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PARACLINICAL EVALUATION OF HEART MUSCLE AFTER PERICARDIECTOMY IN RABBIT

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ABSTRACT

Although medical management is a standard treatment of pericardium disease, some cases require surgical intervention such as pericardiectomy. The aim of this study was to examine probable changes in heart structure, its function and effects of surgery on clinical and par clinical parameters after pericardiectomy of healthy rabbits. Twelve healthy white New Zealand rabbits male in same age, approximately weight of 1.5±0.3 kg were randomly divided into two groups of six (Group A: experimental group and Group B:control group). Experimental group were treated with Pericardiectomy, while the control group undertook Thoracotomy. In order to evaluate Aspartate Amino Transferase (AST), lactate dehydrogenase (LDH), and Creatine Kinase **(**CK) enzyme, blood samples were taken from both groups during first, second, third and fourth weeks following the operation. Four weeks after the operation, the rabbits underwent necropsy finding and degree of adhesion and pathologic degree were evaluated. Using SPSS 19.0 Trial Version, Oneway and Mann-Whitney analysis tests were carried out for each group for each week following the intervention. There was a significant difference between CK – MB level of control

and experimental groups in 1st week. Significant difference was observed in CK – MB in experimental groups between 1st & 2nd, 1st & 3rd and 1st & 4th weeks. There was also a significant difference between AST and LDH level of control and experimental groups in 2st week. Difference in AST and LDH was significant between 1st & 2nd, 2nd & 3rd, and 2nd & 4th weeks in experimental groups. Average of Adhesion degree in control and experimental group was 0.5 and 2 respectively (P = 0.000). Mean pathologic degree in control and treatment groups were 1.16 and 2.33 respectively (p =0.028). Pericardiectomy may lead to damage of the heart cells, but since markers of myocardial damage will return to the reference range, this damage is temporary. As it was observed in gross pathology in the fourth week, pericardiectomy may also lead to cardiac and ventricular enlargement.

Key words: Heart Muscle, Pericardiectomy, Rabbit

INTRODUCTION

Pericardium is a fibro-serous sac that surrounds the heart and its main arteries. The pericardium consists of two layers; fibrous layer and serous layer. Potentially many diseases can affect pericardium such as; rough or fibrotic pericarditis, congestive heart failure, constrictive pericarditis regardless of its causes, congenital pericardial defects, infections, cancers, connective tissue disorders including acute hematic fever, allergies and autoimmune diseases, myocardial infarction, metabolic diseases such as gout, and pericardium leakage **[1]**. Pericarditis, fluid aggregation, or development of fibrin in the pericardium space can cause adherence of two visceral and parietal walls of pericardium, which lead to elevated pressure around the heart. This condition is called Cardiac Tamponade which interferes with heart's pumping action; both systolic and diastolic functions and reduces cardiac output and peripheral blood pressure **[2]**. Although medical management is a standard treatment of pericardial disease, some cases may require surgical intervention such as pericardiectomy in cases that the regeneration and resortation of pericardium is impossible **[3]**. Pericardiectomy will increase cardiac output, promote oxygen supply, increase in end-diastolic volume, but could lead to ventricular enlargement and myocardial growth **[2, 4]**. It could also affect cardiac output and heart rate **[5]**. Pericardiectomy can also cause lower blood volume in the lungs **[6]**. Furthermore, Pericardiectomy is the treatment of choice for constrictive pericarditis **[7, 8]**, even though it does interfere with the Right Ventricular (RV)

systolic function **[9]**. However, insufficiency was not observed in echocardiography of the Regional Wall Motion **[10]**. The aims of this study was to examine probable changes in heart structure and function as well as the effects of surgery on clinical and paraclinical parameter after pericardiectomy in healthy rabbits. We also examined the necropsy and biochemistry factors in the study.

MATERIALS AND METHODS

In our study all rabbits were kept according to the norms of the Islamic Azad University faculty of veterinary medicine Tehran Iran laboratory of animal experimentations; this study was approved by the committee of ethics in research with animals in Islamic Azad University.

