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Detection of NFKB1 Gene in Patients with Type 2 Diabetes Mellitus and Non Diabetics Using Polymerase Chain Reaction Method

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Abstract

A metabolic condition called type 2 diabetes mellitus is characterized by elevated blood sugar levels because of reduced insulin release by β pancreatic cells. The primary form of NF-kB is Nuclear Factor Kappa Beta subunit-1 (NFKB1), which is a gene that encodes the DNA binding protein (p50). The NFKB1 gene contributes to the oxidative stress and mild inflammatory processes that might exacerbate diabetes. The enzymatic procedure known as Polymerase Chain Reaction (PCR), multiplies a nucleotide sequence in order to identify if type 2 diabetes mellitus or non-diabetic has the NFKB1 gene. The purpose of this study was to detect the presence of the NFKB1 gene in type 2 diabetes mellitus and non-diabetes. The study used a descriptive research method using a purposive sampling technique conducted at the molecular biology laboratory of the Sekolah Tinggi Ilmu Kesehatan Nasional. The respondents in this study were 9 patients with type 2 diabetes mellitus who were prolanes of Puskesmas Wonosari I Klaten and 9 non-diabetic patients in PKK Kadilangu RT 2/RW 1, Baki, Sukoharjo. From the results of the study, it can be seen that the NFKB1 gene was detected after electrophoresis and visualized at 176 bp (base pair). The qualitative presence of the NFKB1 gene in DNA is still detectable, but the level of gene expression in the soil of transcription and translation is not yet known.

Keywords: Type 2 Diabetes Mellitus, *NFKB1* Gene, PCR

Introduction

Multifactorial polygenic diabetes mellitus (Type 2 Diabetes) is believed to arise from the interplay of multiple genes and environmental variables (Rheinheimer *et al*. 2017). Insulin resistance and beta cell dysfunction are linked to inflammation in type 2 diabetes mellitus, expanding the scope of immune metabolism (Ali *et al*. 2022). In Type 2 diabetes, high blood sugar is initially caused by body cells that are unable to respond to insulin optimally, or it can also be a condition called insulin resistance. When insulin resistance develops, this hormone becomes less effective and naturally increases insulin production. Over time, insulin production may decrease because pancreatic beta cells cannot keep up with demand (Webber 2021).

The transcription-related factor the regulation of several genes encoding mediators of inflammation, the cell cycle, apoptosis, viral replication, and a variety of autoimmune illnesses is dependent on Nuclear Factor Kappa Beta (NF-kB) *(Behera et al*. 2020). Five members of the NF-kB family have been identified, and among them is NFKB1 (p105/p50), NFKB2 (p100/p52), RelA (NFKB3/p65), RelB and c-Rel (Sun and Zhang 2007). NFKB1 gene mapped at 4q23-q24, consists of 24 exons. *Nuclear Factor kappa Beta subunit-1* (NFKB1) is a protein-coding gene and is the main form of NF-kB. This gene encodes a protein 105 kD which can perform cottranslation processing by the 26s

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proteasome to produce a 50 kD protein. The 105 kD Protein is a specific transcription inhibitor of the Rel protein and the 50 kD protein is the DNA-binding subunit of the protein complex NF-kappa-B (NF-kB) (Gene, Factor, and Subunit 2023). According to (Raza *et al* 2022), the NFKB1 gene is implicated in the processes of mild inflammation and oxidative stress that might result in difficulties in diabetic conditions. One such mechanism is connected to risk factors for diabetic nephropathy in Type 2 diabetes mellitus. (Oguntibeju 2019). As a risk factor for diabetic nephropathy in Type 2 diabetes mellitus, the NFKB1 gene first appears in a hyperglycemic condition, when an excess of glucose in the blood damages the small blood capillaries in the kidneys (Yuniarti et al. 2021). Long-term hyperglycemia will activate the NFKB1 gene, causing oxidative stress and inflammatory reactions that will in turn trigger the release of proinflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF-α). If glomerular capillaries and kidney alterations are impacted by an inflammatory reaction and an overabundance of cytokines (Jin *et al*. 2023).

