IDENTIFICATION OF POINT MUTATION OF TRNA GENES IN 20 TYPE 2 DIABETES MELLITUS JAVANESE PATIENTS IN YOGYAKARTA, INDONESIA

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Abstract—Background: Molecular information of type 2 diabetes mellitus (DMT2) patients in Javanese tribe was still not available. One of the factors contributing in this disease is genetic. Point mutations in mitochondrial DNA are associated with the condition of DMT2. This study aimed to identify the A3243G mutation of tRNA gene in DMT2 Javanese patients from Wates Hospital at Yogyakarta, Indonesia. Methods: This descriptive study was done in laboratory with PCR and sequencing. From 160 patients in one month in Wates Hospital (Yogyakarta, Indonesia), was chosen randomly to get 100 patients. From 100 patients that were done with DNA isolation, there was 20 DNA samples in the best condition. This research was conducted on March to June 2012 at laboratory of biochemistry FMIPA Universitas Negeri Jakarta and laboratory of Clinical Technology, Faculty of Health Science, Yamaguchi University School of Medicine Result: of 20 DMT2 Javanese patient samples, there was no mutation in a tRNA gene. Conclusion: This research has been successfully identified the nucleotide of the tRNA gene fragment in 20 DMT2 Javanese patients in Yogyakarta and there was no mutation in the tRNA gene.

INTRODUCTION

Type 2 diabetes mellitus (DMT2) is a chronic metabolic disorder that is caused by insulin deficiency and insulin resistance (Lim et al., 2012). In Asia, Indonesia has the most diabetes mellitus cases (Ibrahim et al., 2010). It was predicted there would be a rise of DM patients from 285 million in 2010 to 439 million in the world (Shaw, et al., 2010). The most common type of DM is DMT2. The number of adults with diabetes in Indonesia is expected to rise from 6.9 million in 2010 to 12 million in the year 2030 (Soewondo et al., 2010). The prevalence of urban Indonesia was 5.7% whereas that of impaired glucose tolerance was 10.2% (Soewondo, et al., 2010). DMT2 is influenced by a few factors that consist of environment, food habit, and genetics. Genetic factor is one that has currently been studied in molecular studies. DMT2 is related to the genes in mitochondrial DNA (mtDNA). MtDNA is a circular double chain DNA with the length of 16,569 pairs bases that consists of two rRNA genes, 22 tRNA genes, and 13 subunit protein genes for complex respiration chain, including NADH dehydrogenase 1 (ND1) gene (Anderson et al., 1981). In DMT2 patients, changes in the sequence of base nucleotides, which are called mutation, involve in several mtDNA. The common type of mtDNA mutation found in DMT2 patients is the mutation in tRNA gene, in site of A3243G (Zhong et al., 2000)

Gene mutation generally occurs in DMT2 patients. Related to DMT2, mutations that have been found in Indonesia are T3398C, T3200C, and C3206T

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These different mutation leads to continuous inquiry to find more mt DNA point mutation in DMT2 patients (Puspitaningrum, 2014). Genetic factors can be studied in some tribes in Indonesia, one of which is DMT2 Javanese patients in Yogyakarta.

DMT2 patients in Yogyakarta Province was risen from 150 patients per 10,000 people in 2004 to 3.3% in 2011 (Yogyakarta Province Health Services, 2012). In 2007, the total number of DMT2 patients at Wates Hospital (Yogyakarta Province) was 124 and rose to 160 patients of DMT2 per month in 2012 (Dinas Kesehatan Jawa Tengah, 2008). Despite the large number of patients and its rapid growth, molecular data on DMT2 Javanese patients is still very limited.

So, this research aimed to identify the nucleotide sequence of fragments of tRNA genes in DMT2 patients of the Javanese tribe at Yogyakarta. Through sequencing technique, the nucleotide of tRNA gene of mtDNA from PCR products could be known as a whole and also its mutation or other changes.

**METHODS**

This research had been submitted to Faculty of Medicine Universitas Indonesia ethics commission with numbers of 532/PT02.FK/ETIK/2012.

