

Analysis of Malondialdehyde Level in Leprosy Patients



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Abstract

Background: Leprosy is a chronic disease caused by *Mycobacterium leprae*. Oxidative stress (OS) is a condition associated with an increased rate of cellular damage induced by the oxygen derived oxidants commonly known as reactive oxygen species (ROS). ROS are capable of damaging cellular constituents generated in excess during the chronic, inflammatory, and neurodegenerative disease process of leprosy. Malondialdehyde (MDA) is end product of lipid peroxidation by ROS and serves as marker of cellular damage. **Aim:** To analyse the difference of MDA level in Paucibacillary (PB) and Multibacillary (MB) leprosy. **Methods:** 17 new cases of leprosy patients that were diagnosed by clinical and laboratory examinations. We conducted blood samplings and measurements of plasma MDA level with HPLC method. **Results:** In this study, there was increased the mean of MDA level in MB compared with PB and significant statistically ($p < 0,05$). **Conclusion:** Tissue damage due to OS in leprosy patients is more severe in MB group than PB group

Keywords: leprosy, malondialdehyde, oxidative stress.

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. It usually affects the skin and peripheral nerves. It can also affect muscles, eyes, bones, testes, and other internal organs.^[1,2]

Leprosy is common in developing countries including Indonesia as a result of the country's limited ability to provide adequate services in the scope of health, education, socio-economic in the community so it is still a contagious disease that causes complex problems.^[3] Leprosy has different clinical manifestations in each individual. Based on Ridley and Jopling's grouping of leprosy, it is divided into 5 types based on clinical, histopathological and immunological aspects, namely tuberculoid (TT) type leprosy, borderline tuberculoid type (BT), mid borderline type (BB), borderline lepromatous type (BL) and lepromatous type (LL), while WHO divides leprosy into paucibacillary (PB) type leprosy and multibacillary type leprosy (MB).^[4] Clinical manifestations of leprosy can also be affected by inflammation in tissues mediated by the immune response. Products such as tumor necrosis factor- α (TNF α), nitric oxide (NO) and the presence of reactive oxygen species (ROS), play an important role in the immune response to infection but on the other hand can also cause tissue damage.^[5]

ROS are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they

function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA.^[6] Overproduction of ROS arising either from mitochondrial electron-transport chain or from excessive stimulation of nicotinic adenine dinucleotide phosphate (NADPH) oxidase. This oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages.^[6,7]

The major defense against microbial infection is the macrophage system.^[8] Microbial killing by macrophages is associated with a burst of respiratory activity that leads to the production of a variety of molecules and free radicals called reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radicals.^[9]

The mechanism of microbial killing within phagocytes involves ROS, if not adequately scavenged by antioxidants, this will lead to undesired peroxidation of lipids and other macromolecules. Free radicals react with lipids containing double carbon bonds. The prime targets are polyunsaturated fatty acids (PUFA) in membrane lipids. This reaction causes the separation of hydrogen from carbon and the addition of oxygen produces peroxy lipid (LOO) and lipid hydroperoxide (LOOH). LOOH unstable and degraded by free radicals to form MDA.^[10,11] (figure 1) MDA in plasma serves as a marker of cellular damage due to free radicals.^[7] Considering this, the study was planned to analysis the

status MDA plasma level in new patients with leprosy and differences based on PB and MB leprosy types especially in the city of Medan in the country of Indonesia.

Methods

This study conducted from April until October 2017. This was designed as a cross-sectional observational analytic study involving 17 newly diagnosed cases of leprosy were recruited from the outpatient clinic of the department of dermatology, H. Adam Malik General Hospital and Dr. Pirngadi Hospital Medan. Each subjects signed informed consent were included in this study that had met inclusion and exclusion criteria. Samples was taken from venous blood and malondialdehyde level was measured using High Performance Liquid Chromatography (HPLC).

The results were statistically analyzed by Shapiro-Wilk tests. Using T-independent test and quantitative data were analyzed using mean and SD. p values less than 0.05 were considered significant.

