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Detection Of Glukokinase (GCK) Gene In Type 2 Diabetes Mellitus And Non Diabetics Respondents Using The Polymerase Chain Reaction Method

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ABSTRACT

Diabetes Mellitus is a chronic medical condition in which there is an increase in glucose levels in the blood because the body cannot produce insulin. The glucokinase gene (GCK) is in charge of encoding the glucokinase enzyme which plays a role in glucose metabolism. The GCK gene has a vital role in regulating blood glucose levels in the body. This study was designed to detect the presence of GCK gene in 9 respondents of type 2 diabetes mellitus in the diabetes prolanists of Puskesmas Wonosari 1 Klaten and 9 non-diabetic respondents in PKK Kadilangu Village Rt 2 Rw 1 using the Polymerase Chain Reaction (PCR) method. PCR results were visualized by electrophoresis using agarose gel concentration of 1.5%. The results of the study can be seen that the GCK gene was detected in 9 samples of type 2 diabetes mellitus and 9 non-diabetic samples with a product length of 206 bp. Qualitative detection of GCK genes from DNA isolate samples was successful in this study, but information on gene expression levels in the realm of transcription and translation was not available yet available in this study, due to limitations in testing equipment and reagents, future research can conduct quantitative gene expression analysis using the Quantitative Real-Time PCR (qPCR) method.

Keywords: diabetes mellitus, GCK gene, PCR

INTRODUCTION

Diabetes Mellitus is a long-term medical condition that arises when blood glucose levels rise due to the body's inability to produce or effectively utilize insulin. Insulin, a crucial hormone produced by the pancreas gland, facilitates the transportation of glucose from the bloodstream into the body's cells, where it is converted into energy. Insufficient insulin or the cells' inability to respond to insulin leads to high blood sugar levels, a prominent feature of Diabetes Mellitus, also referred to as hyperglycemia (Pangestika *et al*., 2022).

Reducing the amount of insulin that effectively works will cause an increasing in the concentration of sugar in the blood which will further cause systemic effects. Diabetes Mellitus (DM) is divided into two types, namely type 1 diabetes and type 2 diabetes. Diabetes risk factors that need attention include overweight (obesity), lack of physical activity and heredity. Prevention can be done by anticipating these risk factors and keeping blood sugar levels normal (Agoes, 2010).

The prevalence of diabetes mellitus according to Riset Kesehatan Dasar (Riskesdas) in 2018 nationally was 8.5%, it was increase compare with 2013 with the highest prevalence in 2018, occurred in Yogyakarta, DKI Jakarta, North Sulawesi, and East Kalimantan, while the lowest prevalence was in West Nusa Tenggara province (Kementerian Kesehatan RI. 2020). Diabetes Mellitus (DM) type 2 is the most common type of DM, it is about 90% of all DM cases. In type 2 diabetes, elevated levels of glucose in the blood occur due to inadequate insulin production and the body's resistance to insulin, which is defined as insulin resistance. Type 2 DM often occurs in adults aged 50 years and over, but is increasingly found in children, adolescents, and young adults. The causes of type 2 diabetes are closely related to overweight, obesity, aging, and family history (Pangestika *et al.*, 2022).

The glucokinase (GCK) gene encodes the enzyme glucokinase, which has a role in glucose metabolism. In the pancreas, this enzyme plays a role in the secretion of insulin triggered by glucose, while in the liver, this enzyme is important for taking up glucose and converting it into glycogen. Mutations in this gene can result in changes in enzyme activity, which has been linked to some types of diabetes (Lio and Sugireng, 2019). Glucokinase (GCK) is the main enzyme in controlling insulin release in the cells β pancreas. This enzyme is produced by the glucokinase gene located on chromosome 7p15.3-p15.1 and consists of 10 exons (Li *et al*., 2018).

Polymerase chain reaction otherwise known as *Polymerase Chain Reaction* (PCR) is an enzymatic synthetic process for amplifying nucleotides *Vitro.* The PCR process is a repeating cicle process including denaturation, annealing is the primary introduction step to the corresponding DNA band, and extension by the enzyme DNA polymerase (Yu *et al.*, 2019). Factors that determine the success of PCR are DNA concentration and quality, annealing temprature of both primers, polymerase enzymes, concentration and quality of primers, number of PCR cycles, *deoxynucleotide triphosphate* (dNTP) and other factors such as buffer solutions (Setyawati and Zubaidah, 2021).

