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ORIGINAL ARTICLE

Correlation Between Increasing DNA Copy Numbers That Encode Cortisol Biosynthesis Enzymes and Myocardial Infarction Patients

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ABSTRACT

Background: The dysfunction in the genes that regulate cortisol production may lead to an increase or overproduction of the hormone and thus affect the functioning of the heart, which may lead to Myocardial infarction.

Aim: The aim of our study was to find a correlation between increasing the DNA copy numbers that encode cortisol biosynthesis enzymes and Myocardial infarction disease.

Methods: Between the first of January 2021 and the first of July 2021, 120 blood samples from the patients with the acute myocardial infarction (AMI)—60 samples as controls and 60 patients—of both sexes who were admitted to the Unit of the Cardiac Surgery at AL- Salam General Teaching Hospital, the Intensive Cardiac Care Unit, and outpatient health centers in Mousul, Nineveh province/Iraq, were taken. The CYP11A, CYP17A, and CYP11B1 genes implicated in cortisol biosynthesis were found in this work using qRT-PCR.

Results: The results of this investigation showed a considerable difference between the age groups of AMI patients and healthy control group in means of amplicon copy counts of CYP11A, CYP17A, and CYP11B1 coding genes. The gene coding for the cytochrome CYP17A enzyme was shown to have a significantly higher number of amplicons in all age groups of the patients, but particularly in the second group (46–56 years) in comparison to healthy control group

Conclusion: The results of this study demonstrated for the first time that there were significant correlation between three steroid hormone biosynthesis genes and Acute Myocardial Infarction (AMI) disease by utilizing RT-PCR Technique which revealed a significant increase in the amplicon copy numbers of CYP17 ,CYP11A ,CYP11B genes in all patient's age groups compared with healthy control group.

Keywords: AMI patients, Cortisol biosynthesis, CYP17A , CYP11A and CYP11B1 genes.

INTRODUCTION

Worldwide, one of the major death causes is Myocardial Infarction (MI)[1]. One of its clinical signs is the occurrence of persistent chest pain which appears sharply and may inflict pain to include arms, the left arm more commonly and shoulder, jaw, teeth, neck, and may accompany pain sweating, vomiting, difficulty breathing, palpitations, and the heart may stop abruptly[2,3].

Cortisol is one of the most important corticosteroids and is a steroid hormone that is produced in the body by the Zona fasciculata region in adrenal cortex under the effect of the hormone Adrenocorticotrophic hormone (ACTH)[4]. Cortisol is released in response to psychological stress and stress conditions and is therefore called stress hormone, because it increases blood levels in the case of stress and extreme stress. Cortisol can be defined as one of the significant biomarkers of stress and is related to Type II diabetes and cardiovascular disease (CVD)[5,6]. Cortisol and the rest of glucocorticoids mainly derive from cholesterol in the mitochondria of the Zooona fasciculate, cholesterol is converted into a pregnelonone compound by the enzyme 3β-hydroxysteroid dehydrogenase[7]. Cytochrome P450 enzymes is of high importance in the biosynthesis regarding various compounds, like steroid hormones, lipids such as cholesterol and some acids, cytochrome P450 enzymes are also involved in external substances such as drugs and cancers such as intracellular toxins[8].

There are many genes involved in the construction and secretion of steroids hormones, including the important genes that contributing to cortisol biosynthesis. The most important genes involved in cortisol biosynthesis includes: CYP 11A, CYP17 and CYP11B[9]. CYP11A gene has an important role in accomplishing the first step of cortisol construction by converting cholesterol into pregnelonone[10]. Cytochrome P-45017α (CYP17) is an important gene in cortisol synthesis and is located on chromosome site 10924.3[11] that catalyzes activity regarding 17α – hydroxylase as well as 17,20-lyase enzymes[12]. CYP11B can be defined as one of the significant genes in cortisol synthesis which encodes enzyme 11β hydroxylase that contributes to construction of cortisol, in the zona fasciculate, 11β-hydroxylase converts the 11-deoxy-corticosterone and 11-deoxycortisol to corticosterone and cortisol respectively, and is regulated via adrenocorticotropic hormone secreted by the pituitary gland[13].