One technique for replicating an organism's DNA is the Polymerase Chain Reaction (PCR). The most extensively used method is PCR, which is typically employed for diagnostics requiring very high sensitivity and specificity. PCR is widely utilized for a wide range of purposes, such as gene cloning, DNA computation, the identification of genetic fingerprints, the diagnosis of infectious diseases, and the detection of genetically determined diseases. (Elin *et al*., 2014).

Patients with Type 2 diabetes mellitus and non-diabetics made up the study's responders. Type 2 diabetics are members of the Prolanis diabetes Puskesmas Wonosari I who are 30-60 years old. Puskesmas Wonosari I is one of the health centers Wonosari which is located in Jl. Pakis - Daleman, Dusun I, Bentangan, Kecamatan Wonosari, Kabupaten Klaten, Jawa Tengah. Non diabetic taken from members PKK Desa Kadilangu, RT 2/ RW 1, Baki, Sukoharjo who have no history of diabetes. Based on the above background, this study aims to look at the presence of NFKB1 gene in patients with Type 2 diabetes mellitus and non-diabetes.

Research Methods

Ethical Clearence

This study has passed the ethics of Komisi Etik Penelitian Universitas Muhammadiyah Purwokerto (KEPK-UMP) by Document Registration number KEPK/UMP/57/VIII/2023.

Tools and materials

a. Tools

Tourniquet, holder, mikropipet, tube centrifuge, microcentrifuge, spindown, vortex, microwave, erlenmeyer, tube racks, scales, spatel, ultra low temperature freezer, drybath, screw cap tube, centrifuge tube rack, casting tray, cryogenic box, PCR tube, refrigerator centrifuge PCR thermal cycler, spektofotometer UV-Vis, bowl, monitor, CPU, chamber electrophoresis, Gel doc.

b. Materials

Alkohol 70%, cotton, vacuum tube EDTA, EDTA blood sample, yellow tip, blue tip, collection tube, GS coloumn, alumunium foil, plastic clip, filter paper, aquabidest, PBS (Phosphate Buffered Saline), wash buffer, elution buffer, proteinase-K, GSB buffer, ethanol absolute (32-100%), buffer W1, TE buffer, TBE 1x (Tris-borate-EDTA), DNA ladder, gel red, loading dye, agarose powder, master mix, NFW (nuclease free water), primer forward 5'-GCTGCTGTCATCTGTTGGAA-'3 and primer reverse 5'-CAATGCTTCAGGGATTTGGT-'3 (NCBI, 24 Juni 2023).

Procedure

a. Sample Preparation

A blood sample of 200µl is inserted into a 1.5 ml microcentrifuge tube, then 200µl *Phosphate Buffered Saline* (PBS) is added and homogenized. The sample was added with 20µl proteinase-K , then homogenized

with vortex and incubated at 60°C for 5 minutes (Geneaid Kit).

b. Isolation of DNA

200µl of GSB buffer were added to the sample, and after vortex homogenizing it, it was incubated at 60°C for 5 minutes, with the tube being inverted every 2 minutes. Additionally, the sample was promptly homogenized by vortexing for 10 seconds while in spindown after being incubated into the DNA binding stage, which is accomplished by adding 200µl of ethanol absolute (96–100%) to the sample. The sample is transferred to GS column which has been added to the collection tube by pipetting, then centrifuge at a speed of 14,000 xg for 1 minute. The GS column is then placed into the new collection tube after the flow-through collection tube is disposed of. The sample is added with 400µl buffer W1 to the GS column then centrifuge at a speed of 14,000 xg for 30 seconds, the liquid is removed and reattached GS column into the collection tube. The next step, the sample is added to the wash buffer (ethanol absolute has been added) as much as 600µl into the GS column and dicentrifuge at a speed of 14,000 xg for 30 seconds, the liquid is removed and paired back GS column into the collection tube. The column matrix will then be dried by centrifuging back for three minutes at a speed of 14,000 xg. After transferring the dried GS column into a clean 1.5 ml microcentrifuge tube, 100µl of pre-heated elution buffer is added. The TE buffer is then added to the center of the GS column matrix, and the mixture is allowed to sit at room temperature (15°–25°C) for three minutes to make sure all of the elution and TE buffers have been fully absorbed. Finally, the mixture is centrifuged for 30 seconds at a speed of 14,000 xg to extract pure DNA. Isolate results are saved for next steps (Geneaid Kit).

