Correlation of bacterial index to zinc serum level in multibacillary type leprosy patient

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ABSTRACT

Background: Leprosy Multibacillary type is the leading source of infection because total of bacteria is high. Zinc is an essential trace element of viability of host or pathogen. This research aimed to determine the relationship between bacterial index with zinc serum level in patients with leprosy multibacillary type.

Methods: It was cross-sectional observational analytic research in 31 subjects selected using consecutive sampling and they met inclusion and exclusion criteria conducted in Outpatient Installation at RSUD Dr. Saiful Anwar Malang and RS Kusta Kediri in February to March 2019. Examination of acid-resistant bacteria used Ziehl Neelsen's staining method and measurement based on Ridley and Joplin. Test/examination of zinc serum level was conducted in Prodia Laboratory using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). data were analyzed statistically using program of Statistical Package for Social Sciences (SPSS) version 24.

Results: From 31 research subjects, it was obtained that the lowest bacteria index of 1+ and the highest bacteria index of 6+ with median value of 3.5±2. the average of zinc serum level was 61.81±13.28 μg/dL with the lowest level of 37.00 μg/dL and the highest of 94.00 μg/dL. Based on Spearman correlation test, it was obtained correlation value of - 0.557 (55.7%) and value p = 0.001.

Conclusion: There is a significant correlation between bacteria index and zinc serum level with strong closeness value. Correlation is negative, the higher bacteria index, the lower zinc serum level.

Keywords: bacteria index, leprosy, zinc serum
Cite this Article: Widasmara, D., Wilanti, N.W., Tantari, S.W.H. 2021. Correlation of bacterial index to zinc serum level in multibacillary type leprosy patient. *IJBS* 15(1): 33-38. DOI: 10.15562/ijbs.v15i1.287

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INTRODUCTION

Leprosy, a chronic bacterial infection caused by Mycobacterium leprae mainly attacks skin and peripheral nerves with diverse clinical manifestations. Leprosy has been still health problem in some countries because they drop out value from treatment has been still high in society and complication of physical disability.[1] According to WHO, the highest total patient with leprosy, were in India, followed by Brazil and Indonesia. In 2017, in Indonesia, a new case of leprosy of 15.920 cases with Eat Java Province including endemic region was reported.[2] Total cases of leprosy at RSUD dr Saiful Anwar Malang in 2017 were 277 with percentage of 95% Multibacillary type (MB) and 5% Paucibacillary type (PB).

Leprosy MB type is a vital infection source because many bacteria reach 7000 million bacteria per gram tissue. Assessment of bacterial counts can be carried out by measuring the bacterial index (IB), a semiquantitative measure of acid-resistant bacterial density in slit skin smear (SSS).[3,4] Host immunity factors affect the pathogenesis of leprosy. The high cellular immune system produces a polar leprosy tuberculoid spectrum, characterized by a low Bacterial Index (IB). In contrast, the low cellular immunity system has an opposite lepromatous leprosy spectrum, represented by a high IB.[4,5]

Zinc is a trace element which becomes structural component of main catalytic from hundred enzymes for survival of organisms, both in hosts and pathogens. Our body can't produce zinc. Thus, the source is obtained from the outside, so there is a competition mechanism between bacteria and hosts to control its availability.[6,7] Research by Jain et al. showed that the leprosy type MB had lower serum zinc levels due to the mechanism of redistribution of zinc from blood to various tissues; conducted by the release of endogenous mediator leukocyte/ leucocyte (LEM/IL-1) mediators with continuous phagocytosis *M. leprae* by tissue macrophages.[8,9] Research conducted by Mathur et al. showed that a progressive decrease in zinc serum levels of leprosy type tuberculoid to lepromatous type is likely due to a relationship between serum zinc levels and the number of bacteria associated with zinc consumption hosted by *M.leprae* for survival. As this is the case, research done by Dhana et al. showed

that zinc serum levels of leprosy MB type patients were lower than leprosy PB.[9,10] The purpose of this study was to find out the relationship between bacterial index and zinc serum in multibacillary leprosy patients.

