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HIV-1 clade C infection and Progressive Disruption in the Relationship Between Cortisol, DHEAS and CD4 cell numbers: A two-year follow-up study

Seetharamaiah Chittiprol^{1,*}, Adarsh M. Kumar⁶, H. Ravi Kumar¹, P. Satishchandra², R. S. Bhimasena Rao², V. Ravi³, A. Desai³, D. K. Subbakrishna⁴, Mariamma Philip⁴, K. S. Satish⁵, K. Taranath Shetty¹, and Mahendra Kumar⁶

¹ Department of Neurochemistry, National Institute of Mental Health and Neuro Sciences

² Department of Neurology, National Institute of Mental Health and Neuro Sciences

³ Department of Neuro Virology, National Institute of Mental Health and Neuro Sciences

⁴ Department of Biostatistics, National Institute of Mental Health and Neuro Sciences

⁵ Seva Clinic, Bangalore, India

⁶ Department of Psychiatry & Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL

Abstract

Background—It is well established that there is mutual interaction between the neuroendocrines and immune systems and that the disturbance in any one system could affect the function of the other. While there is a large body of evidence suggesting negative impact of human immunodeficiency virus type 1 B (HIV-1B) infection on both immune and neuroendocrine systems, the consequence of HIV-1 clade C infection (with structural differences from HIV-1B virus) on these systems is not clearly understood.

Methods—We carried out a 2-y longitudinal study on plasma profile of adrenocorticosteroids, including cortisol and DHEAS and their relationship with declining CD4+ cell counts in neurologically asymptomatic HIV-C infected individuals (N=84) in order to understand the impact of HIV-1 clade C infection on adrenocortical dysfunction and its relationship with the progressive decline in the cell mediated immunity.

Results—We found that while plasma cortisol levels increased significantly at baseline in HIV-1C infected individuals compared to those in HIV-negative controls (HIV-1C+, 9.83 ± 0.39 vs. controls, 8.04 ± 0.45 ; $p < 0.01$), there was a significant decrease in DHEAS in HIV-1C+ individuals, compared to that in HIV-negative controls (81.02 ± 4.9 vs. 185.1 ± 12.03 , $p < 0.001$), and consequently a significant increase in cortisol:DHEAS (Cortisol:DHEAS) ratio in HIV-1 clade C infected persons (0.19 ± 0.002 vs. control 0.058 ± 0.006 ; $p < 0.001$). Moreover, in HIV-1 C infected individuals, there was a strong positive correlation between DHEAS and CD4 cells

* Address for correspondence: Dr. Seetharamaiah Chittiprol, PhD, Nationwide Children's Hospital, Research II-WA 2110, 700 Children's Dr., Columbus, OH 43205. Seetharamaiah.Chittiprol@nationwidechildrens.org/ seetha0109@gmail.com, Phone: +1-614-355-2604; +1-858-699-6901, Fax: +1-614-722-5892.

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($r=0.2$; $p<0.05$), and a strong negative correlation between cortisol, as well as Cortisol:DHEAS ratio and CD4 cells ($r=-0.25$; $p<0.01$; and $r=-0.31$; $p<0.001$, respectively).

Conclusions—These findings suggest the persistent and progressive adrenocortical dysfunction during the asymptomatic phase of HIV infection, and that the evaluation of increase in plasma cortisol, a decrease in DHEAS, and an increase in Cortisol:DHEAS ratio may serve as important biomarkers preceding the impending down regulation of CD4 cell counts and progressive decline in the immune system function in HIV-1C infection. Furthermore, these findings may indicate the dysregulation of 3β -hydroxysteroid dehydrogenase (3β -HSD) activity, the enzyme involved in the biosynthesis of cortisol and DHEA through the pregnenolone-progesterone pathway, and that it may offer an opportunity for drug discovery targeting re-regulation of 3β -HSD activity for potential therapeutic application in HIV-1 C infection.

