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Identification and use of polymorphisms to predict possibility in type II diabetes mellitus family using RAPD method

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ABSTRACT

Type II diabetes mellitus (T2D) is a genetic disorder that becomes an unsolved problem. This is because T2D can be a disorder caused by lifestyle changes and is inherited from parents. This study aimed to determine the polymorphism that distinguishes between T2D and non DM patients and the result was predicted to the families that has T2M record. The model in this research was descriptive exploratory and consists of 80 samples (30 samples positive T2D, 30 non DM samples and the others are the member of 5 families). Firstly, the analysis was focused on determining differences in polymorphisms and then the results were applied to the 3 families of T2D using A10 Primer. Difference polymorphism was analyzed using chi-square (cl: 95%). Six bands were obtained from PCR-RAPD which a significantly different band at 997 bp. Analysis of T2D has confirmed that 5 families have a clue band. Based on the analysis this report can be used to screen the possibility of someone to have DM type 2.

INTRODUCTION

T2D is genetic abnormalities have been problematic and unresolved. It has been predicted the high number of diabetes becomes a barrier because it can be compiled with other diseases such as infectious diseases (Covid-19, HIV, Pneumonia, hypertension, etc) [1,2]. Globally, there are 415 million adults suffer from diabetes [3]. people with T2D must also prepare more in the financial support [4], remembering that slowly T2D make complications in several other types of diseases such as kidney failure, blindness, and others. Some studies show that complications can also be very dangerous, such as heart attacks that lead to death[5].

Management of T2D becomes more difficult because it's caused by several factors that support each other [6–9]These factors include genetic factors and environmental factors [10–12]. The genetic factor is a state of DNA inherited from parents including the accompanying factors such as obesity[13]. An environmental factors are habits which include daily activities, food consumption, and lifestyle. The condition of diabetes is also exacerbated by the existence of several lifestyle changes that exist in society[14]. The

prevalence of type 2 diabetes mellitus in Indonesia increased from 5.6 percent from 2013 and is predicted to rise to 6.7 in 2035 [15].Indonesia is also one of the top 10 countries that have the greatest number of people with diabetes mellitus.

RAPD is a method for detecting polymorphism in living things [16]. Previous research on PCR-RAPD was used and found 6 bands that showed polymorphism. Previous studies showed a difference in polymorphism between type 2 DM sufferers and non T2D sufferers [17]. The method used was PCR-RAPD. The purpose of this study was to determine the differences in polymorphism in patients with non-patients with type 2 diabetes with samples in Sidoarjo. Character Polymorphisms was PCR-RAPD can be used to predict someone for having possibility of T2DM. from This study also used samples with families of type 2 DM patients who are expected to strengthen and apply the results of the resulting polymorphisms.

MATERIALS AND METHODS

This research is a descriptive exploratory. Sampling was approved by the Ethics Commission of the Faculty of Dentistry, Airlangga University, no: 090 & 092 / HRECC.FODM/ III / 2020. Samples to be used were 60 people with the distribution of 80 positive T2D (obtained as many as 10 Whole Blood samples from the Tiandte Clinic, Sidoarjo, 20 samples from collections in Molecular Biology Laboratory of the Faculty of Health Sciences, Universitas Muhammadiyah Sidoarjo and 30 people with T2D were obtained from Medical Laboratory Technology students at the University of Muhammadiyah Sidoarjo. The others include in 5 families that have T2D record. The sampling technique used purposive sampling. Inclusion criteria in this study included patients with T2D, patients willing to be subjects of the study, had a history of diabetes mellitus or those patients had blood glucose ≥ 200 mg/dl. DNA isolation was conducted using Gene Aid DNA isolation kit No. Ver.02.10.17 protocol. DNA amplification was carried out with primers A10 (5'- GTGATCGCAG-'3). Primer previously described with polymorphisms existence in T2D (16) . DNA amplification was done with total 15 µL of the reaction mixture containing 2 µL primers, 2 µL of Genomic DNA, 6 µL ddH2O, and 5µL PCR Mix. DNA amplification using PCR in T100 Bio-Rad programmed 50 cycles. The working principle of PCR consists of Pre-denaturation of 94˚C for 5 minutes, denaturation of 94˚C for 1 minute, annealing 36˚C for 1.5 minutes, 72˚C extensions for 2 minutes and final extension 72˚C final for 10 minutes. The result of PCR-RAPD were analyzed with gel 2% Electroforesis. The band was analyzed to generate difference band frequencies by using chi square test (cl: 95%) and the significant different in band using to predict the possibility the person have T2D.

RESULTS AND DISCUSSION (Arial 11)

Molecular analysis to identify an abnormality has often been used, including to detect a possible disease. Molecular method is also often used to identify hereditary diseases such as thalassemia, single Cell anemia protect, and others[18]. Analysis of diabetes mellitus can two use the monocular method more often using methods to detect only certain genes [11,19–21]. More than 120 genes reported include in T2D [22]. But for the use of polymorphism identification using RAPD which is then applied to a case is still rarely done. PCR-RAPD is a well- known method that is inexpensive, easy and simple, however, the method requires optimization so that it produces stable results [16]. PCR-RAPD makes it possible to detect differences in polymorphism in the genome as a whole [23].

