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ORIGINAL ARTICLE

Quantification of Endothelin-1 Gene Expression in the Diabetic Patients' Type II (DMT2) with Cardiovascular Disease

MERVAT KAMIL KADHIM[1], ALAA MOHAMMAD HASSON AL-HUSSEINI[2]
[1,2]University of Al-Qadisiyah, College of Science, Department Of Biology, Iraq
Corresponding author: Mervat Kamil Kadhim, Email: scie.bio.mas.20.33@qu.edu.iq

ABSTRACT

The goal of this study is to see if there's a link between diabetes type 2 problems and endothelin-1 overexpression (ET-1).

Where (90) blood samples from participants were obtained and divided into three groups: a first group representing individuals with (Hypertensive DMT2) and a second group representing (Hypertensive DMT2with CVD,), while the third group was represented by a control group.

mRNA was extracted from blood samples then converted to cDNA by using the (qPCR) technique. When comparing the levels of ET-1 in the (hypertensive DMT2 and hypertensive DMT2with CVD groups) to the control group, the results showed a significant difference ($p<0.05$), The current study analyzed the ET-1 gene in type 2 diabetes and cardiovascular disease, which could be a new target for early CVD therapy in the future.

Keywords: endothelin-1gene, diabetes type II, cardiovascular disease, , endothelial dysfunction

INTRODUCTION

Intensive blood glucose management has been proven in type 2 diabetes studies to postpone the start and progression of microvascular problems. In type 2 diabetes, however, controlling various metabolic variables such as hyperglycemia, free fatty acids, lipids, insulin resistance, and others is required to reduce cardiovascular disease[9]. Diabetes macrovasculopathy causes structural and functional abnormalities in major arteries, resulting in blood flow obstruction, myocardial infarction, hypertension, and mortality [12]. Hyperglycemia plays a major role in the production of ET-1 in diabetes, which contributes to the pathophysiology of endothelial dysfunction and the resulting vascular.[17]

Significant numbers of previous studies indicated a remarkably close relationship between inflammatory markers and cardiovascular outcomes[3] ET-1 has been demonstrated to boost the expression of inflammatory factors such as C-reactive protein and tumor necrosis factor-α, as well as atherosclerosis-related interleukins. [18] Meanwhile, it has been suggested that ET-1, which is derived from endothelial cells, is associated with endothelial dysfunction[4] Interestingly, Endothelial dysfunction has also been linked to future cardiovascular events and has been recognized as a risk factor for atherosclerosis [6] ,Therefore, ET-1 was considered a pro-inflammatory factor. ET-1, which is produced and secreted by ECs, is the most potent endogenous vasoconstrictor. It hastens the pathophysiological course of cardiovascular disease via vasoconstriction[10] Endothelin is thought to be important in hypertension and vascular disease, and atherosclerosis in particular in the context of diabetes[8], The majority of evidence for the role of ET-1 in diabetes has been generated in studies of diabetic nephropathy where the predominant ETA receptor blocker avosentan significantly reduced albuminuria and renal fibrosis [19].

The goal of this study was to look at the involvement of ET-1 in the development of cardiovascular disease in people with type 2 diabetes and hypertension.

MATERIAL AND METHODS

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

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. ET-1 mRNA levels were determined using qRT-PCR using AddScript RT-PCR Syber master (AddBio, Korea). on a Bio-Rad iCycler (Bio-Rad/USA) system To measure the relative expression of target genes The 2–ΔΔCT technique was used. The oligonucleotide primers[10] that were used for the PCR amplifications were purchased from (Macrogene, Korea) and listed as in table 1. As a housekeeping gene, GAPDH was employed.

Table 1: SYBR-Green-based real-time reverse transcription (qRT) PCR primer sets

Primer	Sequence 5'-------3'	
Human-ET-1	F	AAGGCAACAGACCGTGAAAAT
	R	CGACCTGGTTTGTCTTAGGTG
GAPDH	F	CAGTGATGGCATGGACTGTG
	R	CACATCGCTGAGACACCA

Statistical Analysis: The data were statistically evaluated using the Graphpad Prism 7 program. One-way analysis of variance was performed to determine the significant and non-significant variables. Tukey's multiple comparisons test was applied with a significance level of less than 5% for comparing the means.

RESULTS

Efficiency of the assay's amplification Endothelin-1: Figure (1) show melting curve analysis of the amplified products of both ET-1 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 ET-1 gene alongside running with internal reference gene amplification. This also indicates a successful RNA extraction and cDNA synthesis as in figure (2).

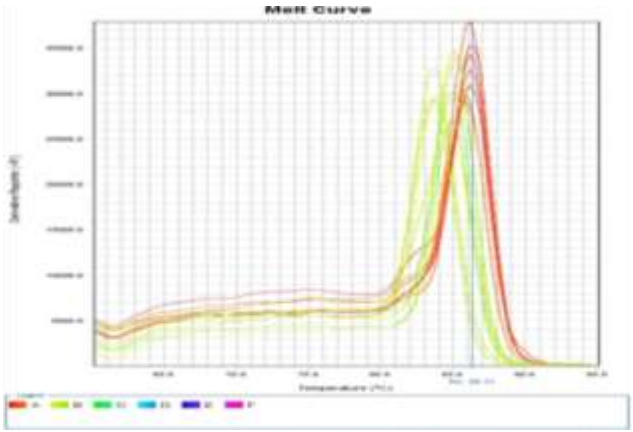

Figure 1: melting curve analysis of the amplified products of both Endothelin-1 and the internal reference genes.

