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Advancing to the Frontier of Innovation in Science

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PROCEEDINGS OF THE 5TH ANNUAL BASIC SCIENCE INTERNATIONAL CONFERENCE

“Advancing to the frontier of innovation in science”

ATRIA HOTEL AND CONFERENCE, MALANG, INDONESIA

FEBRUARY 11th-12th, 2015

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Polymorphisms Of *Glutamate Cysteine Ligase* Gene is an Oxidative Stress Biomarker at Pulmonary Tuberculosis Patients

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Abstract – *Glutamate-Cysteine Ligase* (GCL) enzyme plays role in the synthesis of the glutathione antioxidant, as functions to netralize the oxidative stress, due to the presence of heterodimers consisting of the catalytic subunits (GCLC) and the modulator subunits (GCLM). The polymorphism of GCL gene might cause the changes of the function and the activity of GCL enzyme so that the synthesis of glutathione is disturbed. The polymorphism of GCL gene may be used as the biomarker of the susceptibility against the oxidative stress at the molecular level in the pulmonary TB patients. The aim of the study was to examine the polymorphism of GCL gene in the pulmonary TB patients. The research was carried out at the Institute for Lung Health Society (BKPM) and in some Network Public Health Centers around Semarang, from May 2012 to September 2012. The samples venous blood, underwent polymorphism test using *Polymerase Chain Reaction* (PCR) method to examine the DNA strands. Results show that the polymorphism GCLC and GCLM in the TB group were 33% and 30%, respectively, whereas the control group were 8% and 6%, respectively. Statistical analysis showed that there was relationship between the polymorphism levels of GCLC and GCLM and the pulmonary TB patient's susceptibility against the oxidative stress. It was concluded that the genetic variation of *Glutamate-cystein Ligase* (GCL) gene be used as the biomarker for detection of the susceptibility against oxidative stress. Further study is needed to obtain the GCL genes sequence to comprehend the difference in the gene sequences.

1. INTRODUCTION

Pulmonary tuberculosis (pulmonary TB) is an infectious disease that still becomes a great public health problem and causes the highest death in the developing countries including Indonesia. This disease is caused by *Mycobacterium tuberculosis*, that is acid-fast (BTA), Gram-positive bacteria; a and live intracellular. The prevalence of TB in Indonesia in 2009 was around 520,000 individuals [1]. In 2020, TB was predicted to attack 1 billion persons with around 70 million casualties, if the disease could not be controlled [2]. Some researchers have reported that the administration of anti-tuberculosis drug to the pulmonary TB patients suggested that the drug may result in *Reactive Oxygen Species* (ROS) that might generate oxidative stress [3]. The ROS might also be formed from the immunity mechanism against *Mycobacterium tuberculosis* infection. In the pulmonary TB patients, the cellular immune system plays role as the defense mechanisms against *Mycobacterium tuberculosis* infection [4] and this process involves macrophages as the active phagocyt cells that kill *Mycobacterium tuberculosis* bacteria. A *Respiratory burst* mechanism is the important part of the immune system against *Mycobacterium tuberculosis*, the increasingly produced ROS can trigger the oxidative stress [5]. The oxidative stress on the pulmonary TB is the redox imbalance condition between oxidants and antioxidants in the lungs. Glutathione plays the role as the main component in the *respiratory burst* [5], as an antioxidant in protecting the lung cells from inflammation, as well as protecting the cells from the toxic effect of ROS and RNI and having direct antimicrobial effect by improving the immunity and inhibiting the growth of *Mycobacterium tuberculosis* [6]. The glutathione deficiency on pulmonary TB patients was suggested to have caused the disruption of the regulation of the immune cell function and might cause the failure to scavenge the ROS [7]. Glutathione is synthesized by *Glutamate-Cysteine Ligase* (GCL) enzyme. GCL enzyme is formed by heterodimers consisting of the catalytic subunits (GCLC) and the modulator subunits (GCLM) [8]. The genetic variation (gene polymorphism) of GCL can cause the changes of the function and the activity of GCL enzyme that in turn may cause the disruption of the glutathione synthesis, allowing the reduced glutathione level and the phenotype would show the susceptibility against some diseases such as hemolytic anemia, cancers, myocardial infarction, diabetes mellitus and HIV/AIDS [9]; [10]; [11]; [12]; [13]; [14]; [15]. The low of glutathione level in the patients of pulmonary tuberculosis was suspected to correlate with the oxidative stress. The early detection of oxidative stress at the molecular level in pulmonary TB patients has to be performed to know the susceptibility to the oxidative stress and to prevent the occurrence of the vulnerability and even the severity of the pulmonary TB disease.

