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Original Researh Article

Investigation of Myeloperoxidase and Histopathological Changes During Acute and Chronic Myocardial Infarction in Human and Rat

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Our study used a cryo-injury model to induce myocardial infarction in adult male rats by using liquid nitrogen (- 190 Co) and investigate the physiological changes during acute and chronic M.I through checking serum blood biomarker Myeloperoxidase. And doing scoring between the biomarker and the histopathological changes in rat myocardium in different period time. We used a total of 35 adult male rats and divided them into subgroups as following:

- **Acute M.I groups (4/hours, 8/hours and 24/hours) (15 rats).**
- **Chronic M.I (7/days, 14/days and 28/days) (15 rats).**
- **Control groups (healthy) (5 rats).**

On the other hand, the clinical study investigated the physiological changes during AM.I and chronic M.I through checking serum blood biomarker Myeloperoxidase. We used a total of 35 adult male patients complains from M.I and divided them into subgroups as follows:

- **Acute M.I groups (4/hours, 8/hours and 24/hours) (15 humans).**
- **Chronic M.I (7/days, 14/days and 28/days) (15 humans).**
- **Control groups (healthy) (5 humans). From these experimental and clinical studies, the following results were observed:**

1- Increased the concentration levels of Myeloperoxidase (MPO) in all study groups in experimental and clinical and acquired this elevated highly, significant ($p<0.01$) at 4/hours and reach the peak concentration at 8-24 hours of AM.I and persist significant elevated till 28/days of chronic M.I.

2- Histopathological Changes: The histopathological changes during acute and chronic M.I reflect the physiological changes in concentration of Myeloperoxidase. In this result showed highly significant ($p<0.01$) relation between histopathological changes during acute and chronic M.I and serum level of Myeloperoxidase.

Key words: Histopathological, Endothelin-1, Myocardial infarction.

INTRODUCTION

Myocardial infarction (M.I) or acute myocardial infarction (A.M.I) remains a leading cause of mortality and morbidity worldwide (Mallinson, 2010) acute myocardial infarction, commonly known as a heart attack, results from the interruption of blood supply to apart of the heart, causing heart cells death. 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 (- 190 co) (Christenson et al., 2011 ,Leonard, 2007).

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 (Christenson et al., 2011). Several biomarkers have emerged as strong predictors of risk among patients presenting with acute myocardial infarction as Myeloperoxidase which release of nutrophil cells (Christenson et al., 2011). The pathophysiology alteration during myocardial infarction occurs in two stages: early changes at time of acute infarction and late

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changes during myocardial healing and remodeling (Leonard, 2007).

The focus on the role of Myeloperoxidase, as an independent marker of plaque instability that may help in the diagnosis of AM.I and predict the high risk patients to develop major adverse cardiac events and the histopathological changes during acute and chronic myocardial infarction.

Material and Methods

Clinical Study

Patients Groups

A total of 30 Iraqi patients (male) with myocardial infarction (MI), who were admitted to the Iraqi center for heart diseases, AL-Amam Ali hospital and Baghdad teaching hospital (Emergency unit and coronary care unit) were investigated biochemically from September 2011 to January 2012. And patients age between 25–80 years.

These patients were divided into two groups acute MI contain three sub groups, each one contain 5 patients depend on the following time (7/day, 14/day, 28/day) and chronic MI contain three sub groups, each one contain 5 patients depend on the following time (7/day, 14/day, 28/day) according to a clinical examination by physicians and electrocardiogram (E.C.G.).

Control Groups

The control groups included 5 male apparently healthy individuals with no signs of C.H.D. or other diseases, compatible with patients group from the age of the same age groups.

Collection of Blood Samples

For each patient (acute M.I groups, chronic M.I groups and control groups) 2-3 ml of blood were aspiration by syringe 5ml, serum was separated by centrifugation at 3000rpm/10 minutes, collected serum was frozen immediately at -20 C° until used and thawing of each frozen sample only one at a time of the test.

Experimental Study
Laboratory Animals

In the present study, 35 adult male rats (Rattus rattus norvegicus albinos).Weighting 250-300g.were used for induced myocardial infarction by cryo- injury method(Ewout et al., 2005) and animals divided into three groups each group contains 5 rats in case of acute and chronic M.I and sacrified (4/hr., 8/hr., 24/hr.) (7/day, 14/day, 28/day) respectively, all groups compared with 5 male adult rats healthy weighting 250-300g as a control group. These animals were obtained from the animal house in a medical research unit of college of medicine in Baghdad University; these animals were subjected to unified Laboratory circumstances in terms of light, temperature, ventilation and were given water along the duration of the study.

