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Molecular Detection of Mutations in mtCOX1 Gene in Iraqi Patients with Aortic and Mitral Valve Diseases

Ali Jumaa Al-Hussona[1], Faizah A.W. Ahmed[2]

[1]Ministry of Education, Directorate of Education, Dhi Qar / Iraq,
[2]Department of Biology / College of Education for Pure Sciences / University of Basrah/Iraq.

Abstract

Many evidences suggest that aortic and mitral valve diseases are not a direct result of aging but may be linked to various genetic factors. This study was designed to determine the potential role of mutations in certain mitochondrial genes and their association with aortic and mitral valve disease. The study included 31 patients , 16 with aortic valve defect and 15 mitral valve patients in addition to 20 healthy volunteers as comparative group. The results of the molecular analysis showed that there were 11 mutations of those with an aortic valve, seven silent mutations and two mutations recorded for the first time in the present study at the sites m.6922 G>T; p.W340L, m.6690 G> C; p.G263R and deletion mutation at m.6936 delA site recorded at the clinical variation site with accession number SCV000845763 and one insertion mutation at m.6908 insG site and registered with accession number SCV000845764. The results of the study recorded 12 mutations of the mitral valve eight silent mutations and the other significant mutations at sites m.6253 T>C; p.M117T, m.6366G>A; p.V155I, m.6690G> C; p.G263R and deletion mutation m.6607 insT site registered with the accession number SCV000852048.

Keywords*: aortic valve, mitral valve, mtCOX1, novel mutation.*

Introduction

More than 250,000 heart valves defects patients worldwide have been treated by heart valves replacement ([1]). Although heart valve disease is less common than coronary artery disease or hypertension, it is still a common disease and surgical intervention is often required because treatments for infected valves are limited, and studies on valve diseases are still few compared to other heart diseases ([2]). Cardiac valve diseases, both congenital and acquired forms are contributing factors leading to death ([3]). The prevalence of these diseases increases with age, sex, high body mass index, smoking and hypercalcemia ([4]).as well many other factors such as rheumatic fever ([5]).

Most cell energy is produced in mitochondria by the oxidative phosphorylation (OXPHOS) of five multi-secondary complexes([6]). The mutations in the mitochondrial genome, especially in the genes that encode the cytochrome c subunits, associate with many clinical symptoms such as heart diseases ([7]) This complex has a key role in the process of producing energy and works to transfer electrons from cytochrome c to molecular oxygen, turning water into a water molecule ([8]).

Materials and Method

Case-control study was performed on 31 patients with aortic valve and mitral valve replacement operations at the Nasiriyah Heart Center in southern Iraq for the period from 2017 to 2018. The surgery was assessed through a test performed on patients at the same center. The number of patients were 16 (11 males and 5 females). The patients with mitral valve disease were 15 (8 males and 7 females) in addition to 20 comparative samples that were identical in age, sex, ethnicity and did not have a family history heart valves diseases.

The research and laboratory tests were approved by the Ethics Committee of the College of Education for Pure Sciences / Basrah University and the Ethics

Corresponding :
Ali Jumaa Al-Hussona
E-mail: alijalhussona@gmail.com,, Tel:07811649300;

Committee at the Nasiriyah Heart Center and obtained written approval by the patient.

Forty to fifty mg of valve replacement tissue were extracted for mitochondrial DNA. Extraction was performed by gene extraction kit equipped with Geneiad / south of Korea. A pair of primers were used to amplify a piece of the mtCOX1 gene starting at 5909 bp and ending at 6960 bp. The primers were designed by Primer3plus program, http://www.ncbi.nlm.nih.gov. primer3plus and was as follows:

Forward: 5'-CGCCGACCGTTGACTATTCT-3'

Reverse: 5'- GGCCACCTACGGTGAAAAGA-3'

A PCR reaction was performed using 200ng for mtDNA and 5 pmol for each primer and 5 microliter from Master Mix supplied from Bioneer / south of Korea and 5 microliters of deionized distilled water. The first cycle of amplification program (first amplication) lasted for 5 minutes at 94C° and after 30C° Cycle 94C° for 45 seconds and 60C° for 30 seconds 72C° for 30 seconds after final elongation at 72C° for 5 minutes. The results were then transferred to the 2% agarose gel. Samples were sent to Macrogene company, Seoul, South Korea for a nucleotide sequence analysis. Mutation Surveyer program V.5.2.1 was used by Softgenetics, USA to analyze mutations and detect their location on the mitochondrial genome and to determine whether it was nonsynonymous or synonmous.

