ORIGINAL ARTICLE

Association Between Neopterin Levels and Outcome of HIV/AIDS Naive Patients in 30 Days

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ARTICLE INFO

Article history:
Received 23 August 2019
Received in revised form 11 October 2019
Accepted 25 October 2019

Keywords:
HIV/AIDS,
Serum neopterin levels,
Prognostic factor.

ABSTRACT

Introduction: HIV/AIDS is still a major worldwide problem even in Indonesia. Some markers can provide information regarding the description of immune activity in HIV/AIDS. Neopterin produced by macrophages as a catabolic result of Guanosine Triphosphate (GTP) is one of them. Neopterin has been widely studied as a prognostic indicator of the course in HIV/AIDS. In recent study, we aim to find out the association between neopterin levels and outcomes of HIV/AIDS patients.
Methods: A prospective longitudinal analytic study involved 56 samples of HIV/AIDS naive patients. Serum neopterin levels were measured by the Enzyme Linked Immunosorbent Assay (ELISA) method. Outcome is a living condition or death within the first 30 days of the patient being treated. All variables were analyzed in univariate and multivariate test (p value <0.05).
Results: The average age of the study subjects was 39.7 ± 11 years old with predominantly male gender (71.4%). The median serum neopterin level of the study subjects was 168.13 nmol/L with the lowest levels of 11.78 nmol/L and the highest level of 196.95 nmol/L. Outcomes were grouped live and died, each group at 50%. There was a significant positive association between serum neopterin levels and outcomes in 30 days (p=0.02).
Conclusion: Role of neopterin levels still need to be proven as a prognostic factor by evaluating other factors that influence the outcome of HIV/AIDS patients.

Introduction

HIV/AIDS is still a big problem throughout the world. In 2016, there were more than 36.7 million people living with HIV. HIV/AIDS has a fairly high mortality rate and ranks fourth as the cause of death in the world. The number of people living with HIV/AIDS in Indonesia continues to grow every year. Based on the HIV/AIDS progress report data of the Direc-torate General of Disease Control and Environmental Health Ministry of Health of the Re-public of Indonesia until March 2017, there have been many HIV sufferers in Indonesia's large provinces. East Java ranks second after DKI Jakarta, with a total of 33,043 people. HIV infection tends to increase especially in the productive age group 25-49 years and the age group 20-24 years. This is what makes HIV / AIDS still a health problem, in addition to the high incidence and mortality it also causes high maintenance costs.[1,2] The high mortality of HIV/AIDS patients is mostly caused by sepsis with various complications, namely septic shock and failure or multiorgan dysfunction syndrome (3). Predictors of mortality in HIV/AIDS that have been studied include age, sex, stage of disease, CD4+ T-lymphocyte cell count when starting therapy, nutritional status, and medication adherence .[4,5,6,7,8]

In HIV/AIDS, CD4+ T-lymphocyte levels and viral load values are still widely used in as-sessing disease activity and progression. Several other markers can also provide information related to the description of immune activity in HIV / AIDS and have been widely studied. Neopterin is produced by macrophages and is said to be an indicator of macrophage function and cellular immunity.[9,10] Neopterin has been widely studied as one of the markers associ-ated with disease activity in HIV/ AIDS. Neopterin is also considered to be used as a prog-nostic factor of HIV/AIDS. Neopterin is a

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nonspecific but reliable marker of HIV infection.

Neopterin is a light weight molecule which is found to have increased concentration in human body fluids during cellular immune response. If cellular immunity is dominant, cir-culating neopterin levels increase, including those found in viral infections, intracellular bacterial infections, autoimmune disease groups, malignancy and others. At the time of initial HIV infection, increased concentrations of neopterin have been obtained in the blood and urine of patients,[11,12] and increased concentrations of neopterin as one of the early indications of immune activity in HIV pathogenesis. Fuchs et al's study in 1988 showed that in the early stages of HIV infection, where clinical and laboratory markers were generally still in the nor-mal range, neopterin levels had shown an increase.[10,13]

In the study of Mildvan et al conducted in 2005 analyzed neopterin in HIV / AIDS, it was found to be higher in line with disease progression ($p = 0.0009$; HR = 2.94 (95% CI, 1.48-5, 87) (9). Furthermore, the Bipath et al. Study, neopterin showed superior results com-pared to CRP and IL6 as markers of disease activity at CD4+ levels below 200 cells / mm3 and showed a significant negative correlation with CD4+ T-lymphocyte levels in naive HIV/AIDS patients ($p = 0.02$) and who have received antiviral therapy (ART) ($p = 0.001$) (4). Neopterin shows the course and progression of the disease as well as the efficacy of anti-retroviral therapy. Apart from the fact that there are currently available HIV-specific markers, such as viral loads that indicate disease activity, determination of neopterin levels is still useful in relation to easy and inexpensive techniques for showing information related to the sta-tus of immune activity involved in the pathogenesis and prognostic of HIV infection (13). But the role and relationship to the 30-day mortality of HIV/AIDS patients is still unknown.

