HIV-1 TAT: a Potential Diagnostic and Disease Progression Biomarker of HIV/AIDS

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Abstract

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) constitutes a national threat to Indonesia from many perspectives because the number of new cases there is increasing faster than in any of the other countries in Asia. Susceptibility to opportunistic infection leads to high morbidity and mortality rates, especially because HAART is not widely distributed. A diagnosis of AIDS is often confirmed only after the patient is severely immunocompromised. HIV-1 TAT is a HIV regulatory protein which plays an important role in viral replication. Extracellular TAT has been associated with many pathological conditions related to AIDS.

To detect the presence of TAT in plasma as a means of assessing its potency as a diagnostic and progression marker of HIV/AIDS.

An analytic observational study was conducted on 80 HIV(+) patients and 30 control patients in Soetomo Hospital. The diagnosis of HIV was confirmed by reference to medical records. A CD4+ count was effected by flowcytometry, while the HIV-1 TAT plasma level was measured by means of ELISA. The correlation between the CD4+ count and HIV-1 TAT plasma level was analyzed by Pearson.

HIV-1 TAT presented only in HIV(+) patients. Increased HIV-1 TAT plasma level correlates to a decreased CD4+ count (r=-0.912; p=0.000, p<0.049).

HIV-1 TAT is a potential diagnostic biomarker indicating the degree of disease progression of HIV/AIDS.

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Introduction

Human Immunodeficiency Virus (HIV) infection and *Acquired Immunodeficiency Syndrome* (AIDS) constitute two of the most serious global problems. Southeast Asia suffers from the highest prevalence of AIDS in the whole of Asia. Unlike Cambodia, Myanmar and Thailand, where the number of newly-diagnosed cases has decreased, in Indonesia the number of new cases is rising annually leading to a high mortality rate. According to a 2016 UNAIDS report, approximately 790,000 people in Indonesia are living with HIV and 35.000 have died as a result of AIDS. This disease has considerable potential to affect the future of the world as most newly-infected individuals fall within the 15-24 years age range.[1]

The gold standard of any diagnostic method is the detecting of a specific anti-HIV antibody in a patient's blood. Using rapid ELISA, this method is sufficiently sensitive to detect its presence. However, the antibody is only synthetized during the early stages of HIV infection. After the virus enters a CD4+ cell and remains dormant no antibody is produced until the virus begins replicating itself. This causes a patient to give a false-negative result.[2]

HIV-1 *Trans-Activating Transduction* or *Transcriptional Activator* is one of six HIV regulatory proteins (Tat, Rev, Vif, Vpr, Vpu dan Nef) synthetized both early and late in the viral

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replicatus cycle.[3] This minor 14-16 kDa protein plays a crucial role as a trans-activating factor which is very important in gene transcription, including replication and infectivity. HIV-1 TAT is translated early in the replication cycle and does not constitute a building block of a virion, instead being released by the golgi apparatus to the cell surface where it integrates with the cell membrane. When the cell membrane disintegrates during budding and release of a new virion, TAT is released into the blood plasma.[4] Extracellular TAT has been known to interact with many cells and extracellular matrices. The C-end of the TAT amino acid chain possesses an RGD (arginine-glycine-aspartic acid) sequence, allowing TAT to interact with extracellular matrix proteins such as integrin $\alpha_5\beta_1$ (*fibronectin*), integrin $\alpha_v\beta_3$ (*vitronectin*) and various proteins of *vascular endothelial growth factor* (VEGF). The interaction allows TAT to mimic the effect of these extracellular matrix proteins in regulating cell life activity by activating the corresponding signal tranduction.[5,6,7]

Extracellular HIV-1 TAT has been reported as entering a latently infected CD4+ cell and activating the viral replication cycle.[8] When internalized by an uninfected CD4+ cell, HIV-1 TAT induces expression of CXCR4 in cell membranes.[9] HIV-1 TAT was also reported as playing a role in T cell apoptosis and microvascular endothelial cell through caspase activation, HIV-1 TAT was also considered to have a role in HIV neuropathogenesis associated with AIDS-related dementia and the correlation between detected HIV-1 TAT in a HIV patient's brain with encephalitis incidence.[10,11] The ability of TAT to induce TNF and IL-2 suggests TAT's role in many AIDS-related pathological conditions.[12] TAT's roles in p53 inhibition was assumed to be associated with AIDS-related malignancies.[13]

