Correlation of zinc plasma and IgM anti-PGL-1 levels among close contact of leprosy

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Abstract

Background: Previous study showed leprosy patients have low zinc status. Yet the status of zinc in close-contact, which indicated by IgM anti-PGL1 level, have not determined. The aim of the study was to determine the association of zinc plasma and IgM anti-PGL-1 levels among close contact of leprosy patients in Indonesia.

Methods: This was an observational study. Subjects were 186 close-contact leprosy patients. Measurement of zinc plasma status used atomic absorption spectrophotometer while examination of IgM anti-PGL1 level used ELISA (Polyclonal rabbit anti human IgM/HRP/Dako®).

Results: The average level of IgM anti-PGL1 and zinc plasma were 804 unit/ml (± 439.4) and 16.6 µmol/l (± 3.5) consecutively. There was significant correlation between zinc plasma and IgM anti-PGL1 (r = -0.230; p = 0.002).

Conclusion: There is a significant correlation between zinc plasma and IgM anti-PGL1 in close contact of leprosy (Med J Indones. 2012;21:166-9)

Keywords: IgM anti PGL-1, close contact of leprosy, zinc plasma

A study in Northeastern Brazil showed one of leprosy risk factor is poverty as people with low economic status tend to live in slum area and crowded housing.1 Crowded housing is a condition relevant to the transmission of Mycobacterium leprae, the agent of leprosy.2,3 In low income countries, where diets are predominantly plant-based and intake of animal protein are low, diets are often high in phytate. High phytate causes low bioavailability of zinc.4 Zinc is known as one of micronutrient associated with immune function.5

Several previous studies showed that zinc serum levels of leprosy patients is in accordance with the spectrum of leprosy.6,7 Zinc serum level in leprosy patients gradually decreased from paucibacillary (PB) to multibacillary (MB) type, with the lowest level is in MB type.8 The close-contact of leprosy basically have greater risk to be infected with leprosy although they may not yet show the clinical sign of manifestation (which called seropositive). A study in Brazil detected leprosy seropositivity in 39% of household contacts and 66.5% of school children in hyperendemic areas.9 One of methods to detect seropositive people is examining Immunoglobulin M (IgM) anti-Phenolic Glycolipid (PGL)-1 level.10

The current main concern of leprosy control focused on patients with clinical manifestations. Nevertheless, earlier prevention at the stage of seropositive close-contact may reduce new leprosy cases. Research on the relationship of serum zinc levels with leprosy has frequently done, but how the association of zinc status and IgM anti PGL-1 among close contact of leprosy has not yet done.

METHODS

This was an observational study in cross sectional design. The study was conducted on close-contact people, who live in the same house or near leprosy patients in Lamongan (East Java) and in Semarang (Central Java), Indonesia, from 2006 until 2010. Minimum sample size used formula with significance
level of 95% ($Z_{\alpha} = 1.96$), deviation standard of 4.92, and precision of 10%. The amount of study subject was 185.98 and rounded be 186 close-contact people who met inclusion criteria as follows: (1) age of 15 to 50 years old; (2) both male and female; (3) have not clinically shown any leprosy symptoms. Besides, they must not: (1) take anti-leprosy medicine; (2) have any disease which influenced zinc status; (3) suffer from tuberculosis; (4) take any antituberculosis medicine; and (5) consume any anti-immunosuppressant in the last three months before blood samples are taken. They were also willing to join the study by signing informed concern. The ethical clearance for this study was obtained from the Research and Public Service Institute, Airlangga University.

Measurement of zinc plasma status used atomic absorption spectrophotometer at Balai Besar Laboratorium Kesehatan Surabaya. While examination of IgM anti-PGL1 level used enzyme-linked immunosorbent assay (Polyclonal rabbit anti human IgM/HRP/Dako®) at the Institute of Tropical Disease Laboratory, Airlangga University, Surabaya.

Normality of the data of zinc plasma status and IgM anti-PGL1 was determined by the Kolmogorov-Smirnov test, which showed distribution of data not normal. Therefore, statistical analysis to correlate zinc plasma and IgM anti-PGL1 level used Rank-Spearman.