Until now there have been no studies examine the association between neopterin levels and outcomes in 30 days of HIV/AIDS patients in Indonesia. So this study was conducted to determine the relationship between neopterin levels and outcomes in 30 days of HIV/AIDS patients in the infection intermediate care installation room. 30 days is a period that is often used to assess or estimate the outcome of treatment in either death or readmission in patients aged ≥ 65 years or who receive care in health services (14).

Methods

This was an observational analytic study with a prospective longitudinal design. The population of this study was patients in the infection intermediate care installation room of the RSUD Dr. Soetomo Surabaya was diagnosed as HIV. The study sample was collected by consecutive sampling technique.

The inclusion criteria were HIV naive patients which has been confirmed positively by examination of three method, aged 16-60 years, and subject agreed to participate in the study. Patients with chronic kidney disease who has undergone hemodialisys and malignancy who is ongoing or has received chemotherapy were excluded from the study. This study has obtained ethical feasibility from the ethics committee in Health Research Dr. Soetomo General hospital Surabaya and informed consent was obtained from each subject of this study.

Sample Collection

Neopterin is a laboratory parameter that is detected in serum, as a sign of the activation of cellular immune responses that can describe the activity of macrophages/ monocytes. Neopterin levels obtained are stated in nmol/L (15,16). Neopterin level was determined using competitive Enzyme-Linked Immunosorbent Assay (ELISA). The reagent used was Human Neopterin ELISA, IBL International GMBH RE59321, Flughafenstrasse 52a D-22335 Hamburg, Germany to quantitatively measure the level of neopterin in serum, plasma and urine. Examination was done in the clinical pathology installation of the central laboratory Dr. Soetomo general hospital Surabaya. Solid phase enzyme-linked immunosorbent assay (ELISA) based on the basic principle of a competitive ELISA. An unknown amount of antigen in the sample and a fixed amount of enzyme labelled antigen compete for the antibody-binding site (rabbit-anti-neopterin). Both antigen-antibody complexes bind to the wells of the microtiter strips coated with a goat-anti-rabbit antibody. Unbound antigen is removed by washing. The intensity of the color developed after the substrate incubation is inversely proportional to the amount of antigen in the sample. Results of sample can be determined directly using the standart curve.

Outcome 30 days is a condition of a patient's life or death reported within 30 days of receiving treatment in an infection intermediate care installation room, both when hospitalized or when he was discharged from the hospital. Obtained through medical records or interviews by telephone.

Statistical Analysis

Characteristic data are presented descriptively in the form of frequencies and percentages for categorical data types, while for numerical data are presented in medians and ranges or mean and standard deviations. Analysis to determine the relationship of each characteristic with outcome using the unpaired T test for normally distributed numerical data or the Mann-Whitney test for abnormally distributed data. Whereas Chi-Square analysis is used for categorical data if it meets the requirements, or Fisher's test if it does not meet the requirements. Analysis to determine the relationship between neopterin levels and outcomes within 30 days will be followed by multivariate analysis if confounding factors are obtained from the results of bivariate analysis. Statistical analysis using the "R" program. Interpretation of test results based on the value of p, otherwise significant if $p<0.05$.

Results

Characteristic of Subjects

The sample of this study was total of 56 people who met the inclusion and exclusion criteria. Mean age was

40 years old. The comparison of male sex (71.4%) and women (28.6%). The most risk factors are transmission through heterosexuals (62.5%). Based on clinical stage, the most research subjects in the stage 3 (58.9%). The most opportunistic infections (OI) (26.8%) with PCP. Mean Hemoglobin (Hb) levels was 10.1 and the standard deviation was 2.3 g/dL. The mean albumin level of the study subjects was 2.99 and the standard deviation was 0.65 g /dL. Median of CD4+ cell counts was 21.50 cells /mm3. In this study also assessed SOFA scores, and obtained a median value of 3. The conditions of the characteristics subjects are shown in table 1. And the evaluation of each subjects characteristics in the outcome group is shown in table 2.