These previous studies suggest that increased extracellular HIV-1 TAT augments the risk of disease progressivity, opportunistic infection and malignancies.[14] The presence of this protein in blood plasma may also serve as a potential diagnostic marker, or more optimally, as an antibody against HIV, as HIV-1 TAT appears early in the replication cycle. HIV-1 TAT protein is essential to HIV during the replication cycle meaning that the protein is translated from a stable gene which is not prone to mutation.[8,15]

This study aims to assess the potency of HIV-1 TAT as a diagnostic and progressivity marker of HIV/AIDS.

Materials and methods

An Ethical Clearence Certificate was issued by the Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearence Commission, number: 075/HRECC.FODM/VII/2017.

This study was conducted using an analytical observational design on 80 AIDS patients in Dr. Soetomo General Hospital between July and August 2017 with various major restrictions. Of 80 HIV-positive subjects, 17 were excluded for various reasons, including: being too weak to participate in the study or because they were about to be discharged from hospital. The remaining 63 patients were allocated to one of three categories based on their CD4+ count. HIV diagnoses were taken from medical records and confirmed laboratorically, blood specimens being taken for CD4+ count analysis by means of flowcytometry (BD FACSymphony™, Becton Dickinson and Company, New Jersey, United States). 30 HIV-negative patients were included as the control group. Positive patients were assigned to one of three categories on the basis of their CD4+ count being low (<200 cells/mL), moderate (200-500 cells/mL) or high (>500 cells/mL). 30 patients were randomly selected to represent the CD4+ count category and their specimens were processed for HIV-1 TAT analysis using TAT Antigenemia ELISA Assay (AKE00018, Diatheva, Fano, Italy). The kit is designed to determine HIV-1 TAT concentration quantitatively using monoclonal anti-HIV-1 TAT antibodies (ANT0037, Diatheva, Fano, Italy). Infectious specimen preparation included exposure to ultraviolet light and re-suspension in lysis buffer to inactivate virus.[16] Concentrated standard antigen 10x was diluted with Buffer A at a ratio of 1:10 in order to obtain 1x concentration, then diluted to 1:2, again with Buffer A, to create a standard dilution curve. Samples were inserted into each well, followed by standard dillution from A to N. The plate was incubated at 37 °C for 90 minutes. Thereafter, each well was washed five times with 500 µL. *Buffer* B 1x and rabbit monoclonal antibody anti-Tat (Diatheva, Fano, Italy) was added which was dilluted to a ratio of 1:5000 in Buffer A. Following a 60-minute incubation in 37 °C, each well was

washed again five times with Buffer B. Goat IgG antibody anti-rabbit conjugate HRP (Diatheva, Fano, Italy) was diluted 1:1000 in buffer A, and inserted into 100 µL/well before being incubated for 60 minutes at 37 °C. After further washing, chromogenic substrate buffer was added and incubated for 60 minutes at room temperature, while avoiding the presence of light. Absorbancy readings were taken by means of a microplate reader (2105-0010 EnVision™, PerkinElmer, USA) at 405 nm wave length. A Pearson correlation analysis was conducted using a Statistical Package for the Social Sciences (SPSS) 17.0 software for Windows 8.1 by SPSS Inc., Chicago, United States.

Results

The rate of CD4+ count and HIV-1 TAT per group are displayed in Table 1. A Pearson correlation analysis confirmed inverse correlation between the HIV-1 TAT plasma level and the CD4+ count. Similarly, increased HIV-1 TAT plasma level correlates to a decreased $CD4^+$ count (r=-0.912; p=0.000, p<0.049).

CD4+ categories (cells/mL)	N of subjects (%)	X^2 of CD4+ count	X^2 of HIV-1 TAT count
Low (<200)	25	56	36,768.33
Moderate (200-500)	22	334.3	32,364.999
high (>500)	16	615.1	29,038.299
Total	63	335.13	32,723.88

Table 1. Distribution of the CD4+ of positive subjects.

