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Glutathione Peroxidase-1 Pro198Leu Variant in Tuberculosis-infected Type2 Diabetes Mellitus Patients at Pulmonary Polyclinic Medan

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Abstract

BACKGROUND: Glutathione peroxidase-1 (GPx-1) is an antioxidant that plays an important to the body protection system against oxidative stress. The GPx-1 polymorphism that has been identified in individual with several diseases.

AIM: This study aimed to observe the distribution of GPx-1 Pro198Leu variant in tuberculosis (TB)-infected Type 2 diabetes mellitus (T2DM) patients at pulmonary polyclinic Medan. GPx-1 Pro198Leu polymorphism was genotyped in 40 T2DM patients that also infected by TB.

MATERIALS AND METHODS: Analysis of GPx-1 Pro198Leu polymorphism was done using polymerase chain reaction (PCR) and restriction fragment length polymorphism. The PCR products were digested 4 h at 37°C with Apa1 restriction enzyme. The result of Apa1 enzyme digestion was visualized with 4% agarose.

RESULTS: From 40 TB-infected T2DM patients, the frequency of genotypes CC,CT, and TT were, respectively, 82.5%, 17.5%, and 0%. The frequency of C allele was higher than T allele, i.e. 91.3% and 8.7%.

CONCLUSION: It was concluded that in TB-infected T2DM patients at pulmonary polyclinic Medan, the GPx-1 Pro198Leu polymorphism has CC variant higher than CT, whereas the TT genotype was not found. The frequency of the C allele is higher than the T allele.

Introduction

Oxidative stress causes damage to various cell components such as DNA, proteins, carbohydrates, fats, and other macromolecules. Molecular damage, in turn, impacts cell death and is associated with an increased risk of infectious diseases, cancer, diabetes, coronary and cardiovascular disease, and others. Antioxidants are molecules that act as a complex and comprehensive body protection system against oxidative stress [1]. Glutathione peroxidase (GPx) is an antioxidant that plays an important role in maintaining cell survival. This enzyme works to catalyze the destruction of hydrogen peroxide (H_2O_2) and lipid hydroperoxide through glutathione (GSH). GPx is synthesized by a sequence of genes located on chromosome 3p21 consisting of two exons. GPx synthesis is expressed genetically by the genes sequence on the chromosome. Gene expression can change when there is a substitution of a single nucleotide base in the genome of an individual called single nucleotide polymorphisms (SNPs). Polymorphism causes genetic variation in a population [2], [3], [4].

GPx has six isoforms (GPx-1-6). GPx-1 is the most widely distributed and overflow in human cells. The GPx-1 polymorphism that has been identified is the substitution of cytosine to thymine (C> T), so it causes the substitution of proline with leucine occurs in codon 198 in exon 2 (Pro198Leu, rs1050450). Gene polymorphism plays an important role in several diseases [5], [6]. Previous studies have documented Pro198Leu polymorphisms in Type 2 Diabetes Mellitus (T2DM) [7], [8]. In T2DM Caucasian patients showed that the frequencies of genotypes and alleles of the GPx-1 gene were 51% CC, 37% CT, 12% TT, and 70% C and 30% T [9], but in T2DM Iranian showed only CT genotype (100%) [7].

DM is a group of metabolic diseases characterized by chronic hyperglycemia that occurs due to abnormal defects in insulin secretion, insulin activity, or both. Almost 90% of people with DM are T2DM [10]. Previous epidemiological studies have shown that patients with T2DM are susceptible to infection. This happens because of an immune system disorder caused by hyperglycemia. Hyperglycemia causes disruption of vascularization so that it will reduce the ability of cells

to phagocyte. One of the infections that often attacks T2DM patients is a lung infection [11], [12]. Study by Narasimhan *et al*., showed that DM increased the risk of developing tuberculosis (TB) with RR value of 3.00 [13]. According to study conducted by Alisjahbana *et al*. showed that the prevalence of T2DM in TB patients at Indonesia is 13.3% in 2005 [14].

Previous studies in Medan population have proven that GPx-1 Pro198Leu polymorphism has TT and CT variant in TB patients [15], and CC, CT, and TT variant in T2DM patients [16], but until now the study about distribution of the GPx Pro198Leu variant in T2DM patients with TB have not been conducted yet. Therefore, based on the description above, this study aim to observed the distribution of the GPx-1 variant in TB-infected T2DM patients at Pulmonary Polyclinic Medan.

Methods

This study included forty T2DM patients that also infected by TB. The diagnose was based on endocrinologist and pulmonologist. The patient obtained from Pulmonary Polyclinic at Medan. The study was conducted after due approval of Faculty of Medicine, Universitas Sumatera Utara- Haji Adam Malik General Hospital ethics committee No. 447/KEPK FK USU-RSUP HAM/. The patients were asked to sign the informed consent as study patients after reading the objective and benefits of the study.

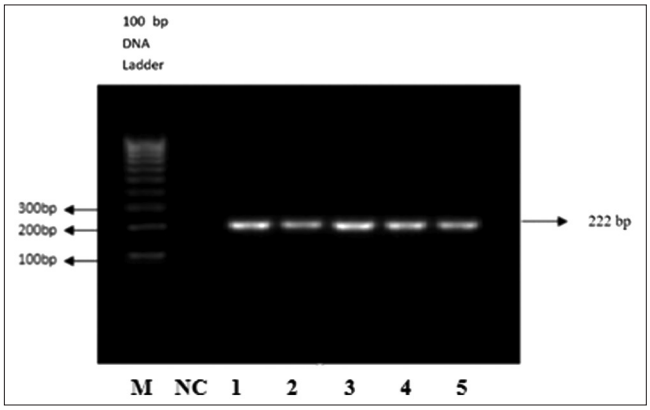

Figure 1: Polymerase chain reaction products analysis of GPx-1 gene visualized in 2% agarose gel electrophoresis. Lane M is a 100 bp DNA ladder, lane NC is a negative control, lanes 1–5 represent GPx-1 gene (222 bp)

