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Genetic Polymorphism In Individuals With Type II Diabetes Mellitus Using PCR-RAPD In Sidoarjo District

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A B S T R A C T

Diabetes Mellitus type II (T2DM) is a metabolic disorder. Its incidence increases from year to year. The control of T2DM incidence is problematic because it is involved genetic and environmental factors. Moreover, it can cause complications in people with infectious diseases. This study aims to determine the polymorphism of sufferers and non-sufferers of T2DM using the Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD) method. This research was descriptive-analytic with a cross-sectional approach. The sample consisted of 60 samples – 30 positive and 30 negative samples taken from several clinics in the Sidoarjo district. The primer used was A18 (5'- AGGTGACCGT-3'). Data analysis used Chi-square with a 95% confidence level. The results produced 17 bands with the length of 197 bp, 239 bp, 269 bp, 319 bp, 390 bp, 530 bp, 588 bp, 686 bp, 777 bp, 972 bp, 1175 bp, 1676 bp, 2780 bp, 3843 bp, 6563 bp, 11072 bp & 18434 bp. The four bands were monomorphic. Two bands that showed significantly different results were 319bp (p=0.035) and 18434 bp (p=0.004). In conclusion, there are significant differences between people with and without T2DM in several fragments, namely 319 bp and 18434 bp bands. Further analysis needs to confirm the genes involved.

INTRODUCTION

Type II diabetes mellitus (T2DM) is a metabolic disorder characterized by increasing blood sugar levels due to insulin resistance so that glucose cannot enter the cells as a source of energy (Ekoe et al., 2018). Based on the International Diabetes Federation (IDF), in 2020, people with diabetes reached 463 million in the world. Half of them do not realize they had diabetes. In addition, diabetes also causes more financial expenditure for treatment (Loviana et al., 2015). T2DM caused an additional 2.2 million deaths by increasing the risk of cardiovascular and other diseases. 43% of the 3.7 million deaths occurred before 70 years (WHO, 2004). The T2DM incidence also exacerbates the comorbidities (Singh et al., 2020). The hematological character also changes according to the characteristics and severity (Kekenusa et al., 2016; Mushlih, 2020; Sebayang, 2016).

T2DM control has not been optimal because genetic and environmental factors affect its incidence (Al-Quwaidhi et al., 2013; Karalliedde & Gnudi, 2016). Genetic factors are inherited from parents, while environmental factors can be habits or lifestyles. In addition, it is influenced by the complex interaction of several genes that regulate energy metabolism in the body. Polymorphisms that occur in genes associated with regulating glucose metabolism have significant implications for T2DM onset (Chen et al., 2013;

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Tsaih et al., 2014). Another factor that also influences T2DM incidence is race (Gray et al., 2015), obesity, age (Evi & Yanita, 2016), and others (Rahayu et al., 2012).

Genotype analysis of people with T2DM is critical to detect possible incidence and preventive measures (Lyssenko & Laasko, 2013). Previous studies could identify polymorphisms in T2DM sufferers using the Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD) method (Zahid et al., 2011). However, the study had several shortcomings, including too few samples, and the genes involved had not been detected. PCR-RAPD is a relatively inexpensive and easy method to identify polymorphisms in individuals (Anggraeni, 2008). In addition, the PCR-RAPD will produce a particular band pattern. This pattern can be used as a fingerprint to detect the possibility of T2DM tendency. This study analyzes the polymorphism in people with T2DM using the PCR-RAPD technique. We used more samples to confirm the band differentiation between T2DM and non T2DM. So that in the future, it can be used to analyze the potential for a person to have T2DM based on genetic markers.

METHOD

This research did at the Molecular Biology Laboratory, University of Muhammadiyah Sidoarjo. The Health Research Ethics Committee (KEPK) approved the feasibility test for sampling, Faculty of Medicine, Airlangga University with numbers 194, 195, & 196 / HRECC. FODM / V / 2019. This paper was descriptive-analytic with a cross-sectional approach. There were 60 samples – 30 T2DM positive and 30 T2DM negative samples. Positive samples were taken from the Sidoarjo Hospital Wounds Clinic & Krian Public Health Center. Meanwhile, negative samples were taken from community volunteers and students at the Muhammadiyah University of Sidoarjo. A positive sample was T2DM diagnosed by a doctor, or random blood sugar (GDA) levels more than 210 mg/dl. Negative samples were determined from the absence of T2DM family history and confirmed by random blood sugar examination less than 180 mg/dl. The venous blood sample was taken using macro sampling (3 ml), then placed in an EDTA tube and stored in a cool box, and immediately taken to the laboratory to be stored in a cold cupboard until it is used. DNA extraction utilized the standard GeneAid protocol and then checked the concentration using a UV Vis Spectrophotometer (Thermoscience evolution 201). The PCR process was carried out with a total volume of 20 μl with a composition of 2 ul DNA (average concentration 200 ng/ul), 10 ul PCR mix, 2 ul primer A 18 (5'- AGGTGACCGT-3') (10 pmol) and the rest was ddH2O. A18 primer produces polymorphic DNA, which has the sequence 5 '- AGGTGACCGT-3'.

