CHANGE OF CD4 COUNT, HSP70 AND ANTI-HSP70 LEVELS IN HIV PATIENTS

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ABSTRACT

The T-CD4 decreased in HIV/AIDS infection through the Heat shock protein 70 (Hsp70) mediated pathway is still unclear. The objective of this study was to disclose the exchange of CD4 count, Hsp70 and anti Hsp70 level in HIV patients. Methods: Fourteen HIV/AIDS infected patients taken by simple random sampling were enrolled in this research. Fourteen persons having a high risk of HIV infection, but still non-reactive, as revealed in HIV serology test, served as control. All subjects and controls were subjected to three-times examinations for CD4 count, Hsp70, anti Hsp70 level. The first examination (day 0) was done two hours after the signing of informed consent while the patients still did not know about their HIV infection status. The second examination (day 7) was carried out when the patients were in acute stress condition after HIV infection diagnosis was informed at the third examination (day 31). The results of this research were analyzed by multivariate analysis. Results: The first examination revealed that CD4 decreased both in HIV infection and non-HIV infection group of less than 1000 cells/mm^3. The decrease progression in the HIV group is faster than that in non HIV infection. This study demonstrated that in HIV infection group Hsp70 had a higher level than that in non HIV infection. The result of the second examination showed that besides the difference of CD4 count in two groups, Hsp70 level increases. It was not only stimulated by biological stress HIV to lymphocytes, but also to other immune cells. Hsp70 level in the second examination was higher than that in the first examination. The result of the third examination revealed that Hsp70 and anti Hsp70 level could be readily produced in large amount and it is enhanced by the CD4 expression cells from the two groups. Hsp70 level increased significantly in HIV group, more than that in non HIV infection at day 31. Hsp70 level in HIV group tended to increase from first, second and third examinations. This research demonstrated a trend of increasing Hsp70 level from acute to chronic stress in HIV infection. In conclusion, HIV not only contributes to T-CD4 lymphocytes depletion, but also stimulates production of Hsp70, a cytoprotection mechanism from stressor in microenvironment.

Keywords: HIV, CD4 count, Hsp70, anti Hsp70

INTRODUCTION

A serious threat in individuals exposed to HIV is the reduction of CD3 or the suppression of immune system. Hsp70 is a chaperonin that is able to interact with various regulator proteins (Smith 1998). Hsp70 has a role as cytoprotector against various interventions to the cells. Hsp70, belonging to the family of chaperone molecules, has a high contribution to innate immunity as cell protector from the effect of stress. HIV that successfully enters the host's body will attempt to enter target cells, affecting the function of body protein, including heat shock protein 70 (Hsp70) (Bailey 2002). The effect of HIV on the change of CD4 count has been disclosed. However, the change that involves Hsp70 and anti-Hsp70 remains unclear, although Hsp70 and anti-Hsp70 have a high important role in the event of immunity damage, particularly T-CD4 lymphocyte, the primary target of HIV infection. In this study, the examination of CD4 count, Hsp70 level, and anti-Hsp70 level in acute and chronic phases were examined.

MATERIALS AND METHODS

This study was conducted in accessible population staying in Infectious Disease Intermediate Treatment Wards, Dr Soetomo Hospital, Surabaya, among HIV-infected patients. This was an observational study by observing and measuring several variables, including CD4 count, Hsp70 and anti-Hsp70 level, in the subject of study according to natural condition. The purpose of this study was to follow the change of CD4 count, Hsp70 and anti-Hsp70 level in HIV-infected patients.
with non-HIV-infected high-risk patients as comparison. Follow-up for 30 days was conducted in each subject of both groups. This study used panel study design. Groups compared were HIV-infected high-risk group and non-HIV-infected high-risk group.