METHODS

The research design used in this research was cross-sectional observation analytic. It aimed to determine the relationship between bacteria index with zinc serum level of patients with leprosy MB type. The research sample was leprosy type MB patients treated at dermatology outpatient clinics in Dr. Saiful Anwar Hospital Malang and Kediri Leprosy Hospital in the period February to March 2019. They were selected by consecutive sampling and fulfilled the inclusion and exclusion criteria.

Inclusion criteria were as follow: all outpatients of leprosy MB type are 15-65 years old with positive bacteria index, either new patients, patients in therapy, release from treatment (RFT), relapse, and willing to be the subject of research and signed informed consent. Exclusion criteria were as follow: Patients with reactions in severe medical conditions such as sepsis, pneumonia, HIV, malignancy, tuberculosis, diabetes mellitus, post-surgery, burns and fractures, Patients with a history of atopic dermatitis, psoriasis vulgaris, severe acne vulgaris, lichen planus, iktiosis, acrodermatitis enteropathies, Patients with liver, kidney and gastrointestinal dysfunction, female patients who were pregnant, lactating or using hormonal contraception. Patients were with alcohol consumption of more than 10 grams/day and smoking more than 10 cigarettes/day. In the last 2 weeks, patients had taken drugs such as zinc supplements, systemic steroids, penicillamine, ACE inhibitors, quinolone/ tetracycline, and diuretics.

Patients who met criteria signed approval to be a research sample and completed leprosy patient status and data collection sheet was made. After the history and physical examination, a division according to the WHO classification, documentation, and calculation of the bacterial index was

conducted. Investigation of acid-resistant bacterial indicated by Ziehl Neelsen's staining method and measurements based on Ridley and Joplin. Examination of Zinc Serum Levels was conducted at Prodia Laboratory using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) equipment. The data obtained were included in the data collection sheet, analyzed using Statistical Package for Social Sciences (SPSS) version 24. Correlation tests were carried out, namely the Spearman correlation test. If the p-value of the correlation analysis was less than 0.05 ($p < 0.05$), then the relationship between the bacterial index and serum zinc level was significant.

RESULTS

Characteristics of study participant

In this study, 31 research subjects were diagnosed with leprosy type MB. Based on the age range of the research subjects, the majority of patients in the 15-25 years-old age range were 29.03% (9 people). Based on sex, 67.74% (21 people) were male, and female was 32.26% (10 people). Based on the regional origin, 90.3% of the subjects (28 people) lived in Java and the rest were outside Java. Based on the ethnicity, the Javanese were 54.84% (17 people), the Madurese was 29.03% (9 people). Based on the education level, the most-high education level was Senior High School, which was 38.71% (12 people). While based on the occupation, mostly was entrepreneurs, which was 35.48% (11 people).

Based on the medical history, the majority of on-treatment subjects were 58.06% (18 people). Based on the type of leprosy treatment, most of the patients taking multi-drug treatment (MDT) MB were 80.65% (25 people). Based on the morphological index (IM), most 0% IM subjects were 80.64% (25 people). Based on the Body Mass Index (BMI), most normal weight range subjects were 74.20% (23 people). Based on the Hemoglobin levels (Hb), the majority of the research subjects had normal Hb levels (≥12 g/ dL) of 54.84% (17 people). Based on the albumin levels, the majority of subjects who had albumin levels of ≥53.5 g/dL were 96.77% (30 people).

Characteristics of subjects and levels of zink serum

The mean of zinc serum levels in each group of age, sex, regional origin, ethnicity, status of education, occupation, medical history, type of treatment, IM, BMI, and albumin was not significantly different ($p > 0.05$), while there is a significant difference in zinc serum levels in Hb (p=0.002). Albumin levels cannot be compared because there is only 1 patient who has an albumin level <3.5 g/dL (Table 1).

