Comparison of Total Antioxidant Capacity (TAC) in the Multibacillary (MB) type of leprosy patients before and after 3 months of MDT-MB WHO therapy

Dinie Ramdhani Kusuma[*,1], **Safruddin Amin**[*], **Khairuddin Djawad**[*], **Srivitayani Achmad**[*], **Sitti Rahmah**[*], **Idham Jaya Ganda**[**] **and Agussalim Bukhari**[**]

[*]Department of Dermatology and Venereology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia., [**]Department of Biostatics and Epidemiology, Faculty of Public Health, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

ABSTRACT Aims This research aimed to (1) measure the level of total antioxidant capacity in multibacillary type of leprosy patients before MDT-MB WHO therapy; (2) measure the level of total antioxidant capacity in multibacillary type of leprosy patients after 3 months of MDT-MB WHO therapy; (3) compared the level of total antioxidant capacity in multibacillary type of leprosy patients before and after 3 months of MDT-MB WHO therapy. **Methods** This research was carried out in the subdivision leprosy of dermatology and venerology clinics in Wahidin Sudirohusodo Hospital Education Centre and his network. The sample was collected from blood serum in patients with inclusion criteria during the study period until the sample is sufficient. The method used was prospective cohort with an observational approach, we take the blood serum twice, before and three months after of MDT-MB WHO therapy. The data were analysed using statistical analysis through a normality test of data with Shapiro wilk then continued to see the difference between the two groups with independent t-test and paired sample t-test. **Results:** The results shows that increase levels of total antioxidant capacity not only happened in multibacillary type of leprosy patients before and after 3 months of MDT-MB WHO therapy but also occurred in multibacillary type of leprosy patients with a lower bacterial index after 3 months of MDT-MB WHO therapy, the increase was statistically significant ($p<0.05$). **Conclusions:**The conclusion is there are increased levels of total antioxidant capacity in the multibacillary type of leprosy patients before and after three months of MDT-MB therapy.

KEYWORDS Total antioxidant capacity (TAC), MDT-MB WHO, multibacillary type of leprosy.

INTRODUCTION

Antioxidants can be synthesised naturally, or via a biomolecular process in which can prevent disruption caused by free radicals through inhibition of formation, eradication, and stimulation of their break down. An enzymatic antioxidant is an endogenous antioxidant such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-PX), and glutathione reductase (GSHR). When the defence of antioxidants weakens, the body cells and tissues will be prone to experience disruptions and diseases. Therefore, maintaining antioxidants within normal limit is essential in preventing and managing several diseases.[1-4]

Several studies have assessed antioxidant levels in patients with Morbus Hansen (MH) by measuring their SOD. It was suggested that compared to normal patients, those with MH tended to have significantly decreased level of SOD, catalase, and glutathione. However, after being administered with MDT, the level of SOD, catalase, and glutathione increased in linear with the

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[1]Dinie Ramdhani Kusuma, Jln Panjitilar Negara No. 118, Mataram-Lombok, Nusa Tenggara Barat, Indonesia, 83115 Phone: 62 81808885366 Email: diniekusuma@gmail.com

improvement of a clinical condition in patients with multibacillary leprosy. For those who have not received medications, their SOD serums were decreased.[5,6]

The total antioxidant capacity (TAC) is a capacity of all antioxidants in the biological sample which consists of several components. It is also used as an indicator in the body in the healthy body since its function is to weaken the assault of free radicals. Also, it also shows the information of antioxidant status or any deficiency of antioxidant level.[2,7]

The lack of any antioxidant component will decrease overall antioxidant status thus results in weakened defence mechanism against free radicals and eventually leads to several diseases. The exact mechanism of the reduced amount of these antioxidants in MH patients has not been found. It is suggested due to the inhibition enzyme or the low concentration of protein enzyme caused by SOD gene regression. Some studies have also shown that bacterial liposaccharides could affect SOD gene. PGL-1 which can be found in Mycobacterium leprae can bind to SOD enzyme thus inhibiting SOD activity and causing down-regulation expression of SOD gene in red blood cells and macrophages.[8-12]

MH is a chronic skin infection which is caused by Mycobacterium leprae. The clinical manifestation of this disease is also affected by the inflammation of tissues in which is mediated by immune responses. Products such as tumour necrosis factor-(TNF-), nitric oxide (NO), and reactive oxygen species (ROS) not only have important roles in immune responses against the infection but also can disrupt tissues. The different clinical manifestations in each are associated with the variant of cellular immune responses or cell-mediated immunity (CMI). Other studies also show that oxidative stress in MH is strongly correlated with three things, such as the duration of the disease, the amount of bacterial index, and multibacillary leprosy.[10,13-16]