c. DNA Qualitative Test

Agarose powder is put into erlenmeyer tube and added TBE 10x as much as 50ml, then heated until dissolved in microwave with medium high level for 2 minutes (every 1 minute homogenized). Agarose that has dissolved and the color becomes clear is poured on the agarose casting tray, then cooled to room temperature. The comb is removed slowly and attached to an electrophoretic device (Maftuchah *et al*., 2014). DNA isolate electrophoresis, the sample is inserted as much as 10 µl (5 µl DNA isolate + 3 µl loading dye + 2 µl gel red) into the pitting hole. The cable is connected with a current source to the electrophoresis tank and it is ensured that the magnetic field is closed according to the specified cable. Voltage and running time are set until 90 volts are obtained for 30 minutes (Maftuchah *et al*., 2014).

d. DNA Quantitative Test

DNA isolate was pipetted as much as 20µl and diluted with 3980µl aquabidest and then homogenized by means of divortex for 15 seconds. Absorbance is read using a UV-Vis spectrophotometer, with wavelengths of 260 nm and 280 nm. 4ml of Aquabidest was inserted into cuvette I as a blank and 4ml of diluted DNA sample was inserted into cuvette II. The results of the DNA quantitative test are calculated using the following formula :

- Concentration of DNA
  λ260 x 50 ng/µl x faktor pengencer
- Purity of DNA
  $\frac{\lambda 260}{\lambda 280}$

e. Amplification of the NFKB1 Gene

Before entering into the PCR, pipet as much as 12µl master mix, 2µl primer

forward, 2µl primer reverse, 5µl DNA template and 4µl nuclease free water into the sample cup. The temperature is set on the PCR program, namely pre-denaturation 95°C for 3 minutes, denaturation 95°C for 30 seconds, annealing 61.1°C for 30 seconds, extension 72°C for 1 minute, final extension 72°C for 5 minutes and hold at 4°C. The Primer used for this PCR examination is primer forward 5'-GCTGCTGTCATCTGTTGGAA-'3 and primer reverse 5'-CAATGCTTCAGGGATTTGGT-'3 (NCBI, 24 Juni 2023).

Research Results and Discussion

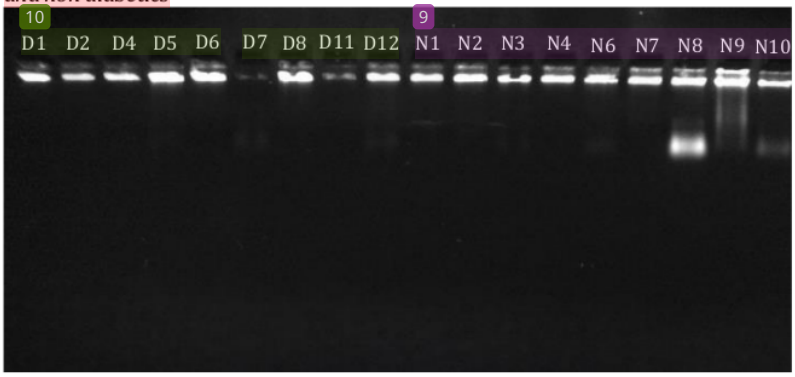
A. Results

The results of FKB1 gene detection were 9 samples in patients with Type 2 diabetes mellitus at prolanis diabetes Puskesmas Wonosari I Klaten and 9 non-diabetic samples in PKK Kadilangu RT 2 / RW 1 Baki, Sukoharjo, which was carried out at the Sekolah Tinggi Ilmu Kesehatan Nasional's Molecular Biology Laboratory. After the isolation phase continued qualitative tests and quantitative tests, with the following results :

1. DNA Qualitative Test

The results of DNA isolation that has been done electrophoresis visualized with Doc Gel can be seen in Figure 1 that all samples were successfully extracted.

