The tumor necrosis factor-α gene polymorphism (-308g/a) in type 2 diabetes mellitus patients with tuberculosis infection

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Abstract. The tumor necrosis factor (TNF)-α gene polymorphism (-308G/A) has been shown influence several diseases. This study aims to analyze the distribution of the TNF-α gene polymorphism (-308G/A) in type 2 diabetes mellitus (T2DM) patients with tuberculosis infection. Forty of T2DM patients with tuberculosis infection were recruited at Balai Pengobatan Lung Disease, Medan. Data collection of characteristics subjects were done through interviews using questionnaires. The blood glucose were measured with a spectrophotometer at a wavelength of 500 nm. Nco1 restriction enzyme was used to digested of gene polymorphism (-308G/A). Genotype frequency of the TNF-α gene was analyzed by direct counting. In this study shown that the TNF-α gene polymorphism (-308G/A) had 38 (95%) of G/G genotype and 2 (5%) of G/A genotype. No A/A genotype shown in this population. This preliminary result indicated that G/G genotype was common genotype in the TNF-α gene polymorphism (-308G/A) in T2DM patients with tuberculosis infection.

1. Introduction
Diabetes mellitus (DM) is a disorder of nutrition metabolism sign with elevated chronic blood sugar levels (hyperglycemic/ BGLs). This hyperglycemia condition can be affected by insulin resistance syndrome, insulin deficiency, or a combination of both. It leads to impaired of nutrition metabolism [1]. Based on data from WHO in 2015 found that about 415 million people suffer from DM and estimated that in 2040 this number will increase to 642 million people. DM and infection of tuberculosis often co-exist, especially in high-risk populations for tuberculosis infection but its still conflict whether DM preceding to tuberculosis infection or tuberculosis infection can leads DM [2]. WHO data shows, eight out of ten countries with the highest tuberculosis infection incidence are also countries with the highest number of DM patients. Indonesia as a country that ranked fourth in the
number of patients with most tuberculosis infection also ranked fourth in the number of patients with DM [3].

There will be an increase of proinflammatory cytokines in disorder of nutrition metabolism and infectious diseases. In DM, the excessive oxidation of glucose will increase reactive oxygen species (ROS). This process trigger lysis of lipid that results in lipid peroxides compounds such as malondialdehyde [4]. An inflammatory response arises in cells which cause the activation of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, interleukin (IL)-6, interleukin (IL)-18, etc. Previous study shown that the association between IL-6 level and diabetes mellitus [5] and the other study shown that association between TNF-α level and diabetes mellitus [6].

The TNF-α is a cytokine that arise in inflammation condition to regulate activation, differentiation, and proliferation of cell. The cytokine was regulating the continuity of the cell. The TNF-α structure in the form of 17 kilodaltons (kDa) protein was constructed from 185 amino acids. Coding of TNF-α genes are on chromosome 6 (6p21.3). TNF-α synthesis is regulated by specific TNF-α gene sequences. Single nucleotide polymorphism (SNPs) or substitution of one nucleotide base in the TNF-α gene squences influence the synthesis or levels of TNF-α. The SNIPs of TNF-α gene was postulated as a key genetic factor in several diseases. In the gene polymorphism (-308G/A) shown that the substitution of a guanine (G) nucleotide by an adenine (A) nucleotide [7].

The previous studies in Iran shown that there was an association between of TNF-α gene polymorphism (-308G/A) with the risk of tuberculosis infection [8,9]. The other previous studies also shown significant associations between the TNF-α gene polymorphism (-308G/A) and the risk of type 2 diabetes mellitus (T2DM) [10,11]. No study of the gene polymorphism (-308G/A) in in T2DM patients with tuberculosis infection has been reported Based on the results of previous research, the current study would like to see how the distribution of TNF-α gene polymorphism (-308G/A) in T2DM patients with tuberculosis infection at Balai Pengobatan Lung Diseases, in Medan city.

2. Method
This study was a cross-sectional design and conducted on February to June 2018 in outpatient at Balai Pengobatan Lung Diseases, Medan City. The study was conducted after all the procedure approved by the ethics committee of the Faculty of Medicine, Universitas Sumatera Utara (USU) with the letter of ethical approval No. 327/KEPK FK USU-RSUP HAM/. All subjects were asked to written informed consent after receiving explain of the benefits of the study.

2.1 Subject selection
A total of forty T2DM patients with tuberculosis infection were obtained from Balai Pengobatan Lung Diseases, Medan City. The disease of subjects are diagnosed by Pulmologist and Endocrinologist according to the criteria of Indonesian Lung Doctor Association [12] and criteria of Indonesian Endocrinology Society [13].

2.2 Sample collection
The venous blood sample (5 ml) was collected from the median cubiti vein of patients. The procedure was using the plain and EDTA vacuum tube syringe. The blood sample was centrifuged for 10 minutes at a rate of 3000 rpm. Serum was separated from the plain blood sample. The level of blood glucose was evaluated within 2 hours after being collected. Whole blood sample was separated from the EDTA blood sample. Whole blood sample was used as a sample to DNA isolation process using the commercial kit (Promega). Isolat of DNA was then stored at a temperature of -80° C for used to the next stage of amputation.
2.3 Level of Blood Glucose Measurement
The blood glucose was measured by the GOD-PAP enzymatic method using glucose assay kit. Examination of the blood glucose was done at Faculty of Medicine Laboratory in Universitas Sumatera Utara (USU) using a spectrophotometer with the wavelength of 500 nm.