The criteria of respondents used in this study were type 2 Diabetes Mellitus patients with an age range of 30-60 years and participated in the diabetes mellitus prolanis program at Puskesmas Wonosari 1 Klaten. For non-diabetic respondents with criteria of not having a history of type 2 diabetes mellitus obtained from PKK Desa Kadilangu Rt 2 Rw 1. According to (Matschinsky & Wilson, 2019) the glucokinase (GCK) gene is expressed at higher levels in type 2 diabetes respondents compared to normal or non-diabetic respondents. This study aims to determine the detection of glucokinase (GCK) genes in patients with type 2 diabetes mellitus and non-diabetes using the *Polymerase Chain Reaction method*.

METHOD

This study used descriptive research method. The population and target sample of this study were 9 respondents of type 2 diabetes mellitus in the prolanis of Puskesmas Wonosari 1 Klaten and 9 non-diabetic respondents in PKK Kadilangu Village Rt 2 Rw 1. This study has passed ethical review with document number KEPK/UMP/05/IX/2023 from Universitas Muhammadiyah Purwokerto. The sampling technique uses purposive sampling, a data

collection technique using primary data, namely by taking blood directly in type 2 diabetes mellitus patients and non-diabetics, DNA isolation then examination is carried out by PCR method, and PCR results are visualized by electrophoresis.

The DNA isolation process begins with sample preparation: The whole blood sample is pipetted as much as 200 µl and inserted into a 1.5 ml microcentrifuge tube. Added 20 µl of Proteinase K mix by pipetting. Incubation is carried out at 60°C for 5 minutes.

Cell insertion, as much as 200 µl of GSB buffer is added to a 1.5 ml microcentrifuge tube and mixed by shaking vigorously. Incubate at 60°C for 5 minutes to ensure the sample is clear. When incubated, the tube is turned over every 2 minutes.

DNA binding, an absolute amount of 200 µl of ethanol is added to the lysate and homogenized by shaking for 10 seconds (if a precipitate appears, separate as much as possible with a pipette). GS column is placed in a 2 ml collection tube, the mixture is transferred (including deposits) to the GS column and centrifuge at a speed of 14,000 rpm for 1 minute. The 2 ml collection tube is removed and the GD column is placed into a new 2 ml collection tube.

DNA washing, as much as 400 µl of Buffer W1 is added to the GS column then centrifuge at a speed of 14,000 rpm for 30 seconds then exhaust flow-through, GS column is placed back on a 2 ml collection tube. A total of 600 µl of Wash Buffer is added to the GS column then centrifuge at a speed of 14,000 rpm for 30 seconds then exhaust flow-through, GS column is placed back on a 2 ml collection tube, then the centrifuge returns at a speed of 14,000 rpm for 3 minutes. The collection tube is discarded and the GS Column is placed in a 1.5 ml microcentrifuge tube.

DNA elution, as much as 200 µl of preheated Elution Buffer. Let stand for at least 3 minutes so that the Elution Buffer is completely absorbed and centrifuge at a speed of 14,000 rpm for 30 seconds to elute pure DNA. Make sure the Elution Buffer is added to the center of the GS column matrix and fully absorbed (Geneaid 2017).

Qualitative test was carried out using 1.5% agarose gel with TBE 1× solvent which was then continued by electrophoresis process. At this stage 5 µl of DNA isolate, 3 µl of loading dye and 2 µl of red gel are mixed and then inserted into the agarose gel well in the electrophoresis chamber. Electrophoresis is carried out for 90 minutes with a voltage of 90 volts and an electric current of 400 amperes.

Quantitative tests were performed by making dilutions of 20 µl DNA isolates plus 3980 µl aquabidest. The absorbance is then read using a UV-Vis spectrophotometer at λ260 nm and λ280 nm.

Detection of the GCK gene by polymerase chain reaction using primers designed by the researcher using the NCBI and Primer3plus software, then confirming the primer suitability using OligoEvaluator and NetPrimer software, with the result of forward primer 5'-CTCGCCTCTGTGTGTGAAATGA-3', reverse primer 5'- TTTGGGCAGGATTTCTCATC -3' with target 206 bp. Perform pipetting consisting of 5 µl master mix, 1 µl forward primer, 1 µl reverse primer, 2 µl Nuclease Free Water, 1 µl DNA template. This stage uses Pre denaturation temperature 95°C for 3 minutes, Denaturation temperature 95°C for 30 seconds, Annealing

temperature 65°C for 30 seconds, Extension temperature 72°C for 1 minute, Final extension temperature 72°C for 5 minutes, and hold temperature 4°C with 34× cycles.

