Indonesian Journal of
Tropical and Infectious Disease
Vol. 1. No. 1 January–April 2010

Research Report

Correlation between soluble urokinase plasminogen activator receptor with CD4 T lymphocyte and WHO clinical staging of HIV infection

Shinta Oktya Wardhani, Niniek Burhan, Gatoet Ismanoe, and **Tri Yudani MR**
Tropical and Infectious Disease Division, Department of Internal Medicine, Brawijaya University

ABSTRACT

The urokinase-type plasminogen activator (uPA) and its receptor play a key role in pericellular proteolysis, cell migration and signal transduction. Previous study showed that suPAR could be used as an independent prognostic marker of disease progression in HIV-1 patients.[1,17] Immune status of HIV patient and progressivity of disease are important parameters used as clinical concideration before initiating anti retroviral treatment and for monitoring treatment effectivity. Recently immune status of HIV patients is determined by CD4 T lymphocyte counting which represents the remaining healthy lymphocyte T expressing CD4 that very expensive and need special laboratory equipment. Destruction and shedding of T lymphocyte, macrophage and natural killer cell will deliver soluble urokinase plasminogen activator receptor, a surface protein which is expressed by those cells and can be measured by ELISA[8,9,11]. This study objective is to determine correlation between suPAR plasma concentration and CD4 T lymphocyte and WHO clinical stagging of HIV infection. Study subjects. Fifty four naieve HIV-1-infected patients (32 males, and 22 females) are participant in a cross sectional study enrolled on 22 November 2007 until 31 july 2008 at the department of infectious disease Saiful Anwar Hospital, Malang, Indonesia. Blood sampling. Two blood samples were drawn before treatment, CD4 counts were measured with an Epics XL-MCL Coulter flowcytometer. EDTA plasma for suPAR measurement was stored at -80°C. Data are presented as mean±standart deviation. P<0.05 is considered significant. Statistical calculations were done using SSPS 15. Patients (n = 54) enrolled and clustered according to WHO clinical stage (I - IV) at inclusion. All HIV-infected patients had measurable levels of plasma suPAR with a median value of 8,9 ng/mL(range 1,65-29,7 ng/mL). Pearson correlation demonstrated a weak but significant negative between suPAR and CD4 T lymphocyte count (p=-0.634, p<.0005). suPAR level positively correlated with the WHO-defined clinical stages (P< .0005, spearman correlation test, r=0,87). There were significant difference between each stage i.e I(1,6± o,61ng/mL), II(3.04±1.03 ng/mL), III (10.53±7.1ng/mL) and IV (20.42±10.81ng/mL) (P< .0005, Spearman test). In addition pearson correlation demonstrated a weak but significant negative correlation between suPAR and CD4 count (p=-0.66; P<.0005). ***There were negative significant correlation*** *between CD4 count and suPAR level, suggested that suPAR could provide as a complementary biological marker for HIV-1 although it can not replace the CD4 count. SuPAR plasma concentration and clinical stage give significantly correlation with WHO clinical staging of HIV infection.*

Key words: *suPAR, HIV, CD4 T lymphocyte, WHO clinical stage*

INTRODUCTION

HIV infection/AIDS is a global pandemic with cases reported from virtually every country. Approximately 40.000 individuals are newly infected each day[1]. Progression of HIV infection is largely dependent on the interaction between tha viral factors and host factors. HIV primarily infect cells which expressed CD4 receptor such as monocyte-macrophage, T lymphocyte, dendritic cell, langerhans and NK cell.[1,2,3,4] It brings about the destruction of those cell through multiple mechanism including apoptosis.[5,6,7,8,9] The loss of CD4 cell population ultimately leads to the inability of infected persons to deal with opportunistic organism.[5,6]

The hallmark of HIV/AIDS infection is to identify immunodeficiency status (stage), because these stage will predict the progression of the HIV infection and treatment response. Imunodefficiency status may be measured through CD4 T lymphocyte count.[3,4] Immune activation in HIV infection is known to be linked positively to HIV-

