RESEARCH
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ESTIMATION OF IL-6(174G>C) GENE EXPRESSION WITH CHRONIC HYPERTENSION PATIENTS IN IRAQI POPULATION

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Abstract
The study aimed to reveal the gene expression estimate for the IL gene (174 G>C) in patients with high blood pressure. The study included 50 blood samples, which 25 were from people suffering from chronic high blood pressure (different races) and their ages ranged between 35 and 68 years, and 25 healthy samples (control group), from different races, and their ages ranged between 17 and 17 years. 53 years old. Studied the estimation of gene expression of the IL-6(174G>C) gene using REAL-TIME-PCR technology (RT-PCR). The statistical results showed that there were highly significant differences between patients and the control group regarding the fold change in IL-6 gene expression. Values were expressed as mean ± standard deviation, which in the control group was (1 ± 0.000), while in the patient group it was (0.644 ± 0.153). The P value for the difference between groups is 0.024, indicating that there is a significant difference (S) between the two groups, as the patient group showed a lower change in IL-6 gene expression compared to the control group. A comparison was made between male and female patients in the study, including the number of cases in each group, the fold change in IL-6 gene expression, and the P value for the difference between groups. There were 14 male and 11 female patients in the study. The average fold change in IL-6 gene expression was (0.648 ± 0.169) in male patients and (0.638 ± 0.142) in female patients. The P value for the difference between male and female patients was 0.961, indicating that there was no statistically significant difference (NS) between male and female patients in the study. The spectrum changes in IL-6 gene expression and the value of the biological function (2-ΔΔCt) for each age group were also studied. In the group of ages under 45 years, the average change in IL-6 gene expression was 0.674 ± 0.192 and in the group of ages 45 to 55 years, the average change in IL-6 gene expression was 0.614 ± 0.132. In the group of ages over 55 years, the average change in IL-6 gene expression was 0.643 ± 0.123. The significance value was 0.975, which indicates that there are no significant differences between the different age groups.
Keywords: IL-6 (174 G>C) gene. Gene Expression, RT-PCR, Hypertension

Introduction

High blood pressure is among the most common medical conditions ([1], high blood pressure is the most common cardiovascular disease that increases the risk of heart, kidney and other diseases [2] as more than a billion people live with (TNH) Hypertension [3] It is considered the number one killer among the causes of death in humans as it [4] [5] causes approximately 9.4 million causes of death in the world [6]. It is recommended to develop a new drug target for HTN complications beyond the traditional interest achieved by lowering blood pressure alone [7]. Globally, the burden of the disease continues to increase [8]. A recent study indicates the need for improvements in the management of HTN in the Middle East, especially in rural communities and younger subjects [9]. -70 times per minute), it pumps blood into the arteries [10]. High blood pressure, also referred to as hypertension, is a condition in which the blood pressure in the arteries is constantly high. Every time a human heart beats, it pumps blood to the entire body through the arteries. High blood pressure can lead to organ damage, as well as many diseases, such as kidney failure (kidney failure), aneurysm, heart failure, stroke, or heart attack, retinal hemorrhage and injury to the glomeruli. The normal level of blood pressure is less than 120/80, where 120 represents the systolic measurement (peak pressure in the arteries) and 80 represents the diastolic measurement (the minimum pressure in the arteries) [11]. Cytokines play a major role in inflammatory processes and have an important role in the development of cardiovascular diseases such as high blood pressure (HTN) and atherosclerosis [12]. Cytokinesis IL-6 has a role in the occurrence of inflammation and the development of chronic hypertension [13]. In view of the significant increase in the prevalence of patients with high blood pressure (HTN) and its effects on mortality and comorbidities, there is a great focus on estimating gene expression in patients with high blood pressure, and studies are continuing to understand the mechanism. Disease [12]. Previous studies and our current study have demonstrated the important role of cytokinesis in gene expression of IL-6, which is an important biomarker for the risk of cardiovascular disease [14].

Patients and Methods

Blood samples from people with high blood pressure and healthy people were collected randomly in areas of Baghdad hospitals were diagnosed by specialists and included samples of different genders. The study included 50 blood samples, including 25 samples from people with chronic high blood pressure whose ages ranged between 35 and 68 years, and twenty-five healthy samples (control group), whose ages ranged from 17 to 53 years. The blood was collected in special 2-ml

tubes containing EDTA, an anticoagulant, which was used in RNA extraction. Patients were diagnosed by specialist doctors

Primers

Three primers were manufactured by the Korean company BIONEER upon request, and were used to detect the G and C alleles in the IL-6 gene (174G>C) for gene expression and were prepared according to the instructions attached by the company (Table 1)

Table 1. primer of IL-6 gene of RT-PCR.