Bacterial index dan zinc serum level

A total of 31 study participant, 9 person have index bacterial 1+ (29.03%), 2+ (25.81%; 8 person), 3+ (16.13%; 5 person), 4+ (19.35%; 6 person), 5+ (6.45%; 2 people), and 6+ (3.23%; 1 person). The bacterial index median was 3.5±2 with the highest bacterial index was 6+ and the lowest was 1+. The mean of the zinc serum level was 61.81±13.28 µg/dL with the lowest zinc serum level of 37.00 µg/dL and the highest of 94.00 µg/dL.

The mean of the zinc serum level in the bacterial index 1+ was 72.56±10.14 µg/dL, 2+ was 58.88± 6.55 µg/dL, 3+ was 63.00±8.46 µg/dL, 4+ was 52.83±8.57 µg/dL, 5+ was 50.50±4.50 µg/dL and 6+ was 59.00 µg/dL. The difference in zinc serum levels in each bacterial index was concluded based on the Kruskal Wallis test analysis results. If the value of $p<0.05$, there is a significant difference, then a further test is carried out. The Kruskal Wallis test results were presented in Table 2. The Kruskal Wallis analysis results showed the value of p=0.037 ($p<0.05$), so it can be concluded that there are differences in zinc serum levels between the treatments so that Mann Whitney further testing was performed. The Mann Whitney test results showed that the lowest zinc levels in the bacterial index 5+ differed significantly from zinc serum levels in the bacterial index 1+, but did not differ significantly from zinc serum levels in the bacterial index 2+, 3+, 4+ and 6+ (Table 2).

The Correlation of Bacterial Index with Zink Serum Levels

In this study, the correlation test used was the Spearman correlation test because one of the data was categorical scale. The correlation of bacterial index and zinc

Table 1.   Mean of Zink Serum Level in the Characteristics of Subjects

Category	n = 31	%	Zink Serum Level (µg/dL)	
			Mean	P
Age (Year Old)				
• 15-25	9	29.03	65.56±13.06	
• 26-35	7	22.58	62.43±18.31	
• 36-45	6	19.35	68.50±8.6	0.104
• 46-55	6	19.35	49.50±6.16	
• 56-65	3	9.68	60.33±4.73	
Sex				
• Male	21	67.74	61.9±15.2	0.971
• Female	10	32.26	61.70±8.65	
Regional Origin				
• Java	28	90.3	68.0±27.1	0.706
• Outside Java	3	9.68	61.1±11.7	
Ethnicity				
• Javanese	17	54.84	58.35±10.65	
• Madurese	9	23.03	66.11±12.08	0.289
• Non-Javanese	5	16.13	65.80±21.59	
Education				
• Elementary School	6	19.35	57.14±9.56	
• Junior High School	7	22.58	55.63±6.67	0.053
• Senior High School	12	38.71	64.00±14.79	
• Bachelor	6	19.35	75.75±15.65	
Occupation				
• Entrepreneurs	11	35.48	64.09 ±16.24	
• Farmers	6	19.35	62.28±12.62	0.683
• Students/College Students	7	22.58	55.83±8.61	
• Unemployed	7	22.58	62.85±13.22	
Medical History				
• *On-treatment*	18	58.06	59.00±14.50	
• RFT ≤ 1 year	7	22.58	70.14±6.67	0.235
• RFT > 1 year	3	9.68	56.00±10.15	
• Relapse	3	9.68	65.00±15.62	
LEPROSY Treatment				
• MDT MB	25	80.65	64.04±11.64	
• MDT MB without dapsone	5	16.13	49.00±16.08	0.051
• Rifampicin-ofloxacin	1	3.23	70.00	
Morphological Index (IM)				
• 0%	25	80.65	62.32±13.42	
• 2%	2	6.45	48.50±12.02	
• 10%	1	3.23	83.00	0.332
• 30%	2	6.45	59.500±0.707	
• 60%	1	3.23	59.00	
Body Mass Index (BMI)				
• Underweight (<18.5 kg/m2)	2	6.45	48.5±16.3	
• Normal weight (18.5-24.5 kg/m2)	23	74.20	62.39±11.30	0.342
• Overweight (25-29,9 kg/m2)	2	6.45	64.00±19.12	
• Obesity (>30 kg/m2)	4	12.90	61.81±13.28	
Hemoglobin (Hb)				
• <12 g/dL	14	45.16	54.1±10.7	0.002**
• ≥12 g/dL	7	54.84	68.2±11.9	

