

Study Of (NF-Kb) Gene Expression As A Biomarker For Development Of Cardiovascular Disease In Type 2 Diabetes

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Abstract

The purpose of this research is to see if there is a link between diabetes type 2 complications and the level of nuclear factor kappa B (NF-κB) overexpression.

Ninety blood samples were collected from individuals and divided into three groups, each group involved thirty samples: the first group representing individuals with (hypertensive type 2 diabetes), the second group representing (hypertensive type 2 diabetes with cardiovascular diseases), and the last group was represented by a control group.

mRNA was extracted from blood samples then converted to cDNA by using the (qPCR) technique. The outcomes revealed a significant increase ($p < 0.05$) in expression of mRNANF-κB in both groups compared to the control group and this over-expression in hypertensive type 2 diabetes with cardiovascular diseases more than hypertensive type 2 diabetes. The NF-κB gene was studied in type 2 diabetes and cardiovascular disease in the current study, and it could be a novel target for early CVD therapy in the future.

Keywords: NF-κB, diabetes type II, cardiovascular disease, qPCR, inflammation

Introduction

Type 2 diabetes and insulin resistance are frequently accompanied by varying degrees of hyperinsulinemia. Diabetes mellitus raises the risk of cardiovascular disease and death compared to normoglycemia.^[1] Diabetes patients have a 2-fold higher (male) and 5-fold higher (female) relative risk of heart failure than age-matched controls, indicating that cardiovascular issues are to blame.^[2] The complicated link between oxidative stress, pro-inflammatory responses, metabolic alterations, and cell death is crucial in the progression of diabetic cardiomyopathy.^[3] The transcription factor NF-κB regulates inflammatory reactions, immunological responses, cell survival, and cell proliferation.^[4] After the landmark finding that the anti-inflammatory drug aspirin inhibits NF-κB and slows degradation of the NF-κB inhibitor, IκB, NF-κB became a leading suspect in the development of insulin resistance and type 2 diabetes.^[7] The canonical NF-κB pathway has been found in cardiac myocytes as a significant mediator of a variety of heart disorders. In the therapy of heart illness, the canonical NF-κB pathway is beneficial.^[5] Persistent hyperglycemia activates the transcription factor NF-κB, which leads to the production of chemokines, cytokines, and cell adhesion molecules. TNF-α, interleukins, Bcl2, TGFβ and other pro-apoptotic genes, and pro-inflammatory proteins are overexpressed by NF-κβ, which is a major risk factor for vascular dysfunction. Overexpression of NF-κβ also causes endothelial cell

calcification, which leads to additional vascular problems and endothelial dysfunction. Inhibition of the NF- κ B pro-inflammatory pathway is a promising new target for the treatment of diabetes-related vascular problems. (6). NF- κ B a critical factor in diabetic vascular injury, is activated by long-term hyperglycemia. When NF- κ B is active, multiple genes are produced, which are pro-inflammatory and the cell adhesion process. Chronic inflammation leads numerous leukocytes to emit pro-inflammatory and profibrotic cytokines, culminating in inflammation and irreversible fibrosis. (8). Factor nuclear End products of Advanced glycation are produced as a result of advanced glycation., active leukocytes release transcription factors such as Nuclear Factor Kabba β (NF- κ B), Interleukin-1b (IL-1b), Tumor Necrosis Factor-alpha (TNF-a), and Interleukin-6 (IL-6)(9). It's been suggested that NF- κ B activation plays a role in chronic diseases, such as DM and its complications. NF- κ B causes aberrant transcription of a number of genes linked to vascular problems and leukocyte recruitment in general, Inflammation and cell adhesion molecules, for example, when it is activated (10). Leukocyte recruitment triggers extracellular matrix remodeling, which can lead to the development of fibrotic tissue. (11).

Variable gene expression patterns reveal pathophysiological processes that take place in different areas of the human body. We examined the expression of NF- κ B, a diabetes-related gene, in T2DM and T2DM with CAD patients to control individuals in this study. Our discoveries will aid in a clearer picture of the disease and its repercussions.

Material and Methods

1- Patients and Study Design

(1 ml) of blood was collected from 90 individuals, divided into three groups, each group included thirty individuals. The first group included type 2 diabetes Hypertensive (Hypertensive DMT2) and the second group type 2 diabetes Hypertensive and cardiovascular diseases(Hypertensive DMT2 CAD) while the last group included healthy individuals as a control group, then immediately drawn blood samples were placed into Dipotassium-EDTA Vacutainer® tubes for use in qPCR Technique.