Samples required from HIV-infected patients were obtained by strata-based sampling selection. To obtain homogeneous sample from HIV-infected high-risk and its comparison, individual matching was conducted regarding age, sex, nutritional status, risk types, and length of contact with high-risk individuals. The factor of HIV transmission affects the course of the disease, which might affect the result of the study (CD4 count). Therefore, the stratification of the risk factors was needed to obtain more homogeneous sub-group (strata). Randomization was conducted in each stratum separately, and the selected subjects were rejoined in matching group. Thus, in this study randomization was conducted within the strata (stratified randomization) using simple randomized sampling. Peripheral blood sampling was performed three times. In the first examination (baseline data), peripheral blood sampling was performed on day 1 when the inclusion criteria were fulfilled but the patient had not known his status of being infected with HIV. The patient declared his agreement to follow research procedure and signed informed consent as subject and agreed to have medical procedure of blood sampling. The second examination was performed on day 7. Day 7 was chosen based on the consideration that the most severe acute stress occurs during the first 7 days after being diagnosed as having HIV infection. The third examination was performed on day 31. Each subject received pre-test counseling and HIV serological post-test.

Blood examination included Hsp70, anti-Hsp70, and CD4 count. To obtain samples that matched the course of HIV infection, follow-up for 30 days was performed in a consideration that acute stress occurred after 2 hours until 15 days, continued with chronic stress after the recognition of the presence of HIV as biologic stressor. Severe biological stress takes place particularly during the first 7 days. During the screening, the examinations of HIV serology, CD4, thoracic x-ray, and others were performed leading to a suspicion of the presence of opportunistic infection. Samples used in this study were blood of HIV-infected patients, which were taken from the vein, and collected within BD vacutainer tube, with or without EDTA. Blood sample was taken three times, first, at the time when the patient declared his willingness to follow the research procedure, but had not known the status of being infected with HIV. Second, on day 7 after knowing the status of HIV-infected. Third, on day-31 after knowing the status of HIV-infected.

For the preparation of Hsp70 and anti-Hsp70 examination, blood sample was taken and frozen at -20 degree C. Examination was performed at once after all samples were collected. Hsp70 was examined using Enzyme-Linked Immunosorbent (ELISA) method, with the kit StressXpress Hsp70 ELISA Kit (Catalog Number: EKS-700) Stressgen Biotechnologies. Anti-Hsp was examined with Enzyme-Linked Immunosorbent (ELISA), using the kit: StressXpress Anti-Human Hsp70 (IgG/A/M) ELISA Kit (Catalog Number: EKS-750) Stressgen Biotechnologies. Inclusion criteria were high-risk group, including heterosexual with changing partners, homosexual, intravenous narcotic users, husband/wife of positively serologically-proven HIV-infected patients, age 15-65 years, marriage status, CD4 count of > 200 cells/mm3 with no opportunistic infection or malignancy, CD4 count of < 200 cells/mm3 with no opportunistic infection or malignancy, provide statement of willingness to participate in the study, either as the subject of study or accepting of medical procedure of blood sampling, by signing the informed consent. The grades of T-CD4 lymphocyte reduction were as follows: mild if CD4 > 500-1000 cells/mm3, moderate if CD4 > 200-499 cells/mm3, severe if CD4 < 200 cells/mm3, and very severe if CD4 < 100 cells/mm3. Exclusion criteria were as follows: The patient was unwilling to participate, the presence of opportunistic infection or malignant diseases, the presence of other disease impeding the measurement or interpretation, such as diabetes mellitus, chronic renal failure, and hepatic cirrhosis; the presence of condition disturbing the feasibility; the patient was not communicative, the patient was in critical condition, the patient had no permanent residence that leads to follow-up difficulties.

