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tRNA^{leu} A3243G Gene Mutation of Mitochondrial DNA as a Risk Factor for Diabetic Retinopathy in Type 2 Diabetes Mellitus in Bali

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ABSTRACT

Introduction: Diabetic retinopathy is one of the chronic complications in patients with diabetes mellitus caused by progressive microangiopathy. Various types of risk factors can affect the occurrence of diabetic retinopathy, one of which is mitochondrial DNA mutations in the tRNA^{leu} A3243G gene that is common in T2DM. This study was conducted with the aim to identify whether the mutation of the tRNA^{leu} A3243G gene acts as a risk factor for diabetic retinopathy in T2DM patients in Bali.
Material and Methods: This study used a case control design with 35 T2DM patients with diabetic retinopathy and 35 T2DM patients without diabetic retinopathy. The techniques used to identify these mutations are PCR and DNA sequencing.
Results: Based on the results obtained, no mutations were found in the tRNA^{leu} A3243G gene in the entire sample. Therefore, the relationship analysis of the two variables cannot be identified.
Conclusions: Mutations of the tRNA^{leu} A3243G gene that were not successfully identified in this study can be concluded not play a role as a risk factor for diabetic retinopathy.

Keywords: Diabetic Retinopathy, Diabetes Mellitus, tRNA^{leu} Gene Mutation, Mitochondrial DNA

INTRODUCTION

The health problem that occurs in Indonesia today is the increasing prevalence of non-communicable diseases compared to infectious diseases. Non-communicable diseases have become the top ten causes of death nationally and globally. One of them is Diabetes Mellitus (DM) which is a metabolic disease that lasts progressively, chronic, and related to the function of insulin hormone. People with diabetes reached 415 million in 2015 and increased to 425 million people in 2017. Indonesia is in the sixth position with the highest number of diabetic population in the world and becomes the third highest cause of death after stroke and coronary heart disease.[1,2] There are two classifications of DM, Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). But T2DM has a higher prevalence with a 95% incidence proportion of the world's population.[3] Complications of diabetes that can occur are macrovascular and microvascular complications. One of the microvascular complications is diabetic retinopathy. Research states that T2DM patients have a 25 times greater risk of developing complications such as diabetic retinopathy.[4]

Diabetic retinopathy is a progressive state of microangiopathy and microvascular damage to the retina. This complication often occurs in patients aged 20-64 years and becomes the most common cause of blindness in diabetics.[5] Diabetic retinopathy is in the fourth position as a cause of blindness after cataracts, glaucoma, and macular degeneration. Risk factors that can affect the occurrence of diabetic retinopathy are hypertension, lipid profile, HbA1c levels, age, duration of diabetes, and genetic factors.[6]

The occurrence of T2DM caused by impaired insulin secretion is related to inhibition of adenosine triphosphate (ATP) production that associated with mutations in the tRNA^{leu} A3243G gene. Study on patients who experience mitochondrial disease due to mutations in the A3243G gene has been conducted in various countries including Indonesia. Research in Taiwan found the presence of A3243G mutation that causes diabetes is 0.15%, in Poland successfully identified diabetic mellitus patients with mutation A3243G, in Japan there were 2.9% of DM patients who also had A3243G mutations, in China studies were conducted on MELAS patients and found A3243G mutations in all patients, in UK found 2 out of 268 diabetic patients have A3243G mutation, in Korea found the presence of mutation A3243G in 22.3% of patients who experience mitochondrial disease, in Croatia 10% of patients with T2DM had A3243G mutations, and in Spain 18% of children had three heteroplasmy mutations including A3243G. Studies related to the mutation of the tRNA^{leu} A3243G gene were also conducted in Indonesia. Some studies have not been successful in identifying the occurrence of A3243G mutations in T2DM patients. But a study conducted by Maksum et al, 2010 was able to identify the presence of A3243G mutations in 2 out of 101 samples that experienced T2DM.[7]

Therefore, the study of mtDNA mutations related to T2DM needs to be developed, because it is related to the maternal relationship of T2DM patients with families who are still at risk of experiencing similar things. If the diagnosis can be established early, the development of complications in T2DM, especially diabetic retinopathy, can be prevented and able to handle the early stages to prevent blindness.[8]

MATERIAL AND METHODS

Study Design

This study was a case control study with 70 samples and consisted of 35 T2DM samples with diabetic retinopathy as the case group and 35 T2DM samples without diabetic retinopathy as the control group.

