Usefulness of Alternate Prognostic Serum and Plasma Markers for Antiretroviral Therapy for Human Immunodeficiency Virus Type 1 Infection

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In developing countries, the usability of peripheral blood constituents that are low-cost alternatives to CD4-positive (CD4+) T-cell and human immunodeficiency virus type 1 (HIV-1) RNA estimation should be evaluated as prognostic markers. The aim of our study was to investigate the use of plasma levels of dehydroepiandrosterone sulfate (DHEAS), albumin, and C-reactive protein (CRP) as alternate prognostic markers for antiretroviral treatment (ART) response in place of HIV-1 load measurements. Paired blood samples were collected from 30 HIV-infected individuals before and after initiation of ART, 13 HIV-infected individuals before and after completion of antituberculosis therapy (ATT), and 10 HIV-infected individuals not on either ATT or ART. Because of the nonavailability of samples, the CRP estimation was done for samples from only 19, 9, and 8 individuals in groups 1, 2, and 3, respectively. The measurements of all three markers, i.e., DHEAS, albumin, and CRP, were carried out with commercial assays. The differences in the albumin levels before and after ART or ATT were significant (P < 0.05), while the differences in DHEAS and CRP levels were not significant (P > 0.05). When levels of DHEAS among the individuals who were followed up were analyzed, 13 (44.8%) in the ART group and 9 (69%) in the ATT group showed an increase following treatment. Prior to treatment of HIV-infected individuals, there was a significant positive correlation of CD4+ T-cell counts and a negative correlation of viral load with albumin and DHEAS levels (P < 0.01). Among the three plasma markers we tested, plasma albumin and, to some extent, DHEAS show promise as prognostic markers in monitoring HIV infection.

Morbidity and mortality due to human immunodeficiency virus (HIV) continue to be major problems in developing countries (8). Currently, the availability and affordability of antiretroviral treatment (ART) are improving in these countries (21). Though CD4-positive (CD4+) T-cell estimation and the plasma HIV-1 load continue to be the major prognostic markers of progression, several other peripheral blood constituents may be considered for roles as prognostic markers (1, 4, 20). The serum albumin level is considered one of these alternatives, as it has been shown that a low level of serum albumin, i.e., <3.5 mg/ml after seroconversion is associated with faster HIV disease progression (14). Studies have shown a significant relationship between the CD4 cell count, serum cortisol, and DHEA levels (2, 3). There are reports on the association of high levels of plasma highly sensitive C-reactive protein (CRP) and HIV disease progression (5, 9). The present study was carried out to investigate if plasma levels of either DHEAS, albumin, or CRP could be used as an alternate prognostic marker(s) of ART response in place of viral-load measurement in HIV-infected individuals in south India.

MATERIALS AND METHODS

The study was carried out in the Department of Clinical Virology, Christian Medical College (CMC) Hospital, Vellore, India, a tertiary-care center in south India. Plasma samples collected from 53 HIV-infected individuals and 30 healthy controls after informed consent during the years 2004 through 2006 were used for this study (there were two groups of healthy controls). The HIV-infected individuals were referred to the Department of Clinical Virology from the Infectious Diseases Clinic of CMC or by general-practice physicians for CD4+ T-cell and or viral-load estimation. Paired blood samples were collected from 30 HIV-infected individuals before and after initiation of ART. The HIV-infected group on ART included 24 men and 6 women; the median age was 40 years (range, 24 to 68 years), and the median time on treatment was 16 months. Samples were also collected from 13 HIV-infected individuals before and after completion of antituberculosis therapy (ATT). The HIV group on ATT included eight men and five women, with a median age of 35 years (range, 26 to 55 years). The third group, with 10 HIV-infected individuals not on either ATT or ART, included 5 men and 5 women, with a median age of 40.5 years (range, 19 to 66 years). Blood samples were drawn between 8 and 10 a.m. on the days of sampling. None of the HIV-infected or the healthy individuals had clinical Cushing’s syndrome or Addison’s disease. ART was initiated when the CD4+ T cells were <200 cells/µl or the patient had clinical AIDS, irrespective of CD4 counts. Those who were asymptomatic with CD4+ T-cell counts of <350 but >200 were also offered treatment. The ART regimen given to the individuals was nevirapine (a nonnucleoside reverse transcriptase inhibitor) with two nucleoside reverse transcriptase inhibitors. In the ART group, the first samples were collected before the initiation ART and the second sample after at least 90 days of therapy. In the ATT group, the first sample was collected before the initiation of ATT and the second sample near the completion of treatment (6 months). The diagnosis of tuberculosis was made from clinical findings and one of the three investigative findings, i.e., microbiological, histological, or radiological support. The drugs included in the short-course directly observed treatment regimen for tuberculosis were isoniazid, rifampin, ethambutol, and pyrazinamide. The remaining 10 HIV-infected individuals were not on ART or ATT, as they were asymptomatic.

