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ABSTRACT

The activation of the immune system plays a very important role in HIV infection. This can be measured by Neopterin in human body fluids. The measurement serves to monitor cellular immune activity. The measurement is sensitive and can be performed easily. Neopterin is a catabolic product of guanosine triphosphate, a purine nucleotide. Neopterin belongs to the chemical group known as pteridines. The purpose of this study was to examine the existence of a correlation between Neopterin level with the number of CD4+ T-lymphocytes in the blood of HIV patients. The study was an analytical observational study with cross-sectional design. The samples consisted of 32 stage I HIV-infected patients, who came to the Intermediate Care Unit of Infectious Diseases Dr. Soetomo Hospital from July to September 2014. The examination of neopterin was performed through ELISA and the examination of number of CD4+ T-lymphocytes was performed through flowcytometry (BD FACSArrayTM). The results were statistically analyzed using Pearson’s correlation test followed by regression test. Neopterin levels of HIV-infected patients tended to increase with an average of 14.74 nmol/L while the number of CD4+ T-lymphocytes tended to decrease with an average of 231.81 cells/μL. A negative correlation was found between Neopterin and CD4+ T-lymphocytes in the blood of stage I HIV infection. The decline of CD4+ T-lymphocytes was followed by the increased levels of neopterin in stage I HIV infected patients.

Key words: Neopterin, CD4+ T-lymphocytes, stage I HIV infection
molecular test to determine the amount of viral load in plasma. Both tests are expensive and require skilled health personnel, therefore an alternative parameter is acquired, in order to reduce the economic burden of developing countries.1–4 The alternative test is expected to be cheaper and can be used to monitor the progress of the disease as well as the response of the HIV-infected treatment. Changes in neopterin concentration occurred earlier than the decrease in the number of CD4+ T-lymphocytes and the clinical progress of the disease, therefore the treatment could be used to predict further development towards AIDS.5–7

Neopterin is the catabolic result of guanosine triphosphate (GTP), a purine nucleotide with chemical class known as pteridin. Neopterin is synthesized by macrophages by stimulation of interferon gamma (IFN-Y) cytokines and indicates the proinflammatory immune status, therefore neopterin can be used as the marker of cellular immune system activation.8

The aim of this study was to know the existence of a correlation between Neopterin level with the number of CD4+T-lymphocytes in patients and to predict the number of CD4+ T-lymphocytes when the level of neopterin is known so that the time to start therapy could be determined.

METHODS

The study was an analytical observational study with cross-sectional design. The population in this study was HIV adult patients who came to the Intermediate Care Unit of Infectious Diseases Dr. Soetomo Hospital. This research was conducted from July to September 2014. The sample size was calculated based on the formula of sample size for correlation coefficient in a single sample by Hulley & Cummings.9 Estimated correlation coefficient was \( r = 0.482 \) with \( Z_\alpha = 1.960 \) for significance level of 5% and \( Z_\beta = 0.84 \) for power of the test of 80%, resulting in a minimum sample size of 32 people.

Inclusion criteria in this study were HIV positive patient with three different tests in stage I, not receiving antiretroviral drugs and willing to participate in this study by signing an informed consent. The exclusion criteria in this study were HIV-infected patients who had received antiretroviral drugs, patients with opportunistic infection, patients with hemolysis, jaundice and lipemic

Neopterin level was determined using competitive Enzyme-Linked Immunosorbent Assay (ELISA). Blood samples were centrifuged in centrifuge at 3,000 rpm for five (5) minutes. The serum was then put into microcentrifuge tubes. Once labelled (name of the patient, date of sample collection), the serum was immediately stored at -20°C until the examination was performed. The samples at temperatures between 2–8°C had a stability of 72 hours, while at a temperature of \( \leq -20°C \) (aliquot), the stability was 6 months. The samples with hemolysis, jaundice and lipemic were excluded. The reagent used was Human Neopterin Elisa to quantitatively measure the level of neopterin in serum, plasma and urine. The reagent was used only for research, not for diagnostic purposes.

The examination to determine the number of CD4+ T-lymphocytes was performed using flowcytometry BD FACS Calibur. The samples in the study were 3 mL of venous blood kept in vacum \( K_3EDTA \) tubes. The stability of the samples was less than 30 hours at room temperature (20–25°C). The delivery of samples should be maintained at room temperature. Temperatures should not be too hot (over 37°C) or too cold (less than 4°C). The samples was excluded if it was hemolysed or frozen or 30 hours after sampling. Scale variables of each group such as sex and diagnosis of disease would be presented as frequency distribution and percentage using Pearson correlation test with \( \alpha = 0.05 \) followed by regression test to estimate the number of CD4+ T-lymphocytes based on the level of neopterin already known when the results of the analysis test showed a significant correlation.