Keywords

HIV-1; Clade C; Cortisol; Cortisol:DHEAS (C/D) ratio; DHEAS; CD₄ cell count; CD₄:CD₈ ratio; Body mass index (BMI); HAART

Introduction

Human Immunodeficiency Virus (HIV) infection leading to acquired immunodeficiency syndrome (AIDS) is characterized by the suppression of cell mediated immunity (CMI) associated with pathological changes affecting multiple organs systems including the immune and neuroendocrine systems [1–7]. A large body of evidence suggests that soon after HIV infection, a drastic change in the cytokine profiles occurs that is characterized by a shift of helper T cells from TH1 to TH2 state resulting in apoptosis of CD4 cells [1,8]. While interaction between the endocrine and immune system is well documented [9,10], the significance of the reported endocrine dysfunction in HIV infection [3,11,12] and its role in the decline of immune function and the overall progression of the disease is not clearly understood. Whereas, dysregulation of neuroendocrine functions in general and adrenocorticoids functions in particular have been reported by our group [4,6,13–17] and others [3,7,11,12,18–24] during the asymptomatic phase of HIV-1B infection, there are no reports to date on the impact of the other HIV-1 strains, such as the HIV-1 clade C strain on neuroendocrine functions in the infected asymptomatic patients. Moreover, HIV-1 clade C is the most prevalent strain among the Asian populations, yet there is a lack of studies related with HIV-1 C clade virus infection and neurological complications as well as neuroendocrine abnormalities in the infected individuals. The fact that there are differences in the structures and properties between the two strains (HIV-1 B and C), the findings on the endocrine abnormalities related with HIV-1 B strain [13–15] may not be applicable to those caused by HIV-1C infection. In order to delineate the impact of HIV-1 clade C on the adrenocortical hormone profile, we carried out a prospective longitudinal study on neurologically asymptomatic HIV-1 clade C seropositive individuals. It is proposed that although the dysregulation of neuroendocrine functions may be a consequence of AIDS per se, the altered endocrine functions during the asymptomatic phase may be one of the important contributory factors in the suppression of immune system and in the augmentation of the progression of the disease. In this report, we present our findings on the changes occurring over time in the adrenocortical hormones, cortisol and DHEAS, as well as cortisol:DHEAS ratio, and the progressive change in their relationship to CD4 cell counts, that may be considered among some of the markers for immune status over 2 y follow-up in HIV-1 C infected individuals.

Materials and Methods

Study subjects

HIV-1 clade C seropositive subjects included in this study were community residing women and men living near the study center, the National Institute of Mental Health and Neuro Sciences (NIMHANS) in Bangalore, India. These individuals (n=120) were between 18 and 45 y and included females and males (females: n=68, 57% and males: n=52, 43% respectively). Healthy, age-matched, HIV-1 seronegative control individuals (n=69) were drawn from the non-spousal family members of HIV-1 infected patients, and from those who volunteered to provide blood samples for establishing the lab reference values for the endocrine parameters. The participant's serostatus was confirmed by ELISA and Western blotting technology. Individuals who had a history of CNS infection, highly active antiretroviral treatment (HAART), substance abuse, steroid dependence, as well as women who were pregnant or lactating were excluded from the study. All participants at the time of enrollment into the study underwent comprehensive neurological and physical examinations and the majority of patients were not found to have any major HIV-associated physical symptoms of illness. Although, the exact time of infection and clinical stage of illness could not be predicted, however, based on the patient interview it was found that the approximate time of infection was 2.62 ± 0.17 y before enrollment in the study. At enrollment, the patients were tested and confirmed to be positive for HIV-1C infection.

All subjects enrolled for the study signed an informed consent approved by the NIMHANS Institutional Ethics Committee and the University of Miami Internal Review Board (IRB). As stated above, since HIV-1 seropositive participants knew their sero-status at least 6 months prior to their enrollment in the study, it prevented interference of any acute psychological distress caused by the sudden information of their HIV-1 seropositivity. In order to carry out the investigations, all study participants were admitted as inpatients for overnight stay at the hospital.

Specimen collection

In consideration of the circadian rhythms affecting basal hormones levels, blood specimens were collected between 8.00–10.00 AM, from the ante-cubital vein in chilled EDTA vacutainer tubes. An aliquot of the blood collected in EDTA was used for CD4 cell counts. For hormone assays, plasma was separated within one hour and specimens were aliquoted in vials containing aprotinin as preservative and stored at -80°C until analysis.

