ORIGINAL ARTICLE

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High levels of zinc (Zn) as a protective factor and negatively correlated with IgM anti PGL-1 levels among household contact with multibacillary leprosy patients

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ABSTRACT

Background: Close contacts may develop subclinical leprosy with no symptoms but with *M. leprae* in their blood. Zinc is said to help in the prevention of *M. leprosy* infection. This study aimed to determine the correlation between zinc (Zn) serum levels and IgM anti PGL-1 levels in household contacts of multibacillary type leprosy patients.

Methods: This study is cross-sectional and involves 48 leprosy subjects (33 multibacillary leprosy household contact and 15 non-contact subjects). Subjects were selected based on the inclusion and exclusion criteria. Zinc and IgM anti PGL-1 levels were derived from venous blood examined with ELISA.

Results: This study showed the mean serum zinc level of the contact groups of 60.88 ± 15.92 µg/dl and the non-contact group of 90.00 ± 7.61 µg / dl (p <0.001; CI 95%: 20.38 - 37.85). The median (interquartile range) serum levels of IgM anti PGL-1 in the contact groups was 613.00 (40 - 1433) u/ml and the non-contact groups was 99.00 (14 - 695) u/ml (p = 0.001; 95% CI: 154.162 − 610.116). The correlation analysis between serum zinc levels and IgM anti PGL-1 serum levels r= 0.644 (p<0.05). A high serum zinc level is a protective factor against high IgM anti PGL-1 levels in household contact with leprosy (PR: 0.47; 95% CI: 0.20-1.09).

Conclusion: Zinc levels negatively correlate with IgM anti PGL-1 levels in subjects with household contact with multibacillary type leprosy. A high serum zinc level protects against high IgM anti PGL-1 levels.

INTRODUCTION

To date, leprosy remains a concern. In addition to the stigma attached to the sufferer, progressive physical deformity has a serious socio-economic impact. The high prevalence and new cases in endemic areas do not indicate the ineffectiveness of the leprosy eradication program. Instead, it may indicate the possible presence of subclinical leprosy, which is often found in leprosy household contacts. Household contact may have subclinical leprosy that misses the early detection. Subclinical leprosy indicates a clinically asymptomatic condition, but with the presence of specific antibodies against *Mycobacterium leprae*.[1] Anti PGL-1 antibodies indicate the magnitude of bacteria in an individual, which may reflect subclinical infection or manifestations of leprosy.[2]

Zinc is a mineral essential for the immune system. Zinc deficiency inhibits the activation and production of Th1 cytokines and causes cellular immune dysfunction due to an imbalance of Th1 and Th2 cell function. Zinc is an element necessary for the formation of the hormone thymulin. The experimental animal studies demonstrate that zinc deficiency causes thymus atrophy and reduces the number of thymocyte cells, which can further reduce cellular immune responses. The decrease in the maturation of T-helper 1 (Th1) cells reduces the production of Interleukin-2 (IL-2) and Interferon-γ (IFN-γ). Cytokines IL-2 and IFN-γ play a role in controlling intracellular pathogens.[3,4]

Early detection of subclinical leprosy in household contacts plays an important role in controlling new cases of leprosy. The cellular immune system plays an important role in the pathogenesis of leprosy. Zinc is the second highest micromineral in the body that maintains the cellular immune system.[5–7] This study aims to evaluate the zinc levels among leprosy household contact. This study is expected to provide evidence-based use of zinc as a supplement in subclinical leprosy.

METHODS

This study was an analytic observational study with a cross-sectional design. This study was conducted in the Dermatology and Venereology Outpatient Clinic of Sanglah General Hospital, Denpasar, Bali, from November 2020 to January 2021. The subjects were selected consecutively, with a total of 33 subjects from multibacillary leprosy households and 15 non-contact individuals (control). Variables in this study are IgM anti PGL-1 and zinc levels.

The subjects with an age range of 18-65 years were selected consecutively. Zinc and IgM anti PGL-1 levels were derived from venous blood examined with ELISA. Household contact subjects were individuals with close contact with multibacillary leprosy for at least 3 months, without any symptoms or signs of leprosy, and willing to participate in the study. Subjects consuming zinc supplementation within three months and having disease related to zinc production or elimination were excluded from this study. Variables were analyzed using SPSS version 22 with the descriptive and analytical methods.