Figure 1
Qualitative test results of DNA isolates from blood sampel of patients with type 2 diabetes mellitus and non diabetics



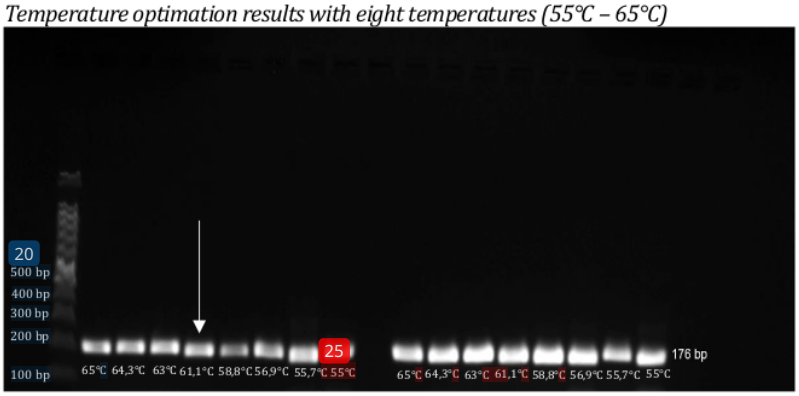
Description :
D1 – D12 : DM Samples
N1 – N10 : Non-DM Samples

2. Temperature Optimization

The results of temperature optimization of NFKB1 gene amplification and obtained the optimal temperature is 61.1°C amplified at 176 bp. Temperature selection based on the firm boundary of the band, the clarity of the band and the absence of double bands.

Figure 2

Temperature optimation results with eight temperatures (55°C – 65°C)

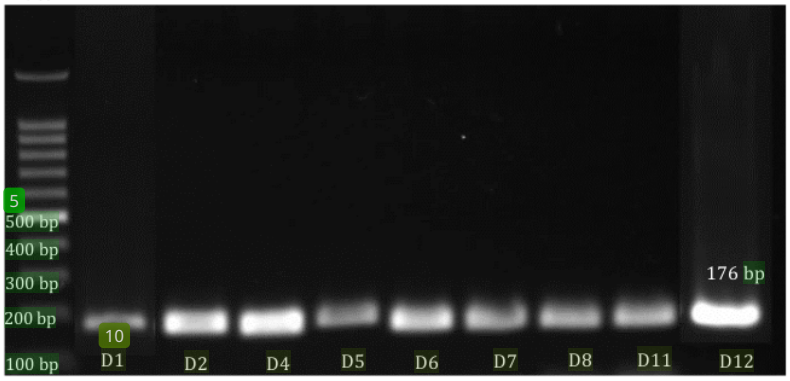


3. PCR visualization of NFKB1 gene in patients with type 2 diabetes mellitus

Figure 3 describing the results of NFKB1 gene visualization in Type 2 diabetes mellitus patients at Puskesmas Wonosari 1 Klaten, where the gene is visualized according to the target of 176 bp.

Figure 3

PCR visualization of NFKB1 gene in type 2 diabetes mellitus patients at Puskesmas Wonosari 1 Klaten

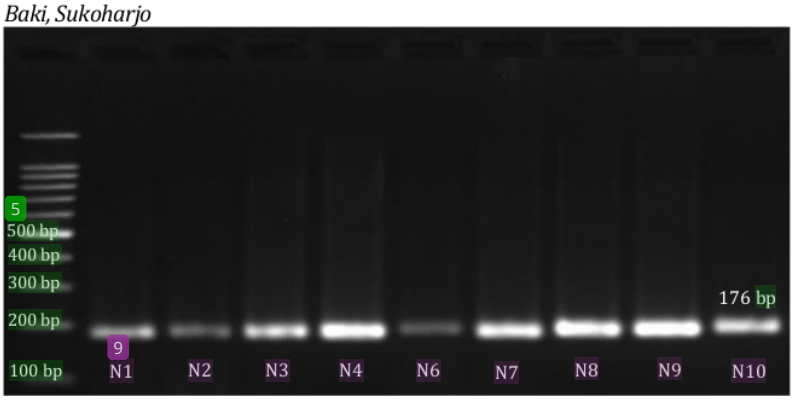


4. PCR visualization of NFKB1 gene in non diabetic patients

Figure 4 describing the results of the visualization of NFKB1 gene in non-diabetic PKK Kadilangu members RT 2 / RW 1, Baki, Sukoharjo, where the gene is visualized according to the target of 176 bp.