Twelve healthy white New Zealand rabbits with same sex and age and approximate weight of 1.5±0.3 kg were randomly divided into two groups of six (Group A: experimental group and Group B: control group). Prior to study, all rabbits were examined clinically and paraclinically and received preventive antiparasetic dose. Clinical examination including heart rate, breathing rate and body temperature was also repeated 24 and 1 hour prior to the surgery. In order to reduce transfer induces stress, subjects were kept in pre operating room for one hour prior the operation and the surgical site was shaved. Subjects were off-fed for 12 hours and received tetracycline prophylactically prior to surgery. Anesthesia was induced with ketamin hydrochloride at a dose of 35 mg/kg and maintained with isofluran in oxygen. Mechanical ventilation was instituted with an approximate tidal volume set at 10mg/kg body weight, with 100/ oxygen, at a rate of 14-16 cycles per minute. In control group rabbits' hearts were exposed using thoracotomy and the duration that their rib cages were open was equivalent to the duration of the pericardiectomy in the experimental group. Rib cage excision was made by a standard incision from manubrium to xiphoid. Thoracic cavity was opened in order to eliminate the negative pressure in the pleura during the pericardiectomy; however the subjects experienced breathing difficulties and were started on artificial respiration using anesthesia-breathing system. Pericardium was held using an Alice Pence and an incision was made from base through the apex and pericardium was separated. Blood and fluids in operation site were removed using suction. After this stage, we attempted to close the rib cage using standard methods. Next, the rabbits were transferred to the recovery room under intensive care for 24 hours, and then were transferred to the hospital's special care unit. To prevent any post operation infection,

Ceftriaxone (40mg/kg) was given daily for total of three days following the operation. Morphine Sulfate (2 mg/kg) was also administered for analgesic. Sutures were removed after two weeks following the operation. In order to evaluate the Aspartate Amino Transferase (AST), lactate dehydrogenase (LDH), and Creatine Kinase **(**CK) blood level changes, blood samples were taken from both groups during first, second, third, and fourth weeks following operation, using syringes and sterile tubes (1 cc sampling). The specimens were then transferred to hospital`s laboratory. In the laboratory, serums were separated using centrifuge and following tests were done. Four weeks after the operation, the rabbits underwent necropsy finding and degree of adhesion and pathologic degree were evaluated. The adhesions were scored according to Abedi et al as shown in **Table 1 [11]**.

Pathologic evaluation also was considered according to Bilge et.al, based on existence of inflammatory cells and cells which are necessary for adhesion (**Table 2**)**[12]**.

Collected data from biochemistry and necropsy findings were analyzed using SPSS 19.0 Version with Lock Code Number: 100-25FC9, and Oneway and Mann-Whitney analysis were carried out for each group for all weeks, to evaluate trend of obtained mean value during each week compared P value of < 0.05 was considered statistically significant.

Table 1: Degree of adhesion was classified from 0 to 4

Degree 0	**without adhesion**
Degree 1	**There is adhesion but it is removed easily**
Degree 2	**Adhesion is removed easily by a sharp tool such as scissors**
Degree 3	**Adhesion is removed with difficulty by a sharp tool**
Degree 4	**Adhesion is severe as it is not possible to remove and it causes heart injury**

Table 2: Pathologic degree was classified from 0 to 5

Degree 0	**Normal**
Degree 1	**Inflammatory cells**
Degree 2	**Fibrin and inflammatory cells**
Degree 3	**Fibroblasts, Fibrin clots**
Degree 4	**Collagen formation**
Degree 5	**Collagen, Fibrin clots**

RESULTS

Proportion of mean value, standard deviation, P–Value for blood enzyme parameters and degrees of adhesion in each groups were shown for whole weeks in **Table 3 to 5**. Differences in proportion of P–Value in experimental groups during four weeks of study were shown in Table 6. There was a

significant difference in CK – MB level between control and experimental groups during 1st weeks **(Table 3)**. Significant difference was observed in CK – MB level of experimental groups between 1st & 2nd, 1st & 3rd, 1st & 4th weeks **(Table 6)**. There was a significant difference in AST and LDH level of between control and experimental groups in 2st weeks **(Table 4, Table 5)**. Difference in AST and LDH was significant between 1st & 2nd, 2st & 3rd, 2st & 4th weeks in experimental groups **(Table 6)**. In control group maximum, minimum **(Figure 1)** and mean degree of adhesion were 0, 1 and 0.5 respectively. In contrast, in experimental group, the least degree of adhesion was 1 and the highest degree was 3 **(Figure 2)** with average of 2 **(Table 7)**. There was a significant difference in adhesion degree of control and experimental groups (P = 0.000).