The treatment of multidrug therapy (MDT)-MB according to WHO consists of dapsone, rifampicin, and clofazimine. Rifampicin is the most effective drug which can decrease the morphological index to zero within approximately five weeks. The use of 600 mg of rifampicin can kill and reduce the amount of living Mycobacterium leprae in multibacillary leprosy, and also can eliminate almost all bacteria.[17]

This study is critical since there has not been any related study which compares the TAS in multibacillary leprosy patients before and three months after being administered with WHO MDT-MB therapy in Makassar, Indonesia.

MATERIALS AND METHODS

The TAC of Sixteen multibacillary leprosy patients which fulfil the inclusion criteria were assessed before and three months after receiving WHO MDT-MB therapy. The numbers were then evaluated.

Measure levels of TAC plasma :

- Sample: Collect blood 5cc from vena cubiti using spoit and add to EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2 - 8°C within 30 minutes of collection. Samples should be aliquoted or must be stored at -80°C for six months use.
- Reagent Provided: Standard Diluent, Streptavidin-HRP, Stop Solution, Substrate Solution A, Substrate Solution B, Wash Buffer Concentrate (30x), Biotin-Conjugate Anti-human T-AOC Antibody.
- Assay Principle: This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). T-AOC is added to the wells pre-coated with the T-AOC monoclonal antibody. After incubation, a biotin-conjugated anti-human T-AOC antibody is added and binds to human T-AOC. After incubation unbound, the biotin-conjugated anti-human T-AOC antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human T-AOC antibody. After incubation unbound, Streptavidin-HRP has washed away during a washing step. The substrate solution is then added, and colour develops in proportion to the amount of human T-AOC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

RESULTS

(Table 1) Shows the distribution of baseline criteria of all samples included in the study. The youngest sample was 12 years old while the oldest was 67 years old, with the mean age of those aged $\leq$ 35 years is 16.75$\pm$3.45 and those aged >35 years is 47.00$\pm$9.01. The number of the sample based on their gender is similar, which is nine patients (50%). Ten (62.5%) subjects were already married, while the remaining six (37.5%) were not. There were four samples (25%) who got a low education, six samples (37.5%) who had middle education, and six samples (37.5%) who received a high education. Out of all subjects, ten (62.5%) patients with multibacillary leprosy showed bacterial index level of +3 and six (37.5%) patients with the same type of disease showed the index number of +4. Half of the patients have normal body mass index (BMI), while a quarter of them was overweight and the remaining 25% were underweight. (Table 2) presents an increased TAC level before MDT-MB WHO therapy although the number was not significant (p=0.131) between a group of patients with a bacterial index of +3 (15.30$\pm$4.32) and a group of patients with a bacterial index of +4 (20.57$\pm$8.52). (Table 3) presents a reduced TAC level after three months of MDT-MB WHO therapy although the number was not significant (p=0.911) between a group of patients with a bacterial index of +3 (17.97$\pm$4.22) and a group of patients with a bacterial index of +4 (17.60$\pm$8.83).

(Table 4) Shows the different mean of TAS level before and after three months of MDT-MB WHO therapy in a group of leprosy patient with a bacterial index of +3. It was found that the number was significantly increased as much as 2.68 U/ml (p<0.001). In a group of those with a bacterial index of +4, the TAS level was significantly decreased as much as 2.97 U/ml (p=0.014) from before to after three months therapy. (Table 5) presented a significant difference of TAC average between a group before receiving therapy (16.28$\pm$5.40) and a group after three-month of therapy (17.90$\pm$4.96) (p=0.036).

(Figure 1) showed the number of the sample which had an increased TAC level from before to after therapy was 12, while samples which had a decreased TAS level were four patients.

