HPA axis activity and neuropathogenesis in HIV-1 clade C infection

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1. ABSTRACT

A disturbance in the activity of the hypothalamic-pituitary-adrenal (HPA) axis has been reported among individuals with HIV-1 infection. However, these studies have been carried out in the West where the infecting clade is clade B. HIV-1 infection is rapidly spreading in various parts of South East Asia, including India, where the HIV-1 infecting clade is largely clade C. An investigation of HPA axis activity in this type of infection is warranted since there are many structural differences between clades B and C. This study was carried out to investigate whether HIV-1 infection clade C interferes with the functions of the hippocampus and thereby affects the HPA axis. We tested the hypothesis that when hippocampus activity is disturbed, it leads to the development of neuropathogenesis in HIV-1 C-clade infected individuals. This study included asymptomatic HIV-1 seropositive individuals (n=117) and, age-matched, HIV-1 seronegative controls (n=29). Neuroendocrine function of the HPA axis was evaluated using plasma levels of cortisol, ACTH, and DHEA-S, both in the morning (0800-1000 hr) and evening (2000-2200 hr). A significant elevation of cortisol levels during A.M. and P.M. hours was observed in HIV-1 infected individuals when compared to the controls. Interestingly, no significant change in ACTH level was observed in HIV-1 seropositive subjects, either during A.M or P.M. Elevated levels of cortisol in HIV-1 seropositive subjects appear to be independent of ACTH and may be the result of a defective negative feedback mechanism. On the other hand, a significant decrease in the plasma levels of DHEA-S was observed during A.M. and P.M. hours in HIV-1 infected individuals, leading to an increased cortisol to DHEA-S ratio. Since increased levels of cortisol and decreased levels of DHEA-S are related to the development of neuropathogenesis, it is hypothesized that a study of the development of neurocognitive deficits among HIV-1 seropositive individuals in India is warranted.

2. INTRODUCTION

It is well established that immediately after HIV-1 infection, the virus crosses the Blood Brain Barrier (BBB) and is localized at high concentration in the hippocampus and to a variable degree in other areas of the brain (1-3). The hippocampus plays an important role in controlling both endocrine and neurocognitive functions. The neuroendocrine function of the hippocampus is mediated through the hypothalamo-pituitary adrenal (HPA) axis. Several lines of evidences from West, where HIV 1 clade B infection is predominant, suggest adverse affects of HIV on the neuroendocrine function. Emerging evidence suggests that these effects may be due to molecular mimicry between HIV-1 genome and key control elements of the HPA axis. For instance, gag, a viral protein of HIV-1, and the LTR region exhibit homology with the proopiomelanocortin (POMC) promoter normally regulated by corticotrophin releasing hormone (CRH), and another HIV-1 protein, viral protein R, can interact with glucocorticoid receptors (4). Glucocorticoids, cortisol in man, the end product of the HPA axis, are thought to cause atrophy of the hippocampus (5). It has been reported that the hippocampus has a high density of both type I and type II corticosteroid receptors (6). Hence, it is likely that increased levels of cortisol may exacerbate the destructive effects of other factors in the brains of HIV-1 infected individuals, since glucocorticoids are known to modulate pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (7). HPA axis related hormones, ACTH and cortisol, are important markers of neuroendocrine responses to various infections and stressors. These conditions result in heightened adrenergic responses which stimulate the limbic-HPA axis resulting in an increase in central CRH activity, which in turn stimulates ACTH secretion and is followed by an increase in cortisol produced by the adrenal cortex. Cortisol, in turn inhibits endogenous ACTH and central CRH secretion via negative feedback inhibition. Dehydroepiandrosterone (DHEA), also an adrenal hormone, responds to fluctuating concentrations of ACTH and is released episodically and synchronously with cortisol. In the brain, DHEA has been reported to be an anti-glucocorticoid, an anti-glutamatergic and may act as a neuroprotective agent (8). Such observations have led to the conclusion that any study on glucocorticoid activity should also include studies of DHEA. DHEA exists in plasma in a conjugated, sulphated derivative form (DHEA-SO$_4$).