Mean pathologic degree in control and treatment groups were 1.16 and 2.33 respectively. Minimum and maximum pathologic degree in control and treatment group was zero and 1, 2 and 3 respectively. Collagen formation was not observed in either groups **(Table 8)**. (p =0.028).

Chart 1 depicts differences in P value of studied enzyme in four weeks. Moreover p value differences of adhesion level and pathologic degree in both group were compared **(chart 2) (chart 3)**.

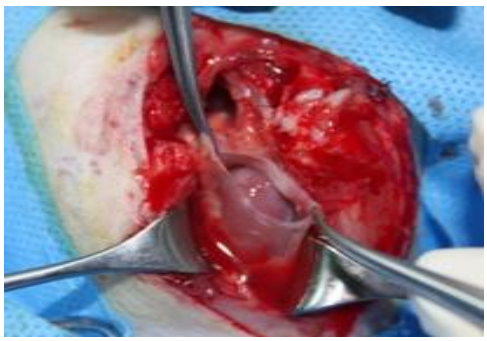


Figure 1: Adhesion Degree 0 in Control Group

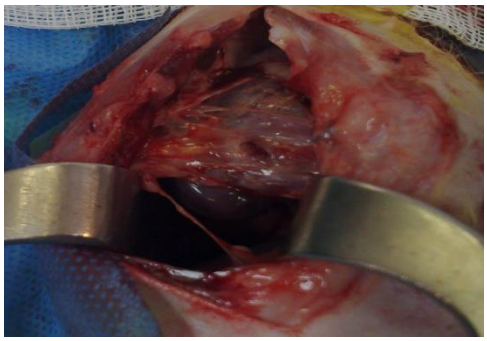


Figure 2: Adhesion Degree 3 a in Experimental Group

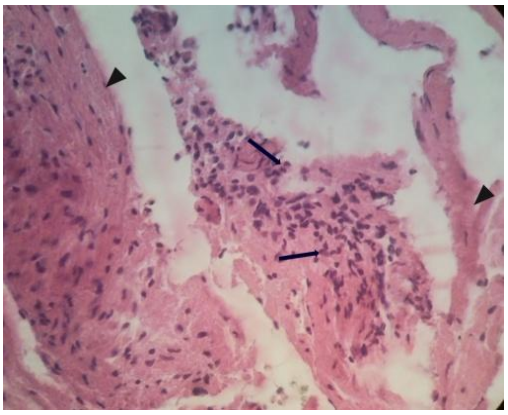


Figure 3: Pathologic Degree 1 in Control Group

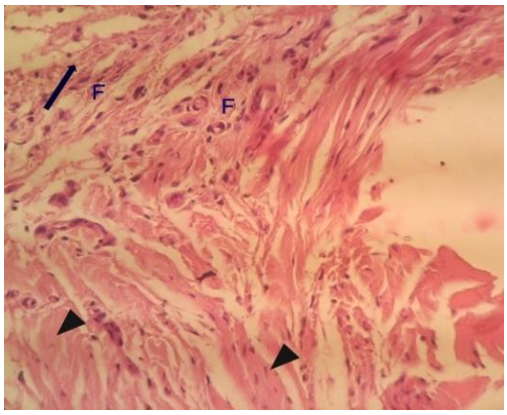


Figure 4: Pathologic Degree 3 a in Experimental Group

Table 3: Proportion of mean value, standard deviation, P – Value of CK - MB during 4 weak in experimental and control group

	Experimental			Control			
	Mean	n	SD	Mean	n	SD	P – Value
CK – MB (week1)	373	6	21.14	244.16	6	48.51	0.007*
CK – MB (week2)	238.16	6	29.81	245.5	6	62.73	1.000
CK – MB (week3)	236.33	6	35.18	241.5	6	80.27	1.000
CK – MB (week4)	232.5	6	38.41	240.16	6	74.97	1.000

Table 4: Proportion of mean value, standard deviation, P – Value of AST during 4 weak in experimental and control group

