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Research Article

Mutations in the *MtCOX2 gene* and Their Association with Aortic and Mitral Valve Dysfunction

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Abstract

Objective: Much evidence suggests that heart valve dysfunction is affected by various genetic factors. So his study is designed to determine the potential role of mutations in certain mitochondrial genes such as (*mtCOX2*) and their association with this pathology.

Materials, methods and results: The study included 31 patients with heart valve dysfunction as well as 20 healthy volunteers as a comparison group.

The results of the analysis of Relay for the gene (*mtCOX2*) documented five mutations for patients with aortic valve three of them effective mutation in the sites 7853G>A and 8072G>A and 8108A>G but in the rest of the mutations are silent, while diagnosed six mutations in patients of the mitral valve all were silent except for the boom extra on-site (7680ins G) which led to the deviation of the reading frame. Scored this heterogeneity was registered in the National Center for Biotechnology Clinical variation (NCBI) with the accession number (SCV000852053).

Conclusion: The occurrence of mutations in the *mtCOX2 gene* such as addition mutations which led to a shift in the reading frame that could represent a predisposition to heart valve dysfunction.

Keywords: Aortic valve; Mitral valve; Mutations; *MtCOX2*; Mitochondria

Introduction

Cardiovascular diseases are considered the number one cause of death worldwide, as confirmed by the statistics of the World Health Organization (WHO). In 2018, nearly 17 million people died because of heart disease, accounting for nearly 31% of all the deaths worldwide, and the organization (WHO) explained that a third of deaths from heart disease were caused by low-and middle-income countries, as for Iraq, according to the same organization's reports, the percentage of deaths due to heart disease reached 33% [1].

The Ventricular Septal Defect (VSD) consider as the most common malformation by 30%, followed by Atrial Septal Defect (ASD) by 10% a Tetralogy of Fallot (TOF) comes in third place with 4%.

As well as heart valve diseases such as aortic valves dysfunction (aortic valve) and mitral valve dysfunction (MV) Mitral Valve, which differ in their etiology depending on the type of and geographical regions [2].

Mitochondrial diseases are defined as disorders affecting the oxidative phosphorylation, respiratory chain or electron transport chain which are located in the inner membrane of the mitochondria in eukaryotic cells while their location is in the plasma membrane in prokaryotic cells. In 1962 the first disease associated with dysfunction of the respiratory chain was described in a woman with severe hypermetabolism [3].

The diagnosis of mutations in the mitochondrial genome has opened a new field in the possibility of more accurate diagnosis of diseases.

Defects in the respiratory chain can be caused either by mutations in the mitochondrial genome or mutations in nuclear genes that affect the constituent subunits or mutations in the genes of the nucleus that encode for proteins necessary for the assembly of respiratory chain units. It is estimated that mitochondrial disorders caused by mutations in nuclear genes are one of the important causes of diseases caused by defects in mitochondria [4].

Working Methods

Thirty-one samples were collected from patients which suffer from heart valve defect (16 patients with aortic valve and 15 patients with mitral valve who underwent open heart operations after being diagnosed at Nasiriyah Heart Center), as well as another 20 cases as comparative samples. All patients or their relatives gave written consent to participate in the research.

0.5 g of heart valves removed during surgery were taken for use in the process of extracting deoxygenated DNA from mitochondria mtDNA and placed in sterile 1.5 ml Eppendorf tubes and directly preserved in freezing-20 M until extraction.

DNA extraction

Deoxyribonucleic acid DNA was extracted from the preserved

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samples. The samples were left to dissolve after being placed in the water bath, and the DNA was isolated using the g SYNCTM DNA Extraction Kit equipped by GENEAID company, and according to the instructions of the producing company to isolate DNA from tissues.

Primers

The Polymerase Chain Reaction (PCR) process was performed using specific primers of the *mtCOX2 gene*. The primers were designed from the GenBank website at the National Biotechnology Information Center (NCBI) http://www.ncbi.nlm.nih.gov.genebank using the Primer3plus program, the primers was used for the first time in this study, and it was prepared by Bioneer as a dried product with different concentrations (picomols) and the primers were dissolved with distilled deionized water DNAse/RNase distilled water to give a final concentration of 100 pmol/ul as a storage and prepare solution a concentration of 10 pmol/ul for experiments (Table 1).

