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Glutathione and F2 Isoprostane Level in the Blood of the Patient of Pulmonary Tuberculosis at Makassar, South Sulawesi, Indonesia

[1]Ari Yuniastuti, [2]Irawan Yusuf, [2]Muhammad Nasrum Massi and [2]Budu

[1]Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Negeri Semarang, Semarang, Indonesia
[2]Faculty of Medical, Hasanuddin University, Makassar, South Sulawesi Indonesia

Abstract: The infection of *Mycobacterium tuberculosis* causes the increasing of *reactive oxygen species* (ROS). The increasing of ROS causes the increasing usage of endogenous antioxidant glutathione continuously for ROS scavenger, so, there will be as decreasing amount of glutathione. The imbalance of antioxidant and imbalance of antioxidant and free radicals causes oxidative stress. This research aimed for revealing the correlation of glutathione and F2 Isoprostane level of pulmonary tuberculosis patients. The analytic observational research methods with research design used was *cross sectional*. The subjects of this research were 103 pulmonary tuberculosis patients, as the inclusion-exclusion criteria. The research was done in Balai Besar Kesehatan Paru Masyarakat (BBKPM) or The Institutional Lung Health Center and Labuang Baji Hospital, Makassar. The examination of glutathione and F2- isoprostane levels was carried out by using the related Elisa kits. The result of the research showed that there is glutathione level for about (0.049±0.036) mM and F2- isoprostane level for about (97.321±25.920) pg/ml. The results of statistical exam showed that there is a meaningful correlation between the level of glutathione and F2- isoprostane with significant value of p=0.05 and pearson correlation value about -0.717, which means if the level of glutathione is high, the level of F2- isoprostane is low & vice versa. In conclusion, the correlation between glutathione and F2- isoprostane level of the pulmonary tuberculosis patients was a very strong correlation (r=1).

Key words: Glutathione · F2- isoprostane · Pulmonary Tuberculosis

INTRODUCTION

Pulmonary tuberculosis is an infectious disease caused by the bacteria of *Mycobacterium tuberculosis* in the lungs and become the biggest deaths cause in the world. WHO [1] stated that there will be approximately 8 million new cases and 3 million deaths because of pulmonary tuberculosis annually. So, that WHO have some strategies with the target is to reduce TB death to 95% and TB incidence to 90% compared with 2015 data [2].

The infection by *Mycobacterium tuberculosis* inducts the existence of *Reactive Oxygen Species* (ROS) in the lungs through the process of *Respiratory burst* macrophage phagocytosis which is signed by the increasing usage of oxygen which will be reduced by *NADPH oksidase* to become *superoxide* (O_2^-) and *hydrogen peroxide* (H_2O_2) [3]. The increasing of ROS can initiate the oxydative stress where there is an imbalance of redox system between oxydative and antioxidant, it causes the damage of the tissue [4]. The increasing of ROS to the patients of pulmonary tuberculosis is the process related to the severity level of the disease [5].

The oxidative stress for the patients of pulmonary tuberculosis happens because of the increasing of ROS in the lungs, whereas the capacity of endogenous antioxidant detoxification will stay static or reducing, so there will be an imbalance of oxidant and antioxidant in the body of pulmonary tuberculosis patients [6]. The oxidative stress causes the transduction signal disturbance of *Mycobacterium tuberculosis*, the synthetic disturbance of DNA and RNA, protein

Corresponding Author: Ari Yuniastuti, Department of Biology, Faculty of Matemathics and Natural Sciences, Universitas Negeri Semarang, Jl Raya Sekaran Gunungpati, Semarang. Indonesia., Post Code: 50229. Tel: +628156615624.

synthesis and causes the antituberculosis drug resistance and relates the pathogenesis mechanism [7]. Some reports showed that there is a reduction of glutathione antioxidant [8] and the improvements of *malondealdehyda* (MDA) levels [9] in the body of pulmonary tuberculosis patients.

