Correlation between HIV-1 genotype and clinical progression in HIV/AIDS patients in Surabaya, Indonesia

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Abstract. Several factors such as host and viral factors can affect the progression of HIV/AIDS. This study aims to identify the correlation viral factors, especially the HIV-1 subtype with HIV/AIDS progression. Inpatient HIV/AIDS during the period March to September 2017 and willing to participate are included in the study. Historical data of disease and treatment was taken by medical record. Blood samples were amplified, sequenced and undergone phylogenetic analysis. Linear regression analysis was used to estimate beta coefficient ($\beta$) and 95%CI of HIV/AIDS progression (measured by the CD4 change rate, $\Delta$CD4 cell count/time span in months). This study has 17 samples. The HIV-1 subtype was dominated by CRF01_AE (81.8%) followed by subtype B (18.2%). There was significant correlation between subtype HIV-1 ($p = 0.04$) and body mass index ($p = 0.038$) with HIV/AIDS clinical stage. Many factors were assumed to be correlated with increased rate of CD4, but we only subtype HIV-1 had a significant correlation ($p = 0.024$) with it. From multivariate analysis, we also found that subtype HIV-1 had a significant correlation ($\beta = 0.788$, 95%CI: 17.5-38.6, $p = 0.004$).

1. Introduction

Human immunodeficiency virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) remains a challenge in the health sector in Indonesia. HIV infection takes an average of 8-10 years to develop into AIDS.[1] Many factors influence the disease progression such as host factor, environmental factor and virology factor. Host factors that may affect are age [2], HLA [3] and CYP polymorphism [4], psychosocial [5], gender, ethnic [6] and body mass index (BMI).[7] The environmental factors affect the progression such as mode of transmission [8] and socioeconomic status. Viral factors include viral subtype [9] co-receptor use [10] or the presence of deleterious mutations in the virus.[11] Information on the factors that affect the progression of the disease will be a valuable guide to manage the disease.

HIV can be classified by type, i.e. HIV-1 and HIV-2. HIV-1 itself can be subdivided by groups (M, N, and O) and subtypes (A, B, C, D, F, G, H, J, and K). Many studies have reported a correlation between HIV-1 and HIV/AIDS progression, but the further relevance of subtypes still results in varying outcomes. Moreover, there is limited data on the relationship of HIV-1 subtypes to disease progression in Indonesia. This study aims to determine the viral subtype and its correlation with disease progression.

2. Material and Methods
Inpatient HIV / AIDS at the Airlangga University Teaching Hospital during the period March to September 2017 and willing to participate in the study are included in the study. Historical data of disease and treatment such as Anti Retroviral Therapy (ART) regimen, duration of ART, CD4 levels baseline, opportunistic infections, clinical conditions of the patients, and possible routes of HIV-1 transmission is taken by medical record database. The Institutional Ethics Committees of Airlangga University and Airlangga University Teaching Hospital approved this study. Informed consent was obtained from all patients before sample collection. Ten milliliters of ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood was from each participant. Plasma was then isolated from peripheral blood samples by centrifugation for 10 min at 2,000 rpm. Peripheral blood mononuclear cells (PBMCs) were also isolated by density gradient centrifugation using Histopaque 1077 (SigmaAldrich, St. Louis, MO). DNA was extracted from PBMCs using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich).

The HIV-1 pol gene encoding protease (PR gene) and Reverse Transcriptase (RT gene) were then amplified from DNA extracted from PBMCs by nested polymerase chain reaction using Ex Taq (Takara Bio, Shiga, Japan) and primer sets as follows. To amplify the viral PR gene fragment, the primers DRPRO5 [5′-AGACAGGYTAATTTTTAGGGA-3′]; corresponding to nucleotides (nt) 2074 to 2095 of an HIV-1 reference strain, HXB2 (GenBank accession no.K03455)] and DRPRO2L(5′-TATGGATTTTCAGGCCCAATTITTTGA-3′; nt 2716 to 2691) were for the first PCR, and the primers DRPRO1M (5′-AGAGCCAACAGCCCCACCAG-3′; nt 2148 to 2167) and DRPRO6 (5′-ACTTTTGCCATCCATCC-3′; nt 2611 to 2592) were for the nested PCR. In addition, to amplify the viral RT gene, RT1L(5′-ATGATAGGGGAATTGGAGGTTT-3′; nt 2388 to 2410) and GPR2M (5′-GGACTACAGTCYACTTGTCCATG-3′; nt 4402 to 4380) were for the first PCR and RT7L(5′-GACCTACACTGTCACATAATTGG-3′; nt 2485 to2509) and GPR3L (5′-TTAAATCTACTARCCATGTYTCTCC-3′; nt 4309 to 4285) were for the nested PCR. PCR products amplified at the end-point dilution of DNA templates were subjected to sequencing analysis to examine the genomic fragment of the major viral population in a sample.