Myocardial Infarction Model

Myocardial infarction was induced following a standardized protocol (Ewout et al., 2005). 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 in a temperature- control plate (37 co). Shaving the chest of hair and sterilized by antiseptic solution (Alcohol 70 %), the rat heart was exposed through a 1.5 cm left lateral thoractomy incision. Cryo –injury was produced by an aluminum or a metal probe (0.5 cm in diameter) cooled to – 190 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 in 5-0 and 3-0 silk suture 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 pencillinG (1500 u/ ml) and procaine pencillinG (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 was aspirated from heart puncture 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 aliguotes and immediately frozen at (- 20) c° until used.

Detection of myeloperoxidase (MPO) ELISA

The fluorescence–based Amplex ultra Red reagent assay for the quantitative determination of myeloperoxidase (MPO) in serum human and rats serum has been carried out the kit used was provided be zen invitrogen – 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 killing the animals 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.

Histological Study

All the rats were sacrificed for the final experiments (acute M.I groups, chronic M.I groups and control groups) by killing the animals 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 fixed with 10% formalin. The tissue samples were embedded in paraffin wax and cut into 5µm thick section by using a rotary microtome; the sections were serially rehydrated with 100% and 70% ethanol after deparafinization with xylene. Then, staining with Harris haematoxylin and eosin stain, dehydrated in graded ethanol, cleared in xylene and mounted with Canada balsam (Wang et al., 2000).

Results and Disscusion

A- Statistic analysis in table (1) revealed highly increase in the mean concentration levels of Myeloperoxidase (MPO) of all human study groups in acute M.I (4/hr., 8/hr and 24/hr). (235.91 ±32.94), (268.25±48.11) and (238.48 ±46.77) respectively compared with control group (101.55 ± 6.67).

Table 1: The concentrate levels of Myeloperoxidase (MPO)(ng/ml) in human serum of acute M.I and control

Study Groups	No.	The Concentrate Levels Of Myeloperoxidase (MPO)(ng/ml)	t-test
		Mean ± SD.	
Control	5	101.55 ± 6.67	
4/ hr.	5	235.91 ±32.94	8.94**
8/hr.	5	268.25 ± 48.11	7.67**
24/hr.	5	238.48 ± 46.77	6.48**

** Highly significant at level p < 0.01

B-Table (2) shows highly increase in the means of Myeloperoxidase (MPO) concentration in all human study groups in chronic M.I (7/days, 14/days and 28/days) (193.03 ±32.42), (170.66 ±27.43) and (144.46 ±22.38) respectively compared with control group (101.55 ± 6.67).

Table 2: The concentrate levels of Myeloperoxidase (MPO)(ng/ml) in human serum of Chronic M.I and control

Study Groups	No.	The Concentrate Levels Of Myeloperoxidase (MPO)(ng/ml)	t-test
		Mean ± SD.	
Control	5	101.55 ± 6.67	
7/days	5	193.02 ±32.41	6.18**
14/days	5	170.66 ±27.43	5.48**
28/days	5	144.46 ± 22.38	4.11**

** Highly significant at level p < 0.01

A- Statistic analysis in table (3) revealed highly increase in the means concentration levels of Myeloperoxidase (MPO) of all rat study groups in acute M.I (4/hr.,8/hr and 24/hr). (195.55±18.59), (214.34 ±19.75) and (257.66±30.61) respectively compared with control group (89.79 ±12.76).

Table 3: The concentrate levels of Myeloperoxidase (MPO) (ng/ml) in rat's serum of acute M.I and control

Study Groups	No.	The Concentrate Levels Of Myeloperoxidase (MPO) (ng/ml)	t-test
		Mean ± SD.	
Control	5	89.79 ± 12.76	
4/ hr.	5	195.55 ±18.59	10.48**
8/hr.	5	214.33 ± 19.75	11.84**
24/hr.	5	257.66 ± 30.61	11.32**

** Highly significant at level p < 0

B-Table (4) shows highly increase in the means of Myeloperoxidase (MPO) concentration in all rats study groups in chronic M.I (7/days, 14/days and 28/days) (210.16 ±14.49), (173.69 ±26.61) and (137.58 ±19.37) respectively compared with control group (89.79 ± 12.76)

Table 4: The concentrate levels of Myeloperoxidase (MPO)(ng/ml) in rats serum of Chronic M.I and control

Study Groups	No.	The Concentrate Levels Of Myeloperoxidase (MPO) (ng/ml)	t-test
		Mean ± SD.	
Control	5	89.79 ± 12.76	
7/days	5	210.16 ±14.49	13.94**
14/days	5	173.69 ±26.61	6.36**
28/days	5	137.58± 19.37	4.61**

** Highly significant at level p <0.01

Statiscal Analysis

All results were given as mean ±SD (Standard deviation) and use paired t-test. To compare between the groups, statistical analysis was performed by using SPSS statistical version 11 software package.