Chain Reaction Program (PCR)

The PCR reaction was performed through the program shown in the table below to amplify the gene (Table 2).

Nucleotide sequence analysis

The samples were sent to the Macrogen Company (South Korea)) to conduct a sequence analysis using a genetic analyzer.

Statistical analysis

Statistical analysis was carried out according to the SPSS (Statistical Package for Social Sciences) Program for all transactions and below the probability level $P \leq 0.05$.

The sequences were aligned using the program Mutation Surveyor V.5.2.1 to analyze mutations and determine their types.

Results

The results of the current study showed that the average age of aortic valve patients was 41.65 ± 14.81 and mitral valve patients 41.06 ± 11.19. The comparison group was 41.30 ± 11.05, the results also showed an increase in the percentage of men with a mitral valve of 68.75% compared to females. The percentage of smokers with aortic valve was 37.50%, while those with mitral valve were 46.67% (Table 3).

Analysis of mutations in the *mtCOX2 gene*

The results of electrophoresis on agarose gel with a 2% concentration of a fragment of the *mtCOX2 gene* showed the appearance of bundles with a size of 627bp nucleotide pair between the two sites (7591-8217) after amplification by PCR technology and the identification code of the protein is (Protein ID: AAB58946.1) and according to the Cambridge Reference Sequence (CRS).

Analysis of mutations in the *mtCOX2 gene* of aortic valve patients

The study diagnosed five mutations in the *mtCOX2 gene* and the mutations was distributed between silent mutations such as changes that occurred in the sites: 7657, 8110, and others were influential mutations, as there was a change in the codon leading to the replacement of the resulting amino acid (Homocysteine) at sites:7853, 8027, 8108, as shown in (Figure 1) and (Table 4).

Table 2: *mtcox2* gene amplification program.

Number of cycles	Time	Temperature	
1	5 m.	94°C	Initial denaturation
30	45 se.	94°C	Denaturation
	30 se.	59°C	Annealing
	30 se.	72°C	Extension
1	5 m.	72°C	Final Extension

Table 3: Clinical features of the aortic, mitral valve, and control group.

Parameter	Control (N=20)	AVR patients (N=16)	MVR patients (N=15)
Age (Mean ± SD)	41.03 ± 11.05	41.65 ± 14.81	41.06 ± 11.19
Gender			
Male	13 (65.00%)	10 (62.5%)	9 (60%)
Female	7 (35.00%)	6 (37. 5%)	6 (40%)
P. Value		**0.587**	**0.693**
Smoking			
Smoker	12 (60.00%)	6 (37.50%)	7 (46.67%)
Non-smoker	8 (40.00%)	10 (62.50%)	8 (53.33%)
P. Value		**0.177**	**0.429**

Table 4: Mutations in gene mtCOX2 for aortic valve patients.

GenBank	Frequency	Affect	Source	Boom
LC437948.1	18.75% (3)	Val90Ile	mitomap	m.7853G>A
LC437950.1	6.25%	Silent	mitomap	m.8110 T>C
LC437950.1	31.25% (5)	Ala148Thr	mitomap	m.8027 G>A
LC439255.1	6.25%	Ile175Val	mitomap	m.8108 A>G
LC439256.1	6.25%	Silent	mitomap	m.7657 T>C

Analysis of mutations in the *mtCOX2 gene* of mitral valve patients

The results of comparing the *mtCOX2 gene* sequences of the study samples with the source sequences showed the presence of genetic heterogeneity represented by the presence of point mutations shown in (Figure 1 and 2) resulting from the replacement of Cytosine (C) to Thymine (T) at sites: 7648 and 8137. At sites 7771 and 8014, the adenine nitrogenous base was replaced by guanine, while Thymine (T) was changed to Cytosine (C) at site 7999. As for the site 7680th, the base Guanine (G) has been added (Table 5).