RESULTS

The demographic characteristics of the study subjects can be seen in Table 1. There were no significant differences in sex, age, nutritional status, and exposure to sunlight (p>0.05). Income, zinc levels, and IgM anti PGL-1 levels were significantly different with a p=0.02 (p<0.05). The household contact group and the control group were predominantly women. In the household contact group, the number of female subjects was 72.7%, while the male subjects were 27.3%. Meanwhile, in the non-contact group, there were 80% female and 20% male. The mean age of the households contact group was 35.06 years (SD: 12.7), and the mean age of the non-contact group was 34.13 years (SD= 7.95). Based on these results, there were no significant differences between the contact groups and the non-contact groups. The socioeconomic status of the household contact group was found to be lower than the non-contact group. The household contact group had a lower income. The prevalence of subjects with income less than the regional minimum wage was 78.8%, and subjects with income more than the regional minimum wage was 21.2%, with a mean of IDR (Indonesian Rupiah) 1,434,848 (SD IRD 773,030). Meanwhile, in the non-contact group, the prevalence of subjects with income less than the regional minimum wage was 26.7%, with a mean of IDR 2,036,000 (SD IDR 791,126). These results indicate a significant difference between the two groups with p=0.02 (p<0.05). The nutritional status was assessed with body mass index (BMI), and the mean BMI data were not significantly different between the two groups, with the mean BMI of the households contact group of 21.91 kg/m^2 (SD 2.24) and the non-contact group was 22.15 kg/m^2 (SD 1.29).

The mean serum IgM anti PGL-1 level was 593.27 (SD: 412.71) in the household

Table 1. The demographic characteristics of study subjects.

Characteristics	Contact status N (%), mean ± SD		p-value
	Households contact (N = 33)	Control (N = 15)	
Sex			
Male	9 (27.3)	3 (20.0)	0.59
Female	24 (72.7)	12 (80.0)	
Age (year)	35.06 ± 12.70	34.13 ± 7.95	0.79
Nutritional status			
Underweight	1 (3.0)	0 (0)	
Normal	30 (90)	15 (100)	0.70
Overweight	2 (6.1)	0 (0)	
Mean ± SD	21.91 ± 2.24	22.15 ± 1.29	
Income (rupiah)			
More than minimum wage	7 (21.2)	4 (26.7)	
Less than minimum wage	26 (78.8)	11 (73.3)	0.02*
Mean ± SD	1,434,848 ± 773,030.24	2,036,000 ± 791,126.38	
IgM anti PGL-1 levels			
Mean ± SD	593.27 ± 412.71	211.13 ± 212.88	0.001*
Serum zinc levels			
Mean ± SD	60.88 ± 15.92	90.00 ± 7.61	<0.001*

*Statistically significant if p<0.05; SD= standard deviation

Table 2. Serum zinc and IgM anti PGL-1 levels in the household contact group and non-contact group.

Variables	Mean ± SD Median (*IQR*) (µg/dl)	Mean difference ± SD	95% CI	P-value
Zinc level in contact group	60.88 ± 15.92	29.21 ± 4.33	20.38 – 37.85	<0.001[a*]
Zinc level in non-contact group	90.00 ± 7.61			
IgM anti PGL-1 levels in contact group	613.00 (40 – 1433)	382,139 ± 90.45	154.162 – 610.116	0.001[b*]
IgM anti PGL-1 levels in non-contact group	99.00 (14 – 695)			

*Statistically significant if p<0,05;
[a]Unpaired T test; [b] Mann-Whitney test

contact group and 211.13 (SD 212.88) in the non-contact group. The mean zinc level in the households contact group was 60.88 (SD 15.92) and 90.00 (SD 7.61) in the non-contact group.

Serum Zinc and IgM Anti PGL-1 Levels in Households Contact Group and Non-Contact Group

The statistical analysis of serum zinc level differences between the two groups was conducted with an independent T test because the data were normally distributed. The mean serum zinc level in the contact group was 60.88 ± 15.92 g/dl and in the non-contact group was 90.00 ± 7.61 g/dl. The difference in the mean serum zinc levels in the two groups was 29.121 ± 4,33 g/dl, significantly different with $p<0.05$ ($p<0.001$; 95% CI: 20.38 – 37.85).