Primers used to detect the IL-6 gene		
Technology	Initiator name	Nucleotide Sequence of the IL6 gene primer
RT-PCR	IL-6 Forward	5-GTCTCCTCTGACTTCAACAGCG-3
	IL-6 Reverse	5-ACCACCCTGTTGCTGTAGCCAA-3

Polymerase chain reaction (PCR) to convert RNA into qDNA

The RNA must be converted into cDNA for the purpose of measuring gene expression using a special kit prepared by BIONEER and according to the recommended concentration for each sample. After adding the ingredients together, I mixed the ingredients using the Vortex device, then put them in the Thermo cycle device according to its program, as shown in its manual. After completing the steps and converting the RNA to cDNA, I put them in the freezer to preserve the sample until it is time for use, then we begin the step. Next

Table (2) Components of the reverse transcriptase PCR reaction to convert RNA to cDNA

Size	the components
11u	RNA
2	Oligo dT
5	DNA ase D.W
2	R.T Primer
20	Total

Real-time multiplex chain reaction (RT-PCR)

A quantitative polymerase chain reaction kit produced by the Korean company BIONEER was used, which is a good solution containing the SYPER Green Master dye, prepared for the Quantitative Reverse Transcription Polymerase Chain Reaction qRT-PCR with high-performance steps. Which uses cDNA as a template for replication to detect mature IL-6 mRNA, using primers specific for IL-6 mRNA through amplification. The IL-6 gene was also adopted as a gene to detect and calibrate the results of IL-6 RNA genes. It is useful in achieving the highest accuracy of gene expression according to the Livak $2^{-\Delta\Delta ct}$ method (Schmittgen, 2001). The Cycle Threshold (ct) is the basic rule in qRT-PCR technology, as it expresses the amount of gene expression and is known as the cycle in which the fluorescence of IL-6 mRNA reaches its maximum. Detection of the amount depends on the fluorescent strength of Syber Green, which is represented by the expressive curve. Regarding the amount of gene expression, gene expression was measured by the relative quantitative method, through which the amount of gene expression for the selected sample is measured based on a reference sample that is almost constant and is not affected by various conditions. The sample quantity is measured with the Ct results for the IL-6 calibrator gene, which is known as the House Keeping Gene, and the gene expression values are extracted according to the Livak equation.

After I mixed the reaction contents of the samples and placed them in a qRT PCR plate, multiplexing steps were performed to investigate the amount of expression of the IL-6 (174G>C) genes.

Table (2) Reaction system and cycles for the qRT-PCR device

Cycle	Time	Temperature	Cycle Step
1 Cycle	60 Seconds	95 c	Pre-denaturation
35 Cycle	15 Seconds	95 c	Denaturation
	40 Seconds	60 c	Annealing & Extension
1 Cycle	45 min	60 – 95 c	Melt Curve

Results and Discussion

Table 1 and Figure 1 provide a comparison between the control group and the patient group regarding the fold change in IL-6 gene expression. Values were expressed as mean ± standard deviation, which in the control group was (1 ± 0.000), while in the patient group it was (0.644 ± 0.153). The P value for the difference between groups is 0.024, indicating a significant difference (S) between the two groups, where the patient group showed a lower change in IL-6 gene expression compared to the control group. Genetic phenomena refer to changes in genetic expression in the absence of changes in the DNA sequence itself. It includes post-translational histone modification. And DNA methylation and microbial encoded RNA [7] (miRNAs). Although epigenetic modifications are hereditary and can be transmitted over several generations, they can also be affected by genetic and nutritional factors and may be reversible. Epigenetic events play crucial roles in physiological processes such as cellular differentiation by ensuring that the expression of certain genes is expressed only in certain cell types [15].