Diagnosis of HIV-1 infection and CD4 counts

HIV-1 infection was confirmed by HIV-1 Tridot rapid visual test (J. Mitra & Co Ltd, India) followed by Western immunoblot analysis of serum (Immunitics, USA). Estimation of CD4 cell counts in blood sample was carried out by flow cytometry (FACS counter, Becton Dickinson, San Jose, CA).

Assay of Cortisol and DHEA-SO₄

Cortisol, and DHEA-SO₄ (DHEA-S) were quantified using commercially available kits (Siemens Medical, Los Angeles, CA.) based on enzyme-amplified immuno-chemiluminescence. The analytical ranges of the methods for cortisol, and DHEA-S were found to be 0.2–50 µg/dl and 3–100 µg/dl, respectively. Intra-assay and inter-assay CVs were 5 and 10%, respectively.

Statistical analyses

Plasma levels of hormones are expressed as mean $\pm$ SEM. Student's *t* test was employed to test for the between groups differences in cortisol, DHEAS, cortisol: DHEAS ratio and CD4 cells. For determining the significance of difference, analysis of variance (ANOVA) was computed followed by post hoc test, and a $p < 0.05$ was considered as significant.

Correlations among the study variables were determined using Pearson's product moment correlation coefficients, where the square of the Pearson's 'r' equals the amount of variance accounted between the variables. Repeated measures of ANOVA (RMANOVA) were carried out to evaluate the changes in the levels of hormones and CD4 cell numbers at the follow-up visits.

Results

Clinical Evaluation and Anthropometric Changes

The characteristics of HIV-1 clade C infected patients and controls are presented in Table 1. Among the 120 asymptomatic HIV seropositive participants enrolled for the study, 7 died of HIV related complications, 28 were given antiretroviral treatment (HAART) during the following 2 y, and one patient discontinued his participation in the study. Therefore, the data presented in the respective figures pertain to 84 (70%) subjects who continued to remain asymptomatic during the follow up study period of 2 y.

General health checkup data on organ functions and biochemical profile among the HIV-1C infected patients were not significantly different from the reference values established in the laboratory. However, despite being asymptomatic at the time of recruitment, the body mass index, (BMI, Kg/m²) of HIV infected patients was significantly lower (Table 2) than that in the control group (20.67 ± 0.29 vs 22.04 ± 0.40 , $p < 0.01$). During subsequent follow-up for 2 y, the BMI of HIV-infected patients who remained clinically asymptomatic ($n=84$), did not differ from the initial value determined at the time of recruitment (1st follow-up: 21.16 ± 0.35 ; 2nd follow-up: 21.31 ± 0.36), indicating no further serious deleterious effect of the disease on health during the follow-up period. However, correlational analysis of BMI with endocrine and immunological parameters revealed a significant negative correlation with Cortisol:DHEAS ratio ($r = -0.20$, $p = 0.04$), and positive correlation with DHEAS ($r = 0.18$, $p = 0.04$) and CD4 cell counts ($r = 0.32$, $p = 0.001$).

Neuroendocrines and their relationship with CD4 cell counts at baseline and at follow-up

Basal plasma levels of cortisol in HIV seropositive individuals were found to be higher and DHEAS levels were significantly lower compared to the reference values for the control group established for both hormones at the time of enrolment in the study program (cortisol, $\mu\text{g/dl}$, 9.83 ± 0.39 vs 8.04 ± 0.45 ; $p < 0.01$; DHEAS, $\mu\text{g/dl}$, 81.02 ± 4.9 vs 185.1 ± 12.03 ; $p < 0.001$, respectively) and resulting in higher cortisol:DHEAS ratio among the HIV-1 C positive patients (HIV-1C+, 0.19 ± 0.002 vs controls, 0.058 ± 0.006 ; $p < 0.001$) (Table 2; Figure 1A–C).