Serum Neopterin Levels, Outcome in 30 day, and Correlation Between Variables

All research subjects were examined for serum neopterin levels. The median serum neopterin in the study subjects was 168.13 nmol/L with the lowest levels of 11.78 nmol/L and the highest levels were 196.95 nmol/L. The evaluation of serum neopterin level in the outcome group showed median value of neopterin levels in the living group is 149.56 nmol/L with the lowest levels of 11.78 nmol/L and the highest is 196.95 nmol / L. The median value of neopterin levels in the group died was 175.3 nmol/L with the lowest levels of 108.37 nmol/L and the highest levels of 195.41 nmol/L. The results of Mann-Whitney test p=0.041, there was a significant difference in neopterin levels in the living outcome group and the outcome group that died. Based on the results presented in table 2, common characteristic variables include age, sex, risk factors, clinical stage, haemoglobin levels, albumin levels, CD4+ cell counts did not show significant differences in the living outcome group and the outcome group that died. SOFA scores are obtained as a confounding factor, then the analysis of the association between neopterin levels and outcomes within 30 days of research subjects will be followed by an analysis of discriminant function. The result obtained p=0.02 (Table 3). This means that there was a relationship between neopterin levels and outcomes within 30 days of the research subjects, and the analysis has controlled for SOFA scores as a confounding factor.

Table 3. Discriminant Funtion Analysis of association between neopterin levels and outcome 30 day (controlled SOFA score)

Variabel	p	Significancy
Neopterin Levels	0,02	Significant
SOFA Score	0,01	Significant

Table 1. Characteristics of Subjects

Characteristics	Results (n=56)
Age (Years)	39,7 ± (11)
Mean ± SD	(20-59)
Range (min – max)	
Age Group (Years)	10 (17,9)
20-29 years, n (%)	17 (30,4)
30-39 years, n (%)	17 (30,4)
40-49 years, n (%)	12 (21,4)
50-59 years, n (%)	
Sex	40 (71,4)
Male, n (%)	16 (28,6)
Female, n (%)	
Risk Factor	35 (62,5)
Heterosexual, n (%)	17 (30,4)
Homosexual, n (%)	4 (7,1)
Others (tatoo,IVDU), n (%)	
Clinical Stage	2 (3,6)
Stage 1, n (%)	0 (0)
Stage 2, n (%)	29 (51,8)
Stage 3, n (%)	25 (44,6)
Stage 4, n (%)	
Opportunistic Infection (OI)	9 (16,1)
Oral Candidiasis, n (%)	5 (8,9)
Chronic diarrhea, n (%)	6 (10,7)
Lung TB, n (%)	9 (16,1)
Pneumonia, n (%)	15 (26,8)
PCP, n (%)	10 (17,9)
Cerebral toxoplasmosis, n (%)	2 (3,6)
Without OI, n (%)	
Hemoglobin (Hb) Levels	10,1 ± (2,3)
Mean ± SD	(4,4-15)
Range (min – max)	27 (48,2)
< 10g/dL, n (%)	29 (51,8)
≥ 10g/dL, n (%)	
Albumin Levels	2,99 ± 0,65
Mean ± SD	(1,66-4,54)
Range (min – max)	31 (55,4)
< 3g/dL, n (%)	25 (44,6)
≥ 3g/dL, n (%)	
CD4+ counts	21,50 (0-338)
Median (min – max)	53 (94,6)
< 200 sel/mm3, n (%)	3 (5,4)
≥ 200 sel/mm3, n (%)	
SOFA score	3 (0-7)
Median (min – max)	