Genome-wide association studies

Based on the concept of disequilibrium at the population level. GWAS attempts to determine the association between genetic variants or single nucleotide polymorphisms. (SNPs) are common disease traits in populations.[16] Polymorphisms are found at specific genetic sites and refer to differences in single nucleotides.[17]

The Wellcome Trust Case Control Consortium (WTCCC) study conducted in 2007 was the first study to attempt to understand gene expression or variants associated with HTN using GWAS. However, no significant association between HTN and gene expression was identified [18] and this study did not agree with our study as The results of our study were that there were high differences in gene expression between patients and the control group, as patients appeared to have no gene expression. A study [13] demonstrated the important role played by cytokinesis IL-6 in inflammation that leads to atherosclerosis, and thus atherosclerosis leads to coronary circulatory insufficiency. [13] Chronic diseases such as high blood pressure and diabetes contribute to causing coronary artery disease. The flow of

cellular dynamics that stimulate inflammation plays crucial roles in the onset and development of chronic diseases. A study [13] proved that cytokine IL-6 has a role in the occurrence of inflammation and the development of chronic hypertension, and this study is consistent with the results of our study. Our study also agreed with a study [13] where cellular motility showed higher levels in patients with blood pressure and diabetes compared to the control group. Due to the dramatically increasing prevalence of patients with high blood pressure (HTN) and its effects on mortality and comorbidities, there is a continuing focus on understanding the mechanism of the disease [12]. Previous studies and our current study have demonstrated the important role of cytokine IL-6, which is an important biomarker for the risk of cardiovascular disease [14]. A study [12] agreed with our study that polymorphisms of the IL-6 gene (174G>C) are associated with the risk of developing high blood pressure (HTN) in different population groups Cytokines play a major role in inflammatory processes, with continued research and increasing evidence regarding their role in the development of cardiovascular diseases such as high blood pressure (HTN) and atherosclerosis [12].

Table 1: Comparison between Control and patients' groups

Groups	Fold of Change ($2^{-\Delta\Delta Ct}$) IL-6 Gene Expression	P value
Control	1 ± 0.000	0.024 S
Patients	0.644 ± 0.153	

S : Significant difference between groups (p value < 0.05) .Values as expressed as (mean ± standard deviation)

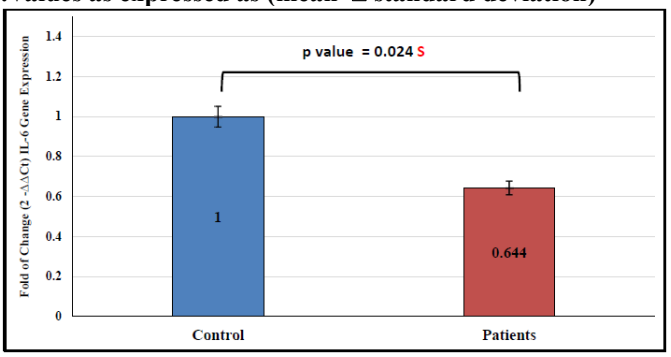


Figure 1: Comparison between Control and patients' groups

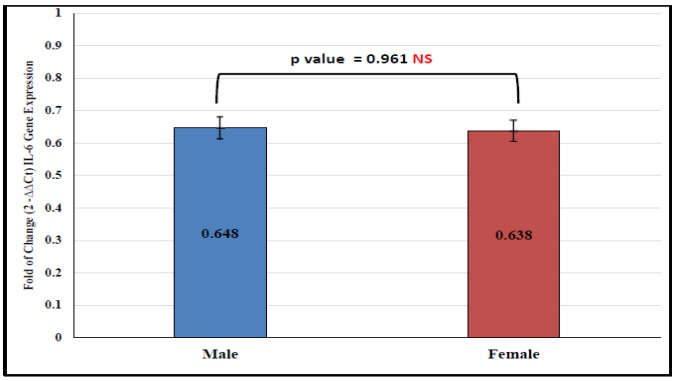
S : Nonsignificant difference between groups (p value < 0.05)

Table 2 and Figure 2 show a comparison between male and female patients in the study, including the number of cases in each group, the fold change in IL-6 gene expression, and the P value for the difference between the groups. There were 14 male patients and 11 female patients in the study. The mean fold change in IL-6 gene expression was (0.648 ± 0.169) in male patients and (0.638 ± 0.142) in female patients. The P value for the difference between male and female patients was 0.961, indicating a Nonsignificant difference (NS) between male and female patients in the study.

Table 2: Comparison between Male and Female in patients' group.

Groups	Number of cases	Fold of Change ($2^{-\Delta\Delta Ct}$) IL-6 Gene Expression	P value
Male	14	0.648 ± 0.169	0.961 NS
Female	11	0.638 ± 0.142	

NS : Nonsignificant difference between groups (p value > 0.05) .Values as expressed as (mean ± standard deviation)



NS : Nonsignificant difference between groups (p value < 0.05)

Figure 2: Comparison between Male and Female in patients' group.