DNA Amplification

The amplification of the tRNA^{leu} mtDNA gene was carried out by PCR technique using forward primer 5'-AGG ACA AGA GAA ATA AGG CCT-3' (nt3130–3149) and reverse primer 5'-AAC GTT GGG GCC TTT GCG T-3' (nt3423–3404), amplify fragments up to 293bp. The PCR process was carried out in an Automatic Thermo Cycler PCR machine for 35 cycles. The initial stage of PCR is initial denaturation at 94^0C for 5 minutes, then enters the PCR cycle program with each cycle consisting of three stages, starting with denaturation at 94^0C for 1 minute, annealing at 55.9^0C for 1 minute, and primary extension at 72^oC for 1 minute. At the end of all cycles, an additional extension process was carried out at 72^0C for 5 minutes. PCR was performed using the GoTaq® Green Master Mix PCR Kit from Promega.

Electrophoresis and Gene Sequencing

Amplification products was analyzed by performing an electrophoresis procedure with 1% agarose gel which aims to visualize DNA fragments. To detect mutations in the tRNA^{leu} A3243G gene, all PCR products were prepared for sequencing at Genetic Science Indonesia, Jakarta and the sequencing results were interpreted through NCBI BLAST Tools and Chromas.

STATISTICAL METHODS

The sequencing results obtained will be analyzed bivariately to prove the relationship between the diabetic retinopathy variable and the mutation of the tRNA^{leu} A3243G gene by performing chi square test if it meets the requirements.

RESULTS

This study was conducted from July to November 2021 at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University. Ethical eligibility permission has been obtained from the ethics committee of the Udayana Faculty of Medicine with number 490/UN14.2.2.VII.14/LT/2021. The total samples used were 70 samples consisting of 35 T2DM samples with diabetic retinopathy and 35 T2DM samples without diabetic retinopathy. This sample is a stored biological material (BBT), so the characteristics data of the sample have been obtained in previous studies.

Polymerase Chain Reaction (PCR) procedure was carried out to amplify the amount of DNA encoding the tRNA^{leu} gene in the DNA isolate samples of type 2 diabetes mellitus patients used in this study. After that, an electrophoresis procedure was carried out to see the results of gene amplification from the previous PCR procedure.