DISCUSSION

(Table 1) Shows the distribution of baseline criteria of all samples included in the study. Indonesian Ministry of Health (2007) has stated that this disease can infect all ages and in epidemic cases, the spread of this disease is almost the same at all ages.[18] The most prevalent age group who gets infected is those aged 25-35 years in the adult group, and 10-12 years in children group. [19] A study by Andy M et al. (2014) showed no significant relationship between age and the incidence of leprosy. Moreover, the

Table 1 Distribution of Patients with MB Leprosy

	CHARACTERISTIC	AMOUNT	PERCENTAGE(%)
Age :	**$\leq$ 35 Year**	8	50.00%
		Mean 16.75±3.45 SD	
	>35 Year	8	50.00%
		Mean 47.00 ± 9.01 SD	
Gender :	**Man**	8	50.00%
	Woman	8	50.00%
Marital Status :	**Yes**	10	62.50%
	No	6	37.50%
Level of Education	**Low**	4	25.00%
	Average	6	37.50%
	High	6	37.50%
Bacterial Index	**+3**	10	62.50%
	+4	6	37.50%
Body Mass Index :	**Underweight**	4	25.00%
	Normal weight	8	50.00%
	Overweight	4	25.00%

Table 2 Total Antioxidant Capacity (TAC) of Leprosy Group Based on Bacterial Index Before MDT-MB WHO

BACTERIAL INDEX	n	Mean ± SD	Median (Min – Max)	p
+3	13	15.30 ± 4.32	15.72 (8.20 - 22.00)	0.131*
+4	3	20.57 ± 8.52	22.41 (11.28 – 28.02)	0.131*
*Result of analysis T Independent Test p<0.05				
Abbreviations: n: amount; p: probabilty				

Table 3 Total Antioxidant Capacity (TAC) of Leprosy Group Based on Bacterial Index After 3 Months of MDT-MB WHO

BACTERIAL INDEX	n	Mean ± SD	Median (Min – Max)	p
+3	13	17.97 ± 4.22	16.97 (11.83 - 24.80)	0.911*
+4	3	17.60 ± 8.83	18.87 (8.20 – 25.72)	0.911*
*Result of analysis T Independent Test p<0.05				
Abbreviations: n: amount; p: probabilty				

multivariate analysis suggested that age was not an independent variable related to the incidence of leprosy. [20] Age usually plays an important role in the incidence and transmission of disease. It is because age might affect immune levels. Leprosy is rare to be found in babies. The incidence is increased in linear with age, with its peak level is those who aged 10-20 years, and the number is decreased afterwards. [21,22] The prevalence is also increased in linear with age with its peak range between 30 and 50 years, the number is also decreased afterwards. [23] These findings fit the theory that has been mentioned previously, in which leprosy infects all group of age.

The number of a subject who was female and male is similar, which is eight patients of each. The study conducted by Malik Yunus et al. (2015) regarding the association between knowledge, gender, dense population, family history, and personal hygiene with the incidence of leprosy showed non-significant result between gender and the incidence of leprosy (p=0.206). [24] Bivariate analysis using chi-square found a p-value of 0.705 between these two variables (OR=0.806 with 95%CI=0.384-1.695). Moreover, multivariate analysis was performed and showed that gender was not an independent variable about the incidence of leprosy. [20] However, the different result showed in The Epidemiology of Leprosy, a chapter in Leprosy, 2nd edition (1994) book by Noorden SK. He suggested that leprosy infects males more than females (3:1). [25] Another reference shows that leprosy in Asia tends to affect males compared to females (1.5:1). Though the disease infects all groups of age, the majority of cases appears in patients aged before 35 years in the endemic area. [26] These findings contradict with what this study found in the subjects.

Half of the subjects had normal BMI, a quarter was underweight, another quarter of which was overweight, while none was obese. Obesity is one of the metabolic syndromes in addition to hyperglycemia, hypertriglyceridemia, and low level of high-density lipoprotein. [43] In metabolic syndrome, there is an increase in glucose transport to adipose tissues. The endothelium cells in adipose tissues stimulate the increase of glucose uptake through glucose transporter. Thus hyperglycemia condi-

Table 4 Total Antioxidant Capacity (TAC) of Leprosy Group Based on Bacterial Index Before and After 3 Months of MDT-MB WHO

BACTERIAL INDEX	n	TAC Level (U/mL) (MDT-MB WHO THERAPY)		Specifity Sig. (2-tailed)
		BEFORE	AFTER	
+3	13	15.29	17.97	p = 0.000*
+4	3	20.57	17.60	p = 0.014*
*Result of Analysis Paired Sample T-Test p<0.05				
Abbreviations: n: amount; p: probabilty				

Table 5 Total Antioxidant Capacity (TAC) in Multibacillary Type of Leprosy Patients Before and After 3 Months of MDT-MB WHO Therapy

