การศึกษาเปรียบเทียบระดับของดีฮัยโดรอีพิแอนโดรสเตอโรนซัลเฟตในเลือดของชายไทย ที่ได้รับการคัดเลือกเข้าเป็นพลทหารกองประจำการกองทัพบก ที่ติดเชื้อและไม่ติดเชื้อเอชไอวี-1

พ.ท.คุณากร คณา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Comparative Study of Serum Dehydroepiandrosterone Sulfate Levels in HIV-1 Infected and HIV-1-Seronegative Royal Thai Army Conscripts

LTC. Khunakorn Kana

A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	COMPARATIVE STUDY OF SERUM DEHYDROEPIANDROSTERONE SULFATE LEVELS IN HIV-1 INFECTED AND HIV-1-SERONEGATIVE ROYAL THAI ARMY CONSCRIPTS
By	LTC. Khunakorn Kana
Field of Study	Health Development
Thesis Advisor	Associate Professor Narin Hiransuthikul, M.D., M.PH., Ph.D.
Thesis Co-Advisor	Colonel Thippawan Chuenchitra, Ph.D.

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พ.ท.คุณากร คณา : การศึกษาเปรียบเทียบระดับของดีฮัยโดรอีพิแอนโดรสเตอโรน ซัลเฟต ในเลือดของชายไทยที่ได้รับการคัดเลือกเข้าเป็นพลทหารกองประจำการ กองทัพบกที่ติดเชื้อและไม่ติดเชื้อเอชไอวี-1 (Comparative Study of Serum Dehydroepiandrosterone Sulfate Levels in HIV-1 Infected and HIV-1-Seronegative Royal Thai Army Conscripts) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.นพ.ดร.นรินทร์ หิรัญสุทธิกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: พ.อ.หญิง ดร.ทิพย์วรรณ ชื่นจิตร

วัตถุประสงค์หลัก: เพื่อศึกษาถึงค่าเฉลี่ยของระดับฮอร์โมน Dehydroepiandrosterone Sulfate (DHEA-S) ในเลือดของพลทหารฯ ที่ติดเชื้อเอชไอวี-1เปรียบเทียบกับพลทหารฯที่ไม่ติดเชื้อเอชไอวี-1

รูปแบบการศึกษา: การศึกษาเชิงวิเคราะห์ ณ จุดเวลาใดเวลาหนึ่ง

สถานที่ทำการวิจัย: สถาบันวิจัยวิทยาศาสตร์การแพทย์ทหาร กรุงเทพมหานคร

วิธีการศึกษา: ทำการศึกษาระดับของฮอร์โมน DHEA-S, ภาวะการติดเชื้อไวรัสตับอักเสบชนิด บีและซี, การติดเชื้อซิฟิลิส ในเลือดของชายไทยที่ได้รับการคัดเลือกเข้าเป็นพลทหารกองประจำการกองทัพบก ผลัดพฤษภาคม 2549 จำแนกเป็นผู้ที่ติดเชื้อเอชไอวีแต่ยังไม่แสดงอาการจำนวน 72 ราย และผู้ที่ไม่ติดเชื้อเอชไอวีจำนวน 199 ราย นำมาวิเคราะห์ร่วมกับข้อมูลเกี่ยวกับการสูบบุหรี่และระยะเวลาของการติดเชื้อเอชไอวี

ผลการศึกษา: ค่ามัธยฐานของระดับฮอร์โมน DHEA-S ในเลือดของกลุ่มที่ติดเชื้อเอชไอวี และกลุ่มที่ไม่ติดเชื้อเอชไอวี เท่ากับ 1.23 ไมโครกรัม/มิลลิลิตร และ 1.42 ไมโครกรัม/มิลลิลิตร ตามลำดับ เมื่อทำการวิเคราะห์โดยใช้สถิติทดสอบ t-test พบว่าระดับฮอร์โมน DHEA-S ในเลือดของกลุ่มที่ติดเชื้อเอชไอวีต่ำกว่ากลุ่มที่ไม่ติดเชื้อเอชไอวีอย่างมีนัยสำคัญทางสถิติ ($p=0.037$)

สรุป: ระดับฮอร์โมน DHEA-S ในเลือดของพลทหารฯ ที่ติดเชื้อเอชไอวี-1 ต่ำกว่าในพลทหารฯที่ไม่ติดเชื้อเอชไอวี-1อย่างมีนัยสำคัญทางสถิติ

สาขาวิชา.....การพัฒนาสุขภาพ... ลายมือชื่อนิสิต.....

ปีการศึกษา...2552..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5175053130 MAJOR HEALTH DEVELOPMENT

KEYWORDS: Dehydroepiandrosterone sulfate /HIV-1 /Thai conscript

LTC Khunakorn Kana: Comparative Study of Serum Dehydroepiandrosterone Sulfate Levels in HIV-1 Infected and HIV-1-Seronegative Royal Thai Army Conscripts. Thesis Advisor: Assoc. Prof. Narin Hiransuthikul, M.D., M.PH., Ph.D. Thesis Co-Advisor: Col. Thippawan Chuenchitra, Ph.D.

Objectives: To determine the serum dehydroepiandrosterone sulfate (DHEA-S) levels in HIV-1 infected Thai military conscripts compare to HIV-1-seronegative Thai military conscripts.

Design: Cross-sectional analytic study.

Setting: Armed Forces Research Institute of Medical Sciences (AFRIMS), a research center

Research Methodology: We studied left-over serum samples of selected sample population. This study included 72 HIV-1 infected and 199 HIV-1-seronegative serum samples of Royal Thai Army Conscripts in round of induction May 2006. The serum samples were tested for serum dehydroepiandrosterone sulfate levels, hepatitis B surface antigen, anti hepatitis C virus antibody, rapid plasma reagin, and HIV-1 subtypes B, E, and D IgG-Capture enzyme immunoassay.

Results: The median serum DHEA-S levels in HIV-1 infected group and HIV-1-seronegative group were 1.23 and 1.42 micrograms/ml, respectively. There was significant difference in serum DHEA-S levels between two groups (p=0.037).

Conclusion: Serum DHEA-S levels in asymptomatic HIV-1 infected Thai military conscripts were lower than serum DHEA-S levels in HIV-1-seronegative Thai military conscripts statistically significant.

Field of Study:.....Health Development... Student's Signature.....LTC. Khunakorn Kana

Academic Year:....2009..... Advisor's Signature.....

Co Advisor's Signature.....COL Thippawan Chuenchitra

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CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

In Human Immunodeficiency Virus (HIV)-infected patients, the immune systems which are responsible for eradicating HIV viruses are 1) Humoral Mediated Immunity (HMI) and 2) Cell Mediated Immunity (CMI). In addition, the endocrine system also has roles in HIV-infected patients. In patients with HIV, opportunistic infections commonly involve the adrenal glands but rarely occupy enough of the gland to cause adrenal insufficiency. Impaired adrenal reserve without overt symptoms of adrenal insufficiency has been described. Subclinical abnormalities in glucocorticoid dynamics are common. HIV-infected patients usually have normal or, even more commonly, elevated basal cortisol levels. HIV-infected patients have decreased basal adrenal androgen levels and impaired adrenal androgen responses to adrenocorticotropic hormone (ACTH) stimulation.[1]

Around the past 15 years, there are evidences that the adrenal androgen dehydroepiandrosterone (DHEA) and its sulfoconjugated derivative (DHEA-S) have some roles in immunity against HIV viruses. The study in murine model found that DHEA can stimulate the interleukin-2 (IL-2) secretion from T lymphocytes. IL-2 is a major cytokine in stimulating CD4 lymphocytes production. The level of change in the cortisol/DHEA ratio could be predictive of progression to AIDS in HIV-infected individuals.[2]

DHEA and DHEA-S are adrenal androgens secreted by the adrenal cortex. DHEA-S is major form of DHEA in the body. The well-established fact that DHEA and DHEA-S concentrations decrease progressively with age has suggested a preventive role for DHEA and/or DHEA-S in ameliorating the signs and symptoms of the aging process.[3]

Because HIV infection is a blood-borne viral infection, HIV-infected patients may have other blood-borne infections such as hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection and syphilitic infection.