Electrophoresis of PCR results was carried out by inserting 5 µl of PCR isolate, 3 µl of loading dye and 2 µl of red gel that has been mixed into this gel well agarose in the electrophoresis chamber. Electrophoresis for 90 minutes was then visualized using Gel Doc. The data analysis technique in this thesis is descriptive based on the detection of the GCK gene in samples of type 2 and non-diabetic diabetes mellitus respondents with a target of 206 bp.

RESULT AND DISCUSSION

This study involved 18 respondents consisting of 9 respondents who had a history of diabetes mellitus at the Puskesmas Wonosari 1 Klaten and 9 respondents who did not have a history of diabetes mellitus at the PKK Kadilangu Village Rt 2 Rw 1. The DNA isolation process was carried out on blood samples of diabetes mellitus and non-diabetes mellitus respondents using the DNA Isolation Mini Genomic Kit (Geneaid). The results of DNA isolation are tested qualitatively by electrophoresis which is then visualized and tested quantitatively using a Uv-vis spectrophotometer to see the purity and concentration of DNA.

DNA isolate can be used as a sample for PCR if the DNA has a purity value. DNA purity can be determined by comparing Optical Density (OD) solutions at different wavelengths using a UV-Vis spectrophotometer. The level of DNA purity is said to be good if the ratio value Optical Density (OD) 260/280 nm obtained between 1.8-2.0, if the OD ratio of 260/280 nm is less than 1.8 the possibility of DNA contaminated with protein while high DNA purity values can occur due to the presence of residual ethanol during the isolation process (Dong *et al.,* 2012). According to (Fatchiyah, *et al.,* 2011) DNA purity levels above 2.0 indicate that impure DNA is also caused by the presence of ethanol remnants at the time of incomplete drying.

Table 1. DM Sample Quantitative Test Results

No	λ 260 nm	λ 280 nm	Concentracion (x 50 ng/µl)	Purity (λ260/√280)
1	0,0221	0,0201	221	1,09
2	0,0166	0,0126	166	1,31
3	0,0171	0,0186	171	0,91
4	0,038	0,0195	380	1,94
5	0,0121	0,0144	121	0,84
6	0,0059	0,0106	59	0,55
7	0,0068	0,0142	68	0,47
8	0,0121	0,0172	121	0,70
9	0,006	0,0133	60	0,45

The lowest DNA purity in the blood sample of diabetes mellitus respondents was 0.45 in sample number 9, while the highest DNA purity was shown by sample no. 4 with a purity value of 1.94, the ratio of DNA purity value of 1.8-2.0 so that it can be said that DNA isolates in diabetes mellitus blood samples have a low level of purity.

Table 2. Quantitative Test Results of Non DM Samples

No	λ 260 nm	λ 280 nm	Concentration (x 50 ng/µl)	Purity (λ260/√280)
1	0,0262	0,0356	262	0,73
2	0,012	0,017	120	0,70
3	0,0121	0,0227	121	0,53
4	0,0085	0,0146	85	0,58
5	0,0087	0,014	87	0,62
6	0,0068	0,0132	68	0,51
7	0,0065	0,0125	65	0,52
8	0,0093	0,0153	93	0,60
9	0,0155	0,0205	155	0,75

DNA purity in the lowest blood samples of non-diabetes mellitus respondents was 0.51 in sample number 6, while the highest DNA purity was shown by sample no. 9 with a purity value of 0.75, the ratio of DNA purity values of 1.8-2.0 so that it can be said that DNA isolates in non-diabetes mellitus blood samples have low purity.

In this study there are differences in results between qualitative tests, purity and DNA concentration which can be caused because contamination with compounds such as proteins, RNA, or other organic compounds can affect qualitative and quantitative test results. These contaminants can affect absorbance at specific wavelengths or increase the measured concentration. It can also be caused by technical factors during the measurement process, such as homogenization and improper pipetting techniques. As a result, DNA breaks off into fragments. This breaking of bonds between molecules can be caused by excessive physical movement during the pipetting process, rough manipulation during the stirring process, excessive centrifugation, or even by exposure to high temperatures or certain chemicals (Aulia *et al.*, 2021).