The statistical analysis of serum IgM level difference between the two groups was conducted with the Mann-Whitney test because the data were not normally distributed. The median value (interquartile range) of serum IgM anti PGL-1 level in the households contact group was 613.00 (40 – 1433) u/ml and 99.00 (14 – 695) u/ml in the non-contact group. The difference was statistically significant different with p-value <0.05 ($p=0.001$; 95% CI: 154.162 – 610.116) (Table 2).

Correlation Test of Zinc and IgM Anti PGL-1 Levels

The correlation analysis between serum zinc and IgM anti PGL-1 levels was conducted with Pearson's test, as seen in Table 3. The median value (interquartile range) of serum IgM anti PGL-1 level in the households contact group was 613.00 (40 – 1433) u/ml and 99.00 (14 – 695) u/ml in the non-contact group. The difference was statistically significant different with p-value <0.05 ($p=0.001$; 95% CI: 154.162 – 610.116).

Prevalence of Serum IgM Anti PGL-1 and Zinc Levels in Households in Contact of Multibacillary Leprosy

The ROC curve analysis was first performed to determine serum IgM anti PGL-1, with a sensitivity of 66.7%, specificity of 66.7%, and a cut-off point of

Table 3. Correlation test of zinc and IgM anti PGL-1 levels.

Variables	Zinc level	
IgM anti PGL-1 level	r	-0.644
	p-value	<0.001*
	N	48

*Statistically significant if p<0.05

Table 4. Risk analysis of serum IgM anti PGL-1 on zinc levels.

	IgM anti PGL-1 level		PR	95% CI	p-value
	Low <283.00	**High**			
Normal zinc level	9 (42.9%)	4 (27.1%)	0.47	0.20-1.09	0.033*
Lower zinc level	12 (57.1%)	23 (85.2%)			

*Statistically significant if p<0.05; PR: prevalence ratio

283.00 u/ml.

The risk analysis found a significant relationship between serum IgM anti PGL-1 and zinc levels in households contacts of multibacillary leprosy ($p=0.033$), and high serum zinc levels were a protective factor for high levels of IgM anti PGL-1 (PR: 0.47; 95% CI: 0.20-1.09) (Table 4).

DISCUSSION

This study demonstrated a lower zinc level in the households contact group with a mean of 60.88 than the zinc levels in the non-contact patient group with a mean of 90.00. There was a significant difference in zinc levels between patients with multibacillary leprosy in household contact and the non-contact group. Zinc is known as the second highest micromineral in the body and is involved in various important processes in the body. The zinc acts as an immunomodulator mediating the natural immune response of neutrophils and NK cells and the cellular immune response. In zinc deficiency, macrophage function of phagocytosis, intracellular antigen production, and cytokine production may be affected.[3,6,8–10] Zinc is said to help prevent *M. leprosy* infection. The zinc deficiency reduces the cellular immune system because of the low immune response of Th1 lymphocytes due to decreased production of IFN-Ɣ, IL2 and TNF- cytokines that help control intracellular pathogens.[6,8,11] In the spectrum of tuberculoid leprosy, it is said that there is a decrease in zinc levels in the lepromatous spectrum and conditions of erythema nodosum leprosum (ENL), it is said that the decrease in zinc levels is

increasing.[12]

This study showed a mean level of IgM anti PGL-1 in the household contact of 613.00 with a mean level of IgM anti PGL-1 level in the non-contact person of 99.0. There was a significant difference in IgM anti PGL-1 levels between multibacillary leprosy household contact compared to non-contact group with $p <0.05$ ($p=0.001$; 95% CI: 154.162 – 610.116). This study showed that the plasma IgM anti PGL-1 levels in the case group were higher than in the control group. Leprosy infection is detected with an increase in the IgM anti PGL-1 antibody titer. Serological examination of anti PGL-1 antibodies is one of the specific diagnostic tests for leprosy and has been widely used to detect subclinical leprosy in household contacts of leprosy patients.[5,13,14] PGL-1 is antigen-specific for *M. leprae* and is used for serological tests to diagnose leprosy. Circulating IgM antibodies have been associated with clinical symptoms and bacterial indices in leprosy patients. Therefore, the higher the IgM anti PGL-1, the higher the infection rate of *M. leprae*.[5,6] Household contacts possess the greatest risk for subclinical leprosy. The risk of family members or contacts who can be infected has a different range according to the type of leprosy. Multibacillary leprosy (MB) has a greater risk of 4-10 times compared to the paucibacillary type (PB), which is two times if both are compared to people of non-household contact.[13,15,16] Frota et al. 2010 compared the seropositivity between household and non-household contacts by measuring the IgM anti PGL-1 titer, demonstrating a significant difference between the two

groups with seropositivity of household contacts of 58.8% and non-household contacts of 15.1%. Healthy individuals with seropositive IgM results are at high risk of developing leprosy.[16] In a study of 60 healthy household contact individuals with seropositive IgM anti PGL-1 results (>605), clinical symptoms began to develop after four years of follow-up. Healthy individuals with seropositivity are 8.65 times more likely to develop leprosy than seronegative individuals.[17]