*p<0.05: there are significant differences based on One Way Anova

**p<0.05: there are substantial differences based on the Independent t-test

serum level has a correlation coefficient of -0.557 (r = -0.557) with a significance value of 0.001 (p<0.05), so it can be concluded that there was a significant negative correlation. In other words, the higher the Bacterial Index, the lower the zinc serum levels (Figure 1). The coefficient value of 0.41 to 0.7 was included as having a strong correlation based on the correlation's strength.

DISCUSSION

Leprosy occurs mostly in the adult age group, the age range of 10-29 years old, related to the level of association or higher activity, and can be younger in endemic areas.[11] Zinc serum levels decreased with age, possibly related to an increased risk of age-related diseases. In this study, there was no significant difference in zinc serum levels with age.[12,13] Based on sex, the majority of leprosy patients were male, this could be due to occupational factors related to contact transmission, cultural factors that made the detection factor in women not optimal. There was a stronger opinion of the immunological response in women due to an increase in estrogen.[11,14] Research by Nepal et al. in Eastern Nepal found the prevalence of zinc serum levels was low in male subjects than female subjects, which may be due to higher body mass in males than in women.[15] In this study, there was no significant difference in zinc serum levels based on sex (p>0.05).

According to Kosasih et al. ethnicity that suffers more leprosy in Indonesia were in Madura. Several theories stated that the spread of leprosy disease seemed to be caused by the displacement of infected populations.[16,17] In this study, there was no significant difference in zinc serum levels based on regional origin and ethnicity (p>0.05). Research by Romero et al. in Spain showed differences in zinc serum levels in agricultural land in each region and/or differences in habitual diet in each place. It was different from the study by Martos et al. which showed that regional origin does not show differences in zinc serum levels.[13] Zinc deficiency can arise from several causes including certain genetic backgrounds.[17]

In this study, the majority of the subjects' education was senior high school. Based on the occupation, the majority of

Table 2. Zinc Serum Levels in the Bacterial Index

Bacterial Index	Median (µg/dL)	p-value
1+	71±10	
2+	57.5±21	
3+	60±5	0.037
4+	55.5±11.25	
5+	50.50±4.50	
6+	59.00	

*p<0.05: there are significant differences based on the Kruskal Wallis test
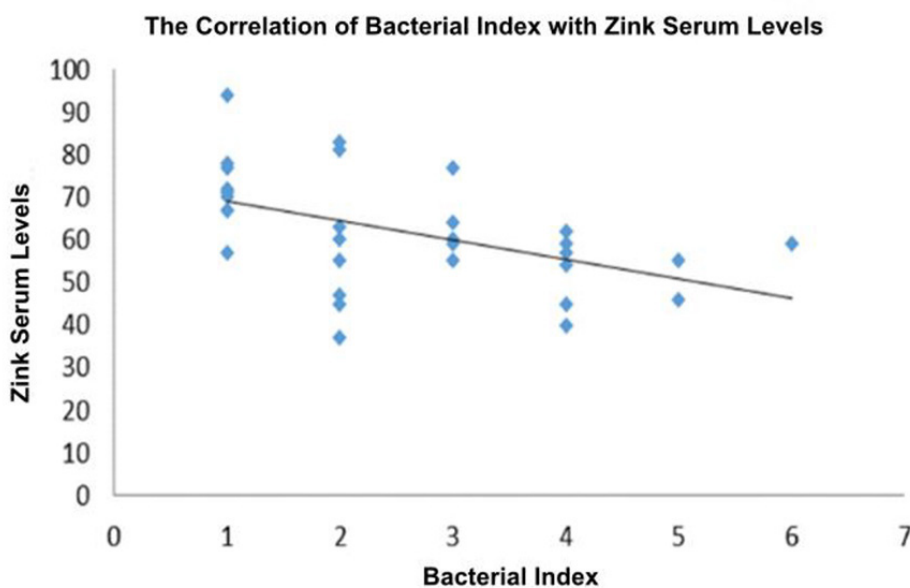
Figure 1. Correlation of bacterial index and zinc serum level