	Experimental			Control			
	Mean	n	SD	Mean	n	SD	P – Value
AST(week1)	80.83	6	10.02	79.16	6	13.25	0.999
AST (week2)	104.33	6	7.74	72	6	23.34	0.003*
AST (week3)	80	6	10.78	72.16	6	23.52	1.000
AST (week4)	81.5	6	10.25	72.33	6	23.71	0.995

Table 5: Proportion of mean value, standard deviation, P – Value of LDH during 4 weak in experimental and control group

	Experimental			Control			
	Mean	n	SD	Mean	n	SD	P – Value
LDH (week1)	206.66	6	23.80	201.66	6	20.89	0.996
LDH (week2)	257.83	6	24.96	200.83	6	20.89	0.004*
LDH (week3)	208.16	6	29.96	201.33	6	20.75	0.990
LDH (week4)	203.33	6	20.65	201	6	20.63	1.000

Table 6: Proportion of P – Value of CK – MB, AST and LDH in experimental group

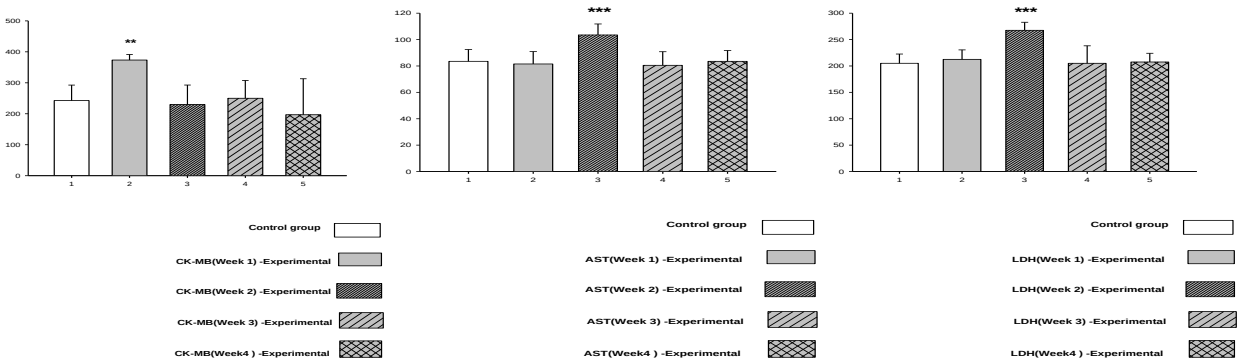
P – Value of CK - MB	Experimental		P – Value of AST	Experimental		P – Value of LDH	Experimental
1st & 2nd weeks	0.011*		1st & 2nd weeks	0.006*		1st & 2nd weeks	0.010*
1st & 3nd weeks	0.044*		1st & 3nd weeks	1.000		1st & 3nd weeks	1.000
1st & 4nd weeks	0.069*		1st & 4nd weeks	1.000		1st & 4nd weeks	0.999
2st & 3nd weeks	1.000		2st & 3nd weeks	0.004*		2st & 3nd weeks	0.013*
2st & 4nd weeks	1.000		2st & 4nd weeks	0.007*		2st & 4nd weeks	0.005*
3st & 4nd weeks	1.000		3st & 4nd weeks	0.999		3st & 4nd weeks	0.997

Table 7: Adhesion rate in experimental and control groups

Rabbit No.	1	2	3	4	5	6
Experimental	1	2	2	3	2	2
Control	0	1	0	0	1	1

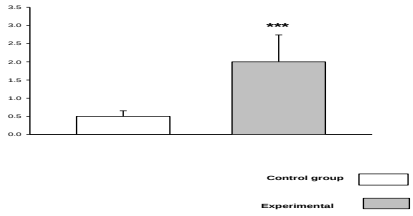
Table 8: Pathologic degree in experimental and control groups

Rabbit No.	1	2	3	4	5	6
Experimental	3	1	2	3	3	2
Control	1	2	2	2	1	0



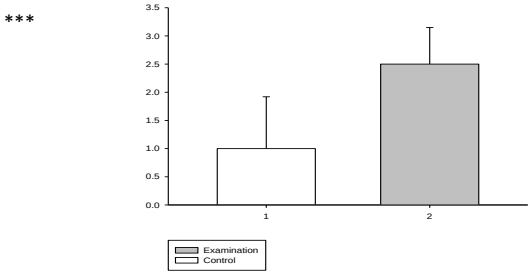
* → 0.01 < p < 0.05 _ ** → 0.005 < p < 0.009 _ *** → 0.000 < p < 0.004