1 replication and negatively to CD4 T-cell depletion.[5,6,7,8] SuPAR is a component of the plasminogen activation system, which comprises urokinase-type plasminogen activator (uPA) and its receptor (uPAR).[10] uPAR is expressed on a variety of different immune cells such as macrophage, T lymphocyte, NK cells, dendritic cell and langerhans cells.[10,11] suPAR is generated by either proteolytic cleavage or shedding from cells.[10,11] SuPAR concentrations are increased and prognostic in a variety of inflammatory including HIV infection.[10] The blood level of the soluble urokinase-type plasminogen activator receptor (suPAR) is increased in untreated human immunodeficiency virus-1 (HIV-1) infection and decreases in HIV-1-infected patients after initiation of highly active antiretroviral therapy (HAART).[12,13,14,15] The plasma concentration of soluble urokinase-type plasminogen activator receptor (suPAR, CD87) is a strong independent predictor of mortality in untreated patients with HIV-1 infection. [15] Plasma concentrations of this immune marker can be quickly and inexpensively measured using a simple enzyme-linked immunosorbent assay (ELISA), which requires much less sophisticated laboratory infrastructure than that needed for CD4 cell count or plasma viral load measurement. Such an assay might therefore be potentially useful in resource-limited settings.[15,16] This study try to determine correlation of suPAR plasma consentration with CD4 T lymphocyte to identify immunodeficiency state of HIV/AIDS infection based on WHO 2006 criteria.

MATERIAL AND METHODS

Study subjects

Fifty four naieve HIV-1-infected patients (32 males, and 22 females) are participant in a cross sectional study enrolled on 22 November 2007 until 31 july 2008 at the department of infectious disease Saiful Anwar Hospital, Malang, Indonesia. All patients enrolling in studies fulfill the inclusion criteria (provide written informed consent and this study was approved by the Research Ethics Committee of the University of Brawijaya; age between 15-50 years old, not pregnant and already confirmed diagnosed suffered from HIV infection). Clinically, we grouiping all the participant based on WHO 2006 criteria.

Blood sampling

Two blood samples were drawn before treatment, CD4 counts were measuresed with an Epics XL-MCL Coulter flowcytometer.[17] EDTA plasma for suPAR measurement was stored at -80°C. Plasma suPAR concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (suPARnostic,™ ViroGates, Lyngby, Denmark) following the manufacturer's instructions. This is a simple double monoclonal antibody sandwich assay that measures total suPAR, including both full-length and cleaved forms of the receptor. In brief, a standard control curve (range 0.6 – 19.3 ng/ml), positive control, and test samples were incubated in duplicates in a 96-well plate pre-coated with anti-suPAR antibody. Following further incubation with a secondary peroxidase-conjugated antibody, the assay was developed by addition of a tetramethylbenzidine (TMB) chromogenic substrate. The reaction was terminated by addition of sulphuric acid and absorbance at 450 nm was determined using a microtitre plate reader. The linear standard curve was used to determine concentrations in positive control and test samples. Samples with concentrations exceeding the highest standard (19.3 ng/ml) were reanalysed using a further 5-fold sample dilution.[18]

Data analysis

Data were analysed using SPSS FOR WINDOWS RELEASE 15.0. As the frequency distribution of values

Table 1. Patients baseline characteristic

Variable	WHO Clinical stagging				p
	Stage 1 (n=16)	Stage 2 (n=10)	Stege 3 (n=13)	Stage 4 (n=15)	
Sex	10 ♂ and 6 ♀	4 ♂ and 6 ♀	9 ♂ and 4 ♀	9 ♂ and 6 ♀	0.841
Age	30.13±6.97	30.50±6.70	29.62±5.22	29.67±5.31	0.983
Total Lymphocyte	**1232.50±337.12**	**1628±674.68**	**1101.54±501.51**	**719.33±413.74**	**0.000 ***
Hb	**11.79±1.43**	**12.42±1.57**	**10.32±1.86**	**10.52±1.78**	**0.007 ***
Albumin	**3.84±0.85**	**3.71±0.95**	**3.37±0.73**	**2.82±0.92**	**0.011 ***
BMI(kg/m^2)	**20.32±2.23**	**18.67±1.67**	**17.56±1.84**	**16.45±1.75**	**0.000 ***
CD_4 (sel/uL)	330.63±113.06	195.40±102.51	128.62±132.14	57.60±94.11	**0.000 ***
suPAR(ng/dL)	1.65±0.61	3.04±1.03	10.53±7.13	20.42±10.81	**0.000***
Source of infection					
• Contaminated needle/drug abuse	10	4	8	8	0.801
• Sexual intercourse	6	6	5	7	

Hb = hemoglobin, BMI = *body mass index,* CD_4 = CD_4 T limfosit, suPAR = *soluble urokinase plasminogen activator receptor.*

was highly right-skewed, the suPaR values were log10-transformed for bivariate analyses (based on the Mann Whitney U or Kruskall Wallis tests to compare medians) Correlation between suPAR consentration and Clinical WHO staging assessed with Spearman analysis and Correlation between suPAR consentration and CD4 T lymphocyte count assessed with Pearson analysis, significant if p<0,05.[19,20]