T2D screening is used to detect whether a person is likely to have this disorder or not [24]. Polymorphisms that were identified indicate a person's probability by looking at the probabilities were generated. In this study, 80 samples were used consisting of 30 positive T2D and 30 negative T2D taken from various places in Sidoarjo Indonesia. PCR-RAPD method produces specific bands. We identified 3 samples have no band. The result of this

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study shows the varied length of the band which 533 bp, 660 bp, 880 bp, 997 bp, 1463 bp, and 1860 bp, (Table 1). Based on Chi-square test shows significantly different at 997 bp (p value: 0.000). A similar analysis using PCR-RAPD was performed and produced a 1000 bp band length with a percentage value of 100% for T2D patients and 20% for controls or negative T2D [25]. In this study likely has a similar result that band position is very near. The difference of count band length may resulted by subjectivity of researcher. The difference in band results may also due to differences in the race used for the study sample [26]. The use of PCR-RAPD for screening T2D is still rarely done.

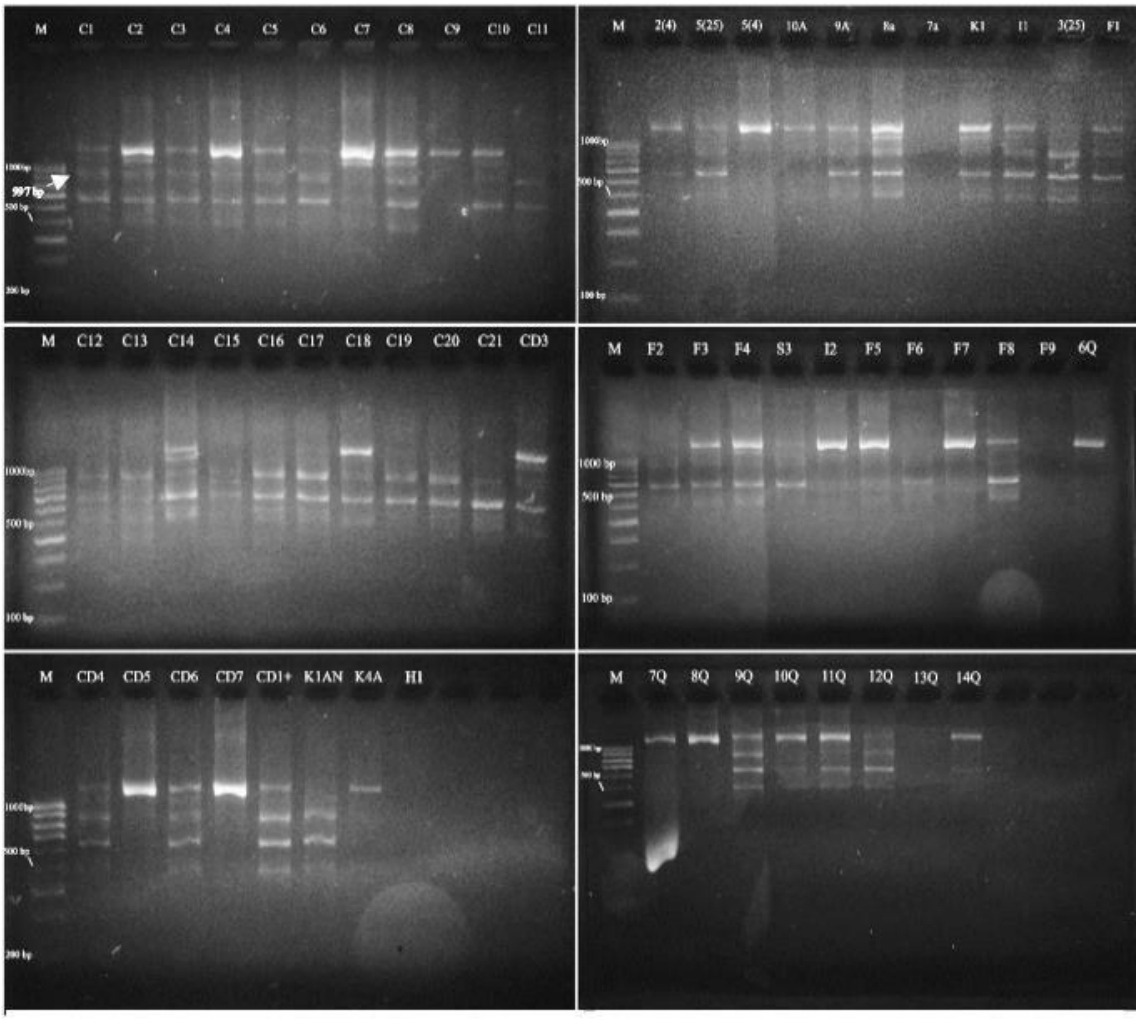

Figure 1. PCR-RAPD visualization on T2D and non T2D.(A) Negative T2D (B) positive T2D; Agarose 2%; marker 100 bp. The white arrow indicates the presence of the T2DM marking band