the subjects' occupation was found to be an entrepreneur, and among the subjects there were also groups that unemployed. Epidemiological studies conducted in Brazil showed that low education levels, family economic status, food deficiency conditions, and nutritional status correlate with leprosy incidence.[11,18] In this study, there were no significant differences in zinc serum levels based on education and occupation (p>0.05).

The majority of the research subjects with on-treatment medical history were treated with MDT MB and the Morphological Index was 0%. Research by Zenha et al. showed that the morphological index would drop rapidly until it reached 0 after MDT was given, this was due to the bactericidal effect of MDT.[19] In this study, there was no significant difference in zinc serum levels based on the medical history, therapy, and morphological index (p>0.05).

The majority of the research subjects' BMI was the normal weight range (18.5–24.9 kg/m²). The statistical tests in Lamongan, East Java in 2017 showed the influence of nutritional status on the increase in leprosy-specific antibody titers in some patients having a BMI <18.5 kg/m². Research by Tamura et al. found a significant correlation between zinc and BMI serum levels, while study by Gjorup et al. and Song et al. reported that there were no significant differences in zinc serum levels between obese and non-obese subjects with zinc levels below the normal range.[19,20]

According to Thamaria et al., the anthropometer's status is less able to describe micronutrient deficiency, including zinc. It needs a combination of laboratory, physical and calculation of nutrient intake.[21] The method for measuring individual-level food intake is quite difficult and requires particular expertise, and become a consideration concerning the differences in the variation of ingredients and nutrients in food, and memory in food recall.[22]

There were significant differences in zinc serum levels in Hb levels (p> 0.05). Anemia is a deficiency of micronutrients namely zinc, because of the effect of zinc on iron metabolism in heme formation, both directly, namely through the role of zinc in protein synthesis including iron-transport proteins namely transferrin, or indirectly where zinc helps carbonic anhydrase stimulate the production of gastric acid which can change the ferric ions into ferrous ions that are easily absorbed by the intestinal mucosa so they can increase the Hb levels.[23,24] Albumin is considered the primary zinc transporter in serum and usually binds about 80% of all zinc serum. A number of studies also show that albumin modulates zinc absorption into cells. Because zinc is bound to albumin, the condition of hypoalbuminemia results in a lower zinc serum concentration.[25,26] In this study, statistically, there was a significant difference between the bacterial index and zinc serum level. MB type leprosy MDT, as is often found in Indonesia, is a more critical infection source than PB the number of bacteria reaches 7000 million bacteria per gram of tissue.[3] The pathogenesis of leprosy is not solely affected by the exposure of *M.leprae* but also affected by host immunity factor because the low cellular immunity system will produce a spectrum of leprosy lepromatous polar disease which is characterized by high IB. Cellular immunity, which functions to increase the microbicidal action of phagocytes to eliminate microbes, decreases leprosy and affects bacterial clearance and microbicidal activity.