Gender Difference in Endocrine Parameters

The data presented in Table 2, also revealed that when the levels of cortisol and DHEAS were expressed as% values of controls, there was 15% increase in cortisol levels in HIV-1 C infected females compared to 32% increase in males. Similarly in female patients, DHEAS levels decreased by 51.6% and cortisol:DHEAS ratio increased by 141.4%, compared to that in males with a 55.1% increase in cortisol and 194.4% increase in cortisol:DHEAS ratio. Moreover, the lower% increase in cortisol:DHEAS ratio was also correlated with lesser decrease in CD₄ cell counts.

Findings from the yearly follow up studies on adrenocortical hormones are presented in Figure 1(A–D). Repeated measures ANOVA analysis reiterated the initial findings on baseline plasma levels of adrenocorticoids during the follow-up visit. During the first year follow up, i.e., at the second visit; there was a further significant increase in plasma cortisol ($\mu\text{g/dl}$, 10.76 ± 0.47 vs 1st visit 9.29 ± 0.45 ; $p < 0.05$), a decrease in DHEAS levels ($\mu\text{g/dl}$, 72.41 ± 4.36 vs 1st visit 89.69 ± 6.20 ; $p < 0.001$), and an increase in cortisol: DHEAS ratio (0.19 ± 0.015 vs 1st visit: 0.13 ± 0.012). However, plasma levels of both cortisol and DHEAS during the second year follow up (3rd visit) remained essentially the same as that found at the 2nd visit (1st year follow up, Fig 1C), but were significantly higher than that at the baseline.

Figure 2(A–C) depicts the correlation between plasma cortisol levels vs CD₄ cell numbers (A), DHEAS levels vs CD₄ cell numbers (B) and cortisol: DHEAS ratio vs CD₄ numbers (C) of asymptomatic HIV-1 C+ patients at baseline, i.e., at the time of recruitment (1st visit). It is evident that while decrease in plasma DHEAS level demonstrates a positive correlation with decrease in CD₄ cell counts, plasma cortisol levels and cortisol: DHEAS ratios were found to have negative correlation with CD₄ counts. In addition, when we determined correlations between CD₄:CD₈ ratio and plasma cortisol levels as well as cortisol: DHEAS (C/D) ratio, it was interesting to find a negative correlation between CD₄:CD₈ ratio and Cortisol ($r = -0.24$, $p = 0.011$) as well as Cortisol: DHEAS ratio ($r = -0.28$, $p = 0.0046$). Similarly% CD₄ decrease also showed significant negative correlation with cortisol ($r = -0.24$, $p < 0.01$) and cortisol:DHEAS ratio ($r = -0.28$, $p < 0.01$).

The data on adrenocortical hormones profile and CD₄ counts during the 2 y follow up among the HIV-1C+ patients who continued to remain asymptomatic and drug naïve ($n = 84$) is presented in Figure 3(A–E). A consistent negative correlation was found between cortisol levels as well as cortisol: DHEAS ratio and CD₄ cell counts, at all three visits of HIV-1+ patients (at the time of enrolment, i.e., 1st visit, and subsequently at the 2nd, and the 3rd years follow up visits). Further, we also examined the cause-effect relationship between the endocrine parameters and CD₄ cell counts. As depicted in Table 4, hormone levels at first visit (cortisol and cortisol: DHEAS ratio) were found to be predictive of a larger% decrease in CD₄ cell counts at the subsequent follow-up visits.