Since 1989, Thailand has established HIV surveillance among ~60,000 the Royal Thai Army (RTA) military conscripts (mostly aged 21 years old) annually. It is believed to be the nationally

representative sample of young Thai men. This ongoing total survey is helpful to be studied for the changes of risk behavioral pattern in this population. Moreover, since 2001, the RTA began recruiting volunteers aged 18-20 years old into the military service. The prevalence of HIV-1 infection among these younger men is a good proxy indicator of the HIV-1 incidence. Because DHEA-S is major form of DHEA in human bodies, we were interested in studying the DHEA-S levels in these asymptomatic HIV-infected young men compare to healthy young men. Determining the true and reliable serum DHEA-S level by adjusting related factors (covariates) which can affect serum DHEA-S level is essential for fully understanding natural changes of this hormone in healthy young men and asymptomatic naive HIV-infected young men. These related factors are blood-borne infections and cigarette smoking.

CHAPTER II

REVIEW OF LITERATURES

The blood levels of DHEA-S in healthy population are in the range of 100-400 micrograms/deciliter or 3-12 micromoles/liter. DHEA and DHEA-S are transformed into Dihydrotestosterone and 17 β-estradiol at peripheral tissues. In the presence, the control and regulation of the release of adrenal sex steroids are not completely understood. However, it is known that adrenal secretion of DHEA and DHEA-S increases in the children at the age of 6-8 years, and values of circulating DHEA-S peak between the ages of 20 and 30 years. Thereafter, serum levels of DHEA and DHEA-S decrease markedly. At 70 years of age, serum DHEA-S levels are at approximately 20% of their peak values and continue to decrease with age. This 70-95% reduction in the formation DHEA-S by the adrenal glands during the aging process results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues. Despite the marked decrease in the release of DHEA as the individual ages, this is not paralleled by a similar decrease in ACTH or cortisol release. The clinical impact of this age-related efficiency in DHEA production is not fully understood but may play an important role in the regulation of immune function and intermediary mechanism, among other aspects of human physiology.[3]

Low endogenous levels of DHEA and/or DHEA-S have been associated with diseases such as lupus[4], cancer, and diabetes. Circulating concentrations of DHEA and DHEA-S resulting from endogenous production or hormone supplementation may also be relevant in psychiatric illness. Drugs such as some central nervous system agents [5], some antihypertensive drugs [6] may significantly increase or decrease circulating concentrations of these adrenal androgens by various mechanisms. The effect of alcohol on

DHEA and DHEA-S concentrations, however, has not been studied extensively, and results of studies are conflicting. Nagata et al reported a trend for increasing serum DHEA-S concentrations with increasing alcohol consumption in post menopausal Japanese women; the model controlled for age and history of hysterectomy (p for trend = 0.01). [7] Cronholm et al studied the effect of alcohol on DHEA-S concentrations. In this study, ethanol 0.72 g/kg was administered orally to 6 healthy men (ages 24-40) in the morning,

and serial samples were obtained through 480 minutes. They found that ethanol decreased DHEA-S concentrations. [8] The majority of studies support the observation that smoking nicotine-containing cigarettes results in elevated concentrations of DHEA and /or DHEA-S by stimulation of release of antidiuretic hormone from posterior pituitary gland and release of ACTH from anterior pituitary gland. [9] Khaw et al [10] evaluated morning plasma hormone concentrations in 233 elderly women (ages 60-79) and reported that DHEA-S concentrations adjusted for age and BMI were approximately 1.5 times higher in smokers than in people who never smoked ($p \leq 0.001$). Vermeulen et al [11] described 1.2- to 1.4- fold higher DHEA-S concentrations in smokers depending on age group. Not all studies concur, however. The results of a study of approximately 1,000 pre- and postmenopausal women showed no evidence of a difference or trend that supported higher DHEA-S concentrations in female smokers. [12] Ortego-Centeno et al [13] reported that young male smokers (n=15) had lower serum DHEA-S concentrations than 17 nonsmokers ($p \leq 0.05$).

There are evidences that the serum concentrations of DHEA-S are changed in patients with infectious diseases. A study in adult men having syphilis pair-matched with 30 normal men showed that serum DHEA-S levels were significantly reduced in syphilitic men compared with normal men, ($p = 0.0018$). [14] There was evidence that the median DHEA-S level was lower in HIV-infected patients who were coinfected with hepatitis

C virus (HCV) compared with HIV-infected patients who were not coinfected with HCV. [15] For hepatitis B virus (HBV) infection, there was no previous study which studied the association between HBV infection and serum DHEA or DHEA-S.

For HIV infection itself, no previous study about association between duration of HIV infection and serum DHEA-S level. Identification of recently infected persons (generally within 6 months of infection) is difficult and has traditionally relied on the prospective testing and longitudinal follow-up of people at risk. A number of methods have been proposed to detect new cases of HIV infection. The methods included HIV-1 p24 antigen test and HIV-1 RNA testing, which has been used to identify recent HIV-1 infection. However, those methods require testing for all HIV-1 seronegative specimens to identify the recent infections. In 2002, Parekh and colleagues described a new assay, the BED-CEIA (HIV-1 subtypes B, E, and D, IgG-Capture enzyme immunoassay), which was shown to have similar sensitivity to multiple HIV-1 subtypes. This laboratory technique can quantitatively measure proportion of Anti-HIV IgG to total IgG in serum. It uses the concept that the newly HIV-infected patients will have low concentrations of Anti-HIV IgG but the long-term HIV-infected patients will have higher concentrations of Anti-HIV IgG. By this test, an optimal normalized optical density (ODn = specimen-OD/Calibrator-OD) cutoff of 0.8 and a seroconversion period of 153 days offered the best combination of sensitivity and specificity for distinguishing between recent and long-term infections.[16]

Progression of HIV infection is marked by severe immunosuppression, especially during the advanced stages, as a result of selective depletion of CD4 lymphocytes. During progression to AIDS, there is a shift from a TH1 to TH2 cytokine profile. [17] It has been suggested that this shift in cytokine profile is in part influenced by the increase in production of cortisol and the reduction of DHEA. [18]

The level of change in the cortisol/DHEA ratio could be predictive of progression to AIDS in HIV-infected individuals. Studies have shown significant relationships among the CD4 cell counts, the development of cachexia, and levels of serum cortisol and DHEA. Although classic clinical symptoms of adrenal insufficiency in HIV-infected patients are seldom seen, but subclinical abnormalities in glucocorticoids dynamics are common. HIV-infected patients usually have normal or, even more commonly, elevated basal cortisol levels. ACTH levels in patients with elevated basal cortisol levels has been found to be normal or elevated. Some of these alterations may be mediated by cytokines; both IL-1 and tumor necrosis factor (TNF) can directly stimulate cortisol secretion, while IL-1 and IL-6 can stimulate ACTH and corticotrophin-releasing hormone (CRH) release. An increase in cortisol levels may also be a direct response to HIV infection itself. [2] An increased cortisol to DHEA ratio has been correlated with body weight loss and HIV-associated malnutrition. [19]

HIV-infected patients have decreased basal adrenal androgen levels and impaired adrenal androgen responses to ACTH stimulation. DHEA has been shown in vitro to inhibit HIV replication; therefore this raised the possibility that the decreased DHEA levels observed in HIV-infected patients might influence the effects of the HIV infection.