Statistical Analysis

Statistical analysis of this study was conducted, using the P.value with 95% confidence intervals (95% CI) by SPSS V.18

Results

Results of the current study showed that the mean age of patients with aortic valve patients was 42.56 ± 15.80 and those with mitral valve 42.60 ± 12.09. Comparison group age was 40.30 ± 11.50, and the results also revealed an increase in the percentage of men with mitral valve which was 68.75% compared to females. The percentage of smokers with an aortic valve was 37.50% and those with mitral valve was 46.67%. Table (1).

Table (1) clinical features of aortic, mitral valve and control group

Parameter	Control (N=20)	AVR patients (N=16)	MVR patients (N=15)
Age (Mean±SD)	40.30 ± 11.50	42.56 ± 15.80	42.60 ± 12.09
Gender			
Male	12 (60.00%)	11 (68.75%)	8 (53.33%)
Female	8 (40.00%)	5 (31.25%)	7 (46.67%)
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		0.180	0.433

The current study found 11 mutation in patients with aortic valve, 7 synonymous mutations at the following sites: m.6446G>A, m.6671T>C, m.6680T>C, m.6170C>T, m.6531C>T, m.6026G>A, m.6272A>G. The mutation allocated in the two sites: m.6922G>T,p. W340L and m.6690G>C,p.G263R were recorded for the first time (Novel) fig.(1). The study recorded a mutation of deletion at m.6936delA site and was registered at the site of clinical variations at the National Center for Biotechnology Information at accession

number SCV000845763 which led to frame shift mutation associated with protein alteration as well as mutation in m.6908insC, recorded with SCV000845765 accession number. table (2).

Table (2) Mutations in mtCOX1 gene for patients with aortic valve

Sequence variation	Amino acid change	Originality	Accession number
m.6922 G>T	W340L	Novel	LC435698.1
m.6446 G>A	Synonymous	mitomap	LC435442.1
m.6671* T>C	Synonymous	mitomap	LC435442.1
m.6680 T>C	Synonymous	mitomap	LC435442.1
m.6936 del A	Frame shift	Novel	SCV000845763
m.6170 C>T	Synonymous	mitomap	LC435696.1
m. 6531 C>T	Synonymous	mitomap	LC435697.1
m.6908 ins C	Frame shift	Novel	SCV000845765
m.6026 G>A	Synonymous	mitomap	LC435699.1
m.6272 A>G	Synonymous	mitomap	LC435699.1
m.6690 G>C	G263R	Novel	LC421988.1

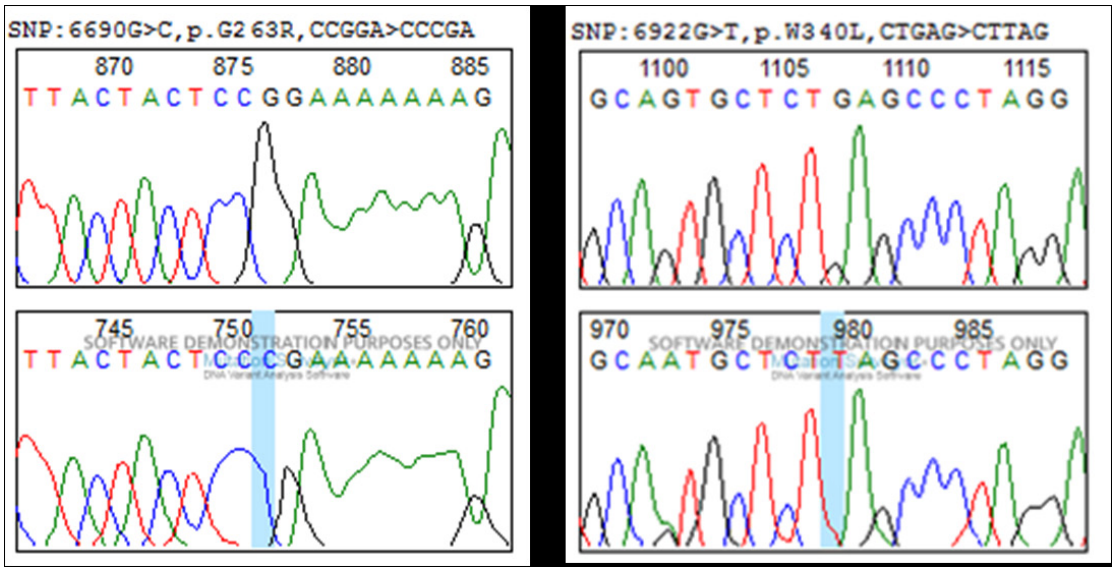
* A common mutation with control samples

The study explored 12 mutation in mitral valve patients, 8 silent mutations at sites: m.5981T>C, m.5987C>T, m.6026G>A, m.6185T>C, m.6257G>A, m.6297T>C, m.6621T>C and recorded the presence of a mutation at m.6690G>C site, which was previously recorded in patients with aortic valve and two mutations affecting the sites: m.6366G>A,p.V155I; m.6253T>C,p. M117T In addition to detecting a mutation in the m.6607delT site, that was registered with the accession number SCV000852048. Table (3).