Changes in CD4 cell counts at the baseline and at follow-up periods

Immune status of HIV-1 C seropositive patients was monitored by CD₄ counts during the 2 y follow up. Thus, according to the CDC classification, the immune status in HIV-1 infected individuals is evaluated on the bases of CD4 cell counts at the baseline, and the patients can be grouped into 3 categories (group 1, CD > 500 ; group 2, CD₄ = 201 to 499; and group 3, CD₄ < 200). Although, at the time of recruitment, the number of HIV-1+ patients was higher in all three groups (group 2, $n = 63$, 53.8%; CD₄: 201 to 499; followed by group 1, $n = 32$, 27.3%; CD₄ > 500 ; and group 3, $n = 22$, 18.8%; CD₄ < 200), than at the time when this study was completed (36 patients discontinued their participation in this study due to different reasons (Table 1), and among those who completed the study the majority of HIV-1+ patients shown in Table 3, belonged to group 2 ($n = 50$, 59.5%; CD₄ = 351 ± 81.3), followed by groups 1 ($n = 30$, 35.7%; CD₄ = 669 ± 142), and group 3 ($n = 4$, 4.76%; CD₄ = 175.5 ± 25.77). Surprisingly, the patients in group 3 who had CD₄ counts < 200 , continued to remain neurologically asymptomatic. The findings on CD₄ counts in 84 subjects were analyzed by repeated measures ANOVA. The fact that the initial number of 22 (18.8%) patients in group 3 were reduced to only 4, suggests that the majority of the patients ($n = 18$) who could not continue to participate in the study were from group 3, and these patients either received HAART or were not able to continue due to disease progression. Interestingly, the four subjects in group 3 who continued to remain asymptomatic during the 2 y follow up were found to have marginal improvement in their CD4 counts during the 2nd

and 3rd visits, i.e., 2 years follow up (CD4= 258±86 and CD4 =215±105 respectively). These findings may indicate the fluctuation in immune status during the progression of disease. However, when the data on CD4 counts in all 84 subjects as a group were compared with CD4 counts at the first visit, a significant progressive decrease in CD4 counts was found (as shown in Figure 1D), indicating the ongoing immunosuppression.

Neuroendocrine profile among HIV-1+ patients who received Highly Active Antiretroviral Therapy (HAART)

At the time of recruitment, none of the patients were receiving HAART, as this was one of the exclusion criteria for this study. Subsequently however, in view of the progression of the disease, particularly those who had CD4 cell count of <200, and/or those who were found clinically ill, they were started on the HAART regimen. The HAART regimen included azidothymidine (AZT), stavudine (STV), lamivudine (LMV) and nevirapine (NVP). Those patients who had systemic tuberculosis were administered the non-nevirapine regimen. During the first year, 3 patients were administered HAART. During the second visit (after 12 months) ten more patients received HAART regimen and between the 2nd and 3rd visits (24 months) another 15 more patients required to be started on HAART treatment in view of clinical deterioration. Hence the data from these 28 patients have been analyzed separately. When biochemical parameters were compared between those who received HAART and those who did not receive HAART, we did not find a significant difference in the neuroendocrine profile between the HAART treated and the untreated groups (data not shown). This may be due to the small sample size and a short duration of follow-up after HAART.

Discussion

The findings of this study demonstrate that HIV-1 clade C infection causes a significant disturbance in the profile of plasma levels of adrenocortical hormones, cortisol and DHEA (measured as DHEA-S), as well as cortisol: DHEAS ratio. We also found that the disturbance in these hormones continues as the disease progresses and is marked by the progressive increase in the levels of plasma cortisol, decrease in DHEAS, and about 3 times increase in cortisol:DHEAS ratio in both males and females, with the concomitant decline in the number of CD4 cells over time.

Although, neuroendocrine dysfunctions, including abnormal hypothalamic-pituitary-adrenal (HPA) axis associated with HIV-1 B infection have been reported in different studies [12–16], studies have been scarce on the impact of HIV-1 clade C virus on the progressive dysfunctions of the neuroendocrine systems over time, particularly, on the adrenocortical hormones. This study was warranted on the premise that the impact of HIV-1 C infection on the neuroendocrine profiles might differ from that caused by HIV-1 B infection because of the difference found in the structure and neurotoxic properties of some of the viral proteins, such as trans activating protein, Tat, between the 2 strains of the virus [45]. However, findings from the present study show concordance to the earlier reports that the decrease in circulating DHEAS, increase in cortisol, and cortisol:DHEAS ratio, and decrease in CD4 cell counts are essentially similar to those reported in men infected with HIV-1B, and that these changes may precede progression to AIDS in those who had CD4 cell counts below 500 [30]. These findings thus implicate that the damage to the adrenal glands is a common complication of infections with HIV-1, irrespective of the strain of the virus.