Optimization of annealing temperature is carried out to achieve the right PCR composition and conditions, resulting in optimal PCR results with the maximum amount of DNA in the specified target area (Aulia *et al*., 2021). In the PCR tool, the annealing temperature variation is done using a gradient program. The primary concentration and annealing temperature that produce the optimal band are then used for PCR (Yuenleni, 2019).

In this study, the annealing temperature that showed the best DNA amplification was at 63°C because it produced the thickest, brighter, clearer and single DNA band compared to other annealing temperatures. The annealing temperature is very influential on the primary binding

process in DNA prints. If the annealing temperature is too high, the primer will not stick well to the template, this is indicated by the thinner the band formed, while if the lower annealing temperature causes the primer to stick to the non-specific binding site, causing unwanted amplification of locus fragments (Rychlik *et al.,* 1991).

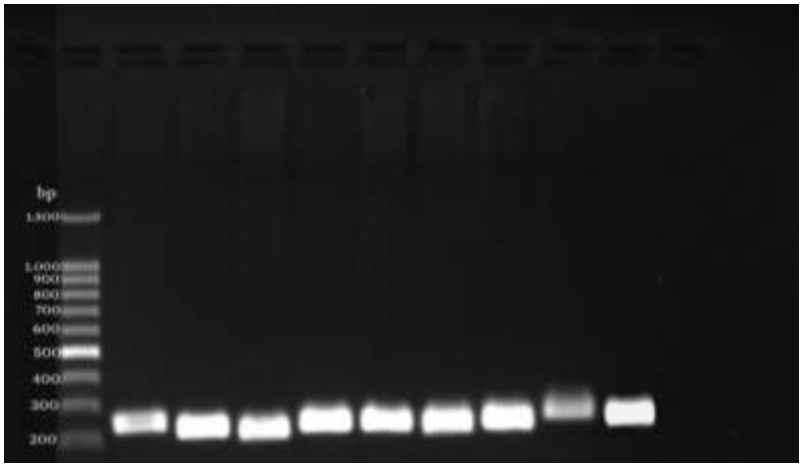

Figure 1. GCK Gene Amplification DM Samples

Amplification of the GCK gene sample of type 2 diabetes mellitus respondents obtained the results of 9 samples showing the presence of the GCK gene according to the target, namely at a product length of 206 bp.

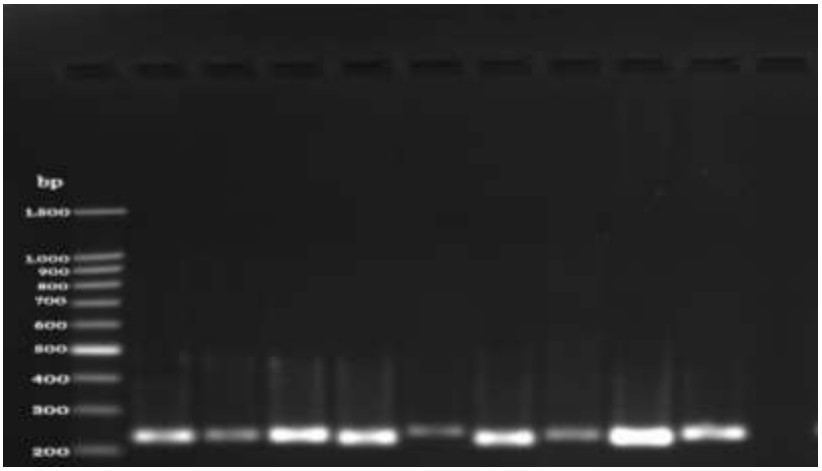

Figure 2. GCK Gene Amplification of Non DM Samples

Amplification of the GCK gene in non-diabetic samples obtained 9 samples showing the presence of the GCK gene at a product length of 206 bp.

Figure 1 shows that the DNA bands are clearly visible and thick, which may occur because the GCK gene is detected with a high amount of amplification product in the diabetes

mellitus samples. High amplification in PCR can occur due to sufficient and high-quality DNA template, ideal annealing temperature, appropriate PCR cycle, and the correct reagent composition. Figure 2 shows thin DNA bands, indicating that the GCK gene is detected in the non-diabetes mellitus samples, but in smaller quantities or with low amplification products. This may be due to technical factors such as too low annealing temperature (resulting in non-specific binding) or too high (inhibiting primer binding), which can lead to low amplification, or insufficient, degraded, or contaminated DNA, which can inhibit amplification.