2.4 Polymerase Chain Reaction (PCR) of TNF-α gene
DNA amplified with polymerase chain reaction (PCR) method. Amplified was done using primer ie [14];

The forward primer 5’-AGGCAATAGGTTTGGAGGCCAT-3’ and
The reverse primer 5’-TCCTCCCTGCTCCGATTCG-3’

Before performing the amplified process, PCR solution was created first (25 µl) according to the standard procedural at Faculty of Medicine Laboratory, USU. The PCR conditions involved an initial denaturation of DNA at 94°C for 4 min, followed annealing process (35 cycles) at temperatures of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min. PCR products were visualized on gel electrophoresis using gel documentation system [15].

2.5 Restriction Fragment Lenght Polymorphism (RFLP)
Five unit of restriction endonuclease Nco1 enzyme was digested the PCR reaction mixture at 37°C for two hours. The RFLP product was visualized by electrophoresis technique at 4% agarose gel [15].

2.6 Statistical Analysis
SPSS version 22 in association with Microsoft Excel 2010 was used for statistical analyzes. G and A were analyzed to see genotype distribution of the TNF-α gene polymorphism (-308G/A) by direct counting.

3. Results and Discussions
This study was done on forty of the T2DM patients with tuberculosis infection. The characteristics of the subjects in this study are shown in Table 1.

| Characteristics                  | mean±SD; %               |
|----------------------------------|--------------------------|
| (N=40)                           |                          |
| Age (years)                      | 53.26±8.77               |
| Gender (male/ female)            | 26(65.0)/14(35.0)        |
| Duration of disease (years)      | 3.61±1.38                |
| Blood glucose levels ad random   | 295.51±57.85             |
| (mg/dl)                          |                          |

Table 1 shows the mean values of age of DM patients with tuberculosis infection was 53.26 years (SD = 8.77). Age is the risk factors for suffering diseases, such as infectious disease and metabolic disorder. This is probably caused by some changes in body composition which was created by the aging process. Aging causes a decline in the immune system and the function of the organs of the body [16].

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 tuberculosis infection, but gender may be a risk factor for DM. Females are more likely to have DM than males [17]. A 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 DM [18]. 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 long duration of DM will cause the parameters of BGLs will get worse [19]. The high level of BGLs showed that DM disease in this group still not well controlled.

The amplified PCR products of TNF-α gene was 107 bp fragment. PCR is a method for detecting gene molecules of samples from blood, urine, saliva and, etc. PCR is an enzymatic method for DNA amplification in vitro. PCR products can be identified using agarose gel electrophoresis. PCR products were read in base pairs. Currently, the PCR-based molecular method has been designed to analyze gene polymorphisms. One of the methods that can be used to find out the TNF-α gene polymorphism is "PCR-Restriction Fragment Length Polymorphism" (PCR-RFLP) [1].

In this research, the product of the TNF-α gene that digested by the Nco1 enzyme can be seen in Figure 1.

![Figure 1. PCR-RFLP product of TNF-α gene (-308G/A) polymorphism on 4% agarose gel electrophoresis.](image)

In Figure 1, it can be seen that after the digestion with Nco1 restriction enzyme, homozygous wild-type G/G showed two 20 bp and 87 bp; heterozygous G/A showed three bands at 20 bp, 87 bp and, 107bp; and homozygous mutant A/A (no found in this study subjects) showed an unbroken gene at 107 bp. Restriction enzymes are compounds that can cut the gene on certain positions at separate locations. This cut will be resulting in some fragment of DNA [1].

In this research, the distribution of the genotype of the TNF-α gene (-308G/A) polymorphism can be seen in Table 2.

| Genotype | N = 40 (%) |
|----------|------------|
| G/G      | 38 (95)    |
| G/A      | 2 (5)      |
| A/A      | 0 (0)      |

In the table 2 can be seen, a high frequency of G/G was found in T2DM with tuberculosis infection patients (95%). No A/A genotype was detected in this study groups. The results of this study are similar to the previous research by Ceylan et al. (2017). Ceylan et al. (2017) found in tuberculosis infection patients had higher G/G genotype than G/A genotype, and no
had A/A genotype [15]. In the research of Matenat et al. (2013) showed that A/A genotype was not found in pulmonary tuberculosis and extra-pulmonary tuberculosis infection groups, but it’s found in control healthy groups [20]. The previous research of the TNF-α gene (-308G/A) polymorphism in T2DM patients at Mexico and Indian show similar results with this study. In the previous studies showed that in T2DM patients had higher G/G genotype than G/A genotype, and A/A genotype was less compared with the others [21,22]. But, previous study inT2DM Egyptian patients showed G/A genotype higher compared with G/G genotype [13]. G/G genotype was homozygous wild type in TNF-α gene. The polymorphism was involved the substitution of a guanine (G) by an adenine (A) in the promoter region of this gene results G/A and A/A genotypes.

Genetic polymorphisms are population specific, affected by ethnic [23]. Different ethnic in Medan city with other city in the world may be a cause G/G genotype was common, G/A genotype was rare, and A/A genotype was not found in this study groups.

4. Conclusion

In this research shows that the G/G genotype was common in Type 2 Diabetes Mellitus patients with tuberculosis infection. Further research with different ethnicities is required to confirm these findings.

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