RESULTS

The first day examination, CD4 count in HIV-infected group vs. that in non-HIV-infected group revealed 250.29 ± 230.15 vs. 598.50 ± 370.70. Hsp70 level in HIV-infected group vs. non-HIV-infected group were 291.82 ± 29.01. Hsp70 was examined using Enzyme-Linked Immunosorbent (ELISA) method, with the kit StressXpress Hsp70 ELISA Kit (Catalog Number: EKS-700) Stressgen Biotechnologies. Anti-Hsp was examined with Enzyme-Linked Immunosorbent (ELISA), using the kit: StressXpress Anti-Human Hsp70 (IgG/A/M) ELISA Kit (Catalog Number: EKS-750) Stressgen Biotechnologies. Inclusion criteria were high-risk group, including heterosexual with changing partners, homosexual, intravenous narcotic users, husband/wife of positively serologically-proven HIV-infected patients, age 15-65 years, marriage status, CD4 count of > 200 cells/mm3 with no opportunistic infection or malignancy, CD4 count of < 200 cells/mm3 with no opportunistic infection or malignancy, provide statement of willingness to participate in the study, either as the subject of study or accepting of medical procedure of blood sampling, by signing the informed consent. The grades of T-CD4 lymphocyte reduction were as follows: mild if CD4 > 500-1000 cells/mm3, moderate if CD4 > 200-499 cells/mm3, severe if CD4 < 200 cells/mm3, and very severe if CD4 < 100 cells/mm3. Exclusion criteria were as follows: The patient was unwilling to participate, the presence of opportunistic infection or malignant diseases, the presence of other disease impeding the measurement or interpretation, such as diabetes mellitus, chronic renal failure, and hepatic cirrhosis; the presence of condition disturbing the feasibility; the patient was not communicative, the patient was in critical condition, the patient had no permanent residence that leads to follow-up difficulties.

The first day examination, CD4 count in HIV-infected group vs. that in non-HIV-infected group revealed 250.29 ± 230.15 vs. 598.50 ± 370.70. Hsp70 level in HIV-infected group vs. non-HIV-infected group were 291.82 ± 107.82 vs. 244.34 ± 29.01. In HIV-infected group there was a moderate reduction of CD4 count (reduced if CD4 > 200-499 cells/mm3) (Figure 1). Hsp70 level and anti-Hsp70 level were found to increase in positive HIV group. In negative HIV group CD4 count was still relatively normal (normal, 410-1590 cells/mm3). Hsp70 and anti-Hsp70 levels in non-HIV-infected group were lower than those in HIV-infected group. The result of CD4, Hsp70 and
anti-Hsp examination on day 7 revealed that CD4 count in HIV-infected group vs. non-HIV infected group was $277.71 \pm 200.58$ vs. $677.86 \pm 488.61$, Hsp70 level in HIV-infected group vs. non-HIV infected group was $1.58 \pm 0.81$ vs. $0.96 \pm 0.40$, and anti-Hsp70 level in HIV-infected group vs. non-HIV infected group was $300.88 \pm 171.88$ vs. $237.89 \pm 33.88$. The results of CD4 count, Hsp70 and anti-Hsp70 examination on day 31 were as follows. The result of laboratory estimation and statistical analysis in HIV-infected group and non-HIV infected group on the mean of CD4 count was $395.14 \pm 265.00$ vs. $598.79 \pm 373.57$, Hsp70 level was $1.70 \pm 0.92$ vs. $0.85 \pm 0.37$, and anti-Hsp70 level was $278.41 \pm 58.91$ vs. $231.36 \pm 47.27$. 

Figure 2. CD4 count, Hsp70 and anti-Hsp70 levels in examination day 7

Figure 1. CD4 count, Hsp70 and anti-Hsp70 levels on day 1

Figure 3. CD4 count, Hsp70 and anti-Hsp70 levels in examination day 31

Figure 4. Change of CD4 count, observation day 1, 7, 31
The changes of CD4 count on days 1, 7, and 31 in HIV-infected group were -0.50, -0.52, -0.42, respectively. In non-HIV-infected group, the examination on day 1, 7, and 31 revealed 0.50, 0.52 and 0.42, respectively. In positive HIV group, from the first day to seventh day examination there had been a reduction of CD4 count, only that the the reduction on day 7 was slightly lower than that of day 1. The reduction of CD4 count was increasing until the the examination on day 31. In negative HIV-group there was a slight increase and then tended to reduce until the examination day 31. Positive HIV group had an increase of Hsp70 level on examination day 7 and 31, as compared to that on day 1. Negative HIV group had a reduction on examination day 7 and reduced more on day 31.