Plasma albumin was measured with the Hitachi 912 analyzer (Roche Diagnostics, Japan). The assay was based on colorimetric principles, in which there was formation of an albumin-bromocresol green complex at pH 4.2, and absorbance was measured at 600 nm.

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The DHEAS levels were measured using an enzyme-linked immunosorbent assay-based technique (Immuno Biological Laboratories, Hamburg, Germany). The assay was carried out according to the manufacturer’s instructions. Briefly, 25 μl of each standard and sample was dispensed into appropriate wells. To each of these wells, 200 μl of enzyme conjugate (anti-DHEAS with horseradish peroxidase) was added. The wells were thoroughly mixed and then incubated at room temperature for 60 min. After the incubation and washing, 100 μl of substrate (tetramethyl benzidine) solution was added. After a 15-min incubation period, 50 μl of stop solution was added. The reading was taken at 450 nm within 10 min of adding the stop solution. A calibration curve was then used to quantify the DHEAS in the sample. Seven standards were included in the assay, i.e., 0 μg/ml, 0.1 μg/ml, 0.5 μg/ml, 1.0 μg/ml, 2.5 μg/ml, 5.0 μg/ml, and 10 μg/ml. All the samples and standards were tested in duplicate, and the mean value was taken as the DHEAS level.

The quantitative determination of CRP was carried out by immunonephelometry (BN ProSpec; Dade Behring, Marburg, Germany) using the CardioPhase highly sensitive CRP reagents (Dade Behring, Marburg, Germany). The manufacturer’s instructions were strictly followed for both the extraction of RNA and the reverse transcriptase PCR.

**Statistical analysis.** Statistical analysis of paired samples with all five parameters were carried out by paired two-tailed t tests using Microsoft Excel software. Correlations of the levels of all three serum markers with CD4 counts and viral loads were analyzed by Pearson’s correlation coefficient test using EPI Info software version 6.04b, and determination of the age-dependent partial correlation coefficient was carried out for all parameters using SPSS software. The comparisons of means of all three markers in the three different groups of HIV-1-infected individuals and healthy controls were carried out by analysis of variance or Kruskal-Wallis one-way analysis of variance tests using EPI Info software version 6.04b and Wilcoxon’s rank sum test.

### RESULTS

The mean albumin, DHEAS, and CRP levels in the healthy controls were 3.73 g/dl, 144 μg/ml, and 3.52 mg/liter, respectively. The means and standard deviations of the CD4+ T-cell count and HIV-1 RNA, plasma albumin, DHEAS, and CRP levels in different groups of HIV-infected individuals and healthy individuals are given in Table 1. The differences in the
albunin levels before and after treatment in both the above-mentioned groups were significant ($P < 0.05$), while the differences in DHEAS and CRP levels were not significant ($P > 0.05$). The levels of DHEAS in individuals on follow-up showed that 13 (44.8%) in the ART group and 9 (69%) in the ATT group had increases following ART and ATT, respectively. This difference was not significant ($P > 0.05$).

There were significant differences in the plasma albumin ($P = 0.044$) and DHEAS ($P = 0.026$) levels seen among healthy individuals and the levels seen during the first visit in HIV-1-infected individuals. The differences in the plasma albumin and DHEAS levels among the three groups of HIV-1-infected individuals were significant ($P < 0.001$) at both visits. There were significant positive correlations between plasma albumin and CD4$^+$ T-cell ($P < 0.05$) and plasma DHEAS ($P < 0.05$) levels when age-dependent partial correlation was also checked. There was no difference in the correlation coefficient controlling for age for these two parameters. The plasma albumin showed a significant negative correlation with the plasma CRP level ($P < 0.01$). The plasma DHEAS level showed significant positive correlation with the CD4$^+$ T-cell count but a negative correlation with the plasma viral load ($P < 0.01$). The correlation between the different plasma markers and the CD4$^+$ T-cell count and HIV-1 RNA level is shown in Table 2.