Analysis of fasting glucose levels was measured by enzymatic colorimetric method using a spectrophotometer. Analysis of GPx-1 Pro198Leu polymorphism was done using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. All measurement process were performed at the Integrated Laboratory of Faculty of Medicine, USU. The following primers were used for amplification reaction was primer forward 5'-TCC AGA

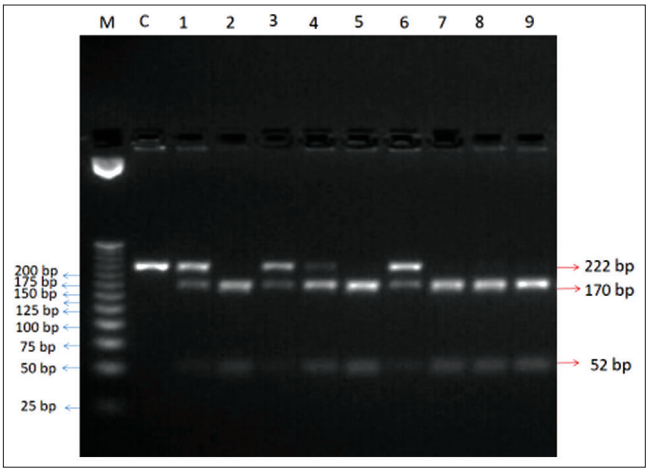

Figure 2: Polymerase chain reaction (PCR)-restriction fragment length polymorphism of glutathione peroxidase-1 Pro198Leu polymorphism visualized in 4% agarose gel electrophoresis. Lane M is a 25 bp DNA ladder, lane C represent PCR product as a control, lanes 2,5,7,8,9 represent homozygote CC (170 bp and 52 bp), lanes 1,3,4,6 represent heterozygote CT (222 bp, 170 bp, and 52 bp). Homozygote TT mutant was not found in this population

CCA TTG ACA TCG AG-3' and primer reverse 5'ACT GGG ATC AAC AGG ACC AG-3'(Promega, Madison, WI USA). PCR was carried out with a primary denaturation step at 94°C for 8 min, continued with 35 cycles of denaturation at 94°C for 30 s, primer annealing at 59°C for 30 s, elongation at 72°C for 30 s, and a final elongation at 72°C for 7 min. The PCR products containing polymorphic site was 222 bp fragment were digested 1 h at 37°C with Apa1 restriction enzyme. The result of Apa1 enzyme digestion was visualized with 4% agarose [16]. The fragment sizes were 170 bp and 52 bp for wild type (CC), 222 bp, 170 bp, 52 bp for CT heterozygote, and homozygote mutant was 222 bp (uncut). Variant was analyzed by direct counting and displayed descriptively using statistical package for social sciences software (SPSS v.21.0).

Results

This study has been conducted on forty TB-infected T2DM patients with the distribution of gender consisted of 14 women and 26 men. The median of age (years) and fasting glucose levels (mg/dl) in the study patients were 54.50 (36–77) and 282 (207–434). PCR and RFLP products from GPx-1 Pro198Leu polymorphism can be seen in Figures 1 and 2 and Table 1.

Discussion

In Indonesia, the study of GPx polymorphism has been conducted. The previous study by Yuniastuti

and Susanti on the population of TB patients at Semarang Public Health Care Center were found homozygote CC and heterozygote CT variant, homozygote TT mutant was not found [15], but study by Sari *et al*. showed there was CC, CT and TT varian in T2DM patients [16]. In this study, there were found the frequency of genotypes CC, CT, and TT were 82.5%, 17.5%, and 0%. Previous study in other country reported that T2DM patients were found all variants [17].

Table 1: Genotype and allele distribution

DM+TB	n	%
Genotypes		
CC	33	82.5
CT	7	17.5
TT	-	-
Alleles		
C	73	91.3
T	7	8.7

The difference results of this study is due to differences in the ethnic of study patients. The people with the different ethnic has specific polymorphism associated with a disease susceptibility cause <1% of all SNPs resulted in variation in proteins. In addition, the demographic and geographical conditions are also suspected as a factor that causes differences in study results. Genetic polymorphisms have proven to be a very important thing in human health studies. Researchers have found SNPs that can help predict a person's response to certain drugs or susceptibility to environmental factors such as poisons. In some studies, the identification of SNPs is used in association with complex diseases such as heart disease, cancer, diabetes, and susceptibility to infectious diseases [3], [18]. The T allele in GPx-1 Pro198Leu has been known to influence the activity of this enzyme in dealing with oxidative stress and this is related to the risk of suffering from certain diseases [5].

Some GPx-1 Pro198Leu studies in Indonesia that have been conducted to assess the enzyme activity alone without observing its association with SNPs in the enzyme gene, or only assess the association of variants of genes with the occurrence of certain diseases without linking to enzyme activity [15], [16], [19]. In the present study, there was also no examination of the GPx activity of the study patients, so that the distribution of gene variants in GPx Pro198Leu related to enzyme activity is still unknown. Further study needs to assess the association of the GPx-1 Pro198Leu gene variant and the GPx levels in the the TB group without T2DM, and the healthy control group, so that it can be assessed the risk of GPx-1 Pro198Leu gene polymorphism against these diseases. We expected that the results of this study became a feedback for further study.

Conclusion

It was concluded that in TB-infected T2DM patients at Pulmonary Polyclinic Medan, the GPx-1 Pro198Leu have CC variant higher than CT, whereas the TT genotype was not found. The frequency of the C allele is higher than T allele.

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