Low serum concentrations of DHEA have been correlated with states of decreased immune function in humans, since concentrations are lowest in early childhood, late adulthood, and as HIV disease progresses. DHEA appears to possess immunomodulating effects, perhaps by enhancing the interleukin-2 (IL-2) from activated T cells as demonstrated in a murine model. A decline in DHEA concentrations, particularly when initially less than 2.01 micrograms/L, might also proved to be a predictor of HIV disease progression. [1] Some of the HIV-associated conditions, such as autonomic and endocrine dysfunction, may play a role in the

balance of the TH1 and TH2. For example, stimulated spleen cells derived from sympathectomized animals secrete less IL-2 and IFN-gamma [20], characteristic of a TH2 predominant response. In vivo and in vitro data suggest that the adrenal hormone dehydroepiandrosterone and its sulfate (DHEA-S) may counteract the effect of glucocorticoids and favor a TH1 response. [21] Low serum levels of DHEA (evidence of endocrine imbalance) have also been associated with progression to AIDS. Mulder et al determined serum DHEA levels in 41 asymptomatic HIV-1-seropositive subjects, who progressed to AIDS within 5 years after entering a cohort study, in 41 HIV-1-seropositive controls, who remained asymptomatic, and in 41 HIV-1-seronegative controls. They found that DHEA levels in the progressors about 5 months before the diagnosis of AIDS were lower than the levels in the nonprogressors after the same follow-up. DHEA levels < 7 nanomoles/liter and CD4 lymphocytes < 500 cells/microliter both proved to be independent predictors for disease progression in HIV-1-infected men. [22] Jacobson et al studied the relationship between serum DHEA and DHEA-S levels and subsequent progression to AIDS in a sample of HIV-infected men from the San Francisco Men's Health Study followed prospectively. They observed an association of subnormal serum DHEA levels with increased risk of progression to AIDS only in patients with CD4 lymphocytes 200-499 cells/microliter (relative hazard = 2.34; 95% confidence interval = 1.18-4.63, p = .01). [23]

In summary, there is little evidence for clinically significant impairment of adrenal steroid excretion in HIV infection. The subtle alterations in the glucocorticoid and androgen synthesis

pathways may be an adaptive response to physiologic stress and may occur with other illnesses. So we wanted to prove that whether the serum DHEA-S levels would be changed in asymptomatic HIV-infected patients.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research Questions

3.1.1 Primary research question:

Is the serum DHEA-S level in HIV-1-infected Thai military conscripts different from

the serum DHEA-S level in HIV-1-seronegative Thai military conscripts?

3.1.2 Secondary research question:

Are there any associations between status of HCV infection, HBV infection, syphilis, recent HIV-1 infection, and cigarette smoking and serum DHEA-S levels?

3.2 Research Objectives

3.2.1 Primary Objective

To determine the serum DHEA-S levels in HIV-1-infected Thai military conscripts compare to HIV-1-seronegative Thai military conscripts.

3.2.2 Secondary Objective

To evaluate the associations between status of HCV infection, HBV infection, syphilis, recent HIV-1 infection, and cigarette smoking and the serum DHEA-S levels.

3.3 Research Hypothesis

Null hypothesis:

There is no difference between mean serum DHEA-S levels in HIV-1-infected group and control group.

Alternative hypothesis:

There is significant difference between mean serum DHEA-S level in HIV-1-infected group and control group.

$$H_0 : \mu_1 = \mu_2$$

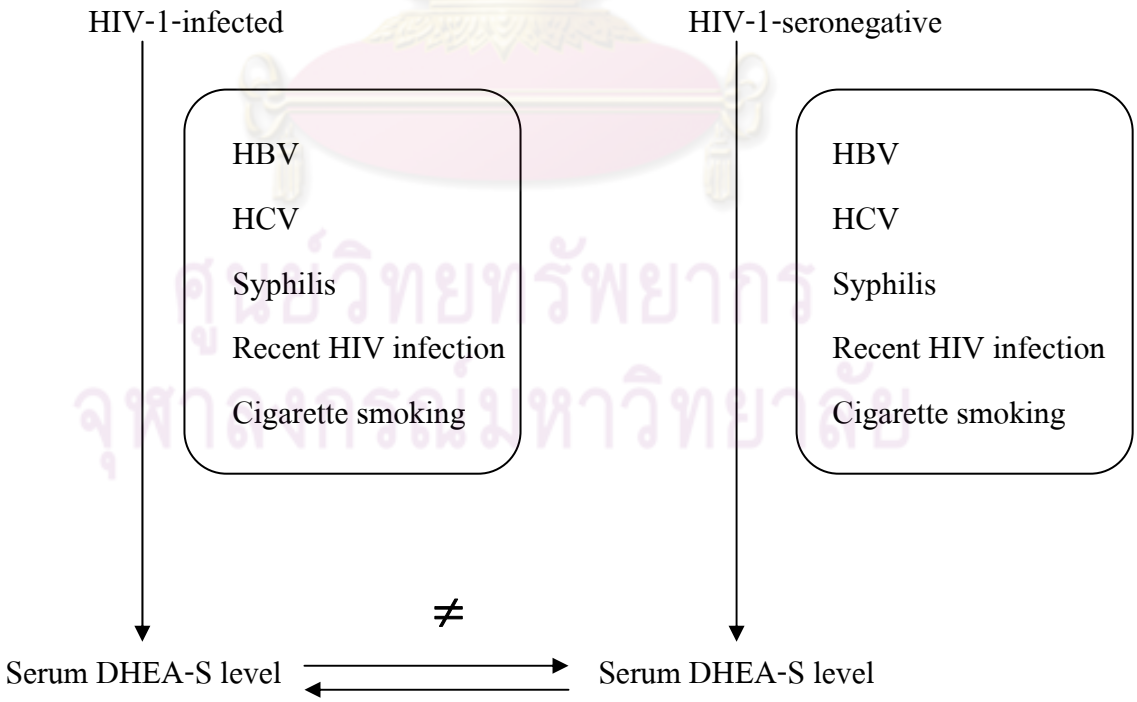
$$H_a : \mu_1 \neq \mu_2$$

Where :

μ_1 = mean serum DHEA-S levels in HIV-1-infected Thai military conscripts

μ_2 = mean serum DHEA-S levels in HIV-1-seronegative Thai military conscripts

3.4 CONCEPTUAL FRAMEWORK (Figure 3.1):



3.5 Operational Definitions

Recent HIV-1 infection:

The HIV-1 infection, which occurred recently (within 153 days after seroconversion) as determined by IgG captured BED-Enzyme Immunoassay (IgG captured BED-EIA). [16] This method are recommended by U.S.A. Centre for Disease Control.

Long-term (chronic) HIV-1 infection:

The HIV-1 infection, which occurred (more than 153 days after seroconversion) as determined by IgG captured BED-Enzyme Immunoassay (IgG captured BED-EIA). [16]

DHEA-S levels:

The levels of DHEA-S in serum which are determined by DRG DHEA-S ELISA EIA-1562. The DRG DHEA-S ELISA-1562 is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of DHEA-S in serum.

HBV infection:

HBV infection is diagnosed by positive result of hepatitis B virus surface antigen (HBs antigen) in serum.

HCV infection:

HCV infection is diagnosed by positive result of anti-hepatitis C virus antibody (Anti HCV) in serum.

Syphilis:

Syphilis is diagnosed by positive result of rapid plasma reagin (RPR) in serum and is confirmed by positive result of Treponema pallidum hemagglutination assay (TPHA).

3.6 Research design

This study was designed as a cross-sectional analytic study to answer the primary research

3.7 Research methods

3.7.1 Population

Target population:

Thai military conscripts (all are men) aged 18-30 years old

Sample population:

Thai military conscripts aged 18-30 years old who were newly recruited into the RTA military service in round of induction in May 2006 are the sample population. The total numbers of them are 29,858 men. They were distributedly filled in all forts around the country on date 1st May 2006. All these young Thai men were physically examined and asked for their underlying disease(s) and all drug treatments they had by physicians in the process of recruitment in April 2006 so only healthy were recruited into the RTA. All these new conscripts got HIV-counseling by well-trained military HIV-counselors in the first week after they came in the forts and informed consent for HIV testing were also obtained. About 1 week later, the new conscripts were blood drawn by the military medical personnels. Then the blood from all forts were sent to the Army Institute of Pathology (AIP) in Bangkok for HIV testing.