Though earlier studies have reported the occurrence of endocrine dysfunctions in HIV-1 clade-B infection, particularly in HPA axis activity (9-13), little is known about neuroendocrine function among the HIV infected population from Asian countries, where the infecting agent is clade C. Further, Neurological complications related with HIV-1 clade C remain largely unknown. However, there are a number of differences between the structures and properties of HIV-1 Clades B and C. For instance, clade C virus almost exclusively uses the chemokine receptor CCR5 for its entry into CD4 cells (14). Among various HIV-1 clade B proteins, Tat has been shown to cause a selective loss of neurons in vitro and in vivo (15, 16). Further, it has been reported that the amino acid content of Tat, specifically: lysine, cytsteine, threonine and serine vary among these two clades (17). Despite these differences, investigations on clade C are scarce. These theoretical considerations form the basis for investigating HPA axis activity among HIV-1 clade C infected individuals. The present investigation was planned to study HPA axis activity in terms of A.M. and P.M. levels of plasma ACTH and cortisol as well as that of DHEA-S among asymptomatic HIV-1 infected individuals in South India, where the infecting clade is C (18).

3. MATERIALS AND METHODS

3.1. Participants

Participants were all community residing women and men living near the study center, the National Institute of Mental Health and NeuroSciences (NIMHANS) in Bangalore, India. Individuals who had a history of CNS infection, substance abuse, steroid dependence, as well as women who were pregnant and/or lactating were excluded from participation in this study. All the participants were 18 to 45 years old and underwent comprehensive neurological and physical examinations. All subjects enrolled for the study signed an informed consent approved by the NIMHANS Institutional Ethics Committee and the University of Miami IRB. Those enrolled in the study (n= 117) were found to be HIV-1 seropositive using ELISA and Western blotting. Healthy, age-matched, HIV-1 seronegative individuals (n = 29), drawn from the non-spousal family members of HIV-1 infected cases, formed the control group. All the HIV-1 seropositive participants knew their serostatus at least 6 months prior to their enrollment in the study. All the subjects were admitted overnight as inpatients to the hospital for the purpose of carrying out investigations.

3.2. Specimen collection

In consideration of the circadian rhythms affecting basal hormones levels, blood specimens were collected during the A.M. (0800-1000 h) and P.M. hours (2000-2200 h) of the day. Blood samples were collected from the ante-cubital vein in chilled EDTA vacutainers (Becton and Dickinson). An aliquot of the EDTA blood was used for CD4 cell counts. Plasma was separated within one hour and specimens were aliquoted in vials containing aprotinin and stored at -80ºC until analysed.

3.3. 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 (Immunitics, USA) analysis of serum. Estimation of CD4 cell counts in blood sample was carried out by flow cytometry (FACS counter, B.D., U.S.A.)