RESULTS

Patient baseline characteristics

There were 54 patient full fill inclusion criteria enrolled in our study. These patients had a median age of 32 (59,3%) years, 32(59%) males dan 22 (41%)females (table 1). After assessed clinical WHO staging, there were 16(29%) patient stage I, 10(18%) stage II, 13(24%) stage III and 15(27%) stage IV. There were gradual CD4 T lymphocyte count depletion in every stage of WHO staging in our patient. CD4 T lymphocyte count 330.63±113.06 in stage I, 195.40±102.51 in stage II, 128.62±132.14 in stage III and 57.60±94.11 in stage IV. All patient showed decreased of BMI (*body mass index*) especially in stage IV. Mean of BMI 20,32 ± 2,23 Kg/m^2 stage I, stage II 18,67 ± 1,67 Kg/m^2, stage III 17,56 ± 1,84 Kg/m^2 and stage IV 16,45 ± 1,75 Kg/m^2. Most of patient infected from using contaminated needles for injecting drugs and sexuall intercourse.

Plasma suPAR concentrations

Detectable levels of suPAR were measured in plasma samples from all 54 patients. The standard curves for each run were linear (mean r2 = 0.995; SD = 0.004) and all positive control readings were consistent with the expected value. The mean suPAR concentration in the patient plasma samples was 1.65±0.61 ng/ml in stage I, 3.04±1.03 in stage II, 10.53±7.13 in stage III and 20.42±10.81 in stage IV.

REFERENCES

1. WHO/UNAIDS, Summary country profile for HIV/AIDS treatment scale up: Indonesia June 2005.
2. Hammer Scott, Management of newly diagnosed HIV infection, N Enl J Med, 353; 16; 2005.
3. Musey Luwy, James Hughes, Timothy Schacker, Theresa Shea, Lawrence Corey, and Juliana Mc Elrat, Cytotoxic-T-Cell Responses, Vial load and disease progession in early Human Immunodeficiency virus type 1 infection, N Engl J Med; 337: 18, 1997.
4. Langford Simone E, Jintanat Ananworanich and David A Cooper, Predictor of diesease progression in HIV infection: a review, AIDS Research and Therapy 2007, 4: 11.
5. Fauci AS, Pantaleo G, Stanley S. Immunopathogenic mechanisms of HIV infection, *Ann Intern Med*, 1996; 124: 654–653.
6. Calles N.R, Evans D, Terlonge DeLouis, Pathophysiology of the human immunodeficiency virus, Weill Medical College of Cornell University. Available at: http://edcenter.med.cornell.edu/CUMC_PathNotes/HIV_Infection/HIV_Infection_04. Di akses September 2008
7. Paranjape RS. Immunopathogenesis of HIV infection. Indian J Med Res 2005; 121: 240–55.
8. Kilbi J.Michael, Eron Joshep, Novel therapies based on mechanism of HIV-1 Cell Entry, N Engl J Med 348; 22, 2003
9. Nasronudin. The Effect of HIV/AIDS Infection Diagnosis to T-CD4 Lymphocytes Apoptosis Mechanism in HIV/AIDS Patients, Psychoneuroimmunoligical Approach. Thesis. Airlangga Univ. Surabaya: 2005.
10. Blasi F, Carmeliet P. uPAR: A versatile signalling orchestrator. Nat. Rev.Mol.Cell Biol. 2002; 3: 750–54.
11. Montuori Nunzia, Maria vincenza Carriero, alvatore Salzano, Guido Rossi, and Pia Ragno, The Cleavage of tha Urokinase Receptor regulates its multiple functions, jbc.org, 2002.
12. Murali Rama, Joshua H.Wolfe, Rebecca Erber, Seto M. Chice, M.R.Murali, Helen G Durkin, Petr Zach and Dominick L.Auci, Altered levels of urokinase on monocytes and in serm of children with AIDS; effects on lymphocyte activation and surface marker expression, J.Leukbio; 64, 1998.
13. Ostrowski. S.R, T. L.Katzenstein, G.Hoyer-Hansen, J.Gerstoft, B.K.Pedersen, H.Ullum, Plasma level of intact and cleaved urokinase receptor decrease in HIV-1-invected patients initiating Highly Active Antiretroviral Therapy, Scandinav J Immunol 2006; 63, 478–486.
14. Sidenius N, Sier CF, Ullum H. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection, Blood 2000; 96: 4091–95.
15. Lawn SD, Myer L, Bangani N, Vogt M, Wood R, Plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR) and early mortality risk among patients enrolling for antiretroviral treatment in South Africa, BMC Infect Dis 2007; 7: 41.
16. Schneider Uffe, Nielsen RL, Pedersen Court, Olsen JE, The prognosis value of the suPARnostic TM ELISA assay in HIV-1 infected individuals is not affected by uPAR promoter polymorphisms, BMC infectious Disease 2007, 7: 134.
17. BD tritest CD3/CD4/CD4 reagent for flowcytometer equipped (BD Catalog no.340385)
18. Missionpharma, SuPARnostic ELISA Kit, www.missionpharma.com, 2007.
19. Santoso,S. Buku Statistik Non Parametrik. Jakarta:Penerbit PT Elex Media Komputindo, 2003.
20. Dajan, A, Pengantar Metode Statistik, Jilid I, Pustaka LP3ES Indonesia, Jakarta, 1995.
21. Kofoed Kristian, Ove Andersen, Gitte Kronborg, Mchae Tvede, Janne Petersen, Jasper Eugen-Olsen and Klaus Larsen, Use of plasma C-reactive protein, procalcitonin, neutrophils, macrophage migration inhibitory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggerring receptor expressed on myeloid cells-1 in combination to diagnose infections: a prospective study, Critical Care 2007, 11: R38.
22. Mangione CM, Gerberding JL, Cummings SR. Occupational exposure to HIV: frequency and rates of underreporting of percutaneous and mucocutaneous exposures by medical housestaff. Am J Med. 1991; 90: 85–90.
23. Nasronudin. Pencegahan penularan infeksi HIV dan AIDS melalui *universal precaution*. HIV & AIDS: Pendekatan Biologi Molekuler, Klinis dan Sosial. **Airlangga University Press, Surabaya; 2007.**
24. Nasronudin. HIV & AIDS: Intervensi HIV dan peran mitochondria. Pendekatan Biologi Molekuler, Klinis dan Sosial. **Airlangga** University Press, Surabaya; 2007.
25. Andersen Ove, Eugen-Olsen, Kofoed kristian, Iversen Johan, Haugaard Steen B; Soluble Urokinase Plasminogen Activator Receptor is a Marker of Dysmetabolism in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy. Journal of medical virology, 2008.
26. Friis Hendrik, Gomo Exnevia, et al. HIV and other predictors of serum folate, serum ferritin and hemoglobin in pregnancy: a cross-sectional study in Zimbabwe, Am J Clin Nutr; 73: 2001.
27. Djoba Siawaya JF, Ruhwald M, Eugen-Olsen J, Walzl G, Correlates for disease progression and prognosis during concurrent HIV/TB infection; Int J Infect Dis. Jul; 11(4): 289–99. 2007.
28. Ditjen PP dan PL 2005, Laporan triwulan pengidap infeksi HIV dan kasus AIDS sampai desember 2005, Jakarta Ditjend PP dan PI, Depkes RI, 2005.