Multidrug therapy does not seem to accelerate bacterial clearance. A decrease in IB often appears after 12 months of therapy and will decrease though we stop the therapy. A study conducted by Zenha et al. showed a reduction in IB after MDT

administration for 12 and 24 months in leprosy patients with MB type.[19] The morphology index will drop rapidly until it reaches 0 after MDT is given, this is caused by the bactericidal effect of MDT, whereas IB takes years to reach 0.[7,28] A slight decrease in IB, it appears that IB cannot be said to be a good parameter to assess the effectiveness of the therapy.[19,27]

Zinc homeostasis in cells, including the immune system, is maintained through strict influx regulation, efflux, and distributions to intracellular organelles. The protein transporter, namely ZIP, which functions in zinc influx into the cytosol, and ZnT which functions in zinc efflux from the cytosol is crucial for this metabolic and functional adjustment.[28,29] Hosts try to limit the availability of zinc, this mechanism is referred to as "nutritional immunity," which describes the competition between bacteria and hosts for this trace element, where pathogens and hosts make efficient strategies to control the zinc availability. Hosts use Natural resistance-associated macrophage protein 1 (NRAMP1) to hold or restrict zinc uptake by pathogens, limiting zinc availability to intracellular pathogens. For *M. leprae*, zinc is essential in the formation of Superoxide dismutase (SOD) and catalase, which provide defense against reactive oxygen species in macrophages. P-type ATPases represent Mycobacterium strategies for intake and to neutralize the effects of toxins from zinc in macrophages.[6,30]

Redistribution of zinc to hosts and zinc utilization by billions of *M. leprae* bacteria for their own metabolism and survival, particularly the leprosy with MB type play an important role. Many enzymes in the metabolic pathway of *M. leprae* are metalloenzymes that are dependent and need zinc, it plays a significant role in decreasing zinc serum levels.[31,32] In leprosy with MB type, there is constant bacillus phagocytosis by macrophages, so this process leads to the production of endogenous leucocyte mediator (LEM)/ IL-1which released from macrophages during constant M. leprosy phagocytosis, causing zinc to be concentrated in serum into hepatocytes, and thus reducing zinc serum levels continuously.[8,9]

In a study carried out by Jain et al. obtained a lower zinc serum levels in leprosy with lepromatous types than the tuberculoid types. A study conducted by Dhana, et al. obtained the average zinc serum levels in leprosy patients with multibacillary, lower than leprosy patients with paucibacillary, where this difference was statistically significant (p<0.05).[8,9] A research by Mathur et al. found a gradual decrease in zinc serum levels from TT type to LL, but the difference was not significant (p>0.05).[9,10,15]

The immune system requires essential micronutrients and trace elements, one of which is zinc for optimal function. Zinc is also an integral part of the thymus hormone molecule, which is thymulin, required for T-cell maturation, so zinc deficiency can disrupt cellular immunity, which can cause increase in morbidity and mortality against viral, microbial and parasitic infections.[31-34] In this study, some weaknesses can cause bias, among others, because in some research references it is found that there is a correlation between food intake and zinc serum levels, while in this study no measurement of intake and variation in food consumption data was collected on research subjects or in collaboration with nutrition department.

CONCLUSION

There is a significantly strong correlation between bacteria index and zinc serum level. Correlation is negative, the higher bacteria index, the lower zinc serum level.

CONFLICTS OF INTEREST

There aren't conflicts of interest

FUNDING

AUTHOR CONTRIBUTION

Dhelya Widasmara responsible for project administration, analysis, conceptualization, and writing the original draft. Nesa Wike Wilanti and Tantri SHW responsible for supporting the project administration, analysis, and writing the original draft. All author had reviewed and agreed for the final draft of the manuscript

Ethical consideration

The research ethics committee has approved the Dr. Saiful Anwar Hospital's study protocol, Malang with ethical clearance references number: 400/018/K.3/302/2019 and Kediri Leprosy Hospital with Number: 002/EC/KEPK RSDH/02/2019. All study procedures in accordance with the Helsinki Declaration of human rights.

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