### DISCUSSION

In our study group of HIV-1-infected individuals, we found that the plasma albumin level and the DHEAS level were significantly lower than in the healthy controls. Though the CRP level was lower in the healthy controls than in the HIV-1-infected individuals, the difference was not significant. However, there was no difference in the albumin, DHEAS, and CRP levels between the healthy controls and the asymptomatic HIV-1-infected individuals who were not on ART or ATT (group 3). The albumin level considerably improved after the initiation of ART. There was a significant increase ($P < 0.001$) in the CD4$^+$ T-cell count, as well, following ART. All 29 individuals in the ART group showed an increase in the CD4$^+$ T-cell count, and 28 (97%) individuals showed a significant reduction in the HIV-1 load. There was an increase in the level of albumin among 24 (83%) of the group 1 individuals. Only three (10%) individuals showed a decrease in albumin after ART. In one individual in whom there was an increase in the HIV-1 load there was a drop in the plasma albumin level. These changes seen in the albumin level following ART showed good correlation with changes in the CD4 level and the HIV-1 load. In those individuals who had HIV infection and tuberculosis, the albumin DHEAS and CRP levels were lower than in those who had been diagnosed with only HIV infection. Among the three plasma markers, only the albumin level had significantly increased after 6 months of ATT alone. Prior to the treatment, there was a significant positive correlation between the CD4$^+$ T-cell counts and albumin and DHEAS levels, but not with CRP, among all three groups ($r = 0.32$ and $P < 0.01$; $r = 0.27$ and $P < 0.01$; $r = -0.014$ and $P > 0.05$). There was a negative correlation between HIV-1 RNA and the DHEAS level, but not with albumin and CRP levels. As expected in this study, there was also a significant negative correlation between the CD4$^+$ T-cell count and the HIV-1 load. However, the value for this correlation was less than that in the previous report for the same population (13a). One of the reasons may be the small sample size and the very high viral load seen in the ATT group.

It has been reported that the baseline serum albumin level can be considered an independent predictor of mortality in HIV-1-infected women and can be used as an additional marker of HIV-1 disease progression (4). Mehta et al. examined the albumin levels among HIV-infected individuals at entry into a community-based cohort, as well as the albumin concentrations measured before and after HIV seroconversion (14). They found that an albumin level of <35 g/liter was associated with faster progression to AIDS. Though the pre-seroconversion albumin levels are not predictive of the outcome of the disease, HIV seroconversion appeared to lower the serum albumin level (14). This increase in the albumin level might have been due to the improvement in the general body mass index (BMI) following better absorption of nutrients from the gut, as there was improvement in the parasitic burden and cytomegalovirus-induced colitis. Reduction in the tumor necrosis factor alpha level following ART (19) might also have reduced cachexia in these individuals, which in turn would have led to an increase in the albumin level.

Among the individuals in the ART group, 83% showed a significant increase in the CD4$^+$ T-cell count and plasma albumin level, with a significant drop in the HIV-1 load. A majority of these individuals also showed significant clinical improvement with weight gain. This effect may be due to control of enteropathy (cytomegalovirus induced) by ART. The increased albumin levels and the effect of ART in the control of enteropathy may not be mutually exclusive.

It is important to look at the feasibility of using the serum/plasma albumin level as a marker for monitoring the response to ART, especially in resource-poor settings. A recent study reported from Africa showed a significant positive correlation between the albumin level, body weight, and the CD4$^+$ T-cell

### Table 2. Correlation of different plasma markers with CD4$^+$ T-cell counts and HIV-1 viral load$^a$

| Marker   | CD4$^+$ T cells | Viral load | Albumin | DHEAS | CRP |
|----------|-----------------|------------|---------|-------|-----|
|          | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ |
| CD4$^+$ T cell | -0.24 | <0.01 | -0.24 | <0.01 | 0.32 | <0.01 | 0.27 | <0.01 | -0.14 | >0.05 |
| Viral load | 0.32 | <0.01 | 0.17 | >0.05 | 0.4 | <0.01 | -0.47 | <0.01 | -0.06 | >0.05 |
| Albumin | 0.27 | <0.01 | -0.24 | <0.01 | 0.40 | <0.01 | -0.06 | >0.05 |
| DHEAS | -0.14 | >0.05 | -0.11 | >0.05 | -0.47 | <0.01 | -0.06 | >0.05 |
| CRP | -0.14 | >0.05 | -0.11 | >0.05 | -0.47 | <0.01 | -0.06 | >0.05 |