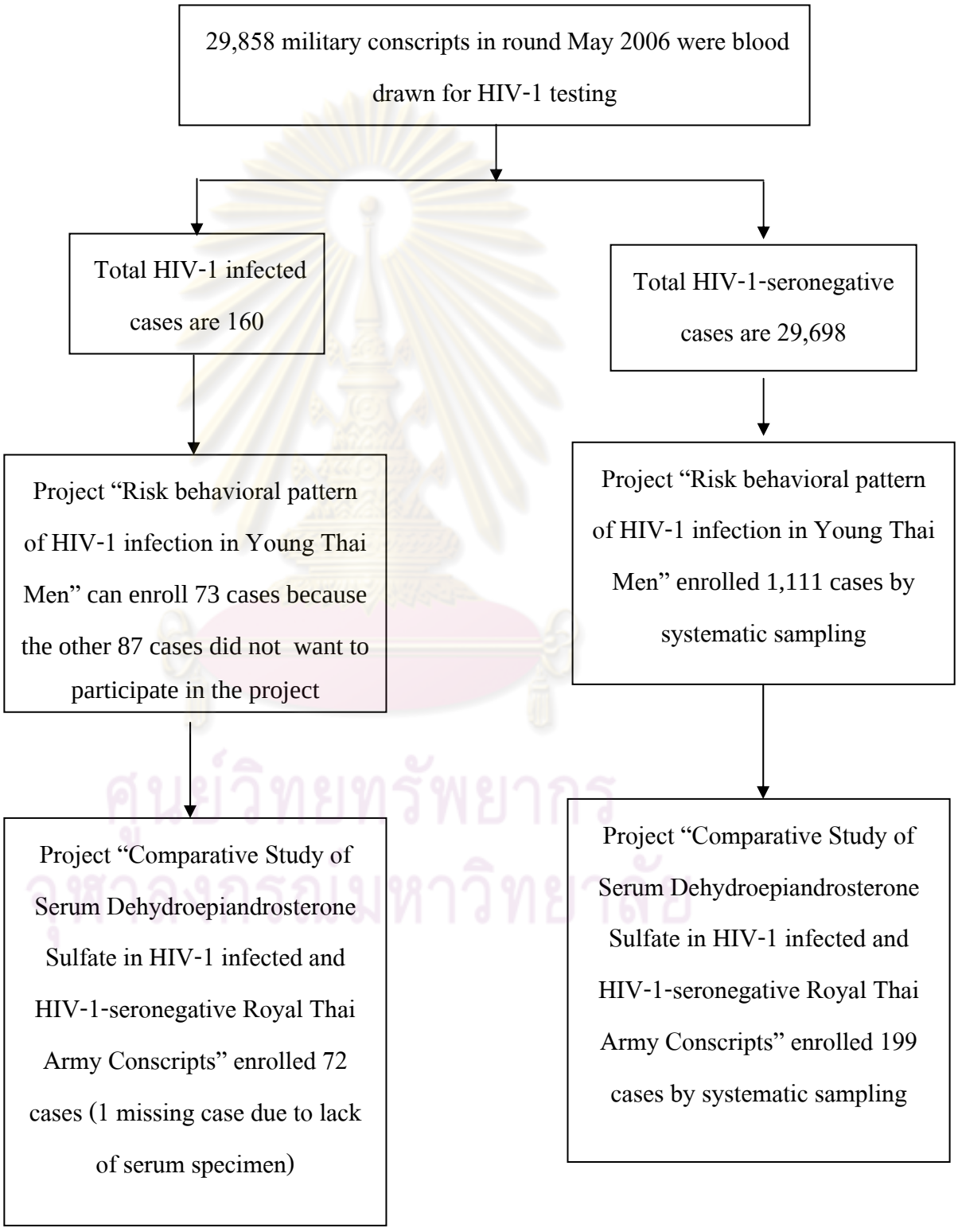
3.7.2 Study procedure

We studied only left-over serum samples of selected sample population so there was no new blood drawing. As the total number of conscripts are 29,858 men, this study will include 72 serum samples of HIV-1-infected conscripts and 199 serum samples of non-HIV-1-infected conscripts. The identification number will be assigned to each serum sample by an officer who is responsible for data base of HIV-1 surveillance program in the RTA military conscripts at AFRIMS. The investigators can not link to the name of conscripts.

We used some data from the project “Risk behavioral pattern of HIV-1 infection in Young Thai Men” which was conducted in this same population by permission from

the principal investigator. These data were age of conscripts, history of cigarette smoking and Anti HCV antibody results. The project “Risk behavioral pattern of HIV-1 infection in Young Thai Men” are conducted in RTA military conscripts all rounds in the period between year 2005 to 2009 by Dr. Ram Rangsin et al. This project has been already approved by the Ethical Committee of the Royal Thai Army Medical Department. We were sure that the conscripts in this study would not drink alcohol within 7 days prior to blood drawing because the rule of RTA did not permit them to drink alcohol in that period.

Figure 3.2: Sampling algorithm



3.7.3 Sample size determination

Hypothesis testing for differences of two means

μ_1 = mean serum DHEA-S levels in HIV-infected Thai military conscripts

μ_2 = mean serum DHEA-S levels in HIV-1-seronegative Thai military conscripts

Hypothesis $H_0: \mu_1 = \mu_2$

$H_a: \mu_1 \neq \mu_2$

Sample size is calculated from this formula:

$$\text{n/group} = 2\left[\frac{(Z_{\alpha/2} + Z_{\beta})\sigma}{\mu_1 - \mu_2}\right]^2$$

α = 0.05 (2-sided)

β = 0.01

According to the study of AJ Kandathil et al, mean DHEA-S level (SD) was 207 (123) micrograms/deciliter (mcg/dl) in normal healthy Indian individuals (n=30) and 83.5 (52) mcg/dl in asymptomatic HIV-infected Indian individuals (n=16). [24]

$$\sigma_{pool} = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{(n_1 - 1) + (n_2 - 1)}}$$

$$= \sqrt{\frac{(30 - 1)(123)^2 + (16 - 1)(52)^2}{(30 - 1) + (16 - 1)}}$$

= 104.37 mcg/dl

$\therefore$ n/group = 26.29

Table 3.1: Sample size calculation in each situation of $\mu_1 - \mu_2$ when $\beta = 0.2$

	$\mu_1 - \mu_2 = 50$ mcg/dl	$\mu_1 - \mu_2 = 100$ mcg/dl	$\mu_1 - \mu_2 = 200$ mcg/dl
n/group =	68	17	4

From the project "Risk behavioral pattern of HIV-1 infection in Young Thai Men", the investigators found that seroprevalence of hepatitis B infection are 6.7-15% (unpublished data). [25] So we increased the sample size in HIV-1-seronegative group to 199 cases to be enough for regression analysis.

3.7.4 Laboratory evaluation

Anti HIV-1 antibody test with confirmation by Western Blot results from AIP laboratory will be used in analyses.

We tested the selected left-over serum samples which are stored in -40 ^{O}C freezer at Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok for

- serum DHEA-S levels using enzyme immunoassay (Immuno-Biological Laboratories, Inc.)
- IgG captured BED-EIA using Calypte^{R} enzyme immunoassay to identify recent HIV-1 infection (within 153 days after seroconversion).
- HBs antigen
- VDRL (RPR)

3.7.5 Data collection

All data will be kept in data software. Data in hard copies will be kept in locker responsible by the principal investigator. The left-over serums will be

transfered to laboratory with only identification numbers. No labeling of individual data is on the serum tubes (anonymous).

3.7.6 Data management and analysis

Table 3.2: Summary of measured variables

Data	type of variables	presentation
Demographic data		
- Age (yr)	Continuous	mean, SD
- History of cigarette smoking	Categorical	N (%)
Outcome variables	Continuous	mean, SD
- Serum DHEA-S	Categorical	N (%)
- IgG captured BED-EIA	Categorical	N (%)
- HBs antigen	Categorical	N (%)
- Anti HCV	Categorical	N (%)
- VDRL		

Chi-square or Fisher's Exact Test will be applied to compare differences in proportions for categorical variables. Student t-test will be used to compare differences among continuous variables. P-values for all tests will be two-sided, with a value <0.05 considered statistically significant. Associations between status of HCV infection, HBV infection, syphilis infection, recent HIV infection, and cigarette smoking to serum DHEA-S levels will be analyzed by regression analysis.