ROS will oxidize *phospholipase* which one of its products, F2-isoprostane, can be used as a sign of oxidative stress appearance [10]. F2- isoprostane is the result of catalyzed arachidonic acid of free radicals, where there is an increasing F2- isoprostane which signs the existence of oxidative stress. F2- isoprostane these days is considered as *in vivo* oxidative stress biomarker which is most reliable, easy to measure and possess high specification and stability [11].

Glutathione is one of the antioxidant which has an important role in protecting cell from the toxic influence of ROS and RNI and also has antimicrobial effect directly with improving the immunity and obstruct the intracellular growth of *Mycobacterium tuberculosis*, which controls the intracellular growth of *Mycobacterium tuberculosis* in the macrophages, has the micro bacterial activity which has a role as the carrier of NO, as the effector molecules of cellular immunity for protecting the body from the infection of *Mycobacterium tuberculosis* [12].

The decreasing of glutathione level in the body of pulmonary tuberculosis patients is suspected causing the disturbance of regulation in immunity cell and causing the failure of ROS scavenger [13]. Up until now, there have never been any reports regarding to the relation of glutathione level and F2-isoprostane in the body of TB patients, therefore, this research was conducted to investigate that relation.

MATERIALS AND METHODS

This research is an analytic observational research with *cross sectional* design. The subject of the research was the Pulmonary Tuberculosis (PT) patients of Balai Besar Kesehatan Paru Masyarakat (BBKP) or The Institutional Lung Health Center dan Labuang Baji Hospital, Makassar. The Pulmonary Tuberculosis patients were chosen based on the order of the visit in *consecutive random sampling* based on inclusion-exclusion criteria.

The investigation of sputum for Acid Fast Bacilli (AFB) was done directly through microscopic examination of sputum films stained with Ziehl Neelson. Blood samples were taken (about 3 ml) from cubital vein from the patients through vacutainer tube (Pyrogens free tube), afterwards, centrifuged serum was taken to analyze the level of glutathione and F2-isoprostane. The level of glutathione and F2-isoprostane was determined by using Elisa technique with human Elisa kits, a product of Cusabio, USA (CSBE12100h).

The data analysis was examined by using Pearson correlation to know the relation between glutathione level and F2-isoprostane. The statistical analysis was carried out using SPSS for window 20 with 95% level of reliability and the value of p = 0.05.

RESULTS

The characteristics of research subjects include the Body Mass Index (BMI) and AFB sputum (Table 1). The data of glutathione and F2-isoprostane level got in the initiation of investigation, specifically before the patients got the Antituberculosis Therapy Medicine (ATM). Next, from those data, we could find the mean of the data and its standard deviation. Based on the *Kolmogorov-smirnov* examination, the data of glutathione and F2-isoprostane level was normal. The result showed that average glutathione and F2-isoprostane level (0.049±0.036) and (97.321±25.920), respectively (Table 2).

The next thing was, to know the correlation between glutathione level and F2-isoprostane towards the body of Pulmonary Tuberculosis patients, *Pearson correlation* statistical exam was executed. Based on its result, it was revealed that there is a meaningful correlation between glutathione level with the level of F2-isoprostane with p=0.05 significant value and -0.717 score of pearson correlation value. It shows a negative correlation, which means if the glutathione level was low, the level of F2-isoprostane would be high and vice versa.

Table 1: Characteristics of Pulmonary Tuberculosis Patients (N=103)

Parameter	Total	P
IMT (kg/m^2)		
< 18.5	60	0.000
18.6-22.9	40	
>25	3	
(mean±sd)	(18.205±2.332)	
AFB Sputum		
(+)	71	0.000
(++)	19	
(+++)	13	
(mean±sd)	(2.560±0.709)	

Table 2: The Glutathione and F2-isoprostane level in Pulmonary Tuberculosis Patients

Parameter	Level	SD	p	r
Glutathione (mM)	0.049	0.036	0.00	-0.717
F2-isoprostane (pg/ml)	97.321	25.920		

Significant value p≤ 0.05

Normal levels of glutathione in blood is about 5-8 mM/l with the highest concentration is in liver related to its important role in detoxification [14].