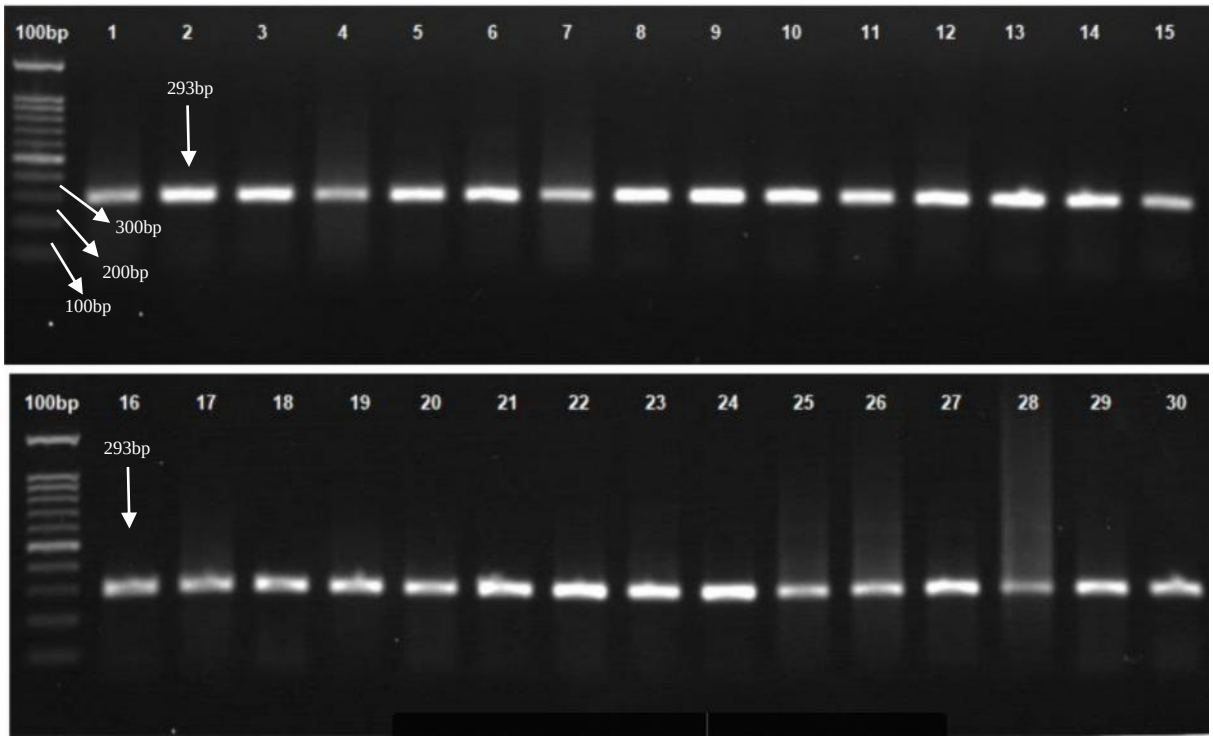

Image 1. Overview of PCR Results through Electrophoresis Gel

Based on the results of the gene sequencing which conducted on the case and control group samples, it was found that there was no mutation of the tRNA^{leu} A3243G gene in all samples. So that the analysis of the relationship between mutations in the tRNA^{leu} A3243G gene and diabetic retinopathy could not be identified because there were no mutations in the entire sample.

Table 1. Results of Analysis of the Relationship between Mutations of the tRNA^{leu} A3243G Gene T2DM Samples With and Without Diabetic Retinopathy

tRNA^{leu} A3243G gene mutation	**DR**	**NDR**	***p value***
Mutation	0 (0%)	0 (0%)	-
No Mutation	35 (50,0%)	35 (50,0%)	-

Homo sapiens mitochondrion, complete genome

Sequence ID: MN692242.1 Length: 16567 Number of Matches: 1

Range 1: 3174 to 3421 GenBank Graphics ▼ Next Match ▲ Pr

Score	Expect	Identities	Gaps	Strand
451 bits(244)	1e-122	247/248(99%)	1/248(0%)	Plus/Plus

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Query  23    AATG-TATCATCTCAACTTAGTATTATACCCACACCCACCCAAGAACAGGGTTTGTTAAG  81
             ||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3174  AATGATATCATCTCAACTTAGTATTATACCCACACCCACCCAAGAACAGGGTTTGTTAAG  3233

Query  82    ATGGCAGAGCCCGGTAATCGCATAAAACTTAAAACTTTACAGTCAGAGGTTCAATTCCTC  141
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3234  ATGGCAGAGCCCGGTAATCGCATAAAACTTAAAACTTTACAGTCAGAGGTTCAATTCCTC  3293

Query  142   TTCTTAACAACATACCCATGGCCAACCTCCTACTCCTCATTGTACCCATTCTAATCGCAA  201
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3294  TTCTTAACAACATACCCATGGCCAACCTCCTACTCCTCATTGTACCCATTCTAATCGCAA  3353

Query  202   TGGCATTCCTAATGCTTACCGAACGAAAAATTCTAGGCTATATACAACTACGCAAAGGCC  261
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3354  TGGCATTCCTAATGCTTACCGAACGAAAAATTCTAGGCTATATACAACTACGCAAAGGCC  3413

Query  262   CCAACGTT  269
             ||||||||
Sbjct  3414  CCAACGTT  3421
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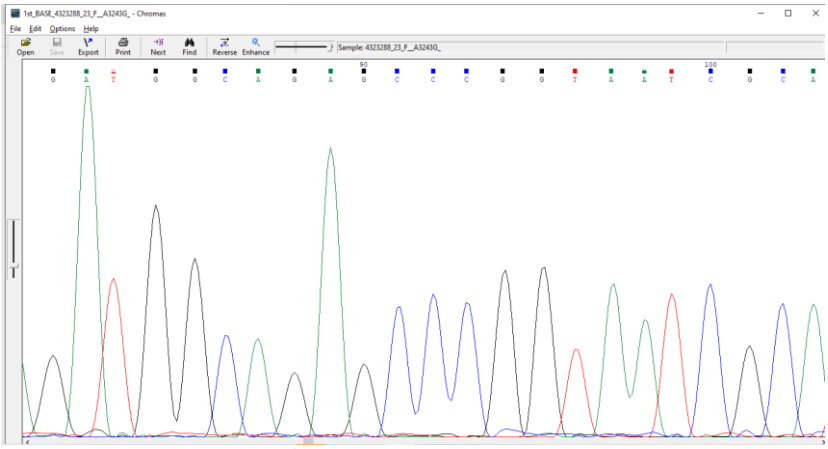

Image 2. Sequencing Results of T2DM Patients With and Without Diabetic Retinopathy

DISCUSSION

Diabetes mellitus is a metabolic disease caused by pancreatic beta cells damage and due to mutations in mitochondrial DNA in genes associated with T2DM, where these mutations affect insulin sensitivity and secretion. Based on the research conducted by the authors related to mitochondrial DNA mutations in the tRNA^{leu} A3243G gene that occurred in diabetic retinopathy patients in Bali, from 70 samples consisting of 35 samples of T2DM patients without diabetic retinopathy as a control group and 35 samples of T2DM patients with diabetic retinopathy as the case group did not find any mutations in the entire sample. So that the analysis of the relationship between tRNA^{leu} A3243G gene mutation and diabetic retinopathy can not be done.