Although, the mechanism involved in HIV-1 related disturbance in the homeostasis between cortisol and DHEAS and cortisol:DHEAS ratio have not been fully elucidated, a similar pattern has been observed in acute and chronic illness, as well as in malnutrition cases among non-HIV patients [48]. In HIV-1 infected patients however, steroid metabolism has been

considered to exhibit a shift from the synthesis of adrenal androgens towards increased synthesis of cortisol [11]. This pattern of high cortisol and reduced DHEA synthesis is thought to be caused by an increase in the activity of the enzymes, 3 β -hydroxysteroid dehydrogenase (3 β -HSD), that may drive the reaction towards increased conversion of pregnenolone to cortisol via 17-OH progesterone pathway, and allowing the limited availability of pregnenolone, along with decreased adrenal 17, 20 lyase activity, needed for the synthesis of DHEA via 17-OH hydroxypregnenolone pathway [47]. Thus the findings of this study (Table 2) as well those reported by others [30,34,41], showing a decrease in DHEAS, increase in cortisol, and cortisol: DHEAS ratio during the asymptomatic phase of HIV infection concur with the altered metabolic pathways of adrenocorticoids.

Moreover, earlier reports by us showing dysregulation of adrenocortical hormones during the asymptomatic phase of HIV infection [4,6,13], and the present observation of altered adrenocortical profile showing more than 20% increase in circulating cortisol levels (table 2; $\mu\text{g/dl}$, from 8.04 ± 0.45 in control to 9.83 ± 0.39 in HIV-1C+; $p < 0.01$), and a concomitant 50% decrease in DHEAS levels ($\mu\text{g/dl}$, 81.02 ± 4.9 ; $p < 0.001$) as compared to that in the control group ($\mu\text{g/dl}$, 185.10 ± 12.03), preceding the decline in CD4 counts to critical level, suggests that these two adrenocortical hormones (cortisol and DHEAS) play an important immunomodulatory role in the regulation of CD4 cell number and HIV-1 disease progression. The interaction between the neuroendocrine and immune systems and its relevance in health and disease is well documented [7,25–27], particularly the immunosuppressive properties of cortisol have been used for therapeutic application in immunomodulation during the organ transplantation and inflammatory conditions [28,29], and the immunoprotectant property of DHEA, although a relatively recent finding, is being increasingly recognized [4,24,30–38]. Thus, it appears that the two adrenocortical hormones, viz., cortisol and DHEA working in tandem, though in antagonistic manner, regulate the mechanisms of homeostasis of the immune functions. In HIV-1 infected patients however, the significance of dysregulated neuroendocrine functions in general, and that of the adrenocorticoids in particular and their relevance in the HIV-1 clade dependent disease progression are not fully understood.

Furthermore, it is interesting to note that our findings of an increase in cortisol: DHEAS ratio at baseline among asymptomatic HIV-1C positive patients (table 2) are in consonant with the findings of a previous report on HIV-1C infected asymptomatic and symptomatic patients showing a significant increase in cortisol: DHEAS ratio [41]. However the findings of persistent changes in hormone profile (increase in cortisol: DHEAS ratio) over 2 y and no further change at the third follow-up visit shown for the first time in the present study (Fig 1D) may be attributed to the nature of the disease progression leading to insufficiency in the adrenal functions. Of further interest is the finding of negative correlation between the cortisol: DHEAS ratio and the CD4 counts both at the time of enrolment in the study and also during the follow up for 2 y (Figs. 2,3). Though the increase in cortisol: DHEAS ratio, and decrease in CD4 cell counts persisted during the first year follow up, the negative correlation between the cortisol: DHEAS ratio and CD₄ count was persistent throughout the study period (baseline + 2 y) of asymptomatic phase of HIV infection.

With regards to the effect of HAART, although recent studies have shown that plasma cortisol levels significantly decrease and DHEAS increase when HIV-positive men are treated with HAART [48], we did not find significant difference in the neuroendocrine profile between the small number of HIV-1 C+ patients who had CD4 cell counts of <200 ($n=4$), and were eligible and received HAART vs those with higher CD4 cell counts (201 to >500) who were not eligible ($n=80$) and did not receive HAART. This lack of difference in neuroendocrine profile between the two groups may be due to the small sample size and a short duration of follow-up after HAART.