Changes in genes regulating cortisol synthesis led to many diseases such as Polycystic ovary (PCOS) in women, breast cancer and Congenital Adrenal Hyperplasia[14–16]. The dysfunction in the genes that regulate cortisol production may lead to an increase or overproduction of the hormone and thus affect the functioning of the heart, which may lead to Myocardial infarction. The aim of our study was to find a correlation between increasing the DNA copy numbers that encode cortisol biosynthesis enzymes and Myocardial infarction disease.

MATERIALS AND METHODS

Between the first of January 2021 and the first of July 2021, 120 blood samples from AMI patients—60 samples as controls and 60 patients—of both sexes who were admitted to the Unit of Cardiac Surgery at AL- Salam Hospital, the Cardiac ICU, and outpatient health centers of Mosul, Nineveh /Iraq, were collected. Samples have been divided into two groups (control and patients) and three age groups (35-45), (46-56) and (≥ 57) years for both genders, (3 ml) of intravenous blood has been withdrawn for patients and healthy and used then for molecular assay.

DNA extraction: Utilizing the Add Prep Genomic Purification Kit Supplemented by (Addprep Korea) in accordance with the manufacturer's instructions, genomic DNA has been isolated from all of the blood samples. The purity and concentration of genomic DNA were measured using nanodrop spectrophotometer (IMPLEN).

Primer's design: Primers sequences were designed in NCBI site as shown in (Table 1). All primers were synthesized by Macrogen company \ Korea as Lyophilized product.

Table 1: Primers used for the detection of cytochrome genes

Primer		Sequences of Primers	Length	Tm
CYP17A	F	5'-CTCCACCCTGCTCTTGTGAT-3'	20	60.50
	R	5'-TCAGGGGTGGAGTAGGAACT-3'	20	60.50
CYP11A	F	5'-	20	60.50

		CCCAGAAGGAGACCGCTAAC-3'		
	R	5'-CAGGCCCAGAGTGAGGTCTA-3'	20	60.50
CYP11B1	F	5'TGCCTTCTGGGGATGTTCAC-3'	20	60.50
	R	5'-GTCTCCTGCAGACGGTGTTT-3'	20	60.50
House Keeping Gen	F	5'-CGGGTCTTTGCAGTCGTATG-3' (20mer)	20	60.50
	R	5'-CTGTTTCTGGGGACTAGGGG-3' (20mer)	20	60.50

TM= Melting Temperature
F= Forward
R= Reverse
qRT-PCR assay
PCR reaction

All of the qRT-PCR reactions have been carried out in 25.0µl volumes in an Eppendrof tube as shown in (Table 2).

Table 2: The qRT-PCR reaction components (25µl) for cytochrome genes amplification.

Components	Volumes
Forward primer 10 (picomoles)	0. 5µl
PCR master mix, 2x	12.5µl
Reverse primer 10 (picomoles)	0. 5µl
Nuclease free water	9.5µl
DNA Template	2µl
Total volume	25

Detection of CYP17A, CYP11A and CYP11B1 DNA copy numbers: The following qRT-PCR programs was utilized to detect CYP17A, CYP11A and CYP11B1 DNA copy numbers as shown in Tables (3,4,5).

Table 3: The qRT-PCR program for CYP17A gene amplification.

No.	Stages	Steps	Temperatures	Durations	Cycle
1	Polymera	1	95 c°	0:2:00	1
2	PCR cyclir	1	95 c°	0:0:15	44
3	PCR cyclir	2	60 c°	0:1:00	44
4	PCR cyclir	3	60 c°	0:1:00	44

Total prog. 2 hrs 2 min 32sec
Total cycles 44

Table 4: The qRT-PCR program for CYP11A gene amplification.

No.	Stages	Steps	Temperatures	Durations	Cycle
1	Polymera	1	95 c°	0:2:0	1
2	PCR cyclir	1	95 c°	0:0:15	44
3	PCR cyclir	2	60 c°	0:1:0	44
4	PCR cyclir	3	60 c°	0:1:0	44

Table 5: The qRT-PCR program for Cyp11B1 gene amplification.