Discussion

The results of the current study showed the distribution of patients by age group with Aortic Valve (AV) or Mitral Valve (MV), the study did not show significant differences, as the percentages indicated that the age group most at risk of aortic valve infection is 48 years and older by 37.5%, so for the age group most affected by mitral valve is (47-37) years by 53.3%. A study [5] showed that the average age of aortic and mitral valve patients compared to the comparison group without significant differences, In a study [6] in Iraq, the results showed that most of the injured were in the sixth or seventh decade of age, and the average age of the sample was (56.18), as for the Study [7], its results showed agreement with the results of the current study, as the study found that the highest incidence was in the age group of 40-30 and amounted to 31%.

The results of the study by gender indicated that there were no significant differences when compared with the comparison group the results showed a higher incidence of aortic valve disease in men by 68.75% than in females while the percentages were close in mitral valve patients. The results of the current study did not agree with

Table 1: Primers of the *mtcox2* gene.

mtCOX2 Primers	Target	Amplicon size bp	Reference
Fwd 5'-ACATGCAGCGCAAGTAGGT-'3	*mtCOX2*	627	This study
Rev 5'-AGGACGATGGGCATGAAACT-'3			

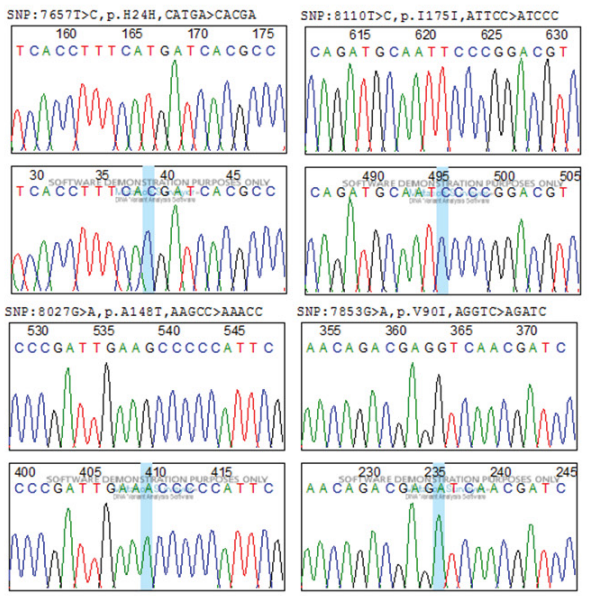

Figure 1: The sequence of nucleotides in the *mtcox2* gene for aortic valve patients compared to (CRS), which represents the upper part in the figure, but the lower part represents the sequence of the study sample and the blue shading represents the mutation site.

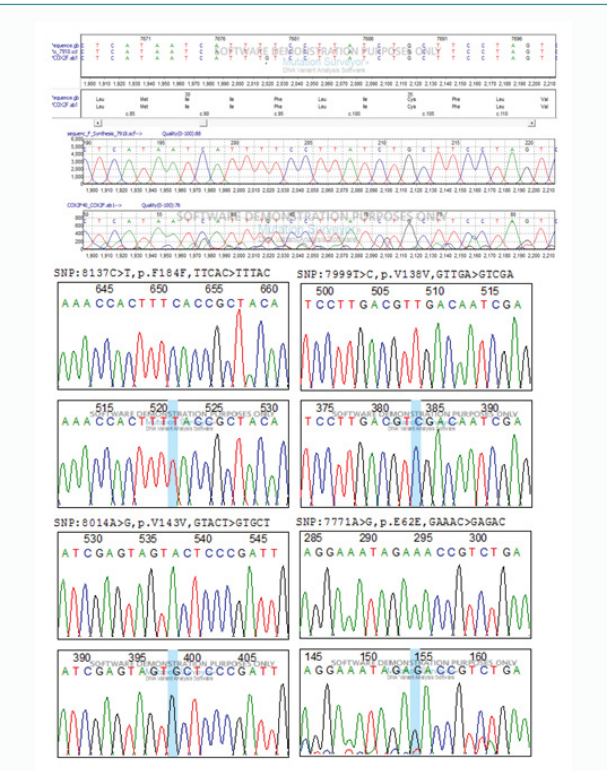

Figure 2: Nucleotide sequencing in the *mtcox2* gene of mitral valve patients compared with (CRS), Which represents the upper part in the figure, the lower part represents the sequence of the study sample, and the shading in blue represents the mutant site.

what he found [8] in his study, as he found an increased incidence of mitral valve stenosis in females compared to males without significant differences. In a study [9], which found that females are more likely to develop aortic sclerosis by 53.8% compared to 46.2% in males, these results are not agreement with the findings of the current study.