3.8 Ethical considerations

The HIV-1 test results will be obtained from the Army Institute of Pathology. The study protocol will be reviewed and approved in advance by the Ethical Committee of the Royal Thai Army Medical Department. The investigators

will take precautions to keep information absolutely confidential. This study will include HIV positive subjects, therefore it will be very important to maintain the confidentiality of their HIV status. All of the documents will be kept in locked study files responsible by the principal investigator at the project center. Each subject will be assigned an identification number. None of the information obtained from this study will be released with name of participants connected to it.

3.9 Expected benefits

The results from this study will give useful knowledges in epidemiology of HIV epidemic and natural changes of this hormone and may lead to implementing DHEA-S to increase immunity in HIV-1-infected patients in clinical trial(s).

3.10 Funding

This research is funded by Armed Forces Research Institute of Medical Sciences (AFRIMS).

CHAPTER IV

RESULTS

Demographic characteristics of HIV-1 infected and HIV-1-seronegative group are shown in Table 4.1.

Table 4.1: Demographic characteristics of HIV-1 infected and HIV-1-seronegative group

	HIV-1 infected group (n=72)	HIV-1-seronegative group (n=199)	p-value
Mean(SD) age in yrs	20.0 (5.8)	18.8 (6.4)	0.15
Cigarette smoking [a]			
-current smoker	54 (76.1%)	127 (63.8%)	0.17
-past smoker	7 (9.9%)	24 (12.1%)	
-never	9 (12.7%)	47 (23.6%)	
-missing case(s)	2 (1.3%)	1 (0.5%)	
HBV infection [a]	13 (18.1%)	13 (6.5%)	0.004
HCV infection [a]	5 (6.9%)	14 (7.0%)	0.979
Syphilis [a]	1 (1.4%)	0	0.096

[a] represents to number of cases (%)

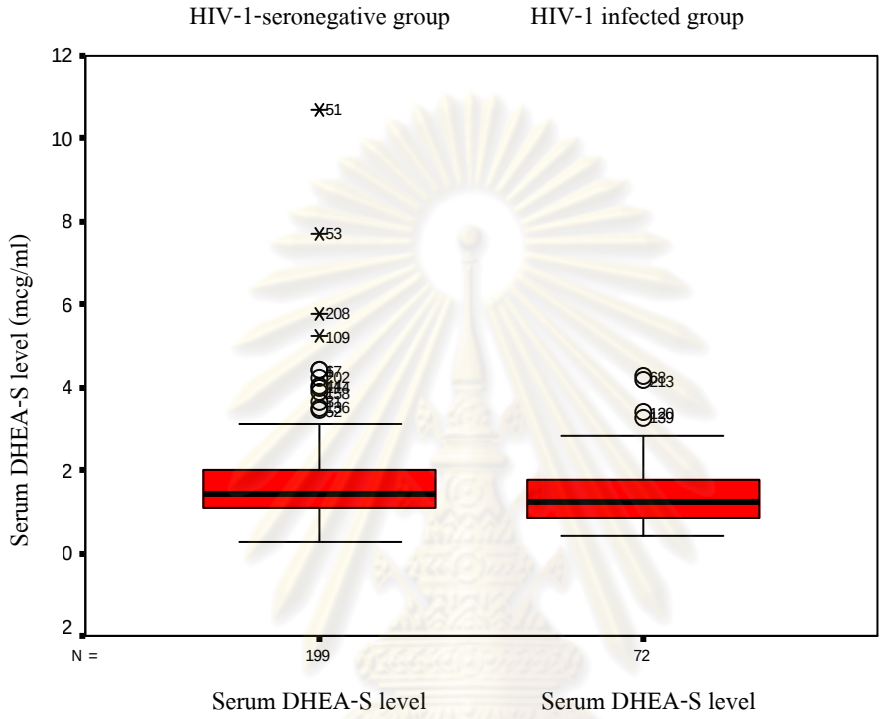
The mean(SD) age of HIV-1 infected group and HIV-1-seronegative group were 20.0 (5.8) and 18.8 (6.4) years, respectively. There was no statistically significant difference in age between groups. There was no statistically significant difference in cigarette smoking habits between groups.

Prevalence of HBV infection was higher in HIV-1 infected group (18.1%) than in HIV-1-seronegative group (6.5%) statistical significantly.

Prevalence of HCV infection in HIV-1 infected group was 6.9% whereas the prevalence in HIV-1-seronegative group was 7.0%. There was no significant difference between groups.
Syphilis was detected in only 1 case in this study.

The results of IgG captured BED-EIA showed that 15% of HIV-1 infected group (11 from 72 cases) were recently infected with HIV-1. The range of serum DHEA-S levels in this subgroup of recently infected cases was 0.43-4.27 mcg/ml.

Figure 4.1: Box plot of serum DHEA-S levels in both groups

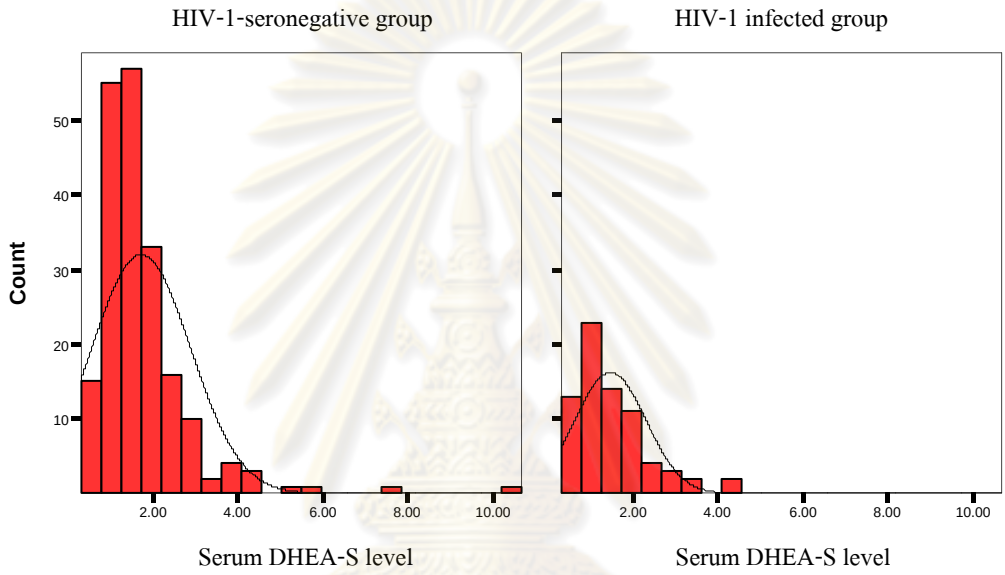


The mean(SD) serum DHEA-S levels in HIV-1 infected group was 1.45 (0.84) mcg/ml and mean(SD) serum DHEA-S levels in HIV-1-seronegative group was 1.71 (1.17) mcg/ml. The median serum DHEA-S levels in HIV-1 infected group and HIV-1-seronegative group was 1.23 mcg/ml and 1.42 mcg/ml, respectively.

When we explored the outliers, we found that there were 10 cases who had serum DHEA-S levels above 4.0 mcg/ml. 8 of these 10 cases were current smokers (5 cases were regular current smokers, the left 3 cases were non-regular current smokers). The others were 1 past smoker and 1 non-smoker.