No.	Stages	Steps	Temperatures	Durations	Cycle
1	Polymera	1	95 c°	0:2:0	1
2	PCR cyclir	1	95 c°	0:0:15	44
3	PCR cyclir	2	60 c°	0:1:0	44
4	PCR cyclir	3	60 c°	0:1:0	44

Statistical analysis: The results were statistically analyzed in the current study using Microsoft Excel, x2 test has been utilized for the statistical comparisons of the groups, P values ≤ 0.05 are considered to be significant.

RESULTS AND DISCUSSION

The results of this work showed a significant difference between the age groups of AMI patients and healthy control group in means of amplicon copy numbers regarding certain coding genes belonging to the super family genes P450 that code a group of enzymes that are crucial for the biosynthesis and production of steroids, hormones, and cholesterol metabolism, like CYP11A, CYP17, and CYP11B1. This difference is shown in Table (6).

Table 6: Computational average number of DNA copies of encrypted genes of CYP17 enzymes, CYP11A,CYP11B for the three age groups.

Age groups (year)	Patients NO.	Gens	Mean
35-45	23	CYP17	2.63413495
		CYP11A	1.93992797
		CYP11B	1.57962797
46-56	24	CYP17	2.97567097
		CYP11A	2.74175147
		CYP11B	2.38145147
57≥	13	CYP17	0.91635873
		CYP11A	2.76040362
		CYP11B	2.40010362

Inborn conditions that affect the adrenal steroidogenesis have been found rather commonly in the pediatric practices and have serious effects on the patient morbidity and mortality[17]. Steroid biosynthesis can be defined as one of the complex processes where cholesterol is converted into steroid hormones with an involvement from numerous cofactors and enzymes. Numerous researches have linked elevated cortisol levels to larger myocardial infarcts[18], ventricular remodeling following AMI[19], and worse mortality rates in those with chronic heart failure[20,21]. Acute coronary syndromes[22] also have a considerable increase in cortisol, and this hormone is linked to shock, left ventricular failure, and hospital mortality after an AMI. Almost all smaller cross-sectional studies[23,24] have found that patients with AMI had elevated cortisol levels, which increase the risk of fatality and morbidity. Additionally,[25,26] showed a correlation between incident cardiovascular disease (CVD) and plasma cortisol, and such results imply that higher morning cortisol is a causative risk factor for CVD.

CYP17A gene code for hydrolase lyase which converts pregnelonone into 17-hydroxy pregnelonone that expressed in different tissues, which include the gonads adrenal cortex and its defects lead to many diseases[27]

In both age groups of patients, but particularly in second group (46–56 years), there had been statistically significant increases in the amplicon copy numbers of gene code for the cytochrome CYP17A enzyme in comparison with the healthy control group (Fig. 1).

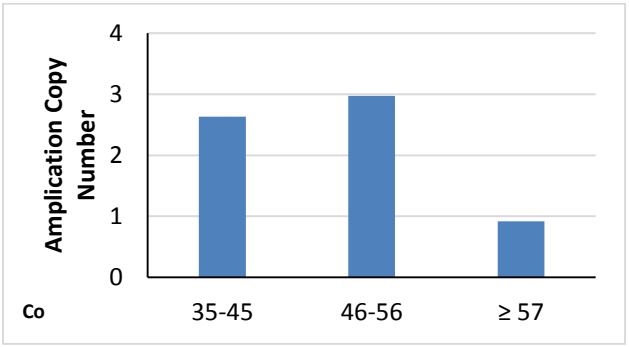

Figure 1: numbers of Amplicon copies of the gene code for the cytochrome CYP17A enzyme in all of the age groups of myocardial infarction patients.