After DNA isolates go through the PCR stage, they can be seen visually through the electrophoresis process. Electrophoresis is a method of chemical analysis that relies on the movement of charged protein molecules in an electric field, especially with respect to their isoelectric points. Based on Figure 1 and Figure 2, DNA bands from samples number 1-9 are seen parallel to markers measuring 206 bp. The movement of molecules in the process of electrophoresis occurs in an electric field and is influenced by the shape, size, charge, and chemical properties of the molecules. The separation is carried out based on the difference in molecular weight and electric charge possessed by macromolecules. When an electric current is flowed through a buffer medium that has been filled with plasma proteins, the protein components will begin to migrate. In the results of the study, the results of the electrophoresis band showed that all samples had similar shapes, sizes, charges, and chemical properties, indicated by the appearance of electrophoresis bands in the same position.

DNA purity test results with an OD ratio of 260/280 nm are presented in Table 1 and Table 2, based on samples calculated concentration and purity, the isolated DNA concentration has a relatively large and non-uniform concentration. For the results obtained on the spectrophotometer is greatly influenced by the solvent components in the DNA solution, because at wavelengths of 260/280 DNA will read the concentration, but in this condition dissolved DNA impurities greatly affect the stability of the concentration of DNA produced. These impurities can be phenol compounds or contaminants that occur during DNA extraction. Based on research conducted (Tarigan, 2016). It is said that these contaminants can be carbohydrates or proteins. With these contaminants, the absorption of 280nm wavelength increases, so that the absorbance ratio is lower which results in the absorbance value being below a predetermined number. The results obtained from the spectrophotometer are strongly influenced by the solvent components contained in the DNA solution.

At wavelengths of 260/280, the concentration of DNA can be read, but the presence of dissolved DNA impurities greatly affects the stability of the concentration of DNA produced. Such impurities can be contaminants that occur during the DNA extraction process. The results of DNA purity in this study may be affected by the use of cuvettes that are dirty and unable to absorb radiation, especially in areas penetrated by UV light. Another factor that can affect the results of DNA quantity testing using a uv-vis spectrophotometer is the calibration of the device. Devices that have not been calibrated may result in deterioration in their quality and result in inaccurate or invalid data (Handoyo and Rudiretna, 2001). The importance of measuring with a nanodrop spectrophotometer to ensure DNA purity is due to the limited number of samples.

Measurement with a spectrophotometer requires a larger number of samples so that with high dilution the results are more heterogeneous.

The glucokinase (GCK) gene detected in diabetes mellitus and non-diabetic samples indicates that the body can maintain glucose balance and respond appropriately to changes in glucose levels. The GCK gene has an important role in converting glucose into glucose-6-phosphate, which is the initial step in glucose metabolism within cells. In individuals who do not have diabetes, this gene helps regulate blood glucose levels by ensuring proper glucose absorption and storage. Glucokinase (GCK) acts as a crucial gene in determining the level of blood glucose in the body. This gene acts as a "glucose sensor" for the pancreas, so when blood glucose levels rise, insulin production also increases. Thus, if the glucokinase gene is functioning normally, blood glucose levels tend to remain under control, not reaching excessive levels.

Based on this, qualitative detection of glucokinase (GCK) genes from DNA isolate samples was successfully carried out in both type 2 and non-diabetic diabetes mellitus samples, but information on gene expression levels in the realm of transcription and translation was not yet available in this study. The qualitative PCR method has limitations, such as only detecting the presence or absence of the target DNA, without providing information about the quantity or concentration of DNA in the sample. Additionally, the results of qualitative analysis are subjective, relying on the visualization of agarose gel electrophoresis to detect DNA bands. It is recommended that future research use the Quantitative Real-Time PCR (qPCR) method, as it is more accurate and specific.

CONCLUSION

Based on the results of the research conducted, several results were obtained that can be concluded that the glucokinase (GCK) gene in respondents with type 2 diabetes mellitus and non-diabetes was detected at a product length of 206 bp by the Polymerase Chain Reaction method.

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