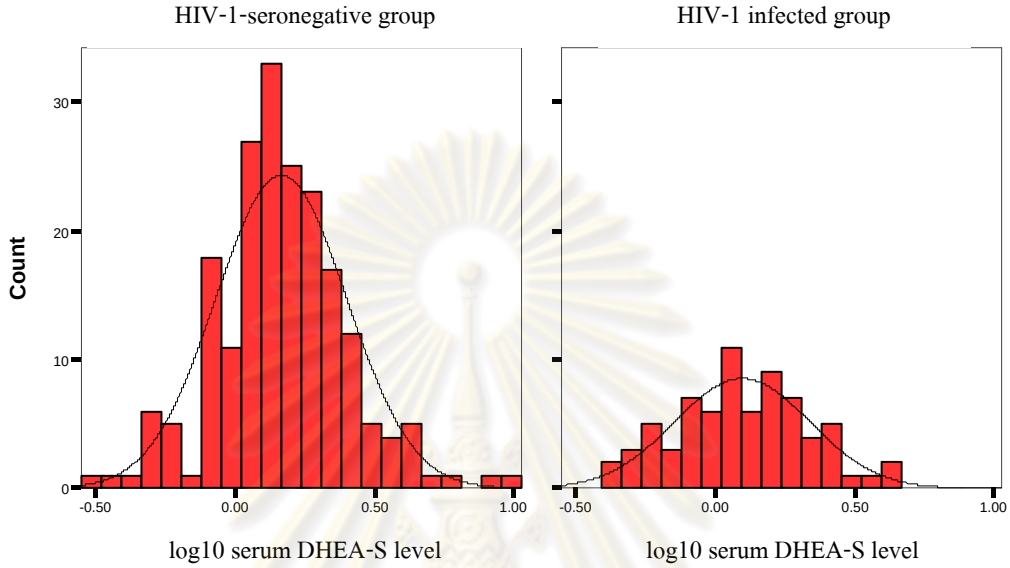
From histogram of serum DHEA-S levels data, the distribution of them was not normal (Figure 4.2). It was positively skewed.

Figure 4.2: Distribution of serum DHEA-S levels data



So we changed them into logarithm10. After changing into logarithm10, the distribution was normal (Figure 4.3).

Figure 4.3: Distribution of log10 of serum DHEA-S levels



Then we tested the difference between groups with independent 2 samples t-test. The results showed that the difference was statistically significant (p = 0.037) (Table 4.2).

Table 4.2: Results of independent samples t-test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
log10 DHEA-S	Equal variances assumed	.644	.423	2.101	269	.037	.06845	.03259	.00429	.13261
	Equal variances not assumed			2.073	122.721	.040	.06845	.03302	.00309	.13382

The mean(SD) serum DHEA-S levels in recently HIV-1 infected group was 1.94 (1.25) micrograms/ml and mean(SD) serum DHEA-S levels in long-term HIV-1

infected group was 1.36 (0.72) micrograms/ml. However, no significant difference between these two groups was found (p=0.163).

There was no significant difference in serum DHEA-S levels between smokers and non-smokers. Table 4.3 showed serum DHEA-S levels in each status.

Table 4.3: Serum DHEA-S levels in each status

Characteristics		n	Median	Minimum	Maximum	p-value	
HIV	non infect	199	1.42	0.28	10.7		
	infect	72	1.23	0.4	4.27		
smoke	current	181	1.38	0.38	10.7	0.247	Kruskal-Wallis test
	past	31	1.24	0.4	4.4		
	never	56	1.52	0.28	4.04		
HBV	non infect	245	1.37	0.28	10.7	0.623	Mann-Whitney U test
	infect	26	1.48	0.4	4.18		
HCV	non infect	252	1.40	0.28	10.7	0.254	Mann-Whitney U t-test
	infect	19	1.26	0.48	5.79		
RPR	negative	270	1.38	0.28	10.7	-	
	positive	1	1.21				

CHAPTER V

DISCUSSION

The present research was a study of determining the serum DHEA-S levels in HIV-1 infected Thai military conscripts in comparison with HIV-1-seronegative infected Thai military conscripts.

This study was designed to control factors affecting on serum DHEA-S levels, i.e. age and cigarette smoking. The strong point of this study is sampling because the military conscripts came from all provinces around the country by lottery sampling. Then the archived serum samples were systematically sampling in this study.

This study explored the associations between status of HCV infection, HBV infection, syphilis infection, recent HIV-1 infection, and cigarette smoking and the serum DHEA-S levels in young Thai men.

Although the total number of HIV-infected cases in round May 2006 was 160, but the project “Risk behavioral pattern of HIV-1 infection in Young Thai Men” can enroll only 73 case. The other 67 case who did not participate in the “Risk behavioral pattern of HIV-1 infection in Young Thai Men” project due to any reasons must be concerned. Consequently, 72 serum samples of HIV-1 infected Thai military conscripts and 199 serum samples of HIV-1-seronegative Thai military conscripts were studied in our study. The other 1 case of HIV-1 infected conscripts group was excluded from the study due to lack of archived serum.

Table 5.1: Serum DHEA-S levels in related studies

Population studied	Serum DHEA-S level	Reference
16 asymptomatic treatment-naive HIV-infected individuals ,30 normal healthy individuals (24 men and 6 women; median age 35 years; range 22 to 58)	Mean(SD) = 83.5(52) mcg/dl, Median = 79 mcg/dl Mean(SD) = 207(123) mcg/dl, Median = 170 mcg/dl	Kandathil et al, 2005 [24]
137 HIV-infected patients (104 men and 33 women; median age 39.1 years for women and 41.8 years for men), not study in normal healthy individual	Median = 202.7 mcg/dl	Mauboussin et al, 2004 [15]
91 normal healthy German men ,aged 20-24 years, not study in HIV-infected individuals	Median = 88-1,017 mcg/ml	Friedrich N et al, 2008 [26]

From data in the study by Kandathil et al, median DHA-S level in the normal healthy individuals was 170 mcg/dl whereas mean DHEA-S level was 207 mcg/dl. In our study, we found that mean difference was only 26 mcg/dl (171-145). The study by Kandathil et al was conducted in India where subtype C of HIV-1 is the predominant strain. [27] Whereas in Thailand, the predominant subtype is subtype E. [28]

To answer primary question, we did univariable analysis. We found that there was significant difference between serum DHEA-S levels in HIV-1 infected and HIV-1-seronegative groups.

We concluded that serum DHEA-S level in HIV-1 infected Thai military conscripts was higher than serum DHEA-S level in HIV-1-seronegative Thai military conscripts statistical significantly.

For secondary research question, we did not find effect of HCV infection or HBV infection on serum DHEA-S levels. This may be caused by low number of HCV-infected cases and HBV-infected cases.

Although no significant difference in serum DHEA-S levels between smokers and non-smokers was demonstrated in this study, but majority of cases who had serum DHEA-S level above 4.0 mcg/ml were current smokers.

LIMITATION

In the present study, we had no data of CD4 lymphocytes count because after the HIV-1 infected conscripts knew their serum HIV testing results, they would go to see physicians individually as they needed. Then they would be blood drawn for CD4 lymphocytes count.

CONCLUSION

In conclusion, the findings from the present study showed that there was evidence that serum DHEA-S levels in asymptomatic HIV-1 infected Thai military conscripts were lower than serum DHEA-S levels in HIV-1-seronegative Thai military conscripts statistically significant.

RECOMMENDATIONS

Further study should be done to follow-up HIV disease progression, CD4 lymphocytes count, serum DHEA-S levels, clinical status and survival of the HIV-infected conscripts with cooperation with the physicians who cared them.

CONFLICT OF INTERESTS

None declared.

REFERENCES

(1) The McGraw-Hill Companies. Lange Endocrine Physiology: Chapter 26. AIDS Endocrinopathies. (online). 2008. Available from : http://www.accessmedicine.com (2008,November 6).

(2) Christeff, N, Gherbi, N, and Mammes, O. Serum cortisol and DHEA concentrations during HIV infection. Psychoneuroendocrinology 22(Suppl.1)(1997): S11-S18.

(3) The McGraw-Hill Companies. Lange Endocrine Physiology: Chapter 6. Adrenal Gland. (online). 2008. Available from : http://www.accessmedicine.com (2008,November 6).

(4) Hedman, M, Nilsson, E, and DeLaTorre, B. Low sulpho-conjugated steroid hormone levels in Systemic Lupus Erythematosus (SLE). Clin Exp Rheumatol. 7(1989): 583-88.

(5) Tollefson, G.D., Haus, E, Garvey, A.J., Evans, M.J., Tuason, V.B. 24 hour urinary dehydroepiandrosterone sulfate in unipolar depression treated with cognitive and/or pharmacotherapy. Ann Clin Psychiatry. 2(1990): 39-45.

(6) Beer, N.A., Jakubowicz, D.J., Beer, R.M., and Nestler, J.E. Disparate effects of insulin reduction with diltiazem on serum dehydroepiandrosterone sulfate levels in obese hypertensive men and women. J Clin Endocrinol Metab. 79,4(1994): 1077-81.