$^a$ Values of the samples collected during the first visit.
count in both pretreatment and posttreatment analyses (18). Another important observation made in this study is the significant increase in the HIV-1 viral load, i.e., 5.69 to 6.33 log copies/ml ($P < 0.01$), and no change in the CD4$^+$ T-cell counts among those who were on ATT, although there was a significant increase in the albumin level. There was significant ($P = 0.003$) increase in the BMI among these 13 individuals following 6 months of ATT. We especially analyzed the BMI for this group as a corollary to the observed albumin increase. An increase or lack of change in the HIV-1 RNA level, insignificant change in the CD4$^+$ T-cell count, and decrease in serum reactive markers were also reported earlier (11, 16). The exact reason for this phenomenon is not known. However, it may be due to the sustained expression of high levels of CD38 related to immune activation, even after ATT; the long-lasting or permanent effects of Mycobacterium tuberculosis on the immune system; or elevated expression of CCR5 induced by the bacteria, encouraging replication of R5 variants (7, 15, 16).

Ferrando et al. have shown that low DHEAS levels can have a negative prognostic effect during the progression of HIV infection and that initiation of antiretroviral therapy can induce an increase in circulating DHEAS (6). Since DHEAS protects against acute lethal viral infection and can inhibit HIV-1 latency reactivation, the increased DHEAS level may be directly inhibitory to HIV replication (10). In our previous study, we investigated the relationship between the lowered levels of DHEAS and HIV infection progression, as well as its effect on the HIV-1 load. We observed a significant negative correlation between the viral load and the DHEAS level ($r = -0.6; P < 0.05$) among HIV-infected individuals as a group (13). In the current study, we looked at the changes in the DHEAS level following ART and ATT in HIV-1-infected symptomatic individuals and its difference from those in asymptomatic HIV-1-infected individuals and healthy individuals. As observed in the previous study, this study also showed a significant difference in the DHEAS levels among HIV-1-infected individuals and healthy individuals. The DHEAS level also had a significant positive correlation with the CD4$^+$ T-cell count ($r = 0.27; P < 0.01$) and a negative correlation with the HIV-1 plasma RNA level ($r = -0.24; P < 0.01$). Unlike the plasma albumin level, our study failed to show any significant change in the group mean levels of DHEAS following ART or ATT. However, when the DHEAS levels in individuals on follow-up were analyzed, 13 (44.8%) in the ART group and 9 (69%) in the ATT group showed an increase, but it was not significant by Wilcoxon’s rank sum test. In order to clearly establish this effect, we need to do a study with a larger sample size.

Acute-phase CRP is a sensitive marker of inflammation and tissue damage (22). There is a report on HIV-1-infected patients without intermittent infection showing higher values of highly sensitive CRP than in the general population (1). This may be a reflection of sustained acute-phase response as a consequence of HIV infection (17). It was also shown that in those with infections, elevated highly sensitive CRP levels fell in response to specific therapy. CRP appears useful for detecting an infectious condition and monitoring of intercurrent infection in HIV-1 antibody-positive patients. Independently of CD4 lymphocyte counts and HIV RNA levels, the plasma CRP levels were associated with HIV disease progression (9). In addition, regardless of progression to AIDS, HIV-infected individuals had a significant increase in CRP over time, and CRP can be considered an independent predictor of mortality in HIV-1-infected individuals (5). In this study, compared to healthy individuals and asymptomatic HIV-1-infected individuals not on ART or ATT, the mean CRP level was high in symptomatic HIV-1-infected individuals who underwent ART or ATT. Similarly, after ART, there was a drop in the mean CRP level. However, in both these groups, the difference was not significant ($P > 0.05$).

In conclusion, of the three plasma markers studied, only albumin showed promise as a prognostic marker in monitoring HIV infection. Our study reported here also supports the findings of Olawumi and Olatunji (18), which showed the usefulness of serum/plasma albumin as another laboratory marker for the monitoring of response to ART. The albumin level, to an extent, may also predict the presence of M. tuberculosis infection, as the level was significantly lower in HIV-infected individuals with tuberculosis infection. The costs for the estimation of CD4$^+$ T-cell counts, HIV-1 loads, and serum/plasma albumin levels are $15, $100, and $2, respectively. Hence, in developing countries, where patient resources are poor, the interval between CD4$^+$ T-cell estimation and HIV-1 load testing may be increased by introducing more frequent testing of the serum albumin level, which will prove economical, while the patients are on ART. For the individuals on follow-up, DHEAS also showed some promise as a prognostic marker. Since this was a pilot study, the usefulness of plasma/serum albumin and DHEAS as surrogate markers needs to be examined in a longitudinal study with a larger number of volunteers.

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