This study is in line with several studies conducted on T2DM patients to identify A3243G mutations. Research by Khan et al, 2020 stated that among the point mutations that occur and associated with a base change from A to G at position 3243 in the tRNA^{leu}(UUR) gene, become the most common causative mutation of T2DM. This study was conducted on five samples with T2DM in Pakistan, where the results obtained were that there was no A3243G mutation found in the tRNA^{leu}(UUR) gene.[9] This study is also in line with research by Ameh et al, 2011 which was conducted on 112 samples of T2DM patients and had a family history of diabetes mellitus. Amplification of the tRNA^{leu}(UUR) gene was carried out to see the presence of A3243G mutations using the PCR-RFLP method. Of the 112 samples studied, there was no mutation found in the A3243G tRNA^{leu}(UUR) gene in the form of homoplasmy or heteroplasmy.[10]

Several studies related to the A3243G mutation with T2DM have also been carried out in Indonesia and have results that are in line with this study. Research conducted by Pranoto, 2007 in Surabaya on 451 T2DM patients with a family history of diabetes mellitus and five people from the entire population experiencing sensory deafness based on audiological examination. DNA samples were obtained from leukocytes in small sizes and were amplified by PCR using two pairs of specific primers to narrow the amplification area. Based on this study, there were no mutations found in all samples with T2DM with a positive family history and T2DM with sensory deafness.[11] Another study was also conducted on the Gorontalo and Javanese tribes by Ishak et al, 2014 which consisted of 20 T2DM patients with an age range of 35 to 60 years and used PCR and sequencing methods. Based on the sequencing results obtained, the A3243G mutation was not found in 20 patients with T2DM and 20 normal controls.[12]

There is study that inversely proportional to this study, which was conducted by Sriwidodo et al, 2008 succeeded in identifying the occurrence of mitochondrial DNA mutations in the tRNA^{leu} A3243G gene in T2DM patients using the PASA method with PCR technique. This study used two tubes containing universal primers D1 and normal primers Dn and the second tube containing universal primers D1 and mutant primers Dmt, characterization of the fragments formed in PASA will result in differences in normal alleles, homoplasmy mutations, and heteroplasmic mutations. The mutation was declared positive if a band measuring 200 bp was seen in the tube containing the mutant primer or Dmt primer. In this study found that 10 T2DM patients had the A3243G mtDNA mutation.[13] This study has similar results to the study by Maksum et al, 2010 which consisted of 101 T2DM patients in Bandung. The sample used was blood cells because the sampling is relatively easy and has been used in previous studies that succeeded in identifying the A3243G mutation. The results obtained were the discovery of two samples with A3243G mutations based on RFLP characterization with the ApaI restriction enzyme.[7]

The absence of mutations in the tRNA^{leu} A3243G gene in diabetic retinopathy patients in this study could occur due to several factors such as the number of samples that were too little or the sample size used was too small. Based on a study conducted in Japan on 240 T2DM patients, it was found that only 2.9% had the A3243G mutation, in addition, a study conducted in China on 207 T2DM patients found that only 0.5% had mutations, and in a worldwide study involving 100,000 T2DM patients, only 5.71% had the A3243G mutation.[14,15] Several studies have stated that DNA samples taken from leukocytes have the lowest incidence of A3243G mutations when compared to DNA samples taken from skeletal muscle, urine sediment, fibroblast tissue in the skin, hair roots, and the cheek mucosa.[16,17,18] The absence of the A3243G mutation can be influenced by various factors, so further research needs to be done to determine the relationship between the A3243G mutation and using a larger sample population and adjust better sampling techniques, so that the genetically inherited A3243G mutation can be detected earlier to prevent one of the microvascular complications, diabetic retinopathy.

CONCLUSIONS

Based on the results of the study, it can be concluded that:

1. No mitochondrial DNA mutation was found in the tRNA^{leu} A3243G gene in 70 samples consisting of 35 T2DM with diabetic retinopathy as a case group and 35 T2DM without diabetic retinopathy as a control group.
2. The relationship between the two variables could not be analyzed so that mutations in the tRNA^{leu} A3243G gene were not a risk factor for diabetic retinopathy.