MDT-MB WHO THERAPY	n	Mean ± SD	Median (Min – Max)	p
SEBELUM	16	16.28 ± 5.40	15.75 (8.20 – 28.02)	0.036*
SETELAH (3 Bulan Terapi)	16	17.90 ± 4.96	17.53 (8.20 – 25.72)	0.036*
***Result of Analysis Paired Sample T-Test p<0.05**				
Abbreviations: n: amount; p: probabilty				

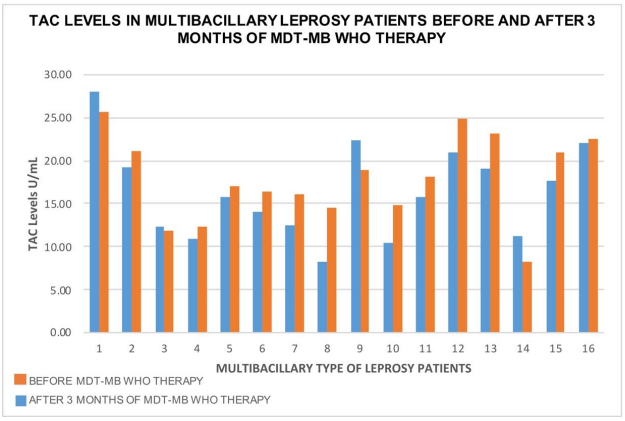
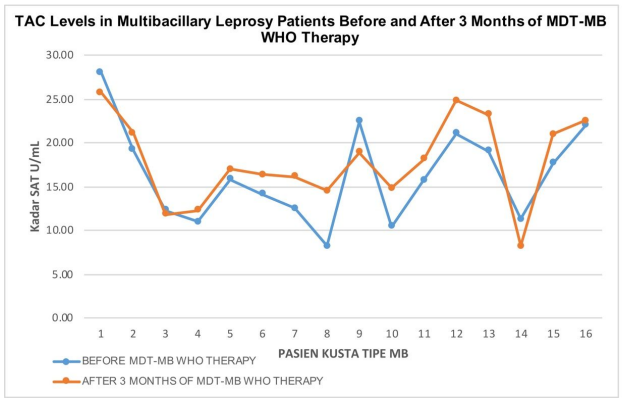


Figure 1: Differences TAC levels in multibacillary leprosy patients before and after 3 months of MDT-MB WHO therapy.



tion increases the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the production of reactive oxygen compound in mitochondria. The increase of ROS produces oxidative stress and activates inflammation signal, hence attracting more macrophage pro-inflammatory cells. [27] The oxidative stress is defined as an imbalance condition between ROS production and the endogenous antioxidant status. [28] The high level of oxidative stress is shown by the low cellular antioxidant status and the high product of lipid peroxidase. In reality, the decrease activities of SOD, catalase, and glutathione peroxidase enzyme are supported by the high level of malondialdehyde (MDA) in plasma. [29] In this study, the lowest BMI was 17.12 kg/m2, and the highest number was 27.99 kg/m^2.

(Table 2) Presents an increased TAC level before MDT-MB WHO therapy between a group of patients with bacterial index. A study conducted by Raka I et al. (2018) in 30 leprosy patients before receiving treatment showed the increase TAS level in leprosy patients with higher bacterial index and decreased SOD level. That particular study suggested that there was a significant increase of oxidative stress in leprosy patient. The decreased SOD level in leprosy patient showed enzymatic stress. [30] Another study by Vijayaraghavan R et al., (2011) in India found a significant decrease in enzymatic antioxidant levels such as SOD, catalase, glutathione peroxidase, glutathione reductase, and glutathione-s-transferase in leprosy patients compared to normal individuals. [31] Also, a study by Prasad et al., (2007) in 100 newly diagnosed leprosy patients found a decreased level of SOD and catalase. The increased MDA level was also presented in leprosy patient with a higher number of the bacterial index. [32] Garad et al., (2014) conducted a study regarding lipid peroxidase and found a decrease in thiol antioxidant level in multibacillary leprosy patients compared to normal people. [33]

Those findings showed similar results with this study regarding the increase in TAS level before therapy in patients with higher bacterial index compared to those with a lower number.