2. METHODS

2.1 Chemicals

Buffer lisis (10 mM Tris H-Cl pH 8,0; 100 mM NaCl; 1 mM dosidium EDTA pH 8,0; 0,5% Sodium Dodecyl Sulfat; 0,4 mg/ml Proteinase K), larutan ss-fenol, 3 M Na-asetat (pH 4,8), ethanol absolute dingin, 0,5 M Tris HCl pH 7,4, 5,0 mM EDTA (disodium), 1 mM NaCl, RNAase (10mg/ml)

2.2 Procedures

The study was an analytical observational research, and case control study design was employed. the pulmonary tb patients with bta (+) were examined by the institute for lung health society (bkpm) and in some network public health centers around semarang (i.e. tlogosari kulon, tlogosari wetan, gunungpati, kagok, and bandarharjo) during the study period. the case group consisted of 25 tb patients without accompanying diseases such as diabetes, coronary heart disease, hypertension and cancer, whereas the control group consisten of 25 healthy persons. the research was carried out from may 2012 to september 2012. the samples were selected based on the first come first served policy to the patients who visited bkpm or network public health centers during the research period (*consecutive sampling from admission*). the polymorphism examination was done through several steps: the dna isolation and purification (*chelex method*), the concentration and the purity measurement of dna as the product of the isolation, the electrophoresis to get the exact total amount of dna as the product of the isolation, the gclc and gclm gene amplification using *polymerase chain reaction* (pcr) which was performed using gclc primer with the base sequence as follows: forward: (5'-TCGTCCCAAGTCTCACAGTC-3') and reverse: (5'-CGCCCTCCCCGCTGCTCCTC-3') and the gclm primer: forward: (5'-CTCAAGGGCAAAGACTCA-3') and reverse: (5'-CCGCCTGGTGAGGTAGACAC-3'), and the restriction enzymes used were *tsp451* and *mspi*, which further the pcr result underwent electrophoresis in the gel agarose 2%. to find the genetic variation differences between gclc and gclm genes among the samples, a *chi-square* (χ^2) is used. the statistical analysis was assisted by spss 12 for windows application program, the significance value was $p < 0.05$, with the confidential level of 95%.

3. RESULTS AND DISCUSSION

During the study period as many as 25 persons were selected as samples of pulmonary TB patients with BTA (+), who were considered to meet the inclusion and exclusion criteria and willing to take part in the research. Further, one person withdrew from the study due to suffering other disease, and therefore the case samples were only 24 individuals. The control group consisted of 25 samples from the volunteers who donored their blood to the Indonesian Red Cross (PMI). The study showed that the number of the female subjects (12 individuals, 50%) were equal to the male subjects (12 individuals, 50%) with the age ranging from 15 years to 60 years. The age range of the case group was 15-60 years with an average of 59.16 years (± 11.62), whereas age range of the control group was 22-60 years with an average of 50.76 years (± 14.56).

Twenty five pulmonary TB sufferers who were treated by the Institute for Lung Health Society (BKPM) and by some Network Public Health Centers around Semarang participated in the research. However, only 24 individuals actively participated in the research, while 1 person withdrew from the research because it was discovered that this person was a diabetic sufferer. The molecular examination was carried out to understand the genetic variation of GCLC and GCLM enzymes using PCR method.