**DISCUSSION**

In regard with CD4 reduction, both in HIV-infected group and in group that is serologically not yet infected with HIV, there was a trend toward CD4 reduction to less than 1,000 CD4/mm$^3$. CD4 reduction rate in HIV-infected group was more dramatic that that in non-HIV infected group. In the first group, CD4 reduction occurred more progressively since T-lymphocyte was the primary target of HIV. In addition to CD4 receptor on its surface, T-lymphocyte is also equipped with two co-receptors, the CCR5 and CXCR4, which tighten the binding with gp120 on the outer surface of HIV. In group that had not been infected with HIV, CD4 reduction may remain possibly due to HIV intervention. Although during serological examination the HIV was found to be non-reactive, it did not mean that the patients were free from HIV infection. Whateoever, the members of the group belonged to high risk individuals (homosexuals, multipartner heterosexual, and alternate intravenous drug users), so that during the HIV serological examination they were still at window period, which occurred at the early stage of infection, which was a seronegative condition in a period between 6 weeks to 6 months post-infection.

Once the contact with T-lymphocyte has occurred, HIV, as a biological stressor, will trigger T-lymphocyte to have stress with its various changes. The change is started with the expression of CD43 receptor (cyalophorin) at the surface of T-lymphocyte. Subsequently, the expressed CD43 receptor became activator, both to T-CD4 lymphocyte itself and to the HIV (Barat 2002; Brown 2003). In addition, rCD43 also had effect on the increase of CD69 and CD40 (Barat 2002; Brown 2003).
HIV that has been present within T-CD4 lymphocyte is also being activated by the effect of rCD43, inducing the formation of TCR-CD3 complex and, along with CD28, it affects HIV to be more active. Such condition results in an increase of transcription activity, protein translation, and more progressive HIV replications, thereby its presence in the circulation will eventually reach a very high number, forming million of HIV genomes per ml. The production during active infection reached \(10^9-10^{11}\) virus particle per day (Barat 2002; Drew 2001; Hirschel 2003). If infection ensues without treatment, the virus count may reach 500 - 1,000,000 HIV-RNA copies per ml. The continuously increasing viremia may attempt to attack the subsequent T-CD4 lymphocytes. In acute infection the reduction takes place dramatically until reaching less than 1,000 CD4/mm\(^3\), and then increases again during seroconversion. In chronic phase it continuously decreases in a rate of 70 cells/ul each year (Hirschel 2003). If CD4 count reaches or passes the critical threshold of < 200 cells/mm\(^3\), the AIDS stage has been entered with or without clinical manifestation.

Once the internalization process of T-lymphocyte by HIV occurs, in addition to changes both through T-CD4 lymphocyte activation and HIV, the emergence of stress protein, including heat shock protein70 (Hsp70) is also taking place. The Hsp synthesis inductor was stress environment, ROS, and the numerous unfolded polypeptides (Fink 1998; Stryer 1995). In this study, predominant stress protein in the first examination was signed by the increase of heat shock protein 70 (Hsp70) level. The predominance of Hsp70 level occurred at the first examination performed at the time when the individuals had not known their HIV serological status. The increase of Hsp70 may result from the influence of stress internal environment triggered by the biological stressor the HIV. HIV biological stressor affected the expression of CD40 receptor at the outer surface of the lymphocyte. The CD40 receptor is aimed for Hsp70 (Lehner 2005). It is therefore reasonable that HIV inductor triggers the increase of Hsp70 in HIV-infected group as demonstrated in the result of this study.

HIV is one of the inductors of Hsp70 and HSF1 synthesis. Activity that disrupting HSF1 affects negative regulatory interaction between Hsp70 and HSF1. Increasing macrophage activity in HIV infection tends to drive the increase of arachidonic acid metabolism that affects the increase of HSF1 activity. Arachidonic acid is the primary mediator and inflammatory response precursor that attempts to adapt to Hsp70 activity within or nearby infected cells. Intracellular Hsp70 induction and accumulation mechanism serve as control and inhibitor of protein misfolding, which may prevent the progressiveness of disease. HIV triggers the increase of Hsp70 level through CD40 receptor. Thus, Hsp70 induction is highly effective in preventing or minimizing HIV infection progressiveness into AIDS.