RESULTS AND DISCUSSION

Quality assurance on the test result of neopterin by ELISA were performed using control 1 and 2 during running. This control material was already provided in the test kit by Gen Way Biotech Inc. Data on intra-assay and inter-assay of were listed in the of Gen Way Biotech Inc. Intra-assay data brochure of neopterin in serum were between 33.1–4 3 nmol/L with a CV at 4.3–11.7%. Inter-assay data of neopterin in serum were between 4.67 to 29.98 nmol/L with a CV at 8.8–13.8%. The result of duplication performed on samples was a mean of CV at 5.215%.

Quality assurance on the test result of the number of CD4+ T-lymphocytes was performed bimonthly using external quality control and for daily, the samples were compared with the test result of healthy individuals.

The characteristics of the subjects were presented in Table 1, in which 15 (46.9%) people were males and 17 (53.1%) people were females. Based on the type of occupation, 14 (43.8%) people were housewives, one (3.1%) person was an employee, one (3.1%) person was a student, two (6.3%) people were sellers, four (12.5%) people were hairdressers, seven (21.9%)
people worked in private sector, one (3.1%) person was a male migrant worker and two (6.2%) people were female migrant workers. Based on the risk factor of transmission, five (15.6%) people were homosexuals, sixteen (50%) people were infected by their spouse, ten (31.1%) people practiced free sex, one (3.1%) person practiced free sex and IVDU.

The results showed that the lowest neopterin was 1.60 nmol/L and the highest was 74.20 nmol/L (mean was 14.74 nmol/L and standard deviation was 16.89 nmol/L) (Table 2).

The results showed that the lowest absolute CD4+ T-lymphocytes was 28 cells/μL and the highest was 559 cells/μL (mean was 231.81 cells/μL and standard deviation was 140.50 cells/μL). The lowest CD4+ T-lymphocytes was 1.45% and the highest was 25.90% (mean was 13.60% and standard deviation was 7.13%) (see Table 2).

Once the level of neopterin and the number of absolute CD4+ T-lymphocytes were determined, statistical analysis using Pearson correlation test was performed to determine the presence of correlation between neopterin and CD4+ T-lymphocytes.

The results of the analysis showed a significant negative correlation between the level of neopterin and an absolute number of CD4+ T-lymphocytes with the value of r=-0.4817 and p=0.005 in stage I HIV-infected patients (see Figure 1). The results of the analysis also showed significant negative correlation between the level of neopterin and the percentage of CD4+ T-lymphocytes with the value of r=-0.4278 and p=0.015 (see Figure 1).

The results of the analysis showed that the estimated number of absolute CD4+ T-lymphocytes (see Table 3) as well as the number of CD4+ T-lymphocytes in percent (see Table 4) were significant, as the value of p<0.5.

Based on the regression equation $y=\beta_0+\beta_1 \times x$ with $\beta_0$ served as the constants, the estimation of the number of CD4+ T-lymphocytes based on the level of neopterin could be determined by the following equation:

**Table 1.** Characteristics of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of people</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>46.9</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>53.1</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>14</td>
<td>43.8</td>
</tr>
<tr>
<td>Employee</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Seller</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>Hairdresser</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Private sector</td>
<td>7</td>
<td>21.9</td>
</tr>
<tr>
<td>Male migrant worker</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Female migrant worker</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual</td>
<td>5</td>
<td>15.6</td>
</tr>
<tr>
<td>Infected spouse</td>
<td>16</td>
<td>50</td>
</tr>
<tr>
<td>Free sex</td>
<td>10</td>
<td>31.3</td>
</tr>
<tr>
<td>Free sex and IVDU</td>
<td>1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**Table 2.** Results of neopterin level

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lowest</th>
<th>Highest</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin</td>
<td>1.60 nmol/L</td>
<td>74.20 nmol/L</td>
<td>14.74 nmol/L</td>
<td>16.89 nmol/L</td>
</tr>
<tr>
<td>Absolute CD4+ T-lymphocytes</td>
<td>28 cells/μL</td>
<td>559 cells/μL</td>
<td>231.81 cells/μL</td>
<td>140.50 cells/μL</td>
</tr>
<tr>
<td>CD4+ T-lymphocytes</td>
<td>1.45%</td>
<td>25.90%</td>
<td>13.60%</td>
<td>7.13%</td>
</tr>
</tbody>
</table>

**Table 3.** The results of the analysis of regression toward absolute CD4+ T-lymphocytes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Constants</th>
<th>β</th>
<th>P</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin</td>
<td>290.981</td>
<td>-4.010</td>
<td>0.005</td>
<td>0.482</td>
</tr>
</tbody>
</table>

**Table 4.** The results of the analysis of regression toward CD4+ T-lymphocytes in percent

<table>
<thead>
<tr>
<th>Variable</th>
<th>Constants</th>
<th>β</th>
<th>P</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin</td>
<td>16.625</td>
<td>-0.181</td>
<td>0.015</td>
<td>0.423</td>
</tr>
</tbody>
</table>
age with the age range between 18–49 years and the mean age of the subjects was 34.37 years. There were three people aged over 50 years. This suggests that HIV infection tends to mostly be found in people of productive age.