With respect to gender differences, although, there were differences found in the hormone profile between HIV-1C infected men and women, and men showing greater decrease (55%) in DHEAS and higher increase in plasma cortisol (32%) compared to that in women (51%, and 15%, respectively, Table 2), the cortisol:DHEAS ratio remained higher in women, mainly because of the low levels of DHEAS in both control women and in those with HIV-1C infection. However, for the final analysis, the data from both genders were combined to delineate the relationship among the hormones levels and between hormones and CD4 cell counts (Figs., 1–3).

The findings of dysregulation of adrenocorticoids metabolism preceding the decline in CD₄ counts among asymptomatic HIV-1C seropositive patients in this study may provide a scope for therapeutic intervention by DHEAS supplementation. These findings based on the metabolic profile may suggest 3 β -HSD as a potential molecular target for drug design aimed to control the dysregulation of adrenocorticoids metabolism among the HIV infected subjects (something comparable with the ‘statin’ inhibition of HMG-CoA reductase as a therapeutic agent for hypercholesterolemia) [43]. Thus addressing the observed changes in the adrenocorticoids profile during the asymptomatic phase may help to restore the cell mediated immunity (CMI) and alter the natural course of disease progression.

In conclusion, the present study clearly demonstrates the dysregulation of adrenocorticoids metabolism resulting in the increase of cortisol:DHEAS ratio during the asymptomatic phase of HIV infection and its negative correlation with CD₄ counts. The finding of increased cortisol:DHEAS ratio preceding the decrease in CD₄ counts, and reaching the critical level to warrant ART administration indicates a scope for therapeutic intervention through immunomodulation by restabilizing the adrenocorticoids profile. In this context, it may be of interest to try the therapeutic efficacy of DHEAS supplementation although with careful monitoring during the asymptomatic phase of HIV infection in order to sustain and possibly resurrect the CMI, which has been down regulated in HIV-1 infected patients. Furthermore, an attempt to decrease in cortisol:DHEAS ratio by down regulation of 3 β -HSD enzyme activity by the candidate molecular target may provide options for newer drug discovery with potential therapeutic applications in disorders of adrenocorticoids metabolism.

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Figure 1.

Plasma levels of cortisol (A), DHEAS (B), cortisol: DHEAS ratio (C) and CD₄ cell counts (D) among asymptomatic HIV seropositives (n=84) during the 2 y follow up. Statistical significance *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ is shown in comparison to the 1st visit; \$: $p < 0.05$ is in comparison to the 2nd visit of HIV-1C infected individuals. Values of base line levels of cortisol, DHEAS and cortisol:DHEAS ratio in the controls are shown in the panels A–C for comparison.

Figure 2.

Correlation between plasma cortisol (A), DHEAS (B), Cortisol: DHEAS ratio (C) and CD4 counts among asymptomatic HIV seropositives ($n = 120$) at the time of recruitment in the study (1st visit). Note the inverse relation between cortisol and CD4 ($r = -0.25$; $p < 0.01$) and cortisol: DHEAS ratio and CD4 ($r = -0.31$; $p < 0.001$) and positive correlation between DHEAS and CD4 counts ($r = 0.20$; $p < 0.05$).

Figure 3.

Correlation between plasma CD4 counts, cortisol, and cortisol:DHEAS ratio in asymptomatic HIV-1 C seropositive individuals over 2 y follow-up. Panels A–F: Correlations between CD4 counts and hormone levels at 1st, 2nd and 3rd visits. Note the negative correlation between CD4 cell counts and cortisol levels, and CD4 cell counts and cortisol: DHEAS ratio at all 3 visits.