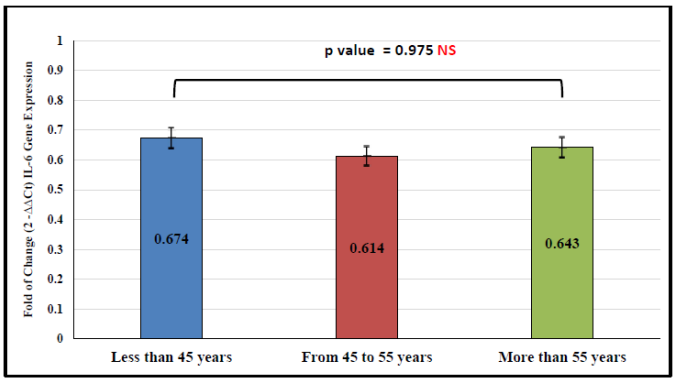
Table 3 and Figure 3 present a comparison between age groups in the study, including the number of cases in each group, the fold change in IL-6 gene expression, and the P value for the difference between the groups. There were 8 cases in the less than 45 years group, 8 cases in the 45 to 55 years group, and 9 cases in the more than 55 years group. The mean fold change in IL-6 gene expression was (0.674 ± 0.192) in the less than 45 years group, (0.614 ± 0.132) in the 45 to 55 years group, and (0.643 ± 0.123) in the more than 55 years group. The P values for the differences between the groups were all greater than 0.05, indicating Nonsignificant differences (NS) between different age groups in the study in terms of IL-6 gene expression.

Table 3: Comparison between Age groups

Groups	Number of cases	Fold of Change ($2^{-\Delta\Delta Ct}$) IL-6 Gene Expression	P value
Less than 45 years	8	0.674 ± 0.192	0.975 NS
From 45 to 55 years	8	0.614 ± 0.132	
More than 55 years	9	0.643 ± 0.123	

NS : Nonsignificant difference between groups (p value > 0.05)

.Values as expressed as (mean ± standard deviation)



NS : Nonsignificant difference between groups (p value > 0.05)

Figure 3: Comparison between Age groups

Figure 4 shows the correlation analysis between age and the Fold change in IL-6 gene expression ($2^{-\Delta\Delta Ct}$). The Pearson correlation coefficient between age and the fold change in IL-6 gene expression is -0.005, which indicates a very weak and Nonsignificant negative correlation. The p-value for this correlation is 0.982, which is greater than 0.05, suggesting that there is no significant correlation between age and the fold change in IL-6 gene expression in the study. This means that age does not appear to be associated with changes in IL-6 gene expression.

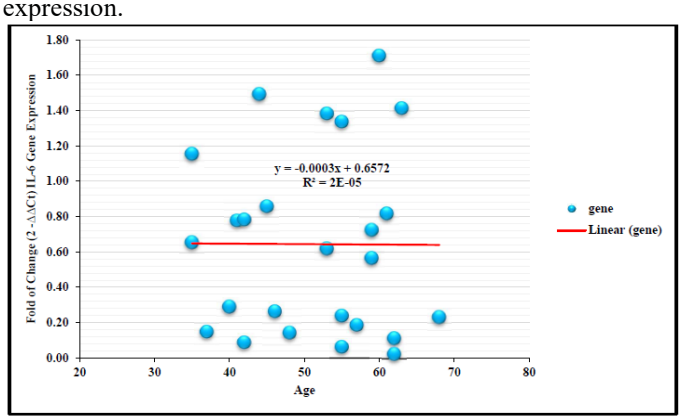


Figure 4: Scatter plot to show the correlation between Age and Fold change in IL-6 gene expression (2-ΔΔCt)

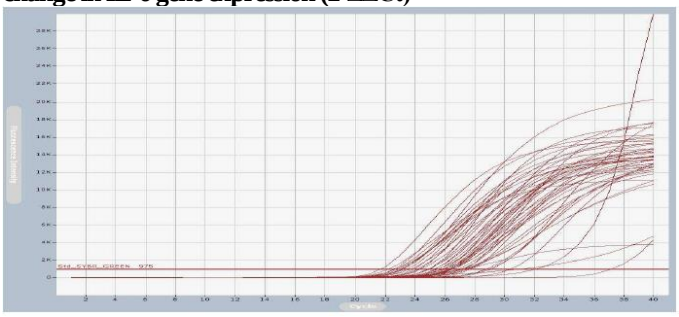


Figure (5) CT value curves for the IL-6 gene

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