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Mutation Analysis Of Rs7903146 Transcription Factor 7 Like 2 (Tcf7l2) Gene In Type II Diabetes Mellitus Sufferers Using The PCR-RFLP Method

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Abstract

Diabetes mellitus (DM) is a series of metabolic abnormalities characterized by increased blood glucose levels (hyperglycemia), which is caused by impaired insulin release, insulin function. The TCF7L2 gene is a gene associated with susceptibility to type 2 diabetes mellitus (T2D). Mutations in the TCF7L2 SNP rs7903146 gene, showed a significant correlation with T2D. The purpose of this study was to analyze the rs7903146 mutation of the TCF7L2 gene in T2D patients in the Sidoarjo area using the PCR RFLP method. This study uses a descriptive research type with a qualitative approach. This research was conducted at the Molecular Biology Laboratory, Faculty of Health Sciences, Muhammadiyah University of Sidoarjo, in July-August 2024. The sampling technique used in this study was non-probability sampling, especially the purposive sampling technique at the Bhayangkara Hospital Pusdik Sabhara Porong for 1 month. The method used for analysis is the PCR RFLP method. This study used 18 samples. In this study, there were no mutations.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Eva, 2019). Poorly controlled diabetes may lead to severe microvascular and macrovascular complications, including nephropathy, neuropathy, retinopathy, and cardiovascular disease. Globally, the prevalence of diabetes continues to increase in both developed and developing countries, making it a major public health concern (Magliano & Boyko, 2021). In Indonesia, national data from Riskesdas 2018 indicate a significant increase in Type 2 Diabetes (T2D), particularly among older adults (Setiaji et al., 2021).

Type 2 Diabetes (T2D) is the most common form of diabetes, accounting for approximately 85–95% of all cases worldwide (Magliano & Boyko, 2021). T2D is primarily characterized by insulin resistance combined with relative insulin deficiency. Although lifestyle factors such as obesity, physical inactivity, high-calorie diet, and smoking play a dominant role in disease progression (Betteng, 2014; Dafriani & Sari, 2021), genetic predisposition significantly contributes to individual susceptibility.

Among the genes associated with T2D, the Transcription Factor 7-Like 2 (TCF7L2) gene is considered one of the strongest genetic susceptibility loci. The single nucleotide polymorphism (SNP) rs7903146 has consistently been associated with increased risk of T2D in various populations. Research conducted in the Minangkabau ethnic group demonstrated a significant association between TCF7L2 variation and T2D incidence (Manaf et al., 2014). However, genetic variation may differ across ethnic groups and geographic regions.

In Sidoarjo, previous research analysing TCF7L2 in families with T2D did not identify significant mutations, suggesting possible population-specific genetic characteristics (Mushlih et al., 2020). Therefore,

this study aims to analyze the presence of mutations at the rs7903146 locus of the TCF7L2 gene in T2D patients in the Sidoarjo region.

In addition to TCF7L2, other genes such as GCKR (glucokinase regulatory protein) have been reported to influence glucose metabolism. The SNP rs780094 of the GCKR gene has shown correlation with T2D risk in Indonesian populations (Qalbissilmi, 2020). These findings indicate that T2D is a multifactorial disease involving complex gene–environment interactions.

METHOD)

This research has passed the ethical clearance at the Research and Health Ethics Commission (KKEPK) Faculty of Dentistry, Airlangga University Surabaya with number 056/HRECC.FODM/V/2024. This study uses descriptive techniques with a qualitative approach. The population in this study were patients with D2T at Bhayangkara Pusdik Porong Hospital. Sampling was done by purposive sampling with the criteria of subjects having a history of D2T as evidenced by supporting documents such as patient medical records, glucose levels >200 mg/dl, male or female aged ≥20 years, and willing to be a research subject using informed consent. The total samples used in this study were 18 samples, this research was conducted in July-August 2024. The research was conducted at the Molecular Biology Laboratory of the D-IV Medical Laboratory Technology Study Programme, Faculty of Health Sciences, Muhammadiyah University of Sidoarjo.