(7) Nagata, C., Kabuto, M., Takatsuka, N., and Shimizu, H. Associations of alcohol, height, and reproductive factors with serum hormone concentrations in

postmenopausal Japanese women: steroid hormones in Japanese postmenopausal women. Breast Cancer Res Treat. 44,3(1997): 235-41.

(8) Cronholm, T., and Sjovall, J. Effect of ethanol metabolism on redox state of steroids sulphates in man. Eur J Biochem. 13(1970): 124-31.

(9) Salek, F.S., Bigos, K.L., and Kroboth, P.D. The influence of hormones and pharmaceutical agents on DHEA and DHEA-S: a review of clinical studies. J Clin Pharmacol. 42(2002): 247-66.

(10) Khaw, K.T., Chir, M.B.B., Tazuke, S., and Barrett-Connor, E. Cigarette smoking and levels of adrenal androgens in postmenopausal women. N Engl J Med. , 26(1988): 1705-09.

(11) Vermeulen, A., Kaufman, J.M., and Giagulli, V.A. Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging and obese males. J Clin Endocrinol Metab. 81,5(1996): 1821-6.

(12) Key, T.J., Pike, M.C., Baron, J.A., Moore, J.W., Wang, D.Y., Thomas, B.S., et al. Cigarette smoking and steroid hormones in women. J Steroid Biochem Molec Biol. 39,4A(1991): 529-34.

(13) Ortego-Centeno, N., Munoz-Torres, M., Hernandez-Quero, J., and Jurado-Duce, A. Effect of tobacco consumption on bone mineral density in healthy young males. Calcif Tissue Int. 60(1997): 496-500.

(14) Parker, C.R .Jr., and Schuster, M.W. Effects of syphilis infection on adrenocortical function in men. Proc. Soc Exp Biol Med. 197,2(1991): 165-7.

(15) Mauboussin, J.M., Mahamat, A., Peyriere, H., Rouanet, I., Fabbro-Peray, P., Daures, J.P., et al. Low plasma levels of dehydroepiandrosterone sulphate in HIV-positive patients coinfected with hepatitis C virus. HIV Med. 5,3(2004): 151-7.

(16) Parekh, B.S., Kennedy, M.S., Dobbs, T., Pau, C.P., Byers, R., Green, T., et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. AIDS Res Hum Retroviruses. 18(2002): 295-307.

(17) Clerici, M., and Shearer, G.M. A TH1 to TH2 switch is a critical step in the etiology of HIV infection. Immunol. Today 14(1993): 107-11.

(18) Clerici, M., Bevilacqua, M., Vago, T., and Villa, M.L. An immunoendocrinological hypothesis of HIV infection. Lancet 343(1994):1552-3.

(19) Christeff, N., Nunez, E.A., and Gougeon, M.L. Changes in cortisol/DHEA ratio in HIV-infected men are related to immunological and metabolic perturbations leading to malnutrition and lipodystrophy. Ann N Y Acad Sci 917(2000): 962-70.

(20) Madden, K.S., Moynihan, J.A., and Brenner, G.J. Sympathetic nervous system modulation of the immune system, III: alterations in T and B cell proliferation and differentiation in vitro following chemical sympathectomy. J Neuroimmunol. 49(1994): 77-87.

(21) Daynes, R.A., Dudley, D.J., and Araneo, B.A. Regulation of murine lymphokine production in vivo, II: dehydroepiandrosterone is a natural enhancer of interleukin 2 synthesis by helper T cells. Eur J Immunol. 20(1990): 793-802.

(22) Mulder, J.W., JosFrissen, P.H., Krijnen, P., Endert, E., Wolf, F.D., Goudsmit, J., et al. Dehydroepiandrosterone as predictor for progression to AIDS in asymptomatic human immunodeficiency virus-infected men. J Infect Dis. 165(1992): 413-8.

(23) Jacobson, M.A., Fissaro, R.E., Galmarini, M., and Lang, W. Decreased serum dehydroepiandrosterone is associated with an increased progression of human immunodeficiency virus infection in men with CD4 cell counts of 200-499. J Infect Dis. 164(1991): 864-8.

(24) Kandathil, A.J., Kannangai, R., David, S., Selvakumar, R., Job, V., Abraham, O.C., et al. Human Immunodeficiency Virus Infection and Levels of Dehydroepiandrosterone Sulfate in Plasma Among Indians. Clin Diagn Lab Immunol. 12,9(2005): 1117-8.

(25) Rangsin, R., et al. Risk Factors for HIV-1 infection Among Young Thai Men. 2009. (Unpublished Manuscript)

(26) Friedrich, N., Völzke1, H., Rosskopf, D., Steveling, A., Krebs, A., Nauck, M., et al. Reference Ranges for Serum Dehydroepiandrosterone Sulfate and Testosterone in Adult Men. Published-Ahead-of-Print on July 3, 2008 by Journal of Andrology.

(27) Ramalingam, S., Kannangai, R., Vijayakumar, T.S., Mathai, D., Abraham, O.C., Subramanian, S., et al. Subtypes and cytokine profiles of HIV infected individuals from south India. Indian J Med Res. 121(2005): 226-34.

(28) Wirachsilp, P., Kantakamalakul, W., and Foongladda, S. Surveillance of subtype and genetic variation of the circulating strains of HIV-1 in Thailand. Southeast Asian J Trop Med Public Health. 5(2007):814-27.

APPENDICES

Appendix A

Identification number _____

Data Entry Form

ข้อมูลที่ขอจากโครงการวิจัย “ ปัจจัยเสี่ยงของการติดเชื้อเอชไอวี-1ในชายไทยวัยหนุ่ม ”

อายุ __ __ ปี

ข้อมูลการสูบบุหรี่ __ __

ผลตรวจ Anti HCV antibody __ __

ผลตรวจ Anti HIV-1 __ __

ข้อมูลผลการตรวจทางห้องปฏิบัติการ

- HBs antigen

- VDRL

- IgG captured BED-EIA

- Serum DHEA-S

Appendix B

Result of DRG DHEA-S ELISA

Plate ID :	01
Assay Date :	10-Feb-10
Operator :	ST

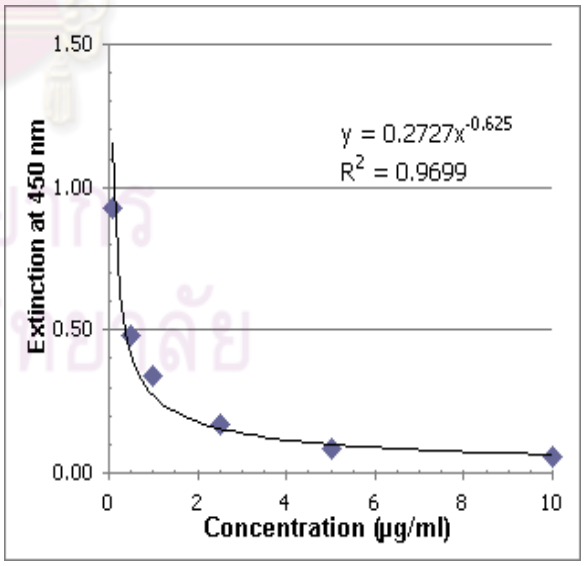
Kit Lot :	30K079-3
Catalog Number :	EIA 1562
Kit Expiration Date :	31-Jul-10
Opened Date :	10-Feb-10

Kit Components	Vol.	Lot	Exp. Date
Microtiterwells	96 wells	30W079	31 Jul 11
Enzyme Conjugate	1 x 25 ml	30D079-3	31 Jul 10
Standard	7 x 1 ml	30S029	28 Feb 11

Kit Components	Vol.	Lot	Exp. Date
Wash Solution	1 x 30 ml	TWP079	31 Jul 11
Substrate Solution	1 x 14 ml	HLS051	31 May 11
Stop Solution	1 x 14 ml	SD079	31 Jul 11

Well ID	Spec ID	OD (450/620 nm)	OD Mean	Plate 01 Concentrations (µg/ml)	Plate 02 Concentrations (µg/ml)
1A	Control	0.322	0.353	0.73	0.66
1B	Control	0.383			

Well ID	Spec ID	OD (450/620 nm)	OD Mean	Concentrations (µg/ml)
1C	Standard 0	1.310	1.254	0
1D		1.198		
1E	Standard 1	0.916	0.928	0.1
1F		0.939		
1G	Standard 2	0.488	0.483	0.5
1H		0.477		
2A	Standard 3	0.328	0.343	1.0
2B		0.358		
2C	Standard 4	0.176	0.173	2.5
2D		0.170		
2E	Standard 5	0.068	0.085	5.0
2F		0.102		
2G	Standard 6	0.058	0.058	10.0
2H		0.058		



Equation	Y =	0.2727	X	-0.6250

Result of DRG DHEA-S ELISA (Cont.)