Ethics

Ethical clearance was given by Health Research Etchical Committee, Faculty of Medicine, University of Sumatera Utara.

Results

In this study we got mean of MDA level was higher in man group (1,568±0,561 µmol/L). The highest MDA level we found in 16-64 years old group (1,447±0,637 µmol/L), type 1 reactions (1,576±0,712 µmol/L) (table 1).

Table 1: MDA levels based on characteristic leprosy patients

Characteristic	MDA level (µmol/L)		
	n	Mean	SD
Age (group)			
0-15	0	0	0
16-64	17(100%)	1,447	0,637
≥65	0	0	0
Gender			
Man	14 (82.4%)	1,568	0,561
Woman	3 (17.6%)	0,886	0,790
Type reactions			
Type 1	4 (23.5%)	1,576	0,712
Type 2	0	0	0
Without reaction	13 (76.5%)	1,408	0,639

Our study showed that there is significantly higher ($p < 0,005$) in the level of MDA in MB leprosy patients, than in PB leprosy patients (table 2).

Table 2: Difference in MDA levels based on type of leprosy patients

Subject	N	MDA level (µmol/L) Mean ± SD	P
MB	3 (17.6%)	1589 ± 0,551	0,04
PB	14 (82.4%)	0,787 ± 0,693	

Discussion

The phenomenon of lipid peroxidation has attracted considerable attention in several pathologic conditions. The major defense against the microbial infection in leprosy is macrophage system. Microbial killing by macrophages is associated with a burst of respiratory activity that leads to production of ROS. The macrophages are rendered inefficient in eliminating bacilli in the borderline or lepromatous forms. This cascade of events could also be responsible for the increase of MDA levels in the leprosy patients, mainly in the cases of the lepromatous form. Prime targets of peroxidation by ROS are the polyunsaturated fatty acids (PUFA) in membrane lipids. PUFA is degraded by free radicals to form MDA. The level of MDA in serum serve as a marker of cellular damage due to free radicals. Since MDA serves as an index of lipid peroxidation, it was estimated in leprosy patients to estimate the extent of lipid peroxidation. This indicates that increased lipid peroxidation due to 'free radical'-mediated injury occurs in leprosy patients. Increased lipid peroxidation can occur if the rate of production of reactive oxygen species is higher or the antioxidant level is low.^[8,12,13] MDA is the major and stable end product formed during the peroxidation of lipids.^[12,14]

In this study showed significantly higher MDA levels from MB than in PB leprosy patients. The macrophages in MB patients show normal phagocytosis; however, they are unable to kill and digest engulfed Mycobacterium leprae because of inadequate superoxide production. Thus, the MB macrophages may not significantly contribute to overall generation of ROS. The systemic increase of MDA levels in the MB patients may be a result of the defect function of monocyte-macrophage and it is well known that cell-mediated immunity is strong in PB.^[7,8]

This is consistent with a study conducted by Garad et al and Trimbake et al, who found that there was a significantly higher MDA levels from MB than in PB leprosy patients.^[9,12] The MDA values increased from tuberculoid to lepromatous, thus indicating increased trend in the mean values in leprosy patients, thus indicating increased lipid peroxidation in leprosy particularly in lepromatous.

This study showed MDA levels from leprosy patients with reaction type 1 higher than without reaction. In this study there was no leprosy reaction type 2.

This is consistent with study conducted by Girish et al, who found that there was a higher MDA levels leprosy patients with reaction (4,89±0,72) than in without reaction (3,19±0,62).^[15] Leprosy reactions also plays a role in causes more severe tissue damage in leprosy patients.

In this study also showed there was increase MDA levels in age group 16-64 years old (1,447±0,637) and MDA levels were higher in male (1,568±0,561) than woman (0,886±0,790). But the authors had yet to find research or theory that further links MDA levels with age and gender.

Conclusion

This study was conducted in patients before the start of the treatment evaluating the levels of MDA in plasma. This study confirms the increases MDA levels significantly higher from MB

than in PB leprosy patients. Further study needed to determine the correlation MDA levels with bacteriology index to prove whether the amount of acid-fast bacilli influence on it.

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