The findings also demonstrated for first time that the copy numbers of this gene's amplicon are significantly higher in comparison to the housekeeping and control genes in all age groups of AMI patients, as seen in Fig. (2), indicating a correlation between this gene and the disease. [28] draw the conclusion that the cytochrome P450 17A1 catalyzes synthesis and metabolism regarding steroid hormone and that it has an important impact on the regulation of blood pressure (BP) and left ventricular hypertrophy pathogenesis. As a result, altered CYP17A1 function

that is caused by genetic variants could affect left ventricular mass and blood pressure.

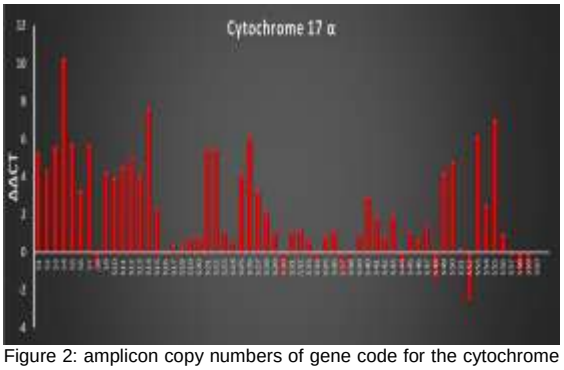

Figure 2: amplicon copy numbers of gene code for the cytochrome CYP17A enzyme in all of the myocardial infarction patient compared to House Keeping Gen.

In particular, the first case report of 17-alpha hydroxylase deficiency, which has been reported in 1966, already alluded to hypertension as a phenotypic characteristic[29]. However, evidence indicating a role of CYP17A1 in cardio-vascular area remains scarce. In line with earlier findings, corticoids and sex steroids are now widely regarded as crucial contributors to development of the hypertension and the resulting damage to target organs[30]. This supports the idea that CYP17A1 represents one of the key enzymes in the development of both conditions.

A study of [31] found a link between increased glucose resistance, lipid levels, and weight gain and a change in CYP17A1 gene. Additionally, [25] found a link between increased morning cortisol and an increased risk of CVD. Which suggests that increased morning cortisol could be a causative factor of risk for the CVD.

In addition the results of the present study have demonstrated a considerable increase of the amplicon copy numbers of the CYP11A enzyme coding gene in all age groups patients of AMI compared with the control healthy group Fig (3) and related with housekeeping gene Fig (4) which indicate the correlation of this enzyme coding gene and AMI risk disease. These findings concur with those of other researchers, such as [32], who came to the conclusion that mitochondrial CYP, which is primarily involved in steroid hormone biosynthesis, can serve as a possible therapeutic target for cardio protective measures. Additionally, [7] found that cortisol is an important component of the circadian system which considerably regulates cardiac function and that excessive cortisol has been related to increased risks of the cardio-vascular events, including arrhythmias, acute coronary syndrome, stroke and sudden cardiac death. CYP27A and CYP11A may play a role in preserving mitochondrial integrity through restricting formation and improving elimination of the toxic oxysterols.

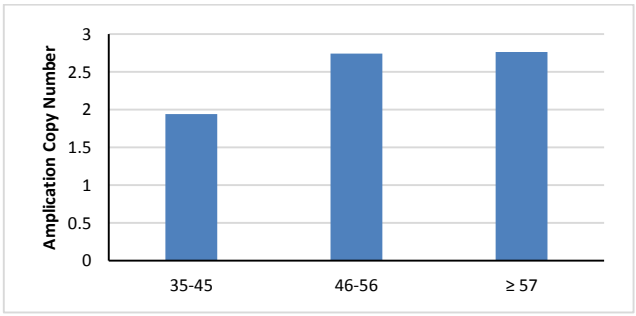

Figure 3: The copy numbers of the amplicon of gene code for the cytochrome CYP11A enzyme in all of the myocardial infarction patient age groups.