RESULTS

The study was conducted in two locations, which are endemic areas of leprosy. Both are located in the northern coast of Java Island, with a relatively equal of socio-economic status, environmental and food patterns. The mean age of subjects was 30 years old (± 8.6) with the majority of respondents were women (74.2%). The average level of IgM anti-PGL1 and zinc plasma were 804 unit/mL (± 439.4) and 16.6 µmol/L (± 3.5) consecutively (Table 1). The majority of zinc plasma levels of the respondents was categorized as normal (82.8 %). There were 3 of total respondents 186 (1.6%) with severe zinc plasma deficiency and the remainders (15.6%) were categorized as marginal zinc plasma deficiency (10.7-13.0 µmol/L). None of the subjects with zinc plasma deficiency was categorized as seronegative (Table 2). We found a negative correlation between zinc plasma levels and IgM anti-PGL1 ($r = -0.230, p = 0.002$).

DISCUSSION

There are several methods to determine zinc status, one frequently used being a measurement of zinc level in plasma or serum, despite of only 0.1% zinc in the plasma. Zinc status in this study was quite good: only 1.6% of severe zinc deficiency and 15.6% marginal zinc deficiency. This would seem contradictory with current theory that prevalence of zinc deficiency in developing countries should be high due to the tendency of high level of phytate consumption. There are several explanation for the result. First, concentration of zinc in plasma and serum is under homeostatic control of the body. Although daily intake of zinc is less, the level of zinc plasma may remains normal for weeks. Second possible explanation came from adult subjects who were not high risk group to zinc deficiency. Most studies on zinc were addressed to children and elderly populations as both were at risk to zinc deficiency.

*Mycobacterium leprae*, as other obligate intracellular mycroorganism, induced mainly cellular mediated immunity. Cellular immune response is the result of macrophage activation characterized by increase of ability to suppress the multiplication or even eliminate bacteria. Humoral immunity to *M. leprae* is activation of B cell lymphocyte either in lymphoid organ and circulation. Stimulation of *M. leprae* antigen will alter B cell lymphocyte into plasma cell, which will produce antibodies. However, humoral immunity in leprosy are ineffective and may lead to complications due to its hyperreaction.

PGL-1 is a specific antigen and it has been using for serological studies in leprosy diagnosis. Level of IgM anti-PGL1 is positively correlated with bacterial index. Therefore, the higher level of IgM anti-PGL1, and the higher level of *M. leprae* infection. Increasing level of IgM anti-PGL1 in close contacts indicates imbalance response between cellular and humoral immunity.

Correlation coefficient of -0.230 indicated that there was an inverse relationship between level of zinc plasma level and IgM anti-PGL1. Our result supported the finding that

<table>
<thead>
<tr>
<th>Variables</th>
<th>Range (min – max)</th>
<th>Mean ± Standard deviation</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>15 – 50</td>
<td>30 ± 8.6</td>
</tr>
<tr>
<td>Body mass index</td>
<td>15.5 – 31.3</td>
<td>21.5 ± 2.9</td>
</tr>
<tr>
<td>Zinc plasma (µmol/L)</td>
<td>10.48 – 26.32</td>
<td>16.6 ± 3.5</td>
</tr>
<tr>
<td>IgM anti PGL-1 (unit/mL)</td>
<td>38.6 – 3037.3</td>
<td>804 ± 439.4</td>
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</tbody>
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mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. Zinc deficiency was associated with a decreased in cellular production of g-interferon and interleukin 2. However, cytokines associated to humoral immunity such as IL-4, IL-6 and IL-10 are not affected. On the other hand, zinc supplementation could modulate T cell to maintain IL-2 level in seropositive contact of leprosy patients. Zinc deficiency may interrupt the process of signal transduction of T cell. The role affects to the lymphocite protein tyrosine kinase/ LcK, protein kinase C and extracellular regulated kinase/ Erk.

In addition, zinc deficiency affected activity of macrophage and other innate immunity to leprosy. Phagocytosis, intracellular killing and cytokines production are all affected by zinc deficiency. M. leprae can adapt and survive in macrophages, thereby disrupting the immune response for several reasons; first, by limiting the hydrolytic ability of macrophages. Second, by limiting access to nutrients as well as the ability of macrophages to process and present antigen. Third, M. leprae able to manipulate the system and causing a failure of endosome maturation.

**REFERENCES**