Table 5: Mutations in the *mtcox2* gene in mitral valve patients.

GenBank	Frequency	Influence	Source	Mutation
LC439257.1	6.66%	Silent	mitomap	m.7648 C>T
LC439258.1	6.66%	Silent	mitomap	m.7999 T>C
	13.30%	Silent	mitomap	m.8137 C>T
SCV000852053	26.66%	Frame shift	Novel	m.7680 ins G
LC439260.1	6.66%	Silent	mitomap	m. 7771 A>G
	6.66%	Silent	Novel	m.8014 A>G

The results of this study are in agreement with the findings of [10] and his group when studying patients with stenosis of the congenital aortic valve, as it was found that the percentage of infected males was higher compared to the percentage of infected females, and the percentage of infected males was about 77.2%.

The results of this study are consistent with the findings of [10] and his group in the study of patients with aortic valve stenosis, as it was found that the percentage of infected in males was higher compared to the percentage of infection in females and the percentage of infected males was about 77.2%.

The results of the study showed that non-smoking patients had a higher percentage than smokers for all studied pathological cases. The results of the statistical analysis showed that there were no significant differences which have statistical indications, and the study recorded that 62% of aortic valve patients and 53.3% of mitral valve patients are non-smokers and these results do not negate the damage caused by smoking and its association with many diseases, especially heart diseases, but this may be due to the sample size and the accuracy of the information provided by the patient.

Molecular analysis

The current study showed the registration of five mutations in the *COX2* gene for aortic valve patients and the mutations were at sites 7853G>A and 8027G>A and 8108G>A led to a change in the amino acids Val190Ile, Au148Thr and Ile175val, respectively, while changes in other sites were recorded silent mutations, Furthermore, the study recorded six mutations in mitral valve patients, five of these mutations were silent and there was no change in amino acids, while an addition mutation was diagnosed at the 7680 ins G site that led to a reading frame deviation, and this mutation was recorded at the site of clinical heterogeneity with the accession number SCV000852053. When comparing the mutations present in the patients with the comparison samples, showed the presence of three common mutations appeared between the comparison samples and the mitral valve patients, While no common mutations were found in aortic valve patients with comparative samples, a study [11] showed that the mutation in the second subunit in the cytochrome (*COX2*), which occurred at site 7671T>A and led to the change of amino acid methionine to the amino acid lysine in a patient with myopathy, and the study indicated the presence of the mutation at high levels in skeletal muscles reached 90%. It is the only clinically affected tissue clinically, while the level of the mutation appeared at a very low level in the blood reached to 6% and the researcher attributed the cause of this defect to the fact that the mutation had affected the process of assembling the fourth complex in the respiratory chain as a result of the presence of this defect in the *COX2* subunit, and the study [12] diagnosed a mutation at the site 7587T>C. In the patients with encephalopathy, the study found a decrease in the activity of the fourth complex of the respiratory chain at a range of up to 35% above normal range and showed that the biochemical activity has decreased significantly upon reaching the threshold limit of the mutation, the study concluded

that this mutation falls on the codon of the starting site and so it will lead to the inability to express the *COX2* protein causing a certain decrease in the activity of *COX2*. In another study, a deletion mutation was diagnosed at site 8042 del AT in the second subunit encoded in the cytochrome produced a protein 72 amino acids shorter than the wild type, which caused the resulting protein to lose its function and had devastating effects on the respiratory function of mitochondria. Because the resulting protein is responsible for binding to Cu copper, and production of a damaged protein will prevent this binding and the non-formation of the stimulating center in the complex [13]. Showed [14] to the presence of a mutation at site 8009G>A of the *COX2* gene in patient with colon cancer.

Conclusion

The Association of mutations in the mitochondrial genome with aortic and mitral valvopathy it can be attributed to a malfunction in the function of mitochondria, and this affects the intracellular calcium balance which leads to calcification of the valves and loss of their function.

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