3.4. Assay of ACTH, Cortisol and DHEA-SO$_4$

ACTH, cortisol, and DHEA-SO$_4$ (DHEA-S) were quantified using commercially available kits (Immulite, Diagnostics product corporation, U.S.A.) based on enzyme amplified immuno-chemiluminescence. The analytical ranges of the methods for ACTH, cortisol, and DHEA-S were found to be 9 - 1250 pg/ml, 0.2 – 50 µg/dl and 3 – 100
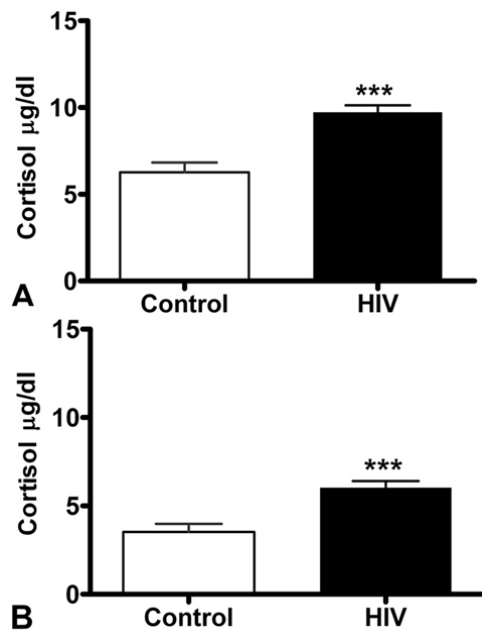
Figure 1. Plasma cortisol levels of HIV-1 seropositive (n=117) and seronegative (n=29) subjects during (A) A.M (0800 – 0900) and (B) P.M. (2000 – 2200) hours of the day. Note the significant increase in cortisol level (p<0.001) among HIV-1 infected subjects during both the A.M. and P.M comparisons.
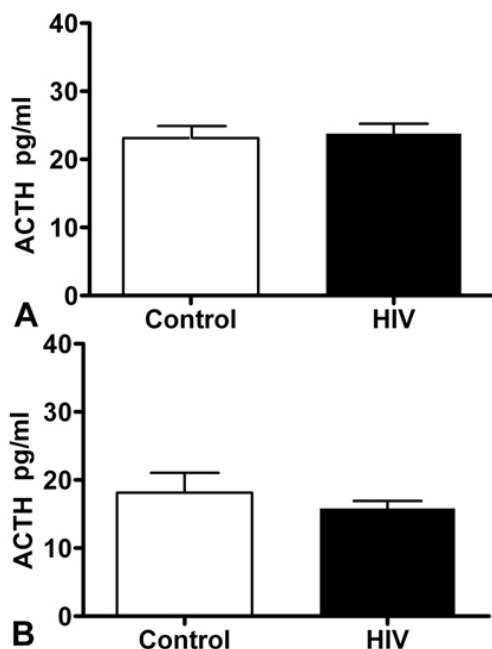

Figure 2. Plasma ACTH levels of HIV-1 seropositive (n=117) and seronegative (n=29) subjects during(A) A.M (0800 – 0900) and (B) P.M. (2000 – 2200) hours of the day. Note that there is no statistically significant difference in ACTH levels observed during the A.M. and P.M. times though the trend of ACTH suppression is noted in P.M. specimens (p>0.005).

μg/dl, respectively. Intra-assay and inter-assay co-efficient of variance (CV) were 5 and 10%, respectively.

3.5. Statistical Analysis

Plasma hormone levels were expressed as Mean ± S.D, student's t-test was employed to test for differences between the groups and analysis of covariance (ANCOVA) was used to adjust for the influence of A.M. on the P.M. measurements. ACTH, cortisol, DHEA-S and Cortisol: DHEA-S ratios of both morning (A.M.) and evening (P.M.) specimens in HIV-1+ and control groups were compared. Cortisol: DHEA-S ratio (C/D) ratios were computed for clinical subgroups based on CD4 counts. In order to maintain normative distribution, all data were subjected to logarithmic transformation. In order to test whether the ratios of the subgroups differed significantly, analysis of variance (ANOVA) was utilized followed by post-hoc testing.

