.

Conclusion: Hemoglobin concentration is directly related to the number of CD4 cells. Measuring CD4 counts using flow cytometry is an expensive technique and as per guidelines is being repeated once in 6 months. Hence the Hb concentration, which is easy to estimate and can be done frequently may be used as a prognostic indicator in resource limited setting.

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INTRODUCTION

Recent statistics indicate that at the end of 2008, 3 people out of 1000 between 15 to 49 years of age were affected with HIV in India (WHO 2012) and more than 80% of them reside in rural areas. Commonly used prognostic markers like CD4 count and viral loads for HIV monitoring are expensive and sophisticated. Therefore, alternatives are needed for resource limited regions like India. Hematological abnormalities are a common complication of HIV infection (Dikshit *et al.,* 2009). Anemia is a frequent complication that occurs in 20-80% of HIV infected persons and is associated with faster disease progression and mortality. HIV infection may lead to anemia in many ways (Obirikorang and Yeboah 2009). Pathogenesis of HIV-associated anemia is unclear and is likely to be multifactorial in nature (Macroft *et al.,* 1999). While CD4 count and HIV-RNA are the gold standard markers for disease monitoring in People Living With HIV/AIDS(PLWHA), when measurement of these parameters is not possible other markers like delayed type hypersensitivity responses (DTH), total lymphocyte count (TLC), haemoglobin(Hb) and body mass index (BMI) become important. Hb levels reflect rapidity of disease progression rates and independently predict prognosis across demographically diverse cohorts. Rates of Hb decrease also probably correlate with falling CD4 count. Hb levels could be measured easily where resources for more sophisticated laboratory markers such as viral load or even CD4 lymphocyte count are not available given that measurement of the CD4 lymphocyte count requires flow cytometry, an expensive technique unavailable in many developing countries. (Obirikorang and Yeboah 2009)

Therefore, the aim of this study is to determine the changes in Hb concentration and its correlation with CD4 counts and also to determine whether Hb alone can be used as an indicator for disease progression in resource limited settings. This will be the first study of its kind done in India, to the best of our knowledge.

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MATERIALS AND METHODS

This study was conducted as a prospective study at a tertiary care research and referral hospital attached to a medical college. 100 patients between the age of 18 to 60 years attending the Antiretroviral therapy (ART) Centre of the hospital were included in the study. Pregnant women and patients on oral iron supplements were excluded. Institutional ethical clearance was taken and informed consent was obtained from the subjects in their own language.

All patients were asked to fill up a demographic questionnaire. 2 ml of venous blood was drawn for CD4 cell count and Hb% once monthly for a period of 3 months. The Hb% was determined using an automated blood analyzer. The definition of anemia chosen for this study was blood haemoglobin less than 12 g/dl[5]. PLWHAs will be placed in three groups according to the Centers for Disease Control and Prevention Criteria (CDC) classification system that emphasizes the importance of CD4+ T lymphocyte testing in clinical management of HIV-infected persons. The system is based on three ranges of CD4 counts (1) ≥ 500 cumm; (2) 200-499 cumm-3; and (3) <200 cumm. The CD4 counts were determined using Becton Dickinson (BD) FASCount system (Becton, Dickinson and Company, California, USA). The results were compiled and descriptive and inferential statistical analyses done. The Statistical software namely SPSS 15.0 was used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

RESULTS

100 patients attending the ART centre of our hospital were studied by measuring their CD4 counts and Hb levels monthly, over a period of 3 months. Majority of these, 45(45%)were from the age group 31-40 years. 51(51%) were males and 49(49%) were females. Of these patients 68(68%) were on ART since 3-7 years, 8(8%) since 1-2 years, 23(23%) since 7-14 years and 1(1%) since more than 14 years. As shown in Table 1, majority of patients had CD4 counts above 200/cumm. Only 2 patients in the 1st month, 1 in 2nd and 2 in 3rd months respectively had their CD4 counts below 200/cumm. Table 2 shows, most had hemoglobin more than 12g/dl i.e. 63% while anemia was found in 37%.

Table 1. Distribution of CD4 count of patients studied

CD4 count	1 month	2 months	3 months	% change
<200	2(2.0%)	1(1.0%)	2(2.0%)	-
200-500	49(49.0%)	57(57.0%)	53(53.0%)	+4.0%
>500	49(49.0%)	42(42.0%)	45(45.0%)	-4.0%
Total	100(100.0%)	100(100.0%)	100(100.0%)	

Table 2. Distribution of hemoglobin mg/dl of patients studied

Hemoglobin mg/dl	1 month	2 months	3 months	% change
<12	37(37.0%)	37(37.0%)	36(36.0%)	-1.0%
>12	63(63.0%)	63(63.0%)	64(64.0%)	+1.0%
Total	100(100.0%)	100(100.0%)	100(100.0%)	

Table 3 and Table 4 show the values of Hb% and CD4 counts obtained over a period of 3 months. Data indicated that the values of both Hb% as well as CD4 fluctuated independently of each other and there was no statistically significant correlation between the two. Pearson correlation is done to investigate whether the difference between the sample correlation co-efficient and zero is statistically significant. Hence according to Pearson correlation depicted in Table 5, as the values are between 0.1 and 0.3, there is a small correlation between CD4 and Hb % (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).