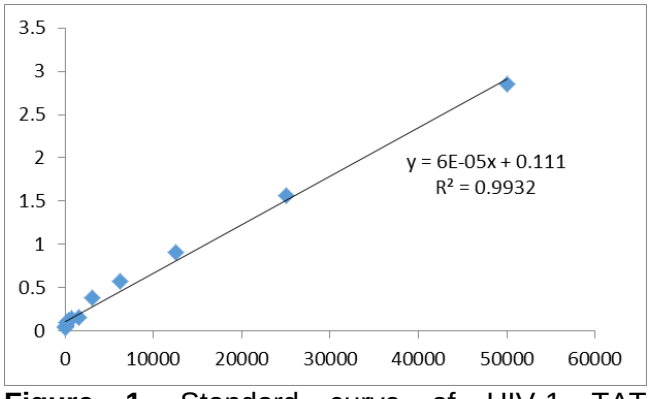

Figure 1. Standard curve of HIV-1 TAT absorbance.

The standard antigen absorbance in ELISA for HIV-1 TAT established the lowest absorbance at 0.037 (eq. 25 pg/ml), while blank absorbance was at 0.058 (eq. 100 pg/ml). The mean of the HIV-1 TAT plasma level on positive subjects was 32,723.88 pg/ml, while the mean of the control group was 87 pg/ml as can be seen in Figure 1.

Discussion

Decreased CD4+ count constitutes a reassuring marker leading clinicians to refer patients for HIV diagnoses.[8,14] An HIV rapid test will confirm diagnosis by detection of anti-HIV antibodies. CD4+ has been the most specific marker in HIV clinical diagnosis because CD4+ cells constitute the sole target of HIV infection. After being used for HIV replication, CD4+ cell will undergo either apoptosis or necrosis, causing depletion of these cells. However, during the early phase of HIV infection, CD4+ count remains stable, although the number of HIV antibodies might still be too low for detection by standard methods. This is the point at which the standard dignostic method faces a challenge.[2]

HIV-1 TAT protein is indispendable to ensure that the RNA elongation process is completed during the replication cycle. Without the presence of TAT, RNA Pol II will only produce either a short transcript or an incomplete one, as it would always stop at 60 basepairs from the elongation starting point. After completing a replication cycle, this protein is actively excreted through independent pathways by golgi apparatus. In this manner, the protein becomes extracellular, although still associated with the cell membrane, until the latter disintegrates and TAT is released into the serum or cerebrospinal fluid. More HIV-1 TAT will be released into the system during budding and the release of new virions.[6,7,16]

These foregoing statements lead to the conclusion that HIV-1 TAT is only present in HIV-infected individuals, specifically during the replication stages. During a latent period, either a window period or when replication is blocked by antiretroviral therapy, the destruction of CD4+ cells is minimized.[17] Consequently, less HIV-1 TAT should be detectable within the system. These hypotheses were addressed in this study.

Using ELISA, the presence of HIV-1 TAT was detected in the blood plasma of HIV-infected individuals but not in that of the HIV-negative individuals constituting the control group,.[18] The sensitivity and reliability of the results cannot be doubted as monoclonal antibodies were

employed in the detection process. Thus, HIV-1 TAT is, indeed, specifically associated with HIV infection and AIDS.

The ELISA method can also determine the quantitative level of HIV-1 TAT by means of a sandwich method antigen-antibody reaction between monoclonal antibodies and the chromogenic substrate. The technology enables the absorbance of this substrate to be read using a microplate reader at a specific wavelength, in this case 640 nm.[19] This study confirmed a significant inverse correlation between CD4+ count and the plasma level of HIV-1 TAT. This finding suggests that the increase of HIV-1 TAT in the plasma is associated with the destruction of CD4+ cells marked by the depletion of CD4+ count. Thus, HIV-1 TAT may also describe the progressivity status of an HIV-infected patient, as well as CD4+ count.

Conclusions

HIV-1 TAT may serve as a marker of disease progression as well as CD4+ count and constitutes a potential marker in any diagnosis of HIV.

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Declaration of Interest

The authors report no conflict of interest.

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