Figure 4

PCR visualization result of NFKB1 gene in non diabetic, in PKK Kadilangu member RT 2 / RW1, Baki, Sukoharjo



B. Discussion

The purpose of this study is to certain if the NFKB1 gene in 9 samples of Type 2 diabetes mellitus patients in prolanis diabetes mellitus Puskesmas Wonosari I Klaten and 9 non-diabetic samples in PKK Kadilangu members RT 2/ RW 1, Baki, Sukoharjo, which were visualized at 176bp.

In Figure 1 of DNA qualitative test from isolation result, visualization of bands with different thickness can be seen. According to (Wijaya, Darmawati, and Kartika 2018), state that the quantity of DNA that is successfully isolated can have an impact on the thickness of the DNA band. The more DNA that is successfully isolated, the thicker the DNA band, and otherwise, if the less DNA is isolated, the thinner the DNA band that is visualized on the agarose gel. In Figure 1 with the code N8, N9, and N10 visible DNA bands are thick and there are excesses and smears (impurities that are under the DNA band). This can be caused because there are still contaminants such as protein or residual compounds in the isolation process (Iqbal, Dwi Buwono, and Kurniawati 2016).

Since the annealing temperature influences the stability of DNA hydrogen bonds and specificity (sequence template), it is a crucial parameter for the success of PCR amplification (Fra[illegible], Travensolo, and Carrilho 2013). The annealing temperature for each pair of primers is determined by taking the melting temperature (Tm) supplied by the supplier, which is the end product of primary synthesis (Aulia *et al*. 2023). Synthesis results for TM primer forward 55.5°C and reverse 53.3°C (contained in the primary sheet), then the calculation of TM temperature estimates. PCR temperature optimization with 8 temperatures with a range of 55°C - 65°C because it follows the temperature range of primary synthesis Tm calculation

results. The results of electrophoretic visualization (Figure 2) show that the primer amplifies the DNA well and shows the presence of appropriate DNA bands targeted at 176 bp. Selection of 61.1°C temperature based on the firm boundary of the band, the clarity of the band, and the absence of double bands. After determining the optimum temperature, PCR amplification was performed at pre-denaturation Temperature 95°C 3 minutes, denaturation 95°C 30 seconds, annealing 61.1°C 30 seconds, extension 72°C 5 minutes, for amplification of NFKB1 gene from Type 2 diabetes mellitus sample (Figure 3) and non diabetes sample (Figure 4) visualized according to the target of 176 bp.

Nuclear Factor kappa Beta subunit-1 (*NFKB1*) is a protein-coding gene and is the main form of NF-kB. NFKB1 gene mapped at 4q24 and encodes the DNA-binding protein (p50) 50kDa which serves as the main regulator of inflammation (Yang *et al.* 2014). The NFKB1 gene contributes to the oxidative stress and mild inflammatory processes that might exacerbate diabetes. The NFKB1 gene in Type 2 and non-diabetic diabetics is a sign of mild oxidative stress and inflammation. According to the study (Raza *et al.* 2022) the NFKB1 gene contributes to the oxidative stress and mild inflammatory processes that might exacerbate diabetes. In Type 2 diabetes mellitus, the NFKB1 gene will be downregulated or expressed low while in non-diabetes the NFKB1 gene is upregulated or expressed high. Patients with Type 2 diabetes mellitus may have low expression of the NFKB1 gene due to increased blood glucose levels, which can lead to oxidative stress and inflammation. These factors can also interfere with gene transcription, resulting in low expression of the gene. In non-diabetic NFKB1 gene is highly expressed because this gene plays the role of activation of mild inflammation signal pathways, where the role of inflammation is a form of the body's immune response so that the gene is highly expressed. However, for the specific mechanism of the cause of decreased and increased NFKB1 gene expression in Type 2 diabetes mellitus and normal (non diabetic) control is still being studied. Gene expression is a mechanism of gene transcription and mRNA that affect the protein produced (Mitsis *et al.* 2020), the expression of this gene was analyzed by PCR quantitatively with RealTime-PCR.

Conclusion

Based on the results of this study concluded that the detection of NFKB1 gene in 9 blood samples of patients with Type 2 diabetes mellitus and 9 non-diabetic samples contained NFKB1 gene visualized at 176 bp.

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