Normal levels of free F2-isoprostane in plasma is (35.6 pg/ml), in urine is 57-390 ng/mg – kreatinin) a and in brain fluid is (23.1 pg/ml) [15].

DISCUSSION

Glutathione (GSH) is a tripeptide which consists of amino acid, glutamate, cysteine and glycine. Glutathione is a co-substrate for glutathione peroxidase enzyme. Glutathione can be reacted to singlet oxygen, superoxide and hydroxyl and able to directly have a role as a scavenger of free radicals. Glutathione is also able to lose or minimize the formation of hydroxy peroxide in the reaction of lipid peroxidase, so, the membrane structure will be more stable [16].

Venketaraman *et al.* [16] reported that there is a decreasing level of glutathione significantly in the peripheral mononuclear blood cells and red blood cells of PT patients. This thing is related to the increasing amount of *Mycobacterium tuberculosis* and the increasing production of proinflammatory cytokines which cause the increasing amount of ROS free radicals. The imbalance of glutathione antioxidant level and free radicals creates oxidative stress. Oxidative stress is known after the usage of F2-isoprostane parameter. Oxidative stress happens inside of the cell whether endothelial cell or in the smooth muscle (Tunica intima or media). The production of F2-isoprostane is also expressed by cells. F2-isoprostane which is detected in the blood of human is the damage of the broken cell wall which spills out the volume of the cell including F2-isoprostane. F2- isoprostane inside of the blood is easily appeared and eliminated compared to the ones inside of a cell [11].

F2- isoprostane has been proven to become the meaningful index to the lipid peroxidase in *in vivo* way. This time, the level of F2- isoprostane can be used as the specific marker in describing the oxidative stress in *in vivo* ways. The method to measure the F2- isoprostane as an oxidative stress in more beneficial because F2-isoprostane is chemically stable, the specific result of peroxidase, formed in *in vivo* ways, detected in body tissue and fluids, increase substantially towards the animal with oxidant injury, not influenced with the amount of lipid inside of a food and sensitive to the antioxidant dosage [17].

F2-isoprostane which is detected in its esterification form in all normal biological tissue and in the free form in all normal biological liquid, indicates the level of oxidative stress [17]. The tissue and the liquid of the body including urine contain less F2- isoprostane. In the oxidative stress condition, isoprostane level is increasing. The level of F2- isoprostane is lower to the subject who consumes less antioxidant supplement, like vitamin E and beta carotene. In that research, it was summed up that F2- isoprostane is the main metabolite which becomes a marker status of the most sensitive endogenous oxidative stress [18].

Result of this study is in accordace with Khorjahani *et al.* (2012) that antioxidant supplementation is an attempt to attenuate any potential increase in oxidative stress [19].

In this research, statistically, the level of glutathione was lower than the level of F2- isoprostane. The result of Pearson correlation exams showed the meaningful relation between glutathione with F2- isoprostane level ($p=0.00$). The Pearson correlation value of -0.717 showed the negative correlation between glutathione and F2- isoprostane level, if the level of glutathione is low then the level of F2- isoprostane is increasing, vice versa. The strength of the correlation is very strong ($r=1$).

Therefore, based on the result of this research and the analysis of the previous research, it can be stated that there was a correlation between the infection of *Mycobacterium tuberculosis* with the low level of GSH and the high level of F2- isoprostane to the body of pulmonary tuberculosis patients.

CONCLUSIONS

There is a correlation between glutathione and F2- isoprostane level to the body of pulmonary tuberculosis patients with very strong correlation ($r=1$). There is a relationship between the the low level of GSH and the high level of F2- isoprostane.

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