Manganese Superoxide Dismutase Gene Ala16Val Polymorphism in Pulmonary Tuberculosis Patients with Diabetes at Balai Pengobatan Lung Disease, Medan City

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Abstract: Manganese Superoxide dismutase (Mn-SOD) gene Ala16Val polymorphism has been shown to be associated with several infectious disease and metabolic disorder. This study to analyze Mn-SOD gene Ala16Val polymorphism in patients with pulmonary tuberculosis (PTB) and also suffer from diabetes mellitus (DM). The study was conducted at Balai Pengobatan Lung Disease, Medan and Integrated Laboratory of Faculty of Medicine, University of Sumatera Utara (USU), Medan. Mn-SOD gene Ala16Val polymorphism was studied in 40 outpatients at Balai Pengobatan Lung Diseases, Medan City. The blood glucose was measured with a spectrophotometer, using commercial glucose kits. Polymerase chain reaction-restriction fragment lengths polymorphisms (PCR-RFLP) technique was done with the BsaW1 restriction enzyme. SPSS version 22 was used for statistical data. Ala and Val were analyzed to see the frequency of genotype of Mn-SOD gene Ala16Val polymorphism by direct counting. Hardy-Weinberg Equilibrium was analyzed by a Chi-square test. The results show that the percentage of Val/Val genotype (57.5%) of Mn-SOD gene was higher compared Ala/Val and Ala/Ala genotypes (37.5%, 5 %) in PTB patients with diabetes. This preliminary study results indicated that Val/Val genotype was common genotypes in the Mn-SOD gene in PTB patients with diabetes at Balai Pengobatan Lung, Medan city.

1 INTRODUCTION

Since the beginning of the 20th century, clinicians have observed an association between pulmonary tuberculosis (PTB) disease and diabetes mellitus (DM), although it is still difficult to determine whether PTB leads to clinical manifestations of diabetes or diabetes preceding PTB. PTB and diabetes often co-exist, especially in high-risk populations for PTB infection (Dooley and Choiasson, 2009). Diabetes can increase the severity and frequency of a disease, including PTB infection (Podell, 2014). Indonesia as a country that ranked fourth in the number of patients with most PTB also ranked fourth in the number of diabetics (WHO, 2016).

Superoxide Dismutase (SOD) is an enzymatic antioxidant and classified as a major antioxidant for the defense of body cells against oxidative stress. Mann and Kleiin first identified SOD antioxidant at the 1938 year. SOD has a role in preventing free radicals and oxidative stress. SOD consists of intracellular, mitochondrial, and extracellular enzymes also referred to as type 1 SOD (Cu Zn-SOD), type 2 SOD (Mn-SOD) and type 3 (EC-SOD) respectively. SOD levels used as parameters of antioxidant status in the body and bio-indicator of oxidative stress conditions (Weisiger and Fridovich, 1973).

Research by Saad et al. (2016) shows an association of EC-SOD gene polymorphism in DM (Saad, et al., 2016). Some studies have found the role of manganese SOD (Mn-SOD) gene Ala16Val polymorphism with diabetes (Pourvali, et al., 2016). Mn-SOD is SOD antioxidant in the mitochondria. Mn-SOD gene is located on chromosome 6q25. The gene polymorphism of this antioxidants was substitution alanine (Ala) to valine (Val). It has been reported that the Mn-SOD Val/Val genotype has lower activity than Ala/Ala genotype (Bresciani, et al., 2013). So better diabetes control was found only in patients with Ala/Ala genotype (Pacal, et al., 2016).
Research of association of PTB with antioxidant gene polymorphism show by Yuniastuti research et al. (2017) was founded the presence of antioxidant gene polymorphism in PTB patients in Semarang city (Yuniastuti, et al., 2017). Based on the results of previous research, the study of Mn-SOD gene Ala16val polymorphism in PTB patients and Mn-SOD gene Ala16val polymorphism in diabetic patients has never been done. But research to find out how Mn-SOD gene Ala16val polymorphism in PTB patients with diabetes has never been done. The current study would like to see how the distribution of Mn-SOD gene Ala16Val polymorphism in patients with PTB and also suffer from diabetes at Balai Pengobatan Lung Diseases, in Medan city.