(Table 3) Presents a reduced TAC level after three months of MDT-MB WHO between a group of patients with bacterial index. A study by Viajayaraghavan R et al., (2009) suggested that multibacillary leprosy patients who have not received MDT therapy tended to have higher bacterial index compared to those who received ones. Besides, a trend of progressive decrease in the bacterial index was seen in patients who received the treatment, from the bacterial index of +4 to +2.[34] The mechanism of decreasing antioxidant system in leprosy patients has not been confirmed. However, it might be due to the enzyme inhibition or low level of enzyme protein concentration due to SOD gene regression. The fact that bacterial lipopolysaccharides might affect the SOD genes also plays an important role. The bacteria itself has PGL-1 which can bind with SOD enzyme thus inhibiting the activity of SOD and resulting in down-regulation of SOD gene expression in red blood cells and macrophages. [10-12] The current studies also tried to find the connection between oxidative stress in leprosy patients with higher bacterial index. This was suggested due to the utilisation of biometal such as zinc, iron, and calcium from the host for bacteria to be survived, hence affecting metalloenzyme like SOD.[10,11] Jyothi et al., in 2007 found similar results where the reduced SOD levels and increased MDA levels happened in leprosy patients, especially significant in multibacillary type patients. These numbers were compared and showed a significant MDA level in multibacillary leprosy patients.[35] In conclusion, the decreased TAC level in multibacillary leprosy patients with higher bacterial index caused by the higher bacterial amount and more variant microorganisms, result in higher oxidative stress and lower endogenous antioxidant production.

(Table 4) Shows the different mean of TAS level before and after three months of MDT-MB WHO therapy in a group of leprosy patient with bacterial index. A study by Ozan et al., (2010) in leprosy patients who have finished MH therapy showed a significant increase in MDA and also GSH and catalase activity compared to control group. This study indicated that oxidative stress not only happened in newly diagnosed cases but also in patients who have finished the therapy.[36] The reduce TAC level in a group with a bacterial index of +4 supports the theory regarding PGL-1 of this bacteria that can affect SOD gene and that this lipopolysaccharide can bind with SOD enzyme thus inhibiting SOD activity. Such study also assessed the progressive decrease of SOD within the leprosy spectrum. [11] Also, it also confirmed other studies which suggest that oxidative stress in leprosy associated with the duration of disease, bacterial index, and multibacillary type of leprosy. [10]

(Table 5) Presented a significant difference of TAC average between a group before and after three months receiving therapy. A study performed by Schalcher et al., (2014) which aimed to measure oxidative stress in a patient during MDT therapy, found an increase in SOD and GSH levels in leprosy patient from before to after three months therapy.42 This result was similar to what happened in this current study.

(Figure 1) Showed the number of the sample which had an increased TAC level from before to after therapy was 12, while samples which had a decreased TAS level were four patients. Out of sixteen patients, four subjects aged between 42 to 52 years experienced a decrease TAS level from before to after three months therapy. The ageing process can explain the production of free radicals follows this. Furthermore, our cells will deteriorate and functionless optimal, these result in the low level of antioxidant in the body. Such findings support the hypothesis suggested by Wellman et al., (2009) regarding different age shows increased oxidative stress levels as well as decreased endogenous antioxidant capacity after the age of 40.[37] Free radicals have contributed to ageing process from the beginning of life. The infamous ageing hypothesis, namely oxidative stress theory was firstly defined by Denham Harman as a free radical ageing theory. It is suggested that this free radical oxygen was formed endogenously as a side product of oxygen metabolism. This theory was then modified with the role of mitochondria in ageing process since it is the primary source of ROS formation.[38-39]

In this study, there was one subject aged 67 years with increased SAT level which was evaluated after three months therapy. Free radicals were responsible for disruption in cellular and tissue level about the aging process. In normal condition, there is an equilibrium between oxidants, antioxidants, and biomolecular. An excessive number of free radicals causes decreased cellular antioxidants that stimulate oxidation hence contributing to cellular dysfunction. Free radicals are the main factors responsible for ageing process and the only factors which can be modified by genetic and environment.[40] Free radicals that originate externally consist of pollutants such as cigarette smoke, vehicle smoke, UV radiation, high cholesterol food, coffee, alcohol, pesticides, etc. Stress or vigorous activities can also trigger the increase of free radicals.[41] This patient had a non-vigorous activity and middle-upper socioeconomic. These two factors might contribute to the increase of TAC level which measured in after three months therapy. Controlling, modifying, and interfering the external free radicals such as having meals three times a day and performing regular activities could not be conducted in this study.