The PCR-RAPD process was carried out using a Biorad T100 thermocycler with predenaturation details 96oC for 5 minutes; denaturation of 96oC for 1 minute; annealing 36oC for 1 minute; elongation at 72oC for 1 minute; post elongation at 72oC for 10 minutes. The process was carried out 40 cycles. The PCR product was then carried out electrophoresis using 2% agarose gel. The data was processed based on the

presence or absence of the resulting band, and the band length is analyzed based on the resulting slope and intercept values. The data were analyzed using the Chi-Square cross-sectional test with a confident level of 95%.

RESULT

The results showed that there were 17 bands produced, namely bands at lengths 197 bp, 239 bp, 269 bp, 319 bp, 390 bp, 530 bp, 588 bp, 686 bp, 777 bp, 972 bp, 1175 bp, 1676 bp, 2780 bp, and 3843 bp. In this study, The four bands were monomorphic. In addition, one band (11072 bp) had the same frequency. The significance difference could be seen in the bands 319bp (p= 0.035) and 18434 bp (p= 0.004). A difference in polymorphisms in T2DM sufferers and controls can be seen in Table 1. The DNA bands from the electrophoresis were then analyzed using 2% agarose gel in Figure 1.

Table 1. Analysis of polymorphisms in people with T2DM and controls

Band length (bp)	Frequency		Percentage		p-value
	Negative	Positive	Negative	Positive	
197	22	19	73.3%	63.3%	0.405
239	30	30	100.0%	100.0%	1
269	9	4	30.0%	13.3%	0.117
319	16	8	53.3%	26.6%	0.035
390	30	30	100.0%	100.0%	1
530	1	0	3.3%	0.0%	0.313
588	30	30	100.0%	100.0%	1
686	30	30	100.0%	100.0%	1
777	29	30	96.6%	100.0%	0.313
972	3	6	10.0%	20.0%	0.278
1175	29	30	96.6%	100.0%	0.313
1676	29	30	96.6%	100.0%	0.313
2780	27	29	90.0%	96.7%	0.301
3843	27	28	90.0%	93.3%	0.64
6563	29	30	96.6%	100.0%	0.313
11072	13	13	43.3%	43.3%	1
18434	11	22	36.6%	73.3%	0.004

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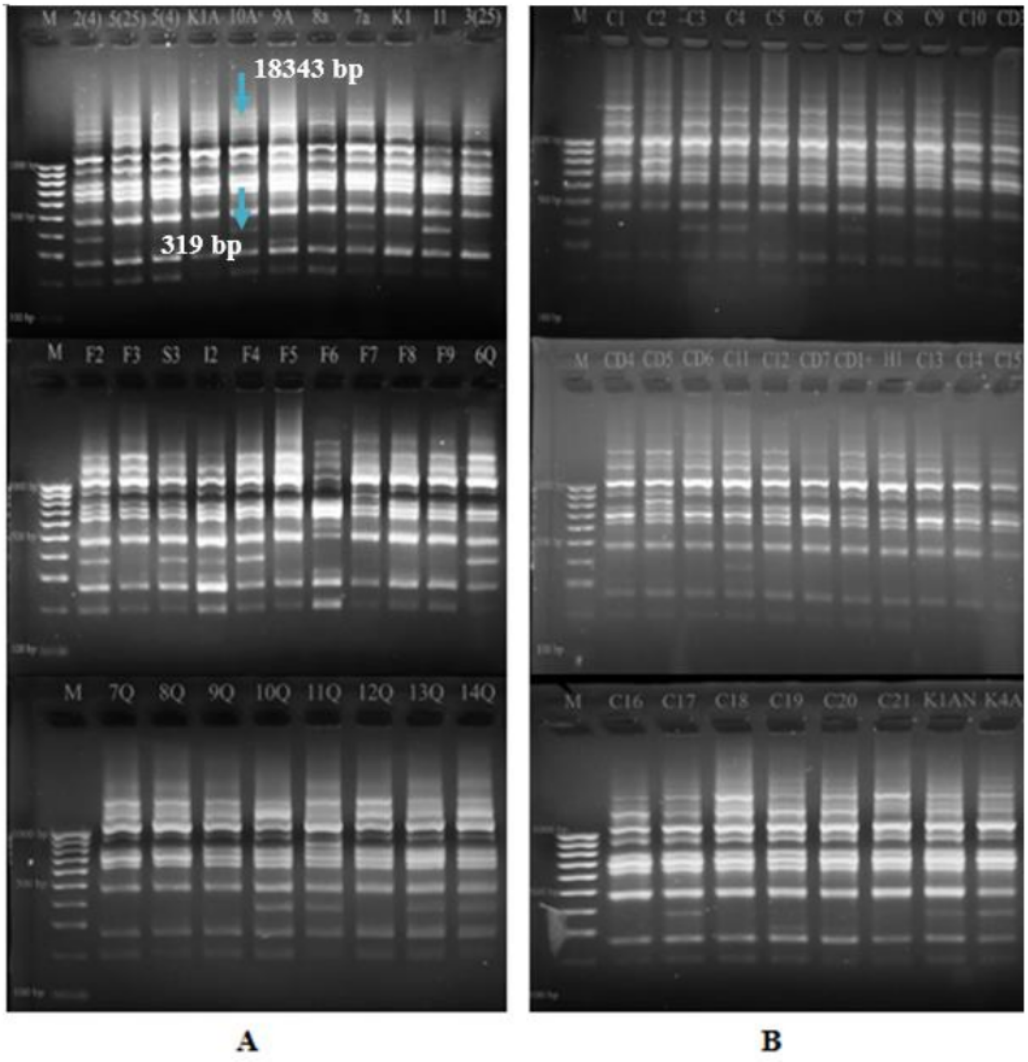