**Selection** - This descriptive research was done in Wastes Hospital for collecting subjects and interviewing to obtain DM2 information and in laboratory with PCR and sequencing. This research was conducted on March to June 2012. From 160 patients a month, 100 DMT2 patients was randomly selected as the sample of this research. Subjects must fulfill the criteria of DMT2 patients from Java descendants (Middle Java or East Java) with age range was 35-60 year old. All 100 DMT2 patients had been drawn for the blood sera. Blood sampling was assisted by health workers and isolation DNA was done. We only found 20 DNA samples in the best condition. It was indicated by the purity index of DNA of 1.75-2 (Puspitaningrum, 2014).

**Technical** - DNA extraction and PCR were held in Biochemistry and Molecular Biology Laboratory, Biochemistry Department, Faculty of Mathematics and Natural Sciences Universitas Negeri Jakarta. Meanwhile the sequencing analysis was held in the Laboratory of Clinical Technology, Faculty of Health Science, Yamaguchi University School of Medicine.

**DNA Extraction**

Mitochondrial DNA extracted from 3 mL of whole blood using Promega Extraction Kit® with modifications. The modifications included the using of isopropyl alcohol to get the DNA. The DNA samples were stored at -20°C before analysis.

**Polymerase Chain Reaction (PCR)**

PCR was done to amplificate tRNA gene of mtDNA using primer of Mt3243 forward 5’-AGG ACA AGA GAA ATA AGG CCT-3’ & Mt3243 reverse 5’-AAC GTT GGG GCC TTT GCG T-3’. The PCR products of 294 bp of tRNA fragment only resulted in a total volume of 50μL. DNA was initially denaturized at 94°C for 3 minute and subjected to 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Amplified products were confirmed by performing electrophoresis in 2% agarose gel.

**Sequencing**

Approximately 2μL of purified PCR product using purification protocol by Qiagen® was amplified in a total volume of 20μL. The amplified gene was precipitated after several times of alcohol washes at 95% and 70%, dried using a vacuum centrifuge, resuspended in Hi-DI formatted, and loaded on to ABI 3130xl Sequence Analyzer, Applied Biosystems for Sequencing. Sequence editing and analyzing were done with the software of Sequencer® 5.0.1. For Machintosh®.

**RESULTS**

The amplified area of 294bp of tRNA gene fragment with reverse and forward primer mt3243 is shown in Figure 1. The blue nucleotide sequence shows the

**Fig. 1.** 294bp tRNA gene fragment product PCR was amplified by primer mt3243 reverse and forward. Blue: coding area of gene 16S rRNA, red: coding area of tRNA , black: coding area of ND1. (12)
coding area of 16SrRNA, the red one for coding area of rRNA, and the black order for ND1 gene area (Gene Bank NCBI).

PCR product of 294bp positive band was running in 2% agarose gel as shown in figure 2. Lane 1 to 20 showed DNA samples (Fig. 2). All sequencing of PCR product showed 100% homolog with human tRNA gene mtDNA on DDBJ (DNA Data Bank of Japan) Figure 3. P01 is a code for one sample which was compared to the data of human tRNA gene of mtDNA using BLASTX (basic local alignment search tools) programme used in online on DDBJ (Fig. 3).

**DISCUSSION**

All 20 DMT2 Javanese patients had been successfully showed for homolog analysis. This means all samples that were analyzed were exactly the same with the nucleotide tRNA gene of human mtDNA in the gene bank data (Gene Bank NCBI) (Fig. 3). There is no SNPs of tRNA gene of mtDNA region that found in this study. It is the same with

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**Fig. 2.** 294bp PCR product of samples P01-P20 was running by 2% Agaraose gel electrophoresis. Lane M is 100bp DNA Ladder. Lane 1- 20 are PCR products of 294bp tRNA gene fragments in DMT2 Javanese patients.

**Fig. 3.** Homology level of tRNA gene fragments - P01 sample

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the study in Gorontalo tribe (Puspitaningrum, 2014). Actually, A3243G mutation is the most common mutation in tRNA region related to DMT2 (Katagiri et al., 1994).