Sequencing analysis of the amplified HIV-1 PR and RT genes was carried out using the BigDye Terminator v3.1 Cycle Sequencing kit with an ABI PRISM3500xL genetic analyzer (Applied Biosystems, Foster City, CA), and data were assembled using Genetyx version 10 software (Genetyx, Tokyo, Japan). The full length of the PR gene (297 bp; nt 2253 to 2549) and the N-terminus of the RT gene (762 bp; nt 2550 to 3311) were sequenced and subjected to subsequent analyses. HIV-1 subtyping was carried out using the Recombinant Identification Program (RIP) available on the HIV sequence database website (www.hiv.lanl.gov/). Also, neighbor-joining (NJ) trees with the Kimura two-parameter model were constructed using theMEGA6.2 software. [12,13] Bootstrap values (1,000 replicates) for relevant nodes were reported on a representative tree. If one of the PR and RT genes failed to be sequenced, the subtype was assigned based on the other gene. Furthermore, if there was a discrepancy in the subtype between PR and RT, it was defined as a recombinant of more than two HIV-1 subtypes and circulating recombinant forms (CRFs).

Linear regression analysis was used to estimate beta coefficient (β) and 95% confidence intervals (CIs) of HIV/AIDS progression (measured by the rate of change of CD4, ΔCD4 cell count/span of time in months).

3. Results

3.1. Demographic Characteristics on the subject

This study includes 17 samples of hospitalized patients at the Airlangga University teaching hospital. Demographic characteristics show predominant male (70.6%) with an average age of 43.35 years. Most samples have transmission routes through heterosexual intercourse (47.1%). The number of secondary infections has a range of 1-6. A total of 23.5% of patients died. The HIV-1 subtype was dominated by CRF01_AE (81.8%) and followed by subtype B (18.2%). The average body mass index of 20.8 kg / m². The median baseline CD4 cell count was 105.4 cells / mm³, and the number of
samples experiencing CD4 failure was 14 (82.3%). The largest ethnic was Javanese (52.9%) followed by Chinese (35.2%) and other ethnics (11.7%)(Table 1). Only 11 of the 17 samples can be amplified, while the rest are not detected. On the 11 samples were sequenced and then performed a phylogenetic analysis (Figure 1).

3.2. Factors related to HIV / AIDS progression
From baseline characteristics and clinical stage, after analysis there was significant correlation between subtype HIV-1 (p = 0.04) and body mass index (p = 0.038) (Table 2). From the analysis in relation to the increasing rate of CD4, it was found only subtype HIV-1 has a significant correlation (p = 0.024). From multivariate analysis, it was found that the subtype HIV-1 still had a significant correlation ($\beta = 0.788$, 95% CI: 17.5-38.6, $p = 0.004$) (Table 3).

| Characteristics                              | All (n = 17) | Clinical Stage (WHO) | $p$  |
|----------------------------------------------|-------------|----------------------|------|
| Age, mean years (SD)                         | 43.35 (13.92)| 36.5 (12.2)          | 0.098|
| Sex, n (%)                                   |             |                      | 0.191|
| Male                                         | 12 (70.6)   | 7 (87.5)             |      |
| Female                                       | 5 (29.4)    | 1 (12.5)             |      |
| Risk factor for HIV infection, n (%)         |             |                      | 0.463|
| Heterosexual intercourse                     | 8 (47.1)    | 3 (37.5)             |      |
| Homosexual intercourse                       | 3 (17.6)    | 1 (12.5)             |      |
| Intravenous drug user                        | 6 (35.3)    | 4 (50)               |      |
| Number of opportunistic infection, mean (Range) | 3.11 (1-6) | 2.5 (1-4)            | 0.136|
| HIV-1 Subtype, n (%)                         |             |                      | 0.04 |
| CRF01_AE                                     | 9 (81.8)    | 2 (25)               |      |
| B                                            | 2 (18.2)    | 2 (25)               |      |
| Body Mass Index, mean kg/m$^2$ (SD)          | 20.8 (4.5)  | 22.8 (5.1)           | 0.038|
| Baseline CD4 count, mean cells/mm$^3$ (SD)   | 105.4 (93.2)| 137.1 (114.6)        | 0.362|
| CD4 Failure, <150 cells/mm$^3$/year, n (%)   | 14 (82.3)   | 5 (62.5)             | 0.063|
| Ethnic                                       |             |                      | 0.073|
| Javanese                                     | 9 (52.9)    | 5 (62.5)             |      |
| Chinese                                      | 6 (35.2)    | 2 (25)               |      |
| Others                                       | 2 (11.7)    | 1 (12.5)             |      |