Sample preparation begins with macrosampling 3cc of EDTA blood, then centrifuged at 3500 rpm for 5 minutes. The blood that has been centrifuged is then taken in the buffy coat section, each of which is taken as much as 200 µL in the tube and carried out coloumn DNA isolation with a TianGen brand kit. Then proceed with the PCR process using a thermal cycler (Bio-Rad T100) with a volume of 20µL consisting of 10µL PCR Mix, 3µL DNA, 5.8µL ddH2O, 0.6µL forward primer (5'- GGT AAT GCA GAT GTG ATG AGA TCT-'3) and 0.6 µL reverse primer (5'-AGA TGA AAT GTA GCA GTG AAG TGC'-3).' With the stages of predenaturation 94˚ for 3 minutes, denaturation 94˚ for 30 seconds, anealling 58˚ for 30 seconds, extention 72˚ for 40 seconds, cycle 30 cycles, post extention 72˚ for 5 minutes. After that, electrophoresis was carried out using 1% agrose gel. and the RFLP PCR process using restriction enzymes was first incubated on a waterbath for 4 hours consisting of, 10 µL pcr DNA, 17 µL ddH2O, 2 µL 10x buffer, 1 µL restriction enzyme, after incubation for 4 hours incubation again for 10 minutes at 80◦. After that, 100volt electrophoresis was carried out for 40 minutes, using 1% agarose gel, 100 ml TBE solution, the marker content consisted of 1 µL loading dye, 3 µL ddH2O, 2 µL marker, for the content consisted of 1 µL loading dye, 3 µL sample, 2 µL ddH2O.

RESULTS AND DISCUSSION

This study aimed to identify the presence of polymorphism at the rs7903146 locus of the TCF7L2 gene in patients with Type 2 Diabetes Mellitus (T2D) in the Sidoarjo region using the PCR-RFLP method. The results showed that all examined samples produced a single DNA band of approximately 300 bp, indicating the absence of detectable mutations in the studied population. These findings suggest that the rs7903146 polymorphism of the TCF7L2 gene may not play a significant role in the genetic susceptibility to T2D in this specific population.

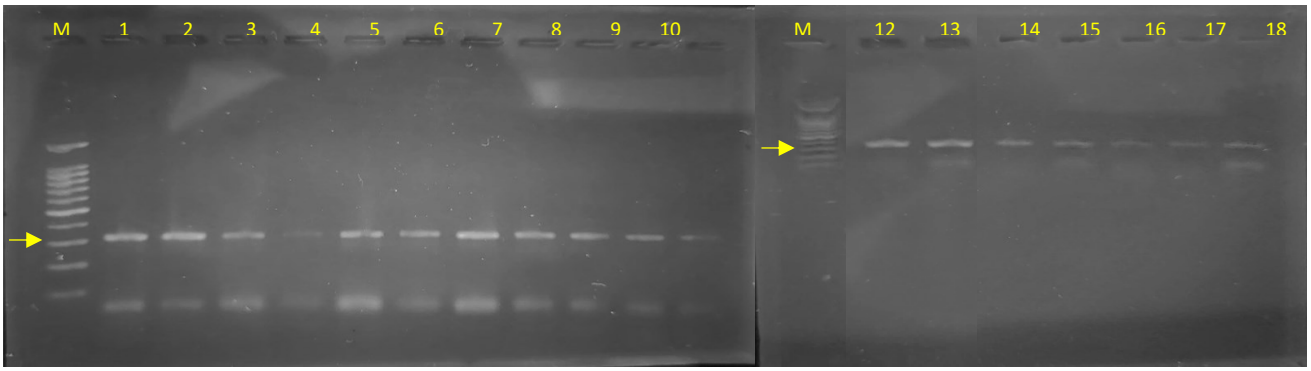

Figure 1. PCR results of TCF7L2 gene in 18 samples of DT2 patients
Electrophoresis of 1% agrose gel concentration, M: Marker. Yellow arrows indicate DNA bands

The TCF7L2 gene has been widely recognized as one of the strongest genetic susceptibility loci for T2D across various ethnic groups. Previous studies have demonstrated that the rs7903146 variant, particularly the T allele, is associated with impaired insulin secretion and increased risk of T2D (Manaf et al., 2014). The mechanism underlying this association is thought to involve altered transcriptional regulation affecting pancreatic β-cell function and glucose metabolism. However, genetic associations are often population-specific and influenced by ethnic background, allele frequency distribution, and environmental exposure. Therefore, the absence of rs7903146 variation in this study may reflect genetic heterogeneity within Indonesian populations.

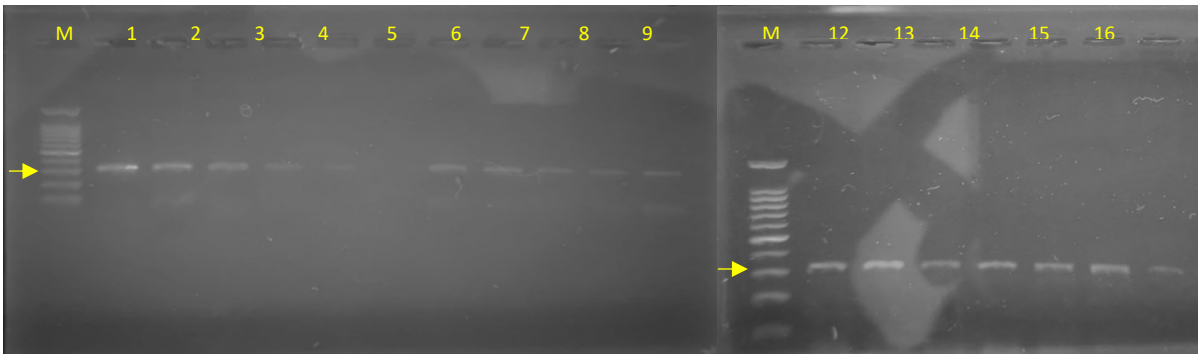

Figure 2. PCR-RFLP results of TCF7L2 gene in 18 samples of DT2 patients.
Electrophoresis 1% agarose gel concentration,
Description: M : Marker. Yellow arrows indicate the TCF7L2 gene target band