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REFERENCES

1. Vidyanto V, Arifuddin A. Determinan peningkatan kadar gula darah pasien interna rumah sakit umum (RSU) Anutapura Palu. Healthy Tadulako Journal (Jurnal Kesehatan Tadulako). 2019;5(1):58-62.
2. Fitriyanti ME, Febriawati H, Yanti L. Pengalaman Penderita Diabetes Mellitus dalam Pencegahan Ulkus Diabetik. Jurnal Keperawatan Muhammadiyah Bengkulu. 2019 Nov 7;7(2):99-105.
3. Suryani N, Septiana H. Diet dan Olahraga sebagai Upaya Pengendalian Kadar Gula Darah pada Pasien Diabetes Melitus Tipe 2 di Poliklinik Penyakit Dalam RSUD Ulin Banjarmasin Tahun 2015. Jurnal Kesehatan Indonesia. 2016 Sep 9;6(2).
4. Lathifah NL. Hubungan durasi penyakit dan kadar gula darah dengan keluhan subyektif penderita diabetes melitus. Jurnal berkala epidemiologi. 2017;5(2):231-9.
5. Utami DR, Amin R, Zen NF. Karakteristik Klinis Pasien Retinopati Diabetik Periode 1 Januari 2014–31 Desember 2015 di RSUP Dr. Mohammad Hoesin Palembang. Majalah Kedokteran Sriwijaya. 2017;49(2):66-74.
6. Annisa Y, Romdhoni F. Perbandingan resiko terjadinya retiopati diabetik antara pasien hipertensi dan non hipertensi yang mengidap diabetes mellitus di RSUD Majenang. MEDISAINS. 2017 Jul 15;15(1):31-8.
7. Maksum IP, Sriwidodo S, Suprijana O, Natadisastra G, Nuswantara S, Noer AS. Identifikasi Mutasi Heteroplasmi A3243G DNA Mitokondria dan Studi Pewarisan Maternal pada Pasien Diabetes Melitus Tipe 2. Bionatura. 2010;12(2):218311.
8. Fischel-Ghodsian N. Mitochondrial DNA mutations and diabetes: another step toward individualized medicine. Annals of internal medicine. 2001 May 1;134(9_Part_1):777-9.
9. Khan NM, Ullah H, Raziq A, Khan AA, Khan MW. Molecular genetic analysis of leucine tRNA in relevance to type 2 diabetes mellitus. Clinical Diabetology. 2020;9(3):167-73.
10. Ameh J, Godwin I, Obi I, Puepet F, Aminu B, Suleiman T. The search for mitochondrial tRNALeu (UUR) A3243G mutation among type 2 diabetes mellitus patients in the Nigerian population. African Journal of Biotechnology. 2011;10(62):13383-9.
11. Pranoto A. A3243G mitochondrial dna mutation does not play an important role among dm population in indonesia. Folia Medica Indonesiana. 2007 Jul 1;43(3):129-35.
12. Ishak AR, Puspitaningrum R, Utari RD, Ferania M, Adhiyanto C, Nitta T, Susanto AB, Yukio H, Yamashiro Y. Mutation of mtDNA ND1 Gene in 20 Type 2 Diabetes Mellitus Patients of Gorontalonese and Javanese Ethnicity. HAYATI Journal of Biosciences. 2014 Dec 1;21(4):159-65.
13. Sriwidodo S, Suprijana O, Subroto T, Maksum IP. Studi Mutasi Titik A3243G DNA Mitokondria Penyebab Maternally Inherited Diabetes and Deafness. Majalah Ilmu Kefarmasian. 2008;5(3):2.
14. Kunsan X, Yanqing W, Songhua W. Mitochondrial tRNA gene mutation diabetes mellitus in Chinese. Chin Med J. 1997;110:372-78.
15. Maassen JA, Kadowaki T. Maternally inherited diabetes and deafness: a new diabetes subtype. Diabetologia. 1996 Apr;39(4):375-82.

16. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. http://dx.doi.org/10.2337/diabetes.51.10.2944. 2002;51:2944-50.
17. Abelardo Solano QF, Playán A, López-Pérez MJ, Montoya J. Genetic diseases of human mitochondrial DNA. salud pública de méxico. 2001 Mar;43(2).
18. Shanske S, Pancrudo J, Kaufmann P, Engelstad K, Jhung S, Lu J, Naini A, DiMauro S, De Vivo DC. Varying loads of the mitochondrial DNA A3243G mutation in different tissues: implications for diagnosis. American Journal of Medical Genetics Part A. 2004 Oct 1;130(2):134-7.

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