Correlation analysis between the serum zinc and IgM anti PGL-1 was conducted with Pearson's test, which resulted in a correlation coefficient (r) of -0.644 and p<0.05. There was a moderate negative relationship between serum zinc and IgM anti PGL-1 levels. The higher the zinc level, the lower the IgM anti PGL-1 level. *M. leprae* is an intracellular bacteria thereby the host's immune defense depends on the cellular immune system. In Th1 and Th2 immunological interactions, the tuberculoid pole represents a clinical form characterized by a small number of bacilli with a granulomatous infiltrate consisting of macrophages and lymphocytes. Milder clinical disease is associated with a lower number of bacilli, and a pattern of immune response of Th1 lymphocytes, i.e., the production of TNFα and IFN-γ activates macrophages and induces the production of iNOS, which destroys bacilli due to the release of free radicals.[18,19] Polar lepromatous, clinically severe disease, is associated with a large number of lesions and a predominance of Th2 lymphocyte responses that induce the production of cytokines such as IL-4, IL-10, and TGF- that inhibit Th1 responses, thereby inactivating the microbicidal response of macrophages, facilitating the bacilli survival.[20] Zinc is said to help prevent *M. leprosy* infection. In the presence of zinc deficiency, a reduced cellular immune system occurs due to the low Th1 lymphocyte immune response.[11] In addition, during *M. leprosy* infection, macrophages are activated to eradicate bacteria by releasing ROS, NO, and RNS, which eradicate intracellular microbes. These free radicals have oxidant activity that damages the tissues. An antioxidant diet can balance the effects of this oxidative stress, and using antioxidants can help improve the disease.

Zinc acts as an antioxidant related to the regulation of metallothionein expression. Zinc is also a structural and catalytic component of superoxide dismutase (SOD), which reduces the oxidative effects of ROS, converting superoxide (O2-, +O2-, +2H+) to hydrogen peroxide (H2O2+O) to minimize chain reactions during cell damage.[8,11,18]

The correlation analysis of this study found a statistically significant relationship between serum IgM anti PGL-1 and zinc levels in multibacillary leprosy household contact (p=0.033), and high serum zinc levels were a protective factor for high levels of IgM anti PGL-1 in household contacts (PR: 0.47; 95% CI: 0.20-1.09). The high zinc levels have a protective factor of 53% against increased levels of IgM anti PGL-1 in a household contact with leprosy. Nutrient deficiency not only causes a decrease in the immune system, but several micronutrients are also important in maintaining the integrity of cellular defenses. A study involving 58 leprosy patients showed different nutritional deficiencies, including decreased serum levels of vitamin A, vitamin E, vitamin C, vitamin D, zinc, selenium, and magnesium.[7,9,11] Zinc is said to help prevent *M. leprosy* infection. In the presence of deficiency, a reduced cellular immune system may occur due to the decreased production of IFN-γ, IL2 and TNF- cytokines that help control intracellular pathogens.[5,8,10,11] We could not evaluate the correlation between zinc levels between before and after contact with leprosy patients. Also, zinc supplementation between household contact and non-contact need to be evaluated. Thus, further investigation regarding this issue is needed.

CONCLUSION

The serum zinc level in multibacillary leprosy household contact was lower than the non-contact group. The IgM anti PGL-1 level is negatively correlated with zinc level. The zinc level is a protective factor against IgM anti PGL-1 level.

ETHICS IN PUBLICATION

Medical Faculty of Universitas Udayana/ Sanglah General Hospital Research Ethic Commission approved this research with the ethical clearance number: 2056/ UN14.2.2.VII.14/LT/2020.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding this publication.

AUTHORS CONTRIBUTIONS

Author AS, as the principal investigator, construct the research method and manuscript construction. Author LMMS and KKW supervised the research and theoretical perspective. Author LMMS also contributed in manuscript submission.

FUNDING

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