**Table 2.** Regression analysis of CD4 change rate.

| CD4 Increasing Rate                           | Univariate | Multivariate |
|-----------------------------------------------|------------|--------------|
| Age                                           | -0.077 (36.1-50.5) | 0.06 (-0.2-0.6) | 0.864 |
| Sex                                           | -0.395 (1-1.5) | 0.129 | 0.401 (-6.4-11.6) | 0.124 |
| Risk factor for HIV infection                 | 0.170 (1.4-2.3) | 0.529 | 7.6 (-0.4-15.6) | 0.062 |
| HIV-1 Subtype                                 | 0.671 (0.9-1.4) | 0.024 | 0.788 (17.5-38.6) | 0.004 |
| Body Mass Index                               | 0.373 (18.1-24.6) | 0.154 | -0.263 (-2.8-0.1) | 0.627 |
| Baseline CD4 count                            | -0.182 (39.7-142.2) | 0.5 | -0.458 (-0.9-0.2) | 0.433 |
| Ethnic                                        | 0.174 (0.19-1.25) | 0.520 | 0.056 (0.297-15.8) | 0.561 |

4. Discussion
Many factors influence the course of HIV / AIDS disease including host factor and virus factor. In this study, one of the host factors that is Body Mass Index (BMI) related to clinical stages of HIV / AIDS, this is not surprising because one of the parameters of WHO clinical assessment of HIV / AIDS is weight loss. However, when associated with death \( (p = 0.477, \text{data not shown}) \) and CD4 cell rate did not show significant correlation \( (p = 0.373) \). Another study stated that the HR of death was 2.2 (CI: 1.6-3.0) for BMI between 16 and 18.4 kg / m\(^2\) and 4.4 (CI: 3.1-6.3) for BMI <16 compared to normal BMI \((\geq 18.5)\).[14] This difference shows that there are more influential factors on HIV / AIDS-related deaths in our study.

Correlation between HIV-1 subtype and disease progression remains controversial. Some studies report the correlation between HIV-1 subtype and HIV / AIDS progression by relating the shortening of time needed by HIV to become AIDS, low rates of CD4 change, high viral load, and HIV / AIDS-related deaths. A meta-analysis shows that people infected with subtype C have HIV / AIDS progression faster than other subtypes, followed by subtypes D, AE, G and A.[15] The study by Chu et al. reported that the low rate of CD4 change was related to the CRF01_AE subtype.[1] The previous studies in Indonesia had reported that this CRF01_AE subtype also has a higher prevalence than other subtypes [16], and in this study, CRF01_AE is also associated with the rate of change CD4 cells in patients who had received ART \( (p = 0.004) \). Ng et al. reported that the viral load was significantly higher in those who got infected with CRF01_AE than with non-CRF01_AE \((P<0.015)\).[17] Viral load testing itself has not been being a routine check-up, so there are limitations in the availability of data. Subtype linkages with HIV / AIDS-related deaths are still contradictory. Costello et al. reported that subtype CRF01_AE had estimated median time to death 7.8 (7.0–9.1) years.[18] While Alaeus et al. reported no difference in survival rates in all subtypes of HIV-1.[19] In this study, the HIV-1 subtype was not associated with death (data not shown).

In conclusion, HIV-1 subtype especially CRF01_AE is related to HIV / AIDS progression. But further research is needed with obtaining more sample quantities and including more factors involved in the correlation with disease progression and maybe we can use viral load as a better marker to reflect disease progression.

**Figure 1.** Phylogenetic analysis of reverse transcriptase (RT) and protease (PR) gene sequences. Phylogenetic trees were generated for newly sequenced HIV-1 PR (A) and RT (B) genes together with the corresponding viral gene of reference HIV-1 strains representing subtype A1 (A1), subtype
A2 (A2), subtype B (B), subtype C (C), subtype D (D), subtype G (G), CRF01_AE (01_AE), CRF02_AG (02_AG), CRF15_01B (15_01B), and CRF33_01B (33_01B). The reference strains of the HIV-1 subtype are in bold and italic. Bootstrap values are shown when the values are > 70.

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