The present findings are consistent with previous research conducted in Sidoarjo, which also reported no significant variation in the TCF7L2 gene among families with T2D (Mushlih et al., 2020). This consistency strengthens the possibility that rs7903146 is not a major contributing factor to T2D susceptibility in this regional population. In contrast, research in the Minangkabau ethnic group showed a significant association between TCF7L2 polymorphism and T2D incidence (Manaf et al., 2014), highlighting the importance of ethnic diversity in genetic studies. Differences in genetic background between populations may explain why certain polymorphisms show strong associations in some regions but not in others.

In addition to genetic variation, T2D is a multifactorial disease strongly influenced by modifiable risk factors. Lifestyle-related behaviors such as high-calorie intake, excessive sugar consumption, physical inactivity, and smoking have been identified as major contributors to insulin resistance and metabolic dysregulation (Betteng, 2014; Dafriani & Sari, 2021). National and global data indicate that the rapid increase in T2D prevalence is closely linked to urbanization and lifestyle transitions rather than purely genetic changes (Magliano & Boyko, 2021). Therefore, the absence of rs7903146 polymorphism in this study may suggest that environmental and behavioral factors exert a greater influence on T2D development in the Sidoarjo population.

It is also important to consider that T2D susceptibility is polygenic, involving multiple genes that regulate glucose homeostasis, insulin signaling, lipid metabolism, and inflammatory pathways. Besides TCF7L2, other genes such as GCKR have been associated with T2D risk in Indonesian populations (Qalbissilmi, 2020). The involvement of alternative genetic loci may partially explain why no mutation was detected in the targeted SNP. Focusing on a single polymorphism may not fully capture the complex genetic architecture underlying T2D in this population.

Methodological considerations may also influence the findings. The relatively small sample size (n=18) limits the statistical power to detect low-frequency alleles. If the minor allele frequency of rs7903146 is low in this population, a larger sample would be required to identify its presence. Moreover, the use of PCR-RFLP, while reliable and cost-effective (Awaluddin et al., 2021; Timbuleng et al., 2021), may have limitations in detecting rare or subtle sequence variations compared to more advanced genotyping techniques such as real-time PCR or sequencing-based approaches.

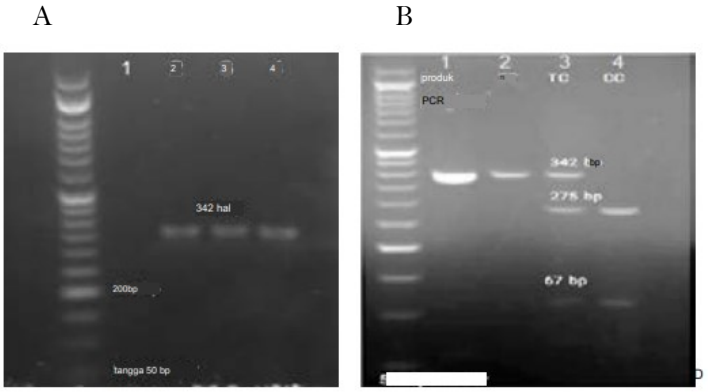

Figure 3. A. 1.5 per cent gel electrophoresis. Lane 1, negative control of PCR product. Lane 2-4 PCR product, B. 3 percent gel electrophoresis of RFLP products. Lane 1, undigested PCR product. Lane 2-4 RFLP products (Foroughmand et al., 2019)

Although no mutation was identified, this result does not exclude the possibility of genetic predisposition to T2D in this population. Instead, it emphasizes the complexity of gene–environment interactions in disease pathogenesis. The development of T2D likely results from the combined effect of multiple genetic variants interacting with long-term lifestyle exposures. Consequently, preventive strategies in the Sidoarjo region should prioritize lifestyle modification programs while future research expands genetic screening to include multiple SNPs and larger cohorts.

CONCLUSION

Based on this research, it is concluded that there is no mutation occurring at mutation 7903146 in the TCF7L2 gene in patients with type 2 diabetes (DT2) in the Sidoarjo region. The method used in this study is PCR-RFLP

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