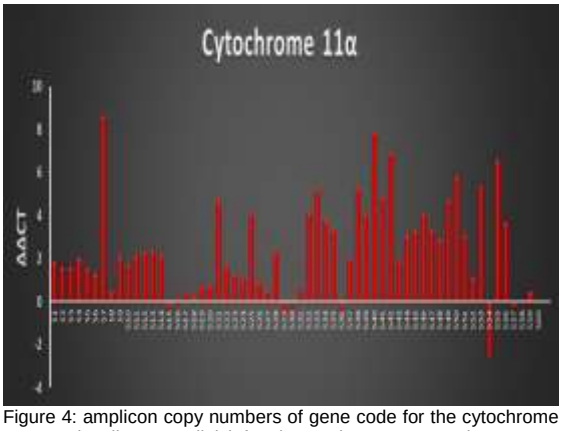

Figure 4: amplicon copy numbers of gene code for the cytochrome CYP11A enzyme in all myocardial infarction patients compared to House Keeping Gen.

Fig (5) revealed that there is a significant increase in the cytochrome 11β enzyme's coding gene amplicon copy numbers between AMI patient's age groups and healthy control groups, while Fig (6) showed the significant increase in the amplicon copy numbers of this gene related to the housekeeping gene and in all patient's age groups, these results confirmed the correlation of this enzyme coding gene and AMI disease.

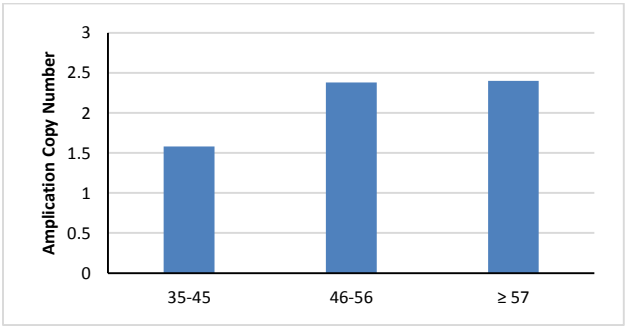

Figure 5: amplicon copy numbers of a gene code for cytochrome CYP11B1 enzyme in all of the myocardial infarction patients' age groups.

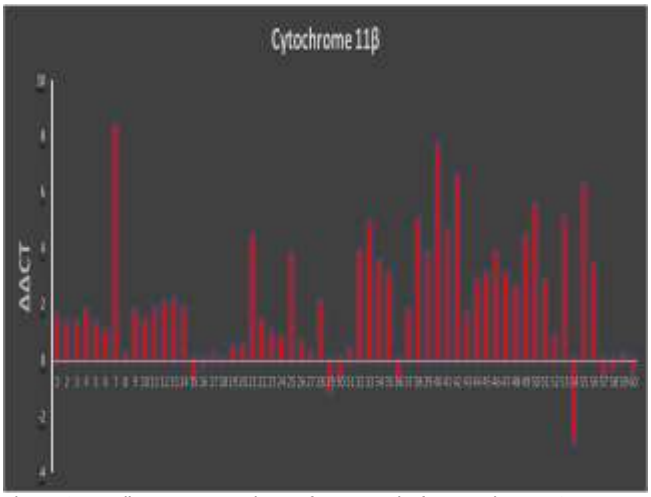

Figure 6: amplicon copy numbers of gene code for cytochrome CYP11B1 enzyme in all of the myocardial infarction patients compared to House Keeping Gen.

The relevance of 11β-hydroxyl steroid dehydrogenase, a potential molecular target of interest for the treatment of the metabolic syndrome and T2DM, is discussed in a study by [7]. The

main factor causing cortisol excess is 11β, and its reduction decreases metabolic irregularities, emphasizes the function of cortisol in regulating circadian rhythm, and details its impact on cardio-vascular system. According to [33], [34], the pathogenesis of CVD like atherosclerosis and myocardial infarction was linked to 11β. This is consistent with the research that was examined, which shows that high levels of cortisol are linked to a higher risk of heart failure and other cardiac diseases.

CONCLUSION

The results of the presented work demonstrated for the first time that there were significant correlation between three steroid hormone biosynthesis genes and Acute Myocardial Infarction (AMI) disease by utilizing RT-PCR Technique which indicated a significant increase in amplicon copy numbers of CYP17, CYP11A, CYP11B genes in all patient's age groups compared with healthy control group.

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