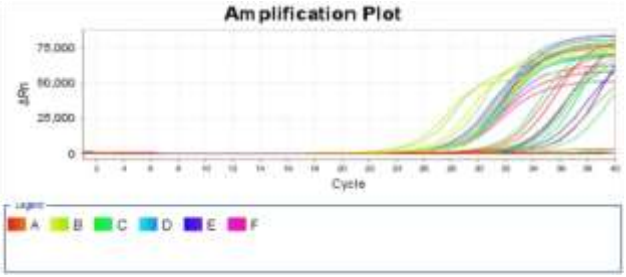

Figure 2: Amplification curve of the tested samples represents for Endothelin-1gene alongside run with internal reference gene amplification.

Analysis of the qRT-PCR gene expression data ET-1: The gene expression of ET-1 was investigated in this study. The results in Figure 1 showed a significant increase in gene expression of ET-1 .The fold change of ET-1 gene was 4.1 and 5.1 fold in the Hypertensive DMT2 and Hypertensive DMT2 with CVD, when compared with control group, respectively.

These results demonstrated asignificant upregulation inthe expression of ET-1 in patients with Hypertensive DMT2 with CVD compared to control ($P \leq 0.05$, Fig.3), only slightly but no significant increase in ET-1 expression was shown in patients with Hypertensive DMT2 when compared to the Hypertensive DMT2 with CVD .

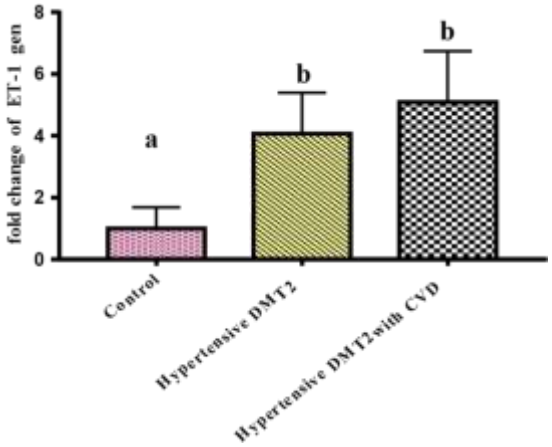

Fig 3: Fold change comparison between the groups expressed ET1 gen. This shows upregulation of Hypertensive DMT2 and Hypertensive DMT2with CVD compared with control group.

DISCUSSION

ET-1 induction is predominantly mediated by hyperglycemia in diabetes, and it contributes to the pathophysiology of endothelial failure and the resulting vascular disease.

These results are supported by studies of **(Ouerd et al[16]; Zhou et al.,[21]; Chen et al.,[1])** in which they demonstrate for the first time that overexpression of endothelial-restricted ET-1 exacerbates atherosclerosis in diabetes through a mechanism involving NADPH oxidase isoform(NOX1) They also claimed that ET-1 concentrations were found to be statistically higher in CVD patients than in non-CVD patients.. They also found that ET-1 mRNA levels were increased in diabetes.

The most likely metabolic shift causing the rise in ET-1 levels is hyperglycemia, while hyperinsulinemia may also play a role when present. Yamauchi et. al.[20] found that increasing the amount of glucose in aortic endothelial cells increased the synthesis of ET-1 protein.

The Studies have indicated that Abnormal endothelial function has been demonstrated in type 2 diabetes, In the natural history of type 2 diabetes, hyperglycemia appears before endothelial impairment[5] . This is related to the activation of protein kinase C (PKC), which promotes endothelium-dependent vasodilator dysfunction by modifying nitric oxide (NO) bioavailability, affecting VEGF expression and activities, and increasing the production of (ET-1)[2].

Studies demonstrate that overexpression of endothelin derived-ET 1 in a model of diabetes accelerated atherosclerosis increases plaque formation by increasing reactive oxygen species (ROS) formation and by promoting immune cell infiltration, predominantly in the perivascular adipose tissue(PVAT). This initial atherosclerotic effect includes NADPH oxidase isoform (NOX1), which has been previously suggested to promote atherosclerosis, especially in the case of diabetes. The interaction between ET and NOX1 opens up new therapeutic opportunities, as both ETA receptor antagonists and NOX inhibitors, a combination therapy approach would be of interest to assess additive or synergistic effects of NOX1 inhibition and endothelin receptor blockade [7].

Hyperglycemia has a major role in the production of ET-1 in diabetes, which contributes to the pathophysiology of endothelial dysfunction and the resulting vascular consequences. [17] Indeed, Overexpression of ET-1 in endothelial cells causes increased production of reactive oxygen species (ROS), decreased nitric oxide bioavailability, increased expression of adhesion molecules, and accelerated atherosclerosis, in addition to its powerful vasoconstrictive effects[15]. It is critical to have a better knowledge of the molecular mechanisms behind ET-1 induction and its downstream consequences on endothelial cell function. Endothelin converting enzyme 1 (ECE1), which converts large ET-1 to physiologically active ET-1, is transcriptionally increased by activation of the proinflammatory transcription factors activator protein 1 (AP-1) and NF-κβ[14] Interestingly, both AP-1and NF-κβ have been linked to the control of EDN1 (ET-1) gene transcription . As a result, an upstream master regulator of AP-1 and NF-κβ might play a key role in modulating ET-1 synthesis in endothelial cells.[13].

Several studies have demonstrated that prolonged, inappropriate elevations in endothelin (ET)-1 levels, as well as other hormones like angiotensin II and fibrotic cytokines like transforming growth factor (TGF), cause collagen synthesis to be activated, which in turn causes an increase in the extracellular matrix protein ECM, which accumulates in diabetes and causes irreparable tissue damage [11].

CONCLUSION

We conclude from the results of our study, and previous studies confirming increased levels gene expression of endothelial-derived gene (ET-1) in type 2 diabetic patients, that ET-1 have a key role in the development of diabetes-related cardiovascular disease. As a result, ET-1 inhibition is a viable option for treating diabetes and its consequences.

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