CONCLUSION

In conclusion, the increasing number of TAS in multibacillary leprosy patients from before to after three months therapy with WHO MDT-MB was identified. TAS of subjects with lower bacterial index in such patients was increased after three months receiving WHO MDT-MB therapy. This finding suggests that therapy of WHO MDT-MB that was given early and routine in three months could increase TAS in multibacillary leprosy patients.

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AUTHORS' STATEMENTS

Competing Interests

The authors declare no conflict of interest.

References

1. Kadifkova Panovska TA, Kulevanova S, Stefova M. In vitro antioksidativno djelovanje nekih Teucrium vrsta (Lamiaceae). Acta Pharmaceutica 2005;55(2):207-214.

2. Winarsi H. Antioksidan alami dan radikal bebas. Yogyakarta: Kanisius 2007;11-23.

3. Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, et.al. Glutathione deficiency is associated with impaired survival in HIV disease. Proceedings of the National Academy of Sciences 1997;94(5):1967-1972.

4. Centers for Disease Control and Prevention. Guidelines for laboratory test result reporting of human immunodeficiency virus type 1 ribonucleic acid determination. Recommendations from a CDC working group. Centers for Disease Control. MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports 2001;50(RR-20):1.

5. Prabhakar MC, Santhikrupa D, Manasa N, Rao OU. Status of free radicals and antioxidants in leprosy patients. Indian journal of leprosy 2013;85(1):5-9.

6. Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Oxidative stress and anti-oxidant status in leprosy patients. Indian journal of leprosy 2003;75(4):307-316.

7. Kusano C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. Journal of cell and molecular biology 2008;7(1)1-15.

8. Wijaya A. Oksidasi LDL, atherosklerosis dan antioksidan in forum diagnosticum 1997;1-15.

9. Kuncahyo I, Sunardi. Uji aktivitas antioksidan ekstrak belimbing wuluh (averrhoa bilimbi, l.) Terhadap 1,1-diphenyl-2- picrylhidrazyl (dpph). Seminar nasional teknologi 2007;E1-E9.

10. Swathi M, Tagore R. Study of oxidative stress in different forms of leprosy. Indian journal of dermatology 2015;60(3):321.

11. Bhadwat VR, Borade VB. Increased lipid peroxidation in lepromatous leprosy. Indian Journal of Dermatology, Venereology, and Leprology 2000;66(3):121.

12. Schalcher TR, Vieira JL, Salgado CG, Borges RD, Monteiro MC. Antioxidant factors, nitric oxide levels, and cellular damage in leprosy patients. Revista da Sociedade Brasileira de Medicina Tropical 2013;46(5):645-649.

13. Lee JY, Aziz N, Gupta S, Agarwal A, Baw CK. Role of oxidative stress in polycystic ovary syndrome. Curr womens health 2010;Rev 6:96-107.

14. Polycarpou A, Walker SL, Lockwood DN. New findings in the pathogenesis of leprosy and implications for the management of leprosy. Current opinion in infectious diseases 2013;26(5):413-419.

15. Passos Vazquez CM, Mendes Netto RS, Barra Ferreira Barbosa K, Rodrigues de Moura T, Pacheco de Almeida R, Duthie MS, et.al. Micronutrients influencing the immune response in leprosy. Nutricion hospitalaria 2014;29(1):26-36.

16. Menaldi SLSW, Bramono K, Indriatmi W, editors. Ilmu Penyakit Kulit dan Kelamin (7th ed). Jakarta: Balai penerbit FKUI 2015;7:73-88.

17. Bryceson A, Pfaltzgraff RE. Treatment. In Medicine in the tropics: leprosy. Edinburgh: Churchill Livingstone 1990;3:77-91.

18. Depkes RI Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan. Buku Pedoman Nasional Pemberantasan Penyakit Kusta. Cetakan XVII Jakarta 2007;XVIII

19. Mansjoer A. Kapita selekta kedokteran. Jakarta: Media Aesculapius 2000;86-92.

20. Muharry A. Faktor risiko kejadian kusta. Jurnal Kesehatan Masyarakat. 2014;9(2):174-182.

21. Vinay K, Smita J, Nikhil G, Neeta G. Human immunodeficiency virus and leprosy coinfection in Pune, India. Journal of clinical microbiology 2009;47(9):2998-2999.