Chart 1: P – Value of CK – MB, AST and LDH in 4 weeks period



* → 0.01 < p < 0.05 _ ** → 0.005 < p < 0.009 _ *** → 0.000 < p < 0.004

Chart 2: Adhesion rate p Value



* → 0.01 < p < 0.05 _ ** → 0.005 < p < 0.009 _ *** → 0.000 < p < 0.004

Chart 3: Pathologic Degree p Value

DISCUSSION

The aim of this study was to examine possible changes in cardiac structure and function after pericardiectomy. Cardiac enzymes are the most important markers measured in the blood, which are known as the myocardial damage markers **[13, 14]**. Although CPK, AST, and LDH are sensitive, CK-MB with CK-MB neuromuscular isoform sensitivity is also of a great value. The special cardiac iso-enzyme CK-MB is known to be a sensitive indicator for cardiac muscle necrosis, and postoperative measurement of its serum level has shown to be useful in screening for post-surgical myocardial damage. Myocardial damage has shown to be the main cause of the rise in CK-MB **[15]**. Following myocardial damage, the CK-MB enzyme starts to increase about 6 hours and peaks usually around 15-20 hours after and returns to its baseline levels within 72 hours of the onset. Thus, following a myocardial infarction, CK-MB is the first enzyme to increase and reaches its peak within 24 hours which returns to its normal status after 3-5 days **[16]**. The reference range of the CK-MB enzyme in rabbits is 140-372 IU/I **[17, 18]**. The mean value of CK-MB in this study increased during the first week of the test and returned to the reference range during the second, third, and fourth weeks; however, no increase in CK-MB level was observed during four weeks of our study in control group. Likewise, mean values were within the reference range increases in test group`s CK-MB. Mean value in first week post operation may result from minor damage to cardiac cells caused by pericardiectomy leading to release of this enzyme to blood stream. Stable level of this enzyme in control group is suggesting that thoracotomy alone does not increase this enzyme`s serum levels. Other cardiac enzymes including LDH and AST are also indicative of myocardium necrosis **[19, 20]**. These enzymes usually peak within 2 to 3 days after the damage and will return to normal state within 5 to 10 days **[21, 22]**.The normal value of LDH and AST enzyme in rabbits are 132-252 IU/I and 10-98 IU/I respectively **[23, 24]**. In this study, the mean value of LDH/AST increased during the second week in test group. However, no increase in LDH and AST blood levels were noted during the first, third and fourth weeks in the control group. The reason for increase in the mean value of LDH and AST during the second week may be due to the damage of myocardium, which results in release of these enzymes in the second week Is unknown. Stable level of these enzymes in control group

is suggesting that thoracotomy alone do not result in elevated levels of LDH and AST in the blood. Adhesion after an open heart surgery is the greatest factor in causing longer operative and perfusion times as well as bleeding and injury to the heart or great vessels **[25]**. In our study, adhesion rate in control group for degree 0 was 50% and for degree 1 was 50%. In contrast this amount for experimental group in first, second and third degree was 16.5%, 67%, 16.5% respectively. These results show that statistically there is a significant difference between these two groups. In a prior study **[12]**, presence of fibroblasts, Fibrin clots and inflammatory cells as well as pathologic degree less than 4, were noted in both control and treatment groups, during a pericardial surgery. In current study, more fibroblast and Fibrin clots were observed in treatment group compared to control group. In contrast, in control group more fibrin and inflammatory cells were observed than that of treatment group. We also showed that although pericardiectomy may not cause severe damage to myocardium, pericariectomy or thoracotomy leads to pathologic degree lower than 4. In conclusion, pericardiectomy can cause myocardial damage, however since cardiac markers return to the reference range, this damage is temporary. As it was observed in gross pathology in the fourth week, pericardiectomy may also lead to cardiac and ventricular enlargement. Further studies are needed to further investigate the cardiac size during the weeks 8 and 12 following pericardiectomy.

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