4. RESULTS

As shown in Figures 1A and 1B plasma levels of cortisol were significantly increased among HIV-1 seropositive individuals when compared to controls for both A.M. and P.M. hours (9.73 ± 4.30 μg/dl vs.6.27±3.0 μg/dl, p <0.001 and 6.03±4.04 μg/dl vs.3.53±2.48 μg/dl, p <0.001; respectively). On the other hand, plasma ACTH A.M. and P.M. levels in the HIV-1+ did not differ from that of control group (23.1±8.72 pg/ml vs. 23.82±15.5 pg/ml, and 18.10±15.84 pg/ml vs. 15.80±11.90 pg/ml, respectively; Figures 2A & 2B). Although no diurnal variation in DHEA-S was seen between the control and HIV-1 seropositive groups, a significant reduction in both the A.M. and P.M. levels of DHEA-S was observed among HIV-1+ individuals when compared to the controls (81.08±54.1 μg/dl vs. 134.7±74.1 μg/dl, p<0.001 and 69.40±46.50 μg/dl vs. 127±70.1 μg/dl, p<0.001; respectively; Figures 3A & 3B). The observed increase in plasma cortisol with concomitant depletion of DHEA-S is reflected as a significant increase in the cortisol to DHEA-S ratio during both A.M. and P.M. hours in the HIV-1+ group when compared to the control group (0.194 ± 0.22 vs. 0.056 ± 0.03, p<0.001 and 0.14±0.22 vs. 0.031±0.02, p<0.001; respectively; Figures 4A & 4B). HIV-1+ individuals were sub grouped on the basis of their CD4 cell count as shown in Table 1. A relationship between a gradual increase in cortisol to DHEA-S ratios and a decrease in CD4 cell count was noted among all groups of patients (Table 1). This strong reverse relationship was observed among all the patient groups irrespective of their CD4 count, with more elevated ratios when the CD4 count was lower.

5. DISCUSSION

The findings presented in this investigation show that HPA axis activity is disturbed in HIV-1 infection in South India, where the infecting clade is clade C. These disturbances in neuroendocrine activity are supported with evidence (Figures 1A and B) that both A.M. and P.M. levels of cortisol are elevated among the infected
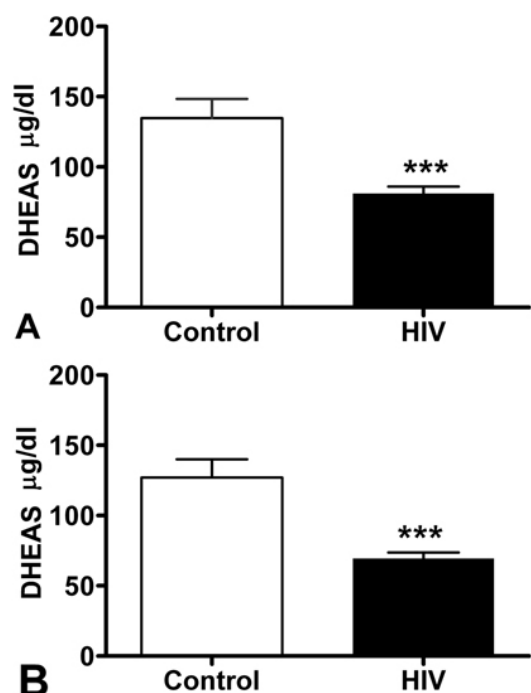
Figure 3. Plasma DHEA-SO$_4$ levels of HIV-1 seropositive (n=117) and seronegative (n=29) subjects during (A) A.M (0800 – 0900) and (B) P.M. (2000 – 2200) hours of the day. Note the significant decrease in plasma DHEA-S level (p<0.001) in both A.M. and P.M. comparisons.
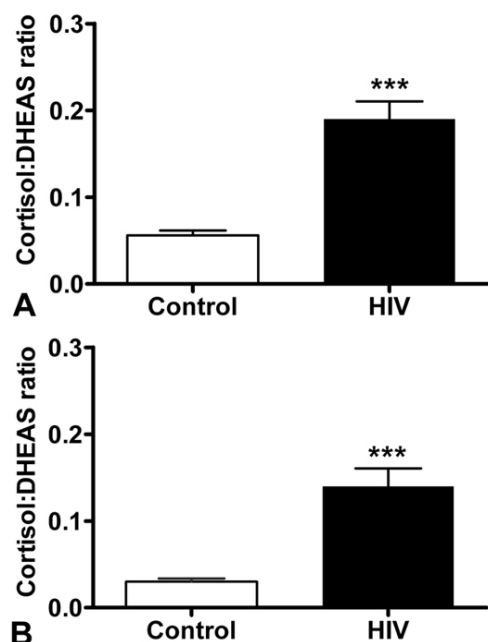