Table 2. Subjects characteristics in the outcome group

Characteristics	Outcome 30 day		P value
	Living (n=28)	Died (n=28)	
Age (Years)			
Mean ± SD	39,29 ± 12,2	40,18 ± 8,9	0,756
Sex			
Male (n/%)	18 / 64,3	22 / 78,6	0,237
Female (n/%)	10 / 35.7	6 / 21,4	
Risk Factor			
Heteroseksual (n/%)	18 / 64,3	17 / 60,7	0,783
Homoseksual (n/%)	9 / 32,1	8 / 28,6	
Others(tatoo,IVDU) (n/%)	1 / 3,6	3 / 10,7	
Clinical Stage			
Stage 1 (n/%)	2 / 7,2	0 / 0	0,060
Stage 2 (n/%)	0 / 0	0 / 0	
Stage 3 (n/%)	17 / 60,7	12 / 42,9	
Stage 4 (n/%)	9 / 32,1	16 / 57,1	
Hemoglobin Levels (Hb)			
Mean ± SD	10,59 ± 1,91	9,62 ± 2,59	0,118
< 10g/dL (n/%)	10 / 35,7	17 / 60,7	
≥ 10g/dL (n/%)	13 / 46,4	11 / 39,3	
Albumin Levels			
Mean ± SD	3,08 ± 0,69	2,89 ± 0,61	0,307
< 3 g/dL (n/%)	15 / 53,6	16 / 57,1	
≥ 3 g/dL (n/%)	13 / 46,4	12 / 42,9	
CD4+ counts			
Median (min-max)	22 (1–338)	21,5 (0-165)	0,780
< 200 sel/mm3 (n/%)	25 / 89,3	28 / 100	
≥ 200 sel/mm3 (n/%)	3 / 10,7	0 / 0	
SOFA score			
Median (min-max)	1,5 (0-7)	3 (0-7)	0,009
Neopterin levels			
Median (min-max)	149,56 (11,78-196,95)	175,3 (108,37-195,41)	0,041

Discussion

We Neopterin and its derivatives are said to be able to interact with the oxidation of ROS. This has been observed in experimental physics-chemical research and cell culture. As the re-sult, neopterin and its derivatives play a role in the signal transduction cascade. In addition, its role in triggering the release of cytokines that can support the development of HIV virus infection. Continuously with the formation of neopterin, IFNγ induces ROS secretion by macrophages. So there is a relationship between neopterin formation and hydrogen peroxide secretion. ROS is involved in numbers of signaling pathways in both normal metabolic condi-tions and pathological mechanisms. Circumstances with high antigen levels and cytokine ac-tivity in HIV/AIDS will end with increased ROS production and weaknesses of antioxidant defenses. So it is said to be very likely an increase in HIV virus replication in response to oxi-dative stress. Based on the literature it is also said that neopterin and its derivatives play a role in the progression of HIV/AIDS where neopterin and its derivatives can activate NF-κB and AP1 factors.[13] Enhancement of NF-κB and AP1 are key transcription factors that orchestrates expression of many genes involved in inflammation, embryonic evelopment, lymphoid differentiation, oncogenesis and apoptosis. Other literature adds that neopterin lev-els are increasingly related to macrophage activation in conditions with a reduced CD4+ cell counts. The HIV virus has the ability to survive, especially in macrophages. Where in chronic infections, macrophages become less sensitive to the induction of apoptosis, and even become a reservoir

against the HIV virus.[17,18]

The above statement can explain that neopterin is an indicator of immune system acti-vation in HIV/ AIDS patients. In addition, neopterin is thought to have a correlation with ROS production so that it can be an indicator of oxidative stress as a trigger for the progres-sion of HIV disease to become more severe as the number of CD4+ T-lymphocyte cells de-creases.[19]

This study shows the results of the analysis of a significant difference in serum neopterin levels in the living group and the deceased group (p=0.041). While the correlation analysis between serum neopterin levels and CD4+ cell counts showed no significant correlation (p=0,132). After an analysis of discriminant function to control SOFA scores as a confounding factor, neopterin levels continued to show a significant relationship with outcomes within 30 days, specifically mortality in HIV / AIDS patients (p=0.02). This is consistent with the study of Sacktor et al (1995) which states that neopterin can predict mortality in HIV patients with evaluation after 3.5 years with a RR of 5.5 (95% CI 2.6-11.9). Another similar study by Bipath et al (2015) which states that neopterin can be a marker of disease progression and outcome of HIV / AIDS patients. In addition, the results are similar to previous studies by Hosp et al in Zambian (2000), but in that study, the periods were longer, patients were followed for a minimum of 3 months to 12 months, and measurements were taken of the initial neopterin levels and after 12 months ($p < 0.05$) (4,20,21).

The limitations of this study was only carried out in a 30 day period. Longer research periods may provide a picture of the association between neopterin levels and other factors on more meaningful outcomes. This study only conducted one measurement of neopterin at the beginning of the study. Measurements made serially will likely provide more meaningful results to describe the relationship of neopterin levels with the progression and outcome of HIV/AIDS.

Conclusion

The results of this study indicate the association between serum neopterin levels and outcomes within 30 days of HIV/AIDS patients. This is in accordance with previous research. However, the role of neopterin levels as a prognostic factor in HIV / AIDS patients still needs to be proven, because there are many other factors that can affect the outcome of HIV/AIDS patients.

Conflict of Interest

The author stated there is no conflict of interest

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