The presence of Hsp70 is induced by the effect of HIV biological stressor that has intervened T-lymphocyte and increased the expression of CD40 receptor. It has been demonstrated from the result of this study that Hsp70 level was predominant and its emergence preceded other protein stress, including cortisol. It was possible since Hsp belongs to small protein (kDa), so that it may emerge earlier and required by HIV as Hsp70 ligand (Mosser 2000). HIV itself requires the role of CD40 receptor in order to bind Hsp70 (Hsp70 as CD40 ligand).

Therefore, in the second examination we observed the increase of Hsp70 level. In the second examination (day 7) mean Hsp70 level was higher than that in the first examination. This finding can be explained if in the first examination the emergence of Hsp70 was induced more by the exposure of HIV biological stressor that has successfully been intervened lymphocyte through the activation of CD40 receptor (Gill 2004). In the examination day 7, the increase of Hsp70 level, in addition to being triggered by HIV biological stressor on T-lymphocyte, it was also triggered by psychological stress and the statement of being HIV-infected, as well as by various producers of some types of other immune and non-immune system cells. CD40 receptor is aimed for Hsp70, but it also present in epithelial cells (Lehner 2005). The subsequent development is that the more the cells involved the more the number of the virus. Production during active infection may reach \(10^9-10^{11}\) virus particles a day (Barat 2002; Drew 2001; Hirschel 2003). The continuously increasing viremia may attempt to attack the subsequent T-CD4 lymphocytes.

In the third examination (day 31) the increase of Hsp70 and anti-Hsp70 levels was predominant since the impact of psychological stress was alleviated by natural coping, which was supported with advising and counseling activities. Hsp70 increased significantly in HIV-infected group in the third examination (day 31), as compared to that in the first and second (day 7) examination. There was a predisposition of Hsp70 increase from acute to chronic stress. Such increase occurred in order to maintain the survival of lymphocyte-T through Hsp70 cytoprotector nature at the early phase.
The increase of Hsp70 in this study resulted from the effect of HIV biological stressor, whose effect tended to be stronger along the course of the time. The stronger effect was related with continuous HIV replication in a mean rate of $10^9-10^{11}$ virus particle a day that augmented the density of HIV (viral load) in the circulation (Barat 2002; Drew 2001; Hirschel 2003). Additionally, there were more T-lymphocyte and the effect of CD4-expressing cells that were simultaneously increasing. HIV that followed the circulation might affect various cells that contained CD4 protein on their surface, such as astrocyte, microglia, monocyte-macrophage, dendritic, and Langerhan's. Those cells were activated to produce Hsp70. Epithelia on the surface of digestive tract were also activated due to the direct effect of HIV gp41 (Pavlakis 1997). The epithelium is also potential to express Hsp70 by having a cytoprotective nature (Bhattacharyya 1999; Nollen 2002).

The third examination was also marked by the increase of anti-Hsp70 level in HIV-infected group, as compared to the group that was serologically not infected yet with HIV. This indicated that immune system cells in subject groups in this study remained providing response against Hsp70. The response was decreasing along the time, particularly in HIV-infected group. This could be observed from the result of the first examination of anti-Hsp70 level in HIV-infected and non-HIV-infected groups. In the second (day 7) and third examination (day 31) anti-Hsp70 was seen to have a predisposition of continuous decrease. This predisposition reflected weaker cells' response in defending homeostasis through GAS (Putra 1999).

CONCLUSION

HIV infection has a role as the inductor of Hsp70 increase through biological stress. In addition to CD4 depletion, in a certain condition HIV is a Hsp70 stimulator that has an important role in cytoprotective mechanism within a stressor-full microenvironment. CD4 is not the only biological parameter in HIV infection. There are other important biological parameters, the Hsp70 and anti-Hsp70. Hsp70, which, as a chaperone molecule, prevents protein aggregation, regulates protein folding rhythm, and maintain functional three-dimensional protein structure. As a ligand, Hsp70 has a protective role against HIV.

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