Table 1**HIV-1 C positive patients characteristics**

Patient details	Number	Percentages
Neurologically asymptomatic HIV seropositives enrolled	120	100
Expired	7	5.83
Patients put on ART*	28	23.3
Number of patients Drop out from the study	1	0.8
HIV-1C positives continued to remain neurologically asymptomatic by the end of two year follow up	84	70
Clinical Outcome at the end of the study (2 nd Follow-up) [#]	Number	Percentages
CMV Infection (Retinitis)	1	0.8
Cryptococcal meningitis	2	1.66
Peripheral neuropathy	9	7.5
Tuberculosis	13	10.8
AIDS Cholangiopathy	1	0.83
Severe Anemia	3	2.5
Diabetes	5	4.16

* Number of subjects on ART at the end of 1st year follow up (n=13)

* Number of subjects on ART at the end of 2nd year follow up (n=28)

[#] Overlapping of outcome/symptoms in same patient.

Table 2

Plasma levels of cortisol, DHEAS and cortisol:DHEAS ratio in controls (HIV-negative) and HIV-1C+ in male and female patients at the enrolment time (baseline values)

	All Controls (N=69)	All HIV-1C (N=120)	P value	Control Males (n=38)	HIV-1 C Males (n=52)	Control Females (n=31)	HIV-1 Females (n=68)
Cortisol (µg/dl)	8.04±0.45	9.83±0.39	<0.01	8.0 ±0.58	10.60±0.67*	8.03±0.74	9.24±0.45*
DHEAS (µg/dl)	185.1±12.03	81.02±4.9	<0.001	222.3±15.42	99.79±7.62*	137.8±16.08	66.66±5.82*
Cortisol:DHEAS ratio	0.058±0.006	0.19±0.002	<0.001	0.04±0.00	0.14±0.01*	0.08±0.01	0.23±0.03*
Body Mass Index (BMI), Kg/m ²	22.04±0.40	20.67±0.29	<0.01	21.59±0.44	21.06±0.43	22.61±0.74	20.37±0.39

A significant difference was found in plasma levels of cortisol and DHEAS as well cortisol:DHEAS ratio between HIV-negative and HIV-1C+ patients. When the hormone data were analyzed separately with respect to gender in both control and HIV-1+ groups, the difference in the levels of hormones between males and females in the two groups remained significant (*p<0.05). There was no significant difference in BMI between the two groups, although, a significant negative correlation was found between BMI and Cortisol:DHEAS ratio (r= -0.20, p=0.04), and positive correlation between BMI and DHEAS (r=0.18, p=0.04) and CD4 cell counts (r=0.32, p= 0.001).

Table 3
Profile of CD4 cell counts and group assignments of HIV-1C+ patients (CDC classification)

CD4 cell numbers	Patient Groups	n	1 st Visit	2 nd Visit	3 rd Visit	P value 1 st vs 2 nd Visit	P value 1 st vs 3 rd Visit	P value 2 nd vs 3 rd Visit
>500	Patient group 1	30	669.1±142.2	533.5±144.9	503.1±146.9	<0.001	<0.001	NS
201–499	Patient Group 2	50	351.5±81.31	314.3±96.90	277.9±154.2	NS	<0.001	NS
<200	Patient Group 3	4	175.5±25.77	258.0±86.47	215.0±105.3	NS	NS	NS

Based on CD4 cell numbers, the patients were divided into three groups and cell numbers were determined at each visit. Although, there was a significant decrease in CD4 cell numbers from 1st visit to the 2nd visit in group 1, and from 1st to 3rd visit in groups 1, and 2, there was no significant change in CD4 cell numbers in group 3 between 1st visit and the subsequent two visits.

Table 4

Plasma levels of cortisol and cortisol: DHEAS ratio at baseline and decrease in CD4 predictability overtime

	% CD4 decrease prediction during 1 st yr Follow-up.	% CD4 decrease prediction during 2 nd yr Follow-up.
Cortisol		
Patient Group 1 (8–12 µg/dl),	4.5%	23.5%
Patient Group 2 (>12 µg/dl),	9.1%	26.5%
Cortisol: DHEAS Ratio		
Patient Group 1 (0.05–0.2)	5.8%	18%
Patient Group 2 (>0.2)	25%	28%

The data showing the levels of plasma cortisol and cortisol:DHEAS ratio as the predictive marker for the % decrease in CD4 cell counts at the follow-up visits among clinically asymptomatic HIV-1C+ patients.