The results showed that the lowest level of neopterin was 1.60 nmol/L and the highest was 74.20 nmol/L (mean was 14.74 nmol/L and standard deviation was 16.89 nmol/L). The lowest level of neopterin in this study was obtained from a patient with the number of CD4+ T-lymphocytes 232 cells/μL. It was likely because the decrease in CD4+ T-lymphocytes was influenced by multiple factors/mechanisms such as direct cell death, integrity loss of plasma membrane due to bulging and tearing by virion, apoptosis, humoral and cellular immune response to HIV, autoimmune mechanism, the target cell death due to hyperactivity Hsp 70.10 The development of HIV infection lead to the activation of chronic immune system. The HIV virus will trigger the activation of B cells, increase the turn over of T cells production of proinflammatory cytokines and increase the number of T cell activation most cytokine levels in serum or plasma are not measurable in normal condition and also in HIV-infection. The option to examine the exceeding cytokine in the blood circulation is performed by the measurement of specific cytokines in plasma or serum, among others by examining neopterin.11 The overview of neopterin reflects immunology process of monocytes/macrophages and dendritic cell which can be seen as a marker of immunological activity in general. Neopterin is the result of responding to the activation of immune system.8 A HIV-infected person will stimulate the immune system that activates Th1 to release interferon cytokine gamma which stimulates macrophages to release neopterin. Increased concentration of interferon cytokine gamma in serum that can activate mononuclear and B cells are found in a HIV seropositive person because the concentration of interleukin-6 increases when infected by HIV virus.5

The longitudinal study showed that the level of neopterin was correlated with the development of certain diseases, Multicenter AIDS Cohort Study reported that serum level of neopterin was a strong predictor of progressive clinical development of AIDS not only of the progressivity but also of the rate of the decrease in CD4+ T-lymphocytes.7

Research conducted by Sanjim Chadna et al12 in New Delhi in 2013 on 100 subjects with HIV seropositive found a significant higher level of neopterin in subjects with CD4+ T-lymphocytes <200 cells/μL compared with subjects with CD4+ T-lymphocytes >200 cells/μL.12

The number of CD4+ T-lymphocytes of patients infected with stage I HIV is influenced by several factors, namely: stress, subtype, viral load and nutrition of the patient. One of the factors that might influence in this study was stress of having been diagnosed HIV positive. The stress due to diagnosed HIV infection may increase corticosteroids from the adrenal cortex having immunosuppressive effects in the lymphoreticular system, thus suppressing the function and the number of lymphocytes, including CD4+ T-lymphocytes.10

A patient with CD4+ T-lymphocytes more than 500 cells/μL is likely due to early stage infection and as a long-term nonprogressors with normal CD4+ T-lymphocytes possibility for infection is still an early stage and as a long-term nonprogressor with number of CD4+ T-lymphocytes within the normal range and stable for years despite not getting anti-retroviral drugs.13,14

The results of the analysis showed a significant negative correlation between neopterin with absolute CD4+ T-lymphocytes (r=-0.482; p=0.005) in patients with stage I HIV. The results also showed a significant negative correlation between neopterin and CD4+ T-lymphocytes in percent (r=-0.428; p=0.015) in patients infected with stage I HIV. These results are similar to those obtained in a previous study conducted by Bipath et al in 201212 which stated that there was a negative correlation between neopterin level and the number of CD4+ T-lymphocytes (r=-0.482; p=0.001). This suggests that the decrease in CD4+ T-lymphocytes is followed with an increased neopterin level in patients infected with stage I HIV.12

The number of absolute CD4+ T-lymphocytes and CD4+ T-lymphocytes in percent could be estimated by neopterin level. However, the accuracy rate was approximately 23.2% for absolute CD4+ T-lymphocytes and 18.3% for CD4+ T-lymphocytes in percent. The equation obtained from the regression analysis could still be used because it was considered as significant although the accuracy rate was low.

CONCLUSION AND SUGGESTION

Based on the study, it can be concluded that: the mean of neopterin level in patients infected with stage I HIV was high at 14.75 nmol/L. The mean of absolute CD4+ T-lymphocytes was considered low, 231.81 cells/μL. The results of regression analysis could estimate the number of CD4+ T-lymphocytes although the accuracy rate was 232%, but significant based on the equation: absolute CD4+ T-lymphocytes = 290.981–(4.010×neopterin level). This study also resulted in a negative correlation between neopterin and CD4+ T-lymphocytes in the stage I HIV-infected blood. These results indicated that the decrease in...
CD4+ T-lymphocytes was followed by an increase in neopterin levels in stage I HIV-infected patients. A further study with a greater population involving patients with HIV of any stage is needed. Additional testing is needed to exclude other infections besides HIV such as a test for hepatitis B and C.

REFERENCES