22. Johnson CM, Lyle EA, Omueti KO, Stepensky VA, Yegin O, Alpsoy E, et.al. Cutting edge: A common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. The journal of immunology 2007;178(12):7520-4.

23. Depkes RI. Profil Kesehatan Indonesia. Depkes RI Jakarta 2015.

24. Yunus M, Kandou GD, Ratag B. Hubungan antara pengetahuan, jenis kelamin, kepadatan hunian, riwayat keluarga dan higiene perorangan dengan kejadian penyakit kusta di wilayah kerja puskesmas kalumata kota ternate selatan. Tumou Tou 2015;1(3):1-8.

25. Noorden SK. The epidemiology of leprosy. Dalam: Hastings RC, Opromolla DVA, penyunting. Leprosy. Edinburgh: Churchill Livingstone 994;2:29-45.

26. Kaminska EC. James, WD, Berger, TG, Elston, DM. Andrews' Diseases of the Skin: Clinical Dermatology. Elseiver Clinics in dermatology 2012;30(4):445-446.

27. Noronha BT, Li JM, Wheatcroft SB, Shah AM, Kearney MT. Inducible nitric oxide synthase has divergent effects on vascular and metabolic function in obesity. Diabetes Journal. 2005;54(4):1082-1089.

28. Barcelo A, Barbe F, De la Peña M, Vila M, Perez G, Pierola J, et.al. Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. European Respiratory Journal 2006;27(4):756-760.

29. Winarsi H, Wijayanti SP, Purwanto A. Aktivitas enzim superoksida dismutase, katalase, dan glutation peroksidase wanita penderita sindrom metabolik. Majalah Kedokteran Bandung 2012;44(1):8-12.

30. Raka I, Rastogi MK. Enzymatic Oxidative Stress Indicators and Oxidative Stress Index in Patients of Leprosy. Nepal Journal of Dermatology, Venereology & Leprology 2018;16(1):35-40.

31. Vijayaraghavan R, Paneerselvam C. Erythrocyte antioxidant enzymes in multibacillary leprosy patients. International Journal of Applied Biology and Pharmaceutical Technology 2011;2(2):409-412

32. Prasad CB, Kodliwadmath MV, Kodliwadmath GB. Erythrocyte superoxide dismutase, catalase activities and hydrogen peroxide induced lipid peroxidation in leprosy. Leprosy review 2007;78(4):391-397.

33. Garad AS, Suryakar AN, Shinde CB. Oxidative Stress and Role of Thiol in Leprosy. International Journal of Pharmaceutical Biological and Chemical Sciences 2014;3(2):22-26.

34. Vijayaraghavan R, Suribabu CS, Oommen PK, Panneerselvam C. Vitamin E reduces reactive oxygen species mediated damage to bio-molecules in leprosy during multi-drug therapy. Curr Trends Biotechnol Pharm 2009;3:428-439.

35. Jyothi P, Riyaz N, Nandakumar G, Binitha MP. A study of oxidative stress in paucibacillary and multibacillary leprosy. Indian Journal of Dermatology, Venereology, and Leprology 2008;74(1):80.

36. Ozan G, Erişir M, Erden G, Ozan ST. Oxidative stress, reduced glutathione levels and catalase activities in leprosy patients. The Febs Journal 2006;273:152.

37. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dynamic medicine 2009;8(1):1.

38. Rahman K. Studies on free radicals, antioxidants, and cofactors. Clinical interventions in aging 2007;2(2):219.

39. Salmon AB, Richardson A, Perez V. Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging. Free Radic Biol Med 2010;48(5):642

40. Fusco D, Colloca G, Monaco MR, Cesari M. Effects of antioxidant supplementation on the aging process. Clinical interventions in aging 2007;2(3):377.

41. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International journal of biomedical science: IJBS 2008;4(2):89.

42. Ribeiro Schalcher T, Borges RS, Coleman MD, Júnior JB, Salgado CG, Vieira JL, Romão PR, Oliveira FR, Chagas Monteiro M. Clinical oxidative stress during leprosy multidrug therapy: impact of dapsone oxidation. PLoS ONE 2014;9(1).

43. Sook-Kyoung Jo, Won-Young Lee, Eun-Jung Rhee, Jong-Chul Won, Chan-Hee Jung, Cheol-Young Park, et.al. Serum γ-glutamyl transferase activity predicts future development of metabolic syndrome defined by 2 different criteria. Clinica Chimica Acta 2009;403(1-2):234-240.