Plate ID :	01
Assay Date :	10-Feb-10
Operator :	ST

Kit Lot :	30K079-3
Catalog Number :	EIA 1562
Kit Expiration Date :	31-Jul-10
Opened Date :	10-Feb-10

Well ID	Spec ID	OD (450/620 nm)	Concentrations (µg/ml)		Well ID	Spec ID	OD (450/620 nm)	Concentrations (µg/ml)
3A	978650	0.220	1.41	/	8A	982091	0.254	1.12
3B	978789	0.161	2.32	/	8B	982156	0.267	1.03
3C	978841	0.239	1.23	/	8C	982236	0.210	1.52
3D	978842	0.341	0.70	/	8D	982328	0.227	1.34
3E	978997	0.144	2.78	/	8E	982441	0.219	1.42
3F	979092	0.424	0.49	/	8F	982556	0.257	1.10
3G	979107	0.482	0.40	/	8G	982676	0.307	0.83
3H	979112	0.237	1.25	/	8H	982799	0.226	1.35
4A	979227	0.217	1.44	/	9A	982906	0.210	1.52
4B	979365	0.312	0.81	/	9B	983023	0.237	1.25
4C	979469	0.310	0.81	/	9C	983234	0.435	0.47
4D	979619	0.114	4.04	/	9D	983319	0.237	1.25
4E	979692	0.135	3.08	/	9E	983372	0.189	1.80
4F	979765	0.302	0.85	/	9F	983499	0.406	0.53
4G	979853	0.215	1.46	/	9G	983593	0.243	1.20
4H	979940	0.281	0.95	/	9H	983683	0.275	0.99
5A	979963	0.173	2.07	/	10A	983784	0.167	2.19
5B	980001	0.223	1.38	/	10B	983932	0.202	1.62
5C	980088	0.062	10.70	/	10C	984048	0.384	0.58
5D	980213	0.126	3.44	/	10D	984145	0.313	0.80
5E	980353	0.076	7.72	/	10E	984250	0.234	1.28
5F	980481	0.195	1.71	/	10F	984370	0.262	1.07
5G	980491	0.187	1.83	/	10G	984486	0.138	2.97
5H	980690	0.159	2.37	/	10H	984576	0.357	0.65
6A	980723	0.210	1.52	/	11A	984582	0.250	1.15

6B	980794	0.310	0.81	/	11B	984592	0.465	0.43
6C	980924	0.224	1.37	/	11C	984694	0.232	1.30
6D	981095	0.391	0.56	/	11D	984929	0.218	1.43
6E	981187	0.215	1.46	/	11E	985079	0.165	2.23
6F	981225	0.243	1.20	/	11F	985100	0.252	1.13
6G	981362	0.166	2.21	/	11G	985123	0.250	1.15
6H	981477	0.267	1.03	/	11H	985202	0.213	1.48
7A	981492	0.244	1.19	/	12A	985364	0.150	2.60
7B	981600	0.163	2.28	/	12B	985487	0.168	2.17
7C	981731	0.108	4.40	/	12C	985536	0.241	1.22
7D	981734	0.110	4.27	/	12D	985598	0.233	1.29
7E	981879	0.180	1.94	/	12E	985747	0.097	5.23
7F	981961	0.315	0.79	/	12F	985782	0.194	1.72
7G	981984	0.238	1.24	/	12G	985867	0.158	2.39
7H	982017	0.336	0.72	/	12H	985979	0.199	1.66

Appendix C

DRG DHEA-S ELISA

Date ☐☐ ☐☐☐ ☐☐ Kit Lot Number ☐☐☐☐☐☐☐

Technologist ☐☐ Plate ☐☐ Catalog Number ☐☐☐ ☐☐☐☐

Opened Date ☐☐ ☐☐☐ ☐☐ Kit Expiration Date ☐☐ ☐☐☐ ☐☐

The assay ☐ Released ☐ Rejected

Project ……

Reagent	Lot Number / Expiration Date		Reconstituted Date / Expiration Date		Note
Microelisa strip plates					
Standard 0-6 (7 vials)					
Enzyme Conjugate					
40X Phosphate Buffer					40X buffer……..…….....ml + DW……...…ml = Wash buffer……..…ml
Substrate Solution					
Stop Solution					

Date ☐☐ ☐☐☐ ☐☐ Number of Specimen ☐☐

Technologist ☐☐ Plate ☐☐ Number of Strip ☐☐

Procedure:

Time

1. นำน้ำยาวางที่อุณหภูมิห้อง (20 – 30 องศาเซลเซียส) ก่อนทำการทดสอบ 60 นาที ☐☐☐☐

2. ใช้หลอดวัด สารมาตรฐาน (0-6), ตัวควบคุม และสิ่งส่งตรวจ ปริมาตร 25 ไมโครลิตร ใส่ในแต่ละหลุมทดสอบ ☐☐☐☐

3. ใช้หลอดวัด Enzyme Conjugate ปริมาตร 100 ไมโครลิตร ใส่ในทุกหลุม ผสมให้เข้ากัน 10 วินาที และวางที่อุณหภูมิห้อง (15-30 องศาเซลเซียส) เป็นเวลา 60 นาที (ไม่ต้องปิดฝา plate) ☐☐☐☐

4. ล้างแต่ละหลุมด้วย Wash Solution 400 ไมโครลิตร ทั้งหมด 3 ครั้ง ☐☐☐☐

5. ใช้หลอดวัด Substrate Solution ปริมาตร 100 ไมโครลิตร ใส่ในแต่ละหลุม และวางที่อุณหภูมิห้อง (15-30 องศาเซลเซียส) เป็นเวลา 15 นาที ☐☐☐☐

6. ใช้หลอดวัด Stop Solution ปริมาตร 50 ไมโครลิตร ใส่ในแต่ละหลุม เพื่อหยุดปฏิกิริยา (ควรวัดภายใน 10 นาทีหลังจากหยุดปฏิกิริยา) ☐☐☐☐

7. นำ plate ไปอ่านค่าการดูดกลืนแสงที่ความยาวคลื่น 450 นาโนเมตร โดยใช้ความยาวคลื่นอ้างอิงที่ 620 นาโนเมตร ☐☐☐☐

Note……..…..

Technologist Reported	☐☐	Date	☐☐☐☐☐☐
Quality Control Officer	☐☐	Date	☐☐☐☐☐☐
Supervisor Reviewed	☐☐	Date	☐☐☐☐☐☐
Direct Reviewed	☐☐	Date	☐☐☐☐☐☐

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Publications :

1. Sangkharomya, S., Nitayaphan, S., Saengdidtha, B., Torugsa, K., Sirisopana, N., and Kana, K., HIV/AIDS prevention and control: an experience of the Royal Thai Army in Thailand. UNAIDS/04.36E (English original, July 2004).
2. Kana, K., Coldren, R., and Lewis, M. Unit-Based Surveillance System. Paper presentation in 15th Asia Pacific Military Medicine conference. Hanoi, Vietnam. May 2005.
3. Kana, K., Tabprasit, S., Chuenchitra, T., Sirisopana, N., and Rangsin, R. HIV-1 Incidence Estimates among Young Thai Men Using IgG-Capture BED- Enzyme Immunoassay (BED-CEIA) during 2005-2006. J Med Assoc Thai 2009; 92 (Suppl 1): S112-6.