2- RNA extraction and qRT-PCR (real-time reverse transcription)

Blood was used to extract total RNA using the extraction kit supplied by (AddBIO, Korea) and reverse transcribed into complementary DNA by using the kit (addBIO, Korea) following the manufacturer's protocol qRT-PCR was used to determine the mRNA levels of NF- κ B using AddScript RT-PCR Syber master (AddBio, Korea). on a BioRad iCycler system (BioRad/USA) , The expression of target genes was analyzed in relation to one another using the $2^{-\Delta\Delta CT}$ technique. The oligonucleotide primers¹⁴ that were used for the PCR amplifications were purchased from (Macrogen, Korea) and listed as in table.1.The housekeeping gene GAPDH was employed..

Table (1) List of primer sets for SYBRGreen-based real-time reverse transcription (qRT) PCR

Primer	Sequence 5'-----3'	
NF- κ B	F	AAGACCCACCCCACCATCAA
	R	AAACTGTGGATGCAGCAGCGGTC
GAPDH	F	CAGTGATGGCATGGACTGTG
	R	CACATCGCTGAGACACCA

3- Statistical Analysis

A one-way analysis of variance was used in the statistical analysis (ANOVA) was used dependent on the Prism 7 software in Graphpad Statistical , P-value of $P \leq 0.05$ was used to determine significance.

Results

1. Efficiency of the assay's amplification NF- κ B

Figure (1) show melting curve analysis of the amplified products of both NF- κ B and the internal reference genes shows a high specific amplification without non-specific reaction or primer dimer. On other hand amplification curve of the tested samples represents for NF- κ B gene alongside running with internal reference gene amplification. This also indicates a successful RNA extraction and cDNA synthesis as in figure (2).

2- - qRT-PCR relative expression

Real-time PCR of peripheral WBCs from 30 healthy subjects 30 (Hypertensive T2D)and 30 (Hypertensive DMT2with CVD) subjects were performed on our selected gene NF- κ B. Information of primers for the selected genes and GAPDH was shown in (Table 1). The gene under study showed differential expression between groups .The fold changes of gene expressions, as determined by real-time PCR, are shown in (Table 2) and (fig 3) where showed a significant ($P < 0.05$) tendency toward an increase in relative expression in Hypertensive DMT2with CVD group and Hypertensive DMT2D when compared to control . This increase was more significant in Hypertensive DMT2who have CVD in comparison with Hypertensive DMT2 without CVD Table 1.

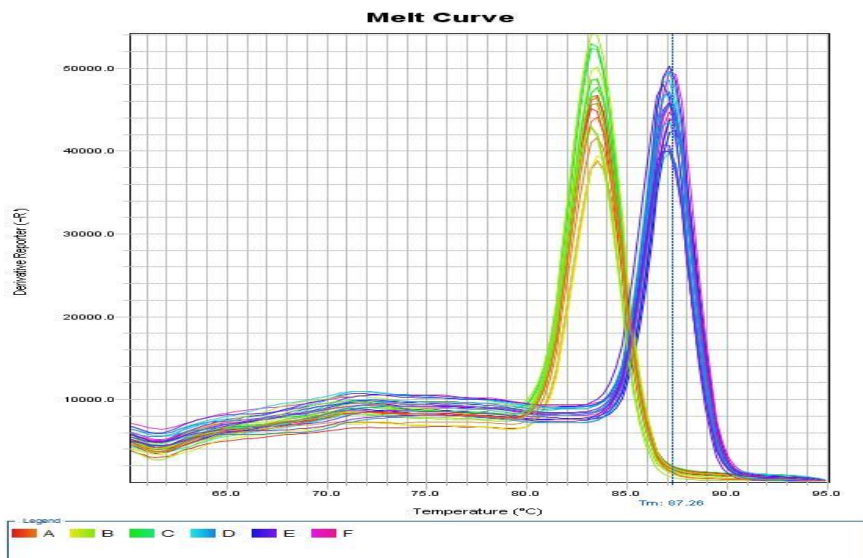


Figure 1: melting curve analysis of the amplified products of both NF- κ B and the internal reference genes



Figure 2: amplification curve of the tested samples represents for NF-κB gene alongside running with internal reference gene amplification.