Table 3. Distribution of CD4 count with hemoglobin

CD4 count		Hemoglobin mg/dl				P value
		<12 mg/dl		>12 mg/dl		
		No	%	No	%	
1 month						
•	<200	2	5.4	0	0.0	0.239
•	200-500	18	48.6	31	49.2	
•	>500	17	45.9	32	50.7	
2 months						
•	<200	0	0.0	1	1.5	1.000
•	200-500	21	56.7	36	57.1	
•	>500	16	43.2	26	41.2	
3 months						
•	<200	2	5.4	0	0.0	0.131
•	200-500	20	54.0	33	52.3	
•	>500	14	37.8	31	49.2	

Table 4. Correlation of mean CD4 count according to Hemoglobin levels

CD4 count	Hemoglobin mg/dl		P value
	<12 mg/dl	>12 mg/dl	
CD4 count at 1 month	538.37±273.12	544.01±242.77	0.915
CD4 count at 2 months	511.51±283.64	525.41±218.29	0.764
CD4 count at 3 months	506.31±281.69	534.34±226.34	0.588

Table 5. Pearson correlation of CD4 count with hemoglobin levels

CD4 vs. Hemoglobin	Pearson correlation	P value
1 month	0.246	0.013*
2 months	0.228	0.023*
3 months	0.256	0.010**

DISCUSSION

Out of the 100 patients studied, 51% were males and 49% were females. For both the genders the cut-off of hemoglobin to say it is anemia was 12 mg/dl. Majority of them were between 31-40 years (45%). All the patients were on ART and most of them (68%) were receiving antiretroviral treatment since 3-7 years. The CD4 counts were less than 200/cumm only in 2 in 1st month, 1 in 2nd and 2 in 3rd months respectively, the rest of the cases had CD4 counts above 200/cumm. Out of those who had CD4 counts above 200/cumm, almost equal number of patients had CD4 counts below 500 and above 500/cumm. Overall, around 37% of patients had anemia and anemia was found to be more in patients with CD4 counts below 500/cumm. Mean CD4 counts with respect to Hb% >12g/dl and <12g/dl (544.01±242.77 and 538±273.12) show that anemia is associated with lesser CD4 counts. Hence small

correlation is obtained between CD4 counts and Hb percentage. As a whole, less number of patients under this study had CD4 counts less than 500, but in those who had low CD4 counts, anemia was significant. In our study, we found that, there was a decrease in the blood haemoglobin levels as the HIV infection progressed and these findings are consistent with those of other studies such as Obirikorang *et al.* (2009). We also found that as the CD4 levels varied, likewise the haemoglobin levels decreased or increased over a period of 3 months which is comparable to the study by Mocroft *et al.* (1999).

The reasons for fall of Hb values in HIV can be manifold. Changes in cytokine production with subsequent effects on haematopoiesis, decreased erythropoietin concentrations, opportunistic infectious agents such as *Mycobacterium avium* complex and parvovirus B- 19, administration of chemotherapeutic agents such as zidovudine, ganciclovir, and trimethoprimsulfamethoxazole as well as myelophthisis caused by cancers such as lymphosarcoma may all contribute. Other mechanisms for HIV-associated anaemia, although uncommon, include vitamin B12 deficiency and the autoimmune destruction of red blood cells. Anaemia has been associated with progression to AIDS and shorter survival times for HIV-infected patients. The ground reality in our country is that PLWHA find it difficult to travel to the designated centres to get their CD4 counts monitored periodically either because of financial constraints, lack of transport, physical inability as a result of HIV infection, comorbid conditions, lack of social support systems, inadequate knowledge, cultural and religious constraints and fear of social stigma. In such a situation, local physicians in the peripheries find it difficult to provide adequate care and support and monitor treatment. In such cases, haemoglobin estimation which is an easy, bedside, simple, cost effective technique that can be done without much expertise at frequent intervals which shall prove to be invaluable to monitor the disease progression as CD4 counts and viral loads are done at 6 months intervals as per National AIDS Control Organization(NACO)(7) guidelines.

Conclusion

There is a small correlation between Hb concentration and the number of CD4 cells. Low Hemoglobin concentration was seen with low CD4 counts. Therefore, anemia can be an indicator in disease progression of HIV infection as measured by the numbers of CD4 cells. Measuring CD4 counts using flow cytometry is an expensive technique and is done once in 6 months as per NACO guidelines. Hence the Hb concentration, which is easy to estimate and can be done frequently may be used as a prognostic indicator in resource limited setting. However, it cannot replace CD4 counts or viral load testing.

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