2 METHOD

This research was performed from February to June 2018 in an outpatient at Balai Pengobatan Lung Diseases, Medan city. The study has received approval from the ethics committee of the Faculty of Medicine, University of Sumatera Utara (USU) with the letter of ethical approval No. 327/FK USU.

Forty of PTB patients who also suffer from DM were diagnosed according to the criteria of Indonesian Lung Doctor Association (PDPI, 2011) and standards of Indonesian Endocrinology Society (PERKENI, 2015) as subjects in this research. The subjects requested to fill out and sign an informed consent after being explained the purpose and benefits of the study. The subjects who 1) took the antioxidants drugs before the examination, 2) had co-infections such as HIV, hepatitis, malignant disease 3) had a positive family history of diabetes mellitus were excluded from this research.

Data collection of characteristics subjects: age, gender and duration of illness were done through interviews using questionnaires. The blood sample were taken three ml from the mediana cubital vein, using the EDTA vacuum syringe. Then, the blood was centrifuged for 10 minutes at a rate of 3000 rpm. The blood glucose was measured with a spectrophotometer at a wavelength of 500 nm, using commercial glucose kits. Analysed of Mn-SOD gene was performed on blood samples of subjects at Integrated Laboratory of Faculty of Medicine, University of Sumatera Utara (USU). DNA for genomics was extracted from whole blood, using the genomic DNA extraction kit from Promega. DNA was amplified by PCR amplification method, using Forward primer 5'-CAC CCC AGC CTG CGG AGA CGG-3' and Reverse primer 5'-CTT GGC CAA CGC CTC CTG GTA CTT-3'. Before performing the amplified process, PCR mix was created first with 25 µl PCR reaction volume consists of 12.5 µl GoTaq® Green Master Mix, one 1 µl Forward primer, 1 µl Reverse primer, 2 µl DNA template, 8.5 µl nuclease free water (Promega, USA).

The PCR conditions involved at 95 °C for 5 min for an initial denaturation of DNA, followed with (95 °C for 45 s (melting), 54 °C for 30 s (annealing) and 72 °C for 30 s) x 30, and a final extension at 72 °C for 5 min. PCR products were analyzed by 2% agarose gel electrophoresis in staining with ethidium bromide. Then, 10 µl of the PCR reaction mixture was digested by the 0.2 µl of restriction endonuclease BsaW1 enzyme at 60 °C in 10 minutes with restriction fragment length polymorphism (RFLP) technique. The RFLP product was visualized by electrophoresis technique at 4% agarose gel. The cutting point of Mn-SOD gene by restriction enzyme results in a 267-bp product (Ala16/ala) or a 267 bp, 183 bp and 84 bp products (Val16/val) (Souiden, et al., 2016). SPSS version 22 was used for statistical data. Ala and Val were analyzed to see frequencies of genotypes of Mn-SOD gene Ala16val polymorphism by direct counting. Hardy-Weinberg Equilibrium (HWE) was analyzed by a Chi-square test.

3 RESULT AND DISCUSSION

This study was done on 40 of the PTB patients with diabetes. The characteristics of the subjects in this study are shown in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N=40 (%)</th>
<th>Mean, SD</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>-</td>
<td>53.26±8.77</td>
</tr>
<tr>
<td>Gender (male/ female)</td>
<td>26/65/14/35</td>
<td></td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>-</td>
<td>3.61±1.38</td>
</tr>
<tr>
<td>Blood glucose levels (mg/dl)</td>
<td>-</td>
<td>295.51±57.8 5</td>
</tr>
</tbody>
</table>

Table 1 shows the mean values of the age of PTB patients with diabetes was 53.26 years (SD = 8.77). Age is the risk factors for suffering diseases, such as infectious disease and metabolic disorder. The condition is probably caused by some changes in body composition which is due to the aging process.
Aging causes a decrease in the immune system and the function of the organs of a body (Rochmah, 2009). In the present research, based on gender found that male in subjects group more than female (65% VS 35%). Gender is not a risk factor for infectious disease, especially PTB infection, but gender may be a risk factor for diabetes. Females are more likely to have diabetes than males (Mihardja, 2009). The proportion of the female in form as central obesity results in a decrease in the action of insulin in the target tissue. It will be leading to an increase in getting diabetes (Flier and Maratos-Flier, 2010). The mean value of the duration of disease was for 3.61 years. The mean level of BGLs in this study subjects was 295.51 mg/dl. The length duration of DM will cause the parameters of BGLs will get worse (Sari et al., 2018). The high level of BGLs showed that DM disease in this group still not well controlled.