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Table 1. Analyses of band resulted from PCR RAPD

Number	Band Length	T2D status		%		chi square
		Negative	Positive	Negative	Positive	p value
1	533	7	12	24.14%	44.44%	0.109
2	660	24	18	82.76%	66.67%	0.165
3	880	12	11	41.38%	40.74%	0.961
4	997	19	1	65.52%	3.70%	0.000
5	1463	7	2	24.14%	7.41%	0.088
6	1860	19	24	65.52%	88.89%	0.038

In this study, we showed that there is a significant difference polymorphism and we used it in 5 families with T2D record. Analysis of the first family shows that there is a band 997 bp in the father and not found in mother. The identification of the band in the two children showed no band found in that position. This shows that alleles that are owned by mothers are likely to be passed to their children. Analysis of the second family showed that the F5 and C21 samples did not find the target band (997 bp). The third family analysis shows the target band appears in mothers who have T2D. The band was not found in fathers who did not experience T2D. Analysis in their children showed the band seemed to be inherited from the father and was not found in the target gene. The analysis in the third family shows that the sample with code 10 shows the target band (997 bp). Sample with code 10 is a positive T2D. While the others sample code did not show a band which are negative T2D.

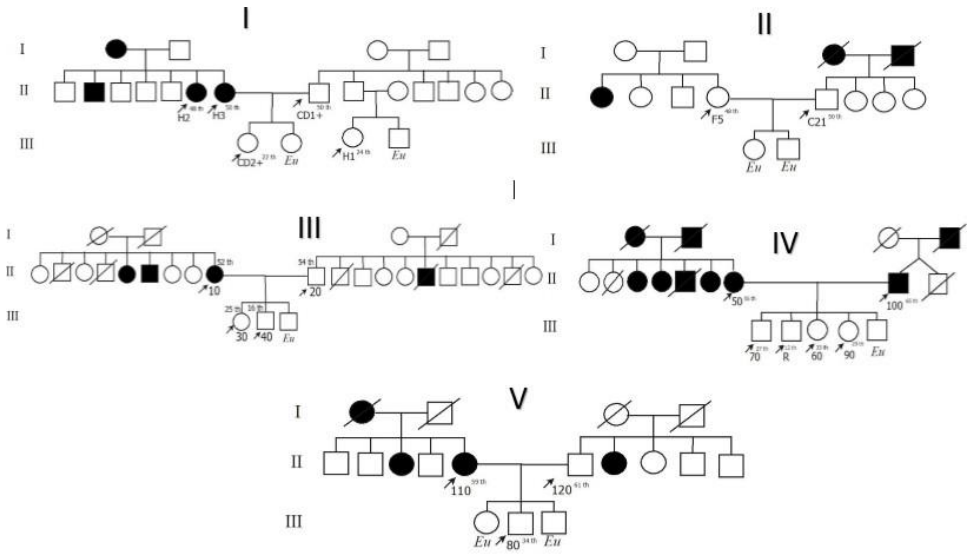

Figure 2. Pedigree of five Family witch used to T2D possibility

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Table 2. Identification 997 bp band and family record

Family	Sample	Gender	Status of T2D	T2M Family Record	Existence of 997 bp band
I	H2	F	yes	yes	yes
	H3	F	yes	yes	no
	CD1+	M	no	no	yes
	CD2+	F	no	yes	no
	H1	F	no	yes	no
II	C21	M	no	yes	yes
	F5	F	no	yes	yes
III	10	F	yes	yes	yes
	20	M	no	yes	no
	30	F	no	yes	no
	40	M	no	yes	no
IV	50	F	yes	yes	yes
	60	F	no	yes	yes
	70	M	no	yes	yes
	90	F	no	yes	yes
	100	M	yes	yes	yes
	R	M	no	yes	yes
v	80	M	no	yes	yes
	110	F	yes	yes	yes
	120	M	no	yes	yes

Analysis of the Fourth family shows that the band appeared in the whole sample, this may cause both families have T2D record so the allele/s may be passed on to all family members. The fifth family shows the target band appears in the whole sample token. This shows the material obtained in father and samples may have potencies of T2D. The emergence of the band is possible due to the inheritance of his parents. This concerns of offspring sample have a potency suffering T2D. Trial identification of polymorphism shows that the method is able to identify exactly 4 out of 5 families used. This indicates that the material used has a high degree of accuracy and can be used to predict the incidence of T2D.

CONCLUSIONS

T2D and non-T2D polymorphisms were obtained, i.e. 533 bp, 660 bp, 880 bp, 997 bp, 1463 bp, and 1860 bp. Basen on the Analysis, band 997 bp distinguished between T2D and non T2D. The use of the PCR method using A10 primers can be used to detect all families with T2D record. Based on the results of this study it is expected that screening by knowing the possibility of someone having DM or cannot be done and reducing the risk of being affected by these metabolic disorders.

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