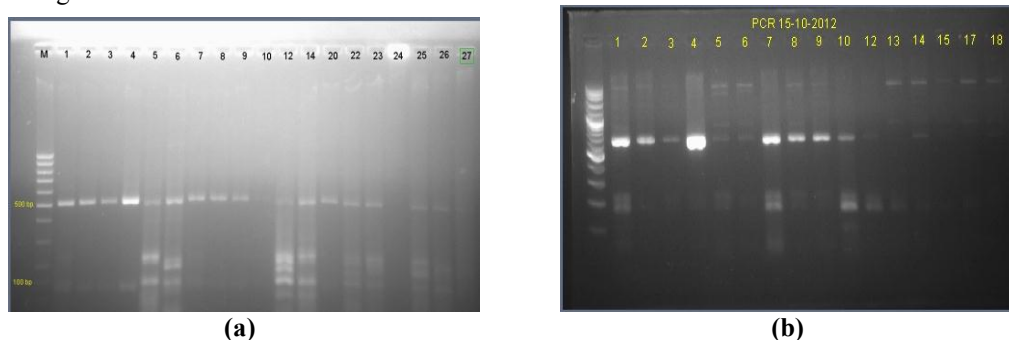


Figure 1 Product electrophoresis PCR-RFLP GCLC (1a) and GCLM (1b) gene

The percentage of the GCLC gene polymorphism was 33% (N=24), while the undetected percentage was 21%. Whereas the percentage of the GCLC gene polymorphism in the healthy people was 8%. (Table 1).

Table 1 The Genotypic Frequency of the GCLC Gene in the Case and the Control Group

Genotypic Frequency	Case (%)	Normal (%)	p value (χ^2 test)
C/C Homozygotic	46	92	0,0036
C/T Heterozygotic	29	8	
T/T Homozygotic	4	0	
Undetected	21	-	

$p < 0.005$

The percentage of the genetic polymorphism of GCLM gene was 30% and the undetected percentage was 30% in pulmonary TB patient are presented Table 2. The percentage of the GCLM gene polymorphism in the healthy people was 6%. The polymorphism was a significant risk factor for oxidative stress in the pulmonary TB patients.

Table 2 The Genotypic Frequency of the GCLM Gene Promoters in the Case and the Control Group

Genotypic Frequency	Case (%)	Normal (%)	p value (χ^2 test)
C/C Homozygotic	40	94	0,0024
C/T Heterozygotic	30	6	
T/T Homozygotic	-	0	
Undetected	30	-	

p < 0.005

Statistical testing using χ^2 test resulted in the value of $p=0.0036$, showing that there was significant correlation between the GCLC gene polymorphism and the pulmonary TB sufferers, and between the GCLM gene polymorphism and the pulmonary TB sufferers at significance value of $p=0.0024$.

When a cell is exposed to an oxidant and oxidative stress happens, the GSH level will decrease, and the GCL gene expression will be more regulated by the response element activation against the oxidative stress on the promoter area. This could initiate the synthesis of GSH and will function as the defender/adaptation mechanism against the oxidative stress. Therefore, the presence of the GCLC and GCLM gene polymorphisms would possibly cause the reduction in the response against the oxidative stress. This would in return cause the reduction in the intracellular GSH production, that might decrease the response against the oxidative stress and as a result the susceptibility to the induction of the oxidants will increase and will damage the tissues. These steps are actually the pathogenesis part of pulmonary TB. In some studies on the GCL gene and its role in various related diseases, several single-nucleotide polymorphisms have been identified in the human GCL gene promoter. This gene has also been correlated with the increase of the susceptibility against several diseases due to oxidative stress.

So far, the bibliographical study did not find any research reporting the polymorphism of GCLC and GCLM genes on pulmonary TB in Indonesia. The current study has limited the focus on the polymorphism of GCLC and GCLM genes. It was suggested to conduct further research to find the exact loci of the GCLC and GCLM genes using a sequencing method. This sort of study would be beneficial to see whether the different location of the GCL gene at a certain position plays role as the risk factor of pulmonary TB in Indonesia, and an advanced research with the larger sample and the more diverse ethnical population could confirm this.

4. CONCLUSIONS

In conclusion, the genetic variation of *Glutamat-cystein Ligase* (GCL) enzyme may be used as the biomarker at the molecular level to detect the presence of oxidative stress on pulmonary TB patients. Further research is required to check the different loci of the varying genes using a sequencing method.

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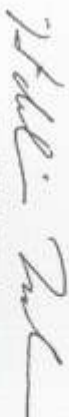
CERTIFICATE

This is to certify that

Ari Yuniastuti

has participated in The 5th Annual Basic Science International Conference,
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