29. KPA 2003, Strategi nasional penanggulangan infeksi HIV/AIDS 2003–2007. Kementrian Koordinator bidang kesejahteraan rakyat, Komisi nasional penanggulangan AIDS, 2003.
30. Ostrowski SR, Katzenstein TL, Piironen T, Gerstoft J, Pedersen BK, Ullum H. Soluble Urokinase Receptor Levels in Plasma During 5 Years of Highly Active Antiretroviral Therapy in HIV-1 Infected Patients. J Acquir Immune Defic Syndr 2004; 35: 337–42.
31. Ostrowski SR. The Soluble Urokinase Receptor in Inflammation-with Focus on HIV-Infection and Malaria. Ph.D.diss. Copenhagen Univ. Denmark; 2004.
32. Ostrowski. S.R, T. Piironen, G.Hoyer-Hansen, J.Gerstoft, B.K.Pedersen, H.Ullum, Reduced release of intake and cleved urokinase receptor in stimulated whole-blood cultures from Human Immunodeficiency Virus-1-infected patients, Scandinav J Immunol 2005; 61, 347–356.
33. Eugen-Olsen J, Gustafson P, Sidenius N, Fischer TK, Parner J, Aaby P, Gomes VF, Lisse I, The serum level of soluble urokinase receptor is elevated in tuberculosis patients and predicts mortality during treatment: a community study from Guinea-Bissau, Int J Tuberc Lung Dis. Aug; 6(8): 686–92, 2002.
34. Kronborg, N. Weis, H. Nielsen, N. Obel, S. S. Pedersen and J. Eugen-Olsen, The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality, Clin Microbiol Infect 2004; 10: 409–415.
35. Ostergaard C, Benfield T, Lundgren JD, Eugen-Olsen J Soluble urokinase receptor is elevated in cerebrospinal fluid from patients with purulent meningitis and is associated with fatal outcome; Scand J Infect Dis. 36(1):14–9 2004.