Table (1) The average of the fold change in NFKB

Control	Hypertensive DMT2	Hypertensive DMT2with CVD
1	1.95	2.163

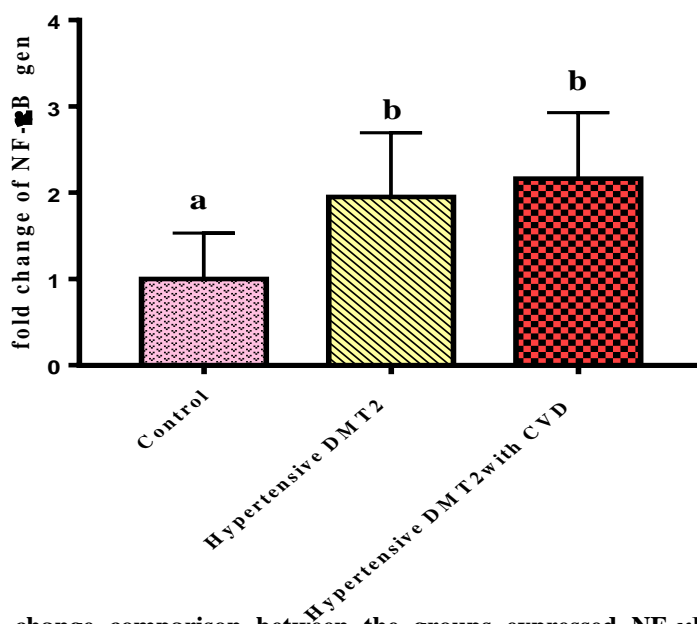


Figure 3: Fold change comparison between the groups expressed NF-κB gene. This shows upregulation of Hypertensive DMT2 and Hypertensive DMT2with CVD compared with the control group.

Values represent Mean ±SE.

different letters indicate to there is significant $p < 0.05$ among groups

similar letters indicate to there is no significant $p > 0.05$ among groups

Discussion

The activation of NF- κ B gene is linked to chronic diseases such as diabetes and its complications. The results of Real-time PCR in this study showed that hypertensive DMT2 with CVD had over-expression of the NF- κ B gene in comparison to DMT2 and the control group. These results supported by studies of (Darwish et al., 2021) ⁽¹²⁾ and (Cong et al., 2015) ⁽¹³⁾ were reported that transcriptional activation of NF- κ B plays an essential role in DMT2 and CAD.

NF- κ B activity is considerably increased under hyperglycemic circumstances, TNF- α , IL1 β -, IL6, CD36, and MCP-1 overexpression promotes endothelial cell death and an inflammatory response by releasing cytokines, TGF- β , chemokines, and vesicular cell adhesion molecules (VCAMs)^{15, 20} Furthermore, overactive NF- κ B is responsible for aberrant DNA transcription and the expression of a number of genes linked to vascular problems. ⁽²¹⁾ Changes in gene expression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), endothelin-1 (ET-1), activated protein C (APC), and transforming growth factor (TGF) are also caused by overactivity of NF- β leading in angiogenesis and vascular cell damage. ⁽²²⁾ According to research, the activation of intracellular pathways, NF- κ B translocation, and its direct effect on the transcription of proteins that promote certain illnesses appear to have a specific origin. The activation of transcription factors and subsequent translation of chemotactic cytokines causes differential recruitment of leukocytes, as well as activation of endothelium, stroma, and adipocytes. As a result, micro-inflammation develops in a chronic, systemic manner. Modifiable risk variables such as nutrition, physical activity, and obesity influence NF- κ B activation. These are the most fundamental molecular pathways. that are shared by diabetes type 2, coronary artery disease, and malignancies generated from epithelial cells. Obesity, specifically DM type II, is a potential risk factor for both CAD and stroke due to the activation of chronic, systemic microinflammation. Periodontitis is a novel, independent risk factor for coronary artery disease that is marked by NF- κ B activation in the same manner as diabetes is. ⁽²³⁾.

Among the numerous other cellular stress pathways are TNF- α , oxidized LDL, the receptor for advanced glycation end-products [RAGE], reactive oxygen species [ROS], members of the protein kinase C enzyme family, and endoplasmic reticulum stress⁽¹⁶⁾. All of these proteins, which are elevated in diabetes mellitus, can activate the NF- κ B transcription factor pathway ⁽¹⁷⁾ NF- κ B impacts the production of pro atherogenic molecules through regulating the expression of pro atherogenic molecules such as surface proteins, cytokenes, and chimokines. Inhibiting this pathways reduces the progression of atherosclerosis in mice^{18,19} \

Conclusion

The current study investigated the up-regulation of NF- κ B gen in Hypertensive DMT2with CVD, which indicated that hyperglycemia in state of DMT2 patient induces activation of NF- κ B gene expression and as a result to that NF- κ B is activated by so many different intracellular pathways. NF- κ B inhibition is extremely likely to be deemed a viable target for the treatment of diabetes and its consequences.

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