The amplified PCR products of the Mn-SOD gene gave a 267 bp fragment. PCR is a method for detecting gene molecules of samples from blood, urine, saliva, etc. PCR is an enzymatic method for DNA amplification in vitro. PCR products can be identified using agarose gel electrophoresis. Currently, a PCR-based molecular method has been designed to analyze gene polymorphisms. One of the ways that can be used to find out genotypes of the Mn-SOD gene polymorphism is the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) (Rodwell, et al., 2015).

In this research, the product of RFLP-PCR of Mn-SOD gene that digested by the BsaW1 enzyme can be seen in Figure 1.

![Figure 1: PCR-RFLP product of Mn-SOD gene Ala16val polymorphism on 4% agarose gel electrophoresis.](image)

At the Table 2 can be seen, the frequency of Val/Val genotype was higher than the Ala/Val and Ala/Ala genotype (57.50% VS 37.50% VS 5%). The results are similar to the previous study by Nakanishi et al. which determines Val/Val genotype is higher than the Ala/Val and Ala/Ala genotype (Nakanishi, et al., 2008). Previous research has found an increase in the frequency of Ala/Val genotype compared to Ala/ala and Val/Val genotypes in the Mn-SOD gene polymorphism of DM patients in the Lebanese population (Zahreddine, et al., 2018).

Table 2: The frequency of genotypes within SOD gene Ala16Val polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala/Ala</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Ala/Val</td>
<td>15</td>
<td>37.50</td>
</tr>
<tr>
<td>Val/Val</td>
<td>23</td>
<td>57.50</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

SOD is an antioxidant as a major defense against oxidative stress that induced by increased reactive oxygen species (ROS). Oxidative stress plays an important role in the pathogenesis of some diseases such as infectious disease, metabolic disorder, etc. In PTB with diabetic patients happen an abnormality in immunity and cell-mediated phagocyte function. The process will produce ROS which will increase oxidant and lipid peroxides. Previous research has shown an increase in lipid peroxides compounds such as malondialdehyde in DM (Sari, et al., 2018). The oxidative stress causes increasing the need for SOD antioxidants. MnSOD, a homotetramer containing one manganese ion per subunit. Specific Mn-SOD gene sequences genetically express Mn-SOD. The Mn-SOD gene is located on chromosome six. If there is a polymorphism in the Mn-SOD gene, it will probably affect the expression level of the Mn-SOD gene (Bresciani, et al., 2013).

Polymorphism is a change of nucleotide sequences in genes that do not cause changes in protein structure but result in variations in protein functions in the cell.
function. Polymorphism can determine susceptibility to disease. The impact of polymorphism is a change in the vulnerability of a population to illness. In this study, the Mn-SOD gene Ala16Val polymorphism of PTB patients with diabetes showed that the Val/Val genotype frequency was higher compared with the wild-type Ala/Ala. Ala/Ala genotype was wild-type of Mn-SOD gene and the occurrence of variations such as Ala/Val heterozygote and Val/Val mutant-homozygote may occur is due to adaptation to the environment that is inherited genetically to next generation (Williams, et al., 1998). Usually, the presence of polymorphism relates only to a person's susceptibility to disease but in this present results have not been able to prove that these genetic variations contribute to the risk of vulnerability to suffer PTB with diabetes. Genotype analysis is required in the healthy control group as a comparison. Hardy-Weinberg Equilibrium was analysis performed by the chi-square test. In this research, the Mn-SOD gene Ala16val polymorphisms in PTB patients with diabetes is consistent with the HWE (p>0.05). The relative proportions of genotype in the population of PTB patients with diabetes constant from one generation to the next. The genotype in this population still in equilibrium is due to the possibility of random marriage, and no migration to the population (Andrews, 2010).

4 CONCLUSION

In this research shows that the Val/Val genotype of Mn-SOD gene polymorphism has a higher percentage than Ala/Val and wild-type homozygote Ala/Ala genotype. It is necessary to conduct a study that compares the group of PTB patients with diabetes with the healthy control group to see the genotype mutant as a risk factor for the development of a disease.

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