Figure 4. Plasma Cortisol:DHEA-S ratios of HIV-1 seropositive (n=117) and seronegative (n=29) subjects during (A) A.M (0800 – 0900) and (B) P.M. (2000 – 2200) hours of the day. Note the significant increase in Cortisol:DHEA-S ratios (p<0.001) among HIV-1 infected subjects both during the A.M. and P.M. comparisons.

individuals. Although the mechanisms of these elevated concentrations were not investigated it is thought that these changes may be mediated by a disturbance in glucocorticoid receptors (6). Since glucocorticoid has been reported to cause atrophy of the hippocampus (5), these findings suggest that HIV-1 infected individuals will suffer from neurocognitive deficits. This is an important finding since unlike HIV-1 clade B infected individuals in the West, there is a scarcity of investigations on neuroendocrine and/or neurocognitive functioning in HIV-1 clade C infected individuals. It is well established that HPA axis activity is initiated by a signal from the hippocampus that stimulates the paraventricular nucleus of the hypothalamus to secrete corticotrophin releasing hormone (CRH) which, in turn, stimulates the anterior pituitary glands to synthesize POMC peptide, resulting in the release of ACTH. It is ACTH that stimulates the synthesis and release of adrenal cortical glucocorticoids. cortisol mobilizes and replenishes energy stores, inhibits growth and the reproductive system, inhibits the immune response, and acts on various neurotransmitters (19). Interestingly, cortisol exerts negative feedback influence inhibiting ACTH and CRH secretion as well as inhibiting hippocampal activity. In this investigation, as shown in Figures 2A and 2B, ACTH levels did not differ from the levels observed in control participants. Since the levels of cortisol in HIV-1+ individuals during AM. and P.M. hours were found to be significantly higher than the control subjects, it may be concluded that negative feedback inhibition by cortisol and/or the sensitivity of the adrenals is increased. Our investigations also demonstrate decreased levels of dehydroepiandrosterone (DHEA), an anti-glucocorticoid, among infected individuals (Figures 3A and 3B). These findings suggest greater cortisol activity which, as mentioned above, has been reported to be neurotoxic at continually high levels, resulting in neuropathogenesis. Moreover, data presented in Table 1 illustrates that the ratio of Cortisol to DHEA-S increases with a decrease of CD4 cells.

It is evident that neuroendocrine, immune and autonomic nervous systems function in an integrated manner through the action of molecular signals such as hormones and cytokines as well as through neural mechanisms (20). The predisposition of the hippocampus and the adrenal cortex, two ends of the HPA axis (21), and the immune system to HIV-1 infection appear to suggest that the HPA axis could be one of the main targets of the virus. Subsequently, it is possible that HIV-1 infection induced hypercortisolemia may result in the exacerbation of neuropathogenesis. There are several reports that support such findings (22-28)but while some of the reports are suggestive of hypercortisolism with altered response to ACTH stimulation among AIDS patients (29, 30) other reports remain ambiguous. It is important to note, however, that many of the earlier reports dealt with clade B infection, prevalent in western countries, and that the present report is perhaps the first from India, where the most prevalent infecting clade is clade C.