Figure 1. Appearance of PCR-RAPD electrophoresis results using primer A18. A. Negative sample (control). B. Positive Samples (T2DM). Agarose 2%. M: Marker 100 bp. Blue arrows indicate bands that are significantly different in individuals with T2DM and negative / control samples

PCR-RAPD is the most widely used technique to detect polymorphisms in an organism, both intra and inter-species. This study had successfully detected polymorphisms in 60 samples and showed several characters that differentiate between T2DM and non T2DM sufferers. The difference laid in the bands 319 bp and 18434 bp (p-value <0.05). This difference was based on chi-square analysis using nominal data. The RAPD analysis depends on whether or not the band comes out when visualizing using the 2%

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gel electrophoresis method. The clearer and more compact the resulting band, the more reliable and can be trusted.

DISCUSSION

In this study, the results were pretty different in the two bands produced. The 319 bp band showed a compact and clear character, while the 18434 bp band showed a faint band. Although the 18434 bp band had a higher significance value, it required a high enough carefulness to read the DNA band to analyze. Unlike the 18434 bp band, the 319 bp band showed a compact character so that it can be used to determine the tendency of T2DM in individuals. Band 319 in the individuals with T2DM and non T2DM showed a clear appearance. The other bands, 239 bp, 390 bp, 588 bp, and 686 bp, were seen in both groups. The appearance of the bands was so compact that they looked similar.

This paper strengthens previous research, which reported that three of 16 primers did not produce amplification patterns. Seven primers produced monomorphic bands. Meanwhile, A10, A18, C5, D20, R3, and R4 produced polymorphic DNA profiles – the most polymorphism was A18 (Zahid et al., 2011). This study also found a band that dominated the negative sample (control) and was not found in people with T2DM. However, statistical tests have not been carried out and only show polymorphisms. A statistical test showed the percentage and significant differences in the appearance of each band. The A18 primer clearly showed the difference in the 319 bp band, which had a compact position and could easily be distinguished from other bands because of its short size. Analysis of the same sample and using different primers (D20) also showed a significant difference in the 576 bp band (Mushlih et al., 2020). This information can explore a potential genetic marker in a society (Kumari & Thakur, 2014).

Genetic information about individuals with and potential T2DM is essential (Shields et al., 2010). This research is the basis for justifying someone who has the potential for DMT2. Further analysis should determine the genes involved based on the differences in the resulting bands. It is because T2DM is very dependent on race, ethnicity, nation, and population. Therefore, the potential T2DM can be different even though they have the same genotype in one gene. Analisis with a PCR-RAPD method can explore the whole genome and not focus on specific gene mutations

CONCLUSION

The results show significant differences between people with and without T2DM in several fragments, namely 319 bp and 18434 bp bands. The 319 bp band shows easier observation results and can predict potential T2DM. The 18434 bp band shows a relatively more difficult band because of its less obvious appearance and higher position. Further analysis of these fragments is necessary to determine the associated genes.

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