The A3243G mutation in tRNA gene of mtDNA was previously reported in Japanese population (Ohkubo et al., 2001). Pakistani population (Sharif, 2010, unpublished data), Chinese Han population, Egyptian population, and Finnish population (Majamaa et al., 1998). However, several investigators did not found this A3243G mutation. Rigoli et al., (1997) failed to detect the A3243G mutation from 231 DMT2 patients in Southern Italian population. Wahid and Naveed (2009) also found no A3243G mutation in 39 DMT2 Pakistani patients. There was also no mutation in 129 MIDD (maternally inherited diabetes and deafness) patients in Polish population (Ma et al., 2001.), 10 DMT2 patients of Tamil Indians (Lepretre et al., 1998), and 112 DMT2 patients of Nigerian population (Kunsan et al., 1998).

The absence of the tRNA gene mtDNA mutation in this study was probably caused by several factors. First, it may be caused by the low number of sample. Ohkubo et al., (2001) found 2.9% of the A3243G mutation in 240 DMT2 patients in Japan, while Sharif (2010) found 1.5% of this mutation in 120 DMT2 Pakistani patients. Kunsan et al. (1998) found 0.5% of 207 DMT2 Chinese patients and only 5.71% of 100,000 DMT2 patients globally were detected A3243G mutation (Maassen et al., 1996). Meanwhile, it was stated that the prevalence of this mutation was approximately 1.5% of diabetic population worldwide (Gerbitz et al., 1995).

Second, all of the DMT2 patients in our study did not show any severe clinical characteristics like in MIDD and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) patient such as acute deafness, obesity, and stroke-like syndrome (Kanaumi et al., 2006). Both of MIDD and MELAS were previously reported as diseases caused by the A3243G mutation (Solano et al., 2000). Relationships between aging and prevalence of DMT2 are the increase of intracellular lipid and insulin resistance because of the decrease of exercise (Shanske et al., 2004).

Next, previous study reported that leucocytes (blood) had the lowest manifestation A3243G mutation of tRNA gene mtDNA compared to skeletal muscle (Krssak et al., 1999), urinary sediment (Kelley et al., 2002), skin fibroblast, hair roots, and cheek mucosa (Schaefer AM, et al., 2007).

It is also reported that the A3243G mutation of the blood was very low in elderly due to the decrease of heteroplasmy levels of mutant mtDNA in leucocytes upon aging (Schaefer et al., 2007). Of these reasons, we still could not state that there is no A3243G mutation of tRNA in DMT2 Javanesse patients prior to further investigation from other tissues.

However, out of the 20 DMT2 patients, there was no A3243G mutation found or other mutations in tRNA genes. There were 2% of 100 DMT2 patients in Indonesia who had A3243G mutation, but the research was done using PCR RFLP (Hart et al., 1996). Therefore, mutation A3243G was rather uncommon among DM patients in Indonesia. The mutation is a heteroplasm, which means it has natural and mutant mtDNA in the same cell (Sue et al., 1998). From this result it could be concluded that DMT2 may be most likely due to environmental interactions.

DNA samples were isolated from white blood cells because these cells were commonly used by many researchers to get mtDNA. However, white blood cells have the lowest mutant DNA among other cells. This may affect mutation finding which is heteroplasmic in nature, such as A3243G mutation of tRNA genes. The level of mtDNA which mutate with nature DNA (wild type) was different in each tissue (Naveed, et al., 2009). The highest mutation level occured in the tissues that actively conducted mitosis such as endocrine (pancreatic) tissue (Lindroos, et al., 2009).

CONCLUSION

This research has been successfully identified the nucleotide of the tRNA gene fragment in 20 DMT2 Javanesse patients in Yogyakarta and there was no mutation in the tRNA gene. Because of limitations in this study, a total of 100 DNA samples were obtained only taken randomly 20 samples with the characteristics of the amount and DNA purity value are best can be analyzed.

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Identification of Point Mutation of tRNA Genes in 20 Type 2 Diabetes Mellitus Javanese

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