HIV-1 Clade C Infection: HPA Axis Activity and Neuropathogenesis

Table 1. Relationship of Cortisol: DHEA-S ratios (C/D ratio) and CD4 counts between control group and HIV-1 seropositive subjects with different CD4 counts

Subjects	Number of subjects	CD4 count (Mean ± SD)	C/D ratio A.M. (Mean ± SD)	C/D ratio P.M. (Mean ± SD)
Control	29	Not done	0.05±0.03	0.03±0.02
Pt group I (CD4 <200)	22	132±50	0.30±0.34 [1]	0.28±0.45 [1]
Pt group II (CD4 200-499)	63	337±87	0.18±0.18 [1]	0.12±0.12 [1]
Pt group III (CD4 >500)	32	665±154	0.14±0.18 [1]	0.16±0.36 [1]

[1] p <0.001

Present findings of elevated plasma cortisol levels among neurologically asymptomatic HIV-1 seropositive subjects when compared to normal subjects (Figures 1A and 1B) are suggestive of a state of hypercortisolism during the early stages of infection. Under normal circumstances, the increase in cortisol brings about the negative feedback inhibition of ACTH secretion to suppress the further release of cortisol. However, in the present context, despite the observed hypercortisolism among HIV-1 infected subjects, ACTH levels remained essentially the same as that of controls (Figures 2A and 2B). The inability of elevated cortisol to down regulate ACTH among HIV-1 seropositive subjects could be due to CNS pathology related to HIV-1 infection (31). The causative factor(s) and/or the mechanism contributing to the disruption of the HPA axis are rather difficult to explain. It is of interest to note that a significant decrease in the expression of glucocorticoid receptors in the medial lobe of the brain and lymphocytes of HIV-1 infected subjects has been previously observed (32). Thus, it appears that the hypercortisolism associated with HIV infection could be a consequence of non-pituitary factors of viral infection. In this regard it is of interest to note that viral agents can stimulate the production of ACTH like peptides from human lymphocytes and also the ability of monocytes to stimulate in-vitro cortisol secretion in cultures of human adrenocortical cells (33, 34). Irrespective of the mechanism(s) leading to hypercortisolism in HIV-1 infection, it is certain that elevated cortisol will also affect the immune system adversely. In this regard, it is important to note that while cortisol is immunosuppressive, DHEA is known for immuno-potentiation (11). Therefore, the measure of both cortisol and DHEA in serum could reflect the impact of these hormones on the immune system. In this context, it is of interest to note that DHEA-SO4, one of the intermediate metabolites of DHEA in plasma, was found to be decreased considerably among HIV-1 seropositive subjects when compared to control (Figures 3A and 3B). The altered homeostasis between the neuropathogenesis caused by cortisol and neuroprotection provided by DHEA in HIV-1 infection attains significance when the ratio of cortisol to DHEA (C/D ratio) is related to CD4 cell count as an index of immunostatus (Table 1). Thus, a definite increase in C/D ratio among HIV-1 infected subjects when compared to control is noted. The present findings of altered cortisol and DHEA-S levels in HIV-1 infected subjects may lead to neuropathogenesis (23, 30, 35, 36). Furthermore, a definite relationship between the C/D ratio and the three CD4 cell count based subgroups was discernible. Thus, HIV-1 seropositive subjects with CD4 counts above 500 were also found to have an elevated C/D ratio when compared to control subjects (Table 1). The observed relationship between C/D ratio and the CD4 counts further confirms the similar trend between low levels of DHEA and low CD4 counts in AIDS patients (37, 38).

In summary, the present study of HPA axis function among HIV-1 seropositive individuals in India, presumably infected with strain C, is the first report from India. It appears that it is not necessarily the severity of AIDS which shifts the pregnenolone metabolism away from both the mineralocorticoid and adrenal androgen pathways toward the glucocorticoid pathway as speculated earlier (39); instead it is the increase in C/D ratio, secondary to metabolic insult to adrenal function during the early stages of HIV-1 infection, that contributes to the progression of the disease leading to AIDS. The observed increase in the basal level of cortisol, C/D ratio and decreased DHEA-S level during the initial stages of HIV-1 infection, apart from reiterating the relationship between the endocrine and immune systems, may also provide an endocrinological indicator for therapeutic intervention much ahead of the decline in CD4 cell count and the onset of AIDS.

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