Histopathological Results

Acute M.I: 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 can be summarized as following:

At 4/hr, the cross section of myocardium show coagulation necrosis is initiated with edema and polymorphonucleus infiltration begins.[Picture 2]. Moreover, at 8/hr, band necrosis in margins, as well as beginning of neutrophil cells infiltration. [Picture 3]

At 24/hr. cardiac muscle fibers necrotized, with loss of nuclei, striation and increased infiltration of neutrophil cells to interstitum. [Picture 4]

Chronic M.I: At the day 7 of M.I the cross section of ventricle showed 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 cardiac muscle fibers showed infiltration of monocyte cells predominant macrophage cells and present fibroblast cells. [Picture 6] On the other hand, during day 28 of M.I the myocardium section shows get increase collagen deposition, decrease cellularity, fibrosis, and cardiac muscle fibers become hypertrophic with the establish fibrotic area. [Picture 7]

Results of the present study revealed highly significant increased in concentration levels of serum Myeloperoxidase (MPO) in acute and chronic M.I in experimental and clinical study versus control groups. (table 1, 2), (3, 4) This finding agrees with previous studies have reported that, in patients with CAD. And acute M.I, plasma MPO levels were higher than levels in healthy subjects (Mehment et al., 2012; Khan et al., 2007; Brennan et al., 2003).

The present study revealed a significant increased in concentration levels of serum Myeloperoxidase (MPO) in acute A.M.I this results agree with (Morrow et al., 2007) that MPO appears to increase early after A.M.I and does identify the patients with ACS earlier than conventional biomarkers such as Troponin or CK-MB (Jong et al., 2011).

Moreover, Valentina et al., (2008) mentioned MPO is a proinflammatory and pro oxidative enzyme that is abundant in rupture plaque, and secreted from activated polymorphonuclear neutrophils and macrophages, also oxidative stress and inflammation play important roles in the pathogenesis of destabilization of coronary artery disease (CAD) lead to acute coronary syndromes (ACS). Infiltration macrophage and neutrophils participte in the transformation of stable coronary artery plaque to the unstable lesion (Morrow, 2007).

On the other hand, MPO able to oxidation of LDL cholesterol into atherogenic form recognized by macrophage scavenger receptors and consumes endothelial derived NO. Bioavailability and impairing its vasodilation and anti-inflammatory properties (Valentina et al., 2008). The experimental results of our study revealed a significant increased in serum levels of MPO in A.M.I at 4/hours and reach the peak levels at 24/hours and persist elevated until 28/days. This results agreement with what know about MPO kinetics release during A.M.I, because myocardium damage causes development inflammatory process and invade the neutrophils and macrophage in these tissue and lead to release large amount of MPO as oxidant substance and generated cytotoxic aldehydes this causes oxidation of common α –amino acid may adversely affect on L.V. remodeling and infarct size. This results reflects our histopathological results study (Matthew, 2007).

Brennan and Colleagues, (2003) show the prognostic usefulness of MPO in patients who presented with chest pain at emergency departments, the found MPO to be helpful as in independent predictor of major adverse cardiovascular events (MACE) (M.I, reinfarction, the need for revascularization, or death) in the following 30 days and at 6 months. Mehamet et al., (2012) find high plasma MPO levels identify patients with a worse prognosis after acute STEMI at 2- years follow-up and useful in determining patients at high risk of death and major adverse cardiovascular events (MACE) who can benefit from further aggressive treatment and closer follow up.

Koening and Khuseyinove, (2007) show MPO may activate matrix metalloproteinases (MMP) and deactivate tissue inhibitors of MMP are promoting weakening and thinning of the fibrous cap. Moreover, Brennan et al., (2003)mention initial MPO levels were an independent predictor for M.I even in patients with negative Troponin.

Histopathological

Various histopathological changes detected during acute myocardial infarction such as at 4 hours that showed edema, and PMN cell infiltration begins.

The edema of the myocardium may have resulted as vascular permeability increase and interstitial oncotic pressure rises because of the leak of intercellular proteins and causes myocytes altered (Leonard, 2007;Avan etal.,2006; Cotran et al.,1994).

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

This finding agrees with Jacob (Jakob, 2004) showed that neutrophils cells activated during inflammation, these cells respond to intracellular signals. In myocardium similar signals of inflammation are generated by endothelial cells and cardiomyocytes.

Some authors mentioned neutrophils cells can interact immuno inflammatory factors to initiate myocardial injury (Deuk and Moo, 2009; Jakob , 2004).

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