Table (3) Mutations in mtCOX1 gene for patients with mitral valve

Sequence variation	Amino acid change	Originality	Accession number
m.6185 T>C	synonymous	mitomap	LC435700.1
m.6257 G>A	synonymous	--	rs281865417
m.6297 T>C	synonymous	mitomap	
m.6690 G>C	G263R	Novel	
m.6026 G>A	synonymous	mitomap	LC435701.1
m.5981 T>C	synonymous	mitomap	LC435704.1
m.5987 C>T	synonymous	mitomap	LC435702.1
m.6221* T>C	synonymous	mitomap	
m.6366 G>A	V155I	mitomap	LC435704.1
m.6607 del T	Frame shift	Novel	SCV000852048
m.6371 C>T	synonymous	mitomap	LC435706.1
m.6253 T>C	M117T	mitomap	LC435707.1

* A common mutation with control samples



Figure(1)Sequencing chromatogram showing the m.6690G>C, m.6922G>T mutation in the mtCOX1 gene.

Discussion

The current study showed that the mean age of aortic and mitral valves patients was less than healthy controls at time of blood sampling for DNA extraction, but the difference was not significant, this is may be due to the small sample size. Mitochondrial genome accumulates mutations with progressing age, but our results showed no significant differences between aortic and mitral valve patients. Our investigation showed that the percentage of male aortic valve infection is significantly higher than that of females. These results are consistent with ([9]). Our findings did not show significant association between smoking and valve defects in the studied cases. Experiments with aortic valves patients identified 11 mutations in the COX1 gene Seven of these mutations were silent. The mutation m.6922 G>T p.W340L a m.6690G>C p.G263R was recorded for the first time and the study recorded a mutation at m.6936 delA site which was registered at the site of clinical variations at the National Center for Biotechnology Information at accession number SCV000845763, it causes frame shift mutation which led to the alteration of the resulting protein in addition to the m.6908 inC, which is registered as well at the clinical variation with the accession number SCV000845765.

For those with mitral valve defect, the study identified 12 variants, 8 of which were silent mutations, while the variant m.6366G>A; altered the amino acid V115I and a deletion mutation at m.6607 delT resulted in frame shift mutation and recorded with SCV000852048. ([10]) found that the defect in the fourth complex in the respiratory chain is associated with many abnormalities that occur in the heart, muscles, and brain tissue. The defect in the complex involves the occurrence of deletions or mutations which are usually associated with age progression due to the presence of ROS free radicals antioxidants tissues due to aging, in his study a mutation at site 6708A>G in the COX1 subunit proved to be a termination code of the premature codon which led to the cessation of protein production component of the COX1 subunit. ([11]) noted that increasing formation of free oxygen radicals (ROS), especially hydrogen peroxide (H2O2), would promote increased calcification of the aortic valve and blood vessels due to increased calcium deposition in the valve. ([12]) found that mitochondrial functions were damaged in infected heart valves. ([13]) proved COX1 activity in patients with heart disease is lower than that in healthy people as well as that oxidative stress and imbalance in the mitochondrial genome are directly involved in several forms of heart disease. ([14]) recoded the mutation 7339A>G in the COX1 subunit in an anemic patient, leading to the formation of a termination codon in the wrong place. The study showed that this mutation resulted in poor oxidative phosphorylation rate in the mitochondria.

The mutations will destabilize the factors of the fourth complex of the respiratory chain in addition to

the mutations in the subunit are not the only defect in one complex but lead to a decrease in the respiratory chain efficiency when the mutation load exceeds a certain threshold ([15]). The presence of mutations will lead to structural changes in the protein produced by the subsidiary COX1 which cause the instability of the components of the fourth complex in the respiratory chain, which affects the biological synthesis of the peptide chains effect required for the functioning of this complex, especially mutations that change the amino acids, and the addition, which has caused a reading frame shift and therefore will lead to a defect in the heart valves efficiency due to lack of COX1 component or a defect leads to increase risk of heart valve defects.

Conclusion

The presence of missense, deletion, and insertion mutations which led to frame shift reading changes and that in turn could cause an aortic and mitral valve defects.

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