Study The Predictive Value Of Troponin – I And Histopathological Changes During Acute And Chronic Myocardial Infarction In Adult Male Rats.

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ABSTRACT

Our study used cryo-injury model to induce myocardial infarction in adult male rats by using liquid nitrogen (-196°C) and investigate the physiological changes during acute and chronic M.I through checking serum blood biomarker (Troponin-I), and doing scoring between the biomarker and the histopathological changes in rats myocardium in different period time. We used a total of 35 adult male rats and divided them in to subgroups as following:

- Fifteen rats for induced acute myocardial infarction (4/hr., 8/hr. 24/hr.).
- Fifteen rats for induced chronic myocardial infarction (7/days, 14/days, 28/days).
- Five rats as a control healthy group.

Troponin-I increased significantly at 4 hours post experimental induction of myocardial infarction and persist significantly high for 7 days then decline later on. The histopathological changes during acute and chronic M.I reflect the physiological changes in concentration of cardiac biomarker (CTnI).

On this results showed highly significant (P<0.01) increase in concentration levels of serum CTnI at 4/hr. post experimental induction of M.I this results means the importance of CTnI as highly specific and sensitive marker of myocardial necrosis, and The histopathological changes during acute and chronic M.I it is close related with physiological changes occur in cardiac biomarker concentration and appear early in blood.

INTRODUCTION

Myocardial infarction (M.I) or acute myocardial infarction (A.M.I) remains a leading causes of mortality and morbidity world wide (1) acute myocardial infarction, commonly known as a heart attack, results from the interruption of blood supply to apart of the heart, causing heart cells die.

The study of certain biomarker and histopathological changes during acute and chronic myocardial infarction can be induced in experimental rats by cryo- injury model by using liquid nitrogen (-196°C) (2,3).

During the past several years, a great achievement has been made in the management of cardiovascular diseases depended on the use experimental animals, this has allowed the development of many effective treatment strategies (2).

Several biomarkers have emerge as strong predictors of risk among patients presenting with acute myocardial infarction as Troponin-I (TnI) which release by damaged Myocardioocytes (2).

The pathophysiology alteration that transpire during myocardial infarction occur in two stages: early changes...
at time of acute infarction and late changes during myocardial healing and remodeling (3).

The focuses on the role of Troponin-I as a predictive marker of myocardial necrosis and reflects the histopathological changes during acute and chronic myocardial infarction.

**MATERIAL AND METHODS**

**Laboratory Animals:**

In the present study, 30 adult male rats (Rattus rattus norvegicus albinos), weighing 250-300 g, were used as the M.I model to induced M.I by cryo-injury model and divided to three groups each group contain 5 rats in case of acute or chronic M.I and sacrificed in the following time (4/hr., 8/hr., 24/hr.), (7/day, 14/day, 28/day) respectively. All groups compared with 5 adult male rats healthy weighing 250-300 g as a control groups, these animals were obtained from the animal house in college of medicine in Baghdad university, during the study, these animals were subjected to unified Laboratory circumstances in terms of light, temperature and ventilation and were given water and standard rat chow continuous.

**Myocardial Infarction Model:**

Myocardial infarction was induced following a standardized protocol: 30 adult male rats weighting 250-300 g. were anesthetized with diethyl ether 10 mg/100 g. under aseptic condition, the rat placed a supine position on a temperature control plate (37°C). Shaving the chest from hair and sterilized by antiseptic solution (Alcohol 70 %), the rat heart was exposed through a 1.5 c.m left lateral thoractomy incision. Cryo-injury was produced with a aluminum or metal prob (0.5 c.m in diameter) cooled to – 196 c° by immersion in liquid nitrogen and was applied left ventricular (L.V) free wall for 15 second periods with a 5 second rest, this procedure was repeated two times and infarct area was visualized.

The muscle layer and skin incision were closed by silk suture size 5-0 and 3-0 respectively and the animals were returned to their cages and carefully monitored for 4 hours post operatively, dressing the incision by use fucidin cream antibiotic and use benzathin penicillinG (1500 u/ml) and procaine penicillinG (1500 u/ml) were given intra-muscularly (0.4 ml per rat) after each operation twice a day for the first 48 hours.

Animals were divided into two experimental groups:

- **First groups:** Acute MI (4/hr., 8/hr., 24/hr.) (15 rats).
- **Second groups:** Chronic MI (7/day, 14/day, 28/day) (15 rats).

**Collection Of Blood Samples:**

From each rat (acute M.I groups, chronic M.I groups and control groups) 3 ml of blood were aspiration from rat heart by syringe 5 ml after use diethyl ether as anesthesia substance, then serum was separated by centrifugation at 3000 rpm. For 10 minutes, then collected serum was divided into (1 ml) small aliquots and immediately frozen at (- 20) c° until used.

### Detection Of Troponin I (cTnI) by ELISA

Immunoenzymometric assay for quantitative determination of Troponin I (cTnI), in rat's serum has been carried out. The kit used was provided by monobind INC.-company – USA.

**Collection Of Tissue Samples:**

All the rats were sacrificed for the final experiments (Acute M.I groups, chronic M.I groups and control groups) by diethyl ether.

The chest was opened and removed the heart, and then the tissue samples of left ventricle from the infarct zone and control groups were collected and then fixed with 10 % formalin.

**Statistics**

Data were collected and analyzed using SPSS version 10(package for social science). All data are expressed as mean ± SD. Comparisons between groups were performed with the use paired t-test. p<0.05 was considered statistically significant (18).

**Results:**

- **A**- Table 1 shows elevated concentration levels of cTnI in rats serum in all study groups of acute myocardial infarction (4/hr., 8/hr and 24/hr.) versus control groups, the mean and standard deviation (7.43 ±675), (4.99 ± 0.70) and (1.60 ± 0.81) respectively compared with control group (0.61 ±0.11) (Table 1).

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>No.</th>
<th>The Concentrate Levels Of Troponin-I (cTnI) ng/ml</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.61 ±0.11</td>
<td></td>
</tr>
<tr>
<td>4/hr.</td>
<td>5</td>
<td>7.43 ±0.68</td>
<td>22.34**</td>
</tr>
<tr>
<td>8/hr.</td>
<td>5</td>
<td>4.99 ±0.70</td>
<td>13.82**</td>
</tr>
<tr>
<td>24/hr.</td>
<td>5</td>
<td>1.60 ±0.81</td>
<td>2.73*</td>
</tr>
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**B** - Table 2 shows increase in the means of cTnI concentration in all rats study groups in chronic myocardial infarction (7/days, 14/days and 28/days) (1.91±0.90), (1.38±0.78) and (0.64±0.29) respectively compared with control group (0.60±0.11).

<table>
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<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.61±0.11</td>
<td></td>
</tr>
<tr>
<td>7/days</td>
<td>5</td>
<td>1.91±0.90</td>
<td>3.24*</td>
</tr>
<tr>
<td>14/days</td>
<td>5</td>
<td>1.38±0.78</td>
<td>20.20</td>
</tr>
<tr>
<td>28/days</td>
<td>5</td>
<td>0.64±0.29</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Significant at the level (p < 0.05)
Histopathological Study:
1- Acute MI: During A.M.I the rats heart muscle (myocardium) show different histopathological changes during serial period of time (4/hr, 8/hr. and 24/hr.) which is can be summarized as:
At 4/hr. a myocardial infarction histopathology is seen the coagulation necrosis with edema and P.M.N. infiltration begins. Picture (2).
Moreover, at 8/hr. there can be seen band necrosis in margins, as well as beginning of neutrophil cells infiltration. Picture (3).
At 24/hr. there is continued cardiac muscle fibers necrosis, with loss of nuclei, striation and increased infiltration of neutrophil cells to interstitium. Picture (4).
2- Chronic MI: At the day 7 of M.I the adult male rats left ventricle cross sections show the beginning of necrosis cardiac muscle fibers, and formation of fibrosis, present fibroblast cells and appear collagen fibers. Picture (5).
Moreover, at day 14 of M.I the myocardium rats sections show infiltration of monocyte cells predominant macrophage cells and present fibroblast cells. Picture (6).
On the other hand, during 28 day of M.I the myocardium section shows get increase collagen deposition, decrease cellularity, fibrosis, and cardiac muscle fibers become hypertrophic with establish fibrotic area. Picture (7).

RESULTS AND DISCUSSION
The analysis of cardiac Troponin-I (CTnI) is considered to be the (gold standard) for non invasive diagnosis of myocardial injury in small animals (22).
Concentration of cardiac injury biomarkers can be altered in samples collected from deeply sedated animals as a result of generalized hypoxia (20). In this study, we used light ether anesthesia shortly before sampling to avoid anesthesia induced generalized hypoxia. Cardiac TnI has replaced traditionally used cardiac biomarkers such as creatine- kinase and its isoenzymes due to its high
sensitivity and specificity for the detection of myocardial injury (21,22).

Our results show highly significant(p<0.01) increased in concentration levels of CTnI at 4 hours post experimental induction of acute myocardial infarction and persist the elevation high (p<0.05) during chronic groups for 7 days then decline later on (Table 1,2).

These results agreement with some studies, show irreversible injury of myocardial cells causes release CTnI in the circulation and these cells are going in to necrosis even if only a few cells are affected (4). These enzyme are considered as cardiac markers, thus they are use as tools for the predictors diagnosis of myocardial infarction (5,6).

Moreover, our results seen to agree with what is known about CTnI is a structural protein of the contractile apparatus, was star appears in blood stream at 4 and 8 hours after A.M.I due to damage cardiomyocytes (7,8).

In present study show increase concentration levels of cTnI in serum rats begins early during myocardial infarction, these finding are in agreement with experimental results of previous reports (23,24) which indicated that concentration levels of rat serum cTnI statistically significant rise early after 30 minutes of MI induced by Isoproterenol model.

Some authors observed the concentration of CTnI increased 20 times higher than the normal blood donor at the time of admission with in 4-6 hours of the start chest pain, such results agree with our results (19).

On the other hand, the haemolysis may interfere with CTn and causes measurement of higher or lower CTn concentration(9).

Present study revealed significantly increased CTnI during chronic M.I at 7/days (Table 1,2), this result agreement with previous reported show persist increase CTnI till 2 week this may due to continuous breakdown of the myofibrillar complex due to large infarct size lead to prolonged elevated of concentration of CTnI in blood stream (8).

On the other hand, the elevated levels of serum cTnI after a week might be due to the release of structurally cell bound troponin and some time probably reflects more-extensive (large) infarction, this fact agree with our results (10).

**Histopathological Study:**

Various histopathological changes detected during acute myocardial infarction such as at 4 hours we see edema, and PMN cells infiltration begins.

The edema of the myocardium may have resulted as vascular permeability increase and interstitial oncottic pressure rises because of the leak of intercellular proteins and causes myocytes altered (3,11,12).

This study revealed necrosis of myocardial cells with loss of nuclei and striations and increase of neutrophils cells to interstitium we see that at 24 hours of A.M.I.

The neutrophils cells activated during inflammation, these cells respond to intracellular signals that identify the invader as foreign. In myocardium similar signals of inflammation are generated by endothelial cells and cardiomyocytes, this finding compatible with our results(13).

Previous studies mentioned neutrophils cells can interact with inflammatory factors to initiate myocardial injury (14,13).

Neutrophils are a primary source of reactive oxygen species (ROS), including superoxide anion and MPO system these causes tissue necrosis and cells death. However, ROS generation can be stimulated by a number of factors release in vivo during M.I such as complement system (C5a), platelet activating factor (PAF), TNF-α, IL-6, IL-8 (13).

The present study shows during chronic M.I various histopathological changes in adults rats left ventricular cross section such as:

- At 7 days we see persist necrosis cardiac muscle fibers, beginning formation of fibrosis, present fibroblast cells and appear collagen fibers.
- The optimal cardiac repair requires containment of the inflammation in the infracted area, extension of the inflammation into the non infracted area could results in expansion of the neutrophil cells infiltration and worsening of the remodeling (13).
- The present histological study revealed present fibroblast cells at day 7 and day 14 of M.I.
- Moreover, during day 14 of chronic M.I we can see Continuous cardiac muscle fibers necrosis, infiltration of monocyte predominant macrophage cells.
- Irreversible injured myocytes do not regenerated, rather, the cell are remove and replaced by fibrous tissue. Macrophage cells invade the infarcted myocardium shortly after neutrophile cells infiltration and remove necrotic tissue (3).
- The inflammatory response and cytokines release from the myocardium are essential components of the host response to A.M.I, and play a crucial role in cardiac repair process (14).
- At 28 days the myocardium section shows establish fibrotic area.

This change may be related with cytokines release such as Transforming Growth Factor (TGF-β) is a multifunctional cytokine that control proliferation and cellular differentiation in most cell (17).

On the other hand, reduced myocardium perfusion might lead to activation of fibroblasts cells in myocardium and consequently lead to excessive collagen deposition and fibrosis (14).

**CONCLUSION**

Highly significant increase in concentration of serum levels of CTnI at AM.I and persist elevated till 7/days during chronic M.I. This results means the importance of CTnI as highly specific and sensitive marker of myocardial necrosis to detect myocardial infarction, and...
The histopathological changes during A.M.I and chronic M.I it is close related with physiological changes occur in cardiac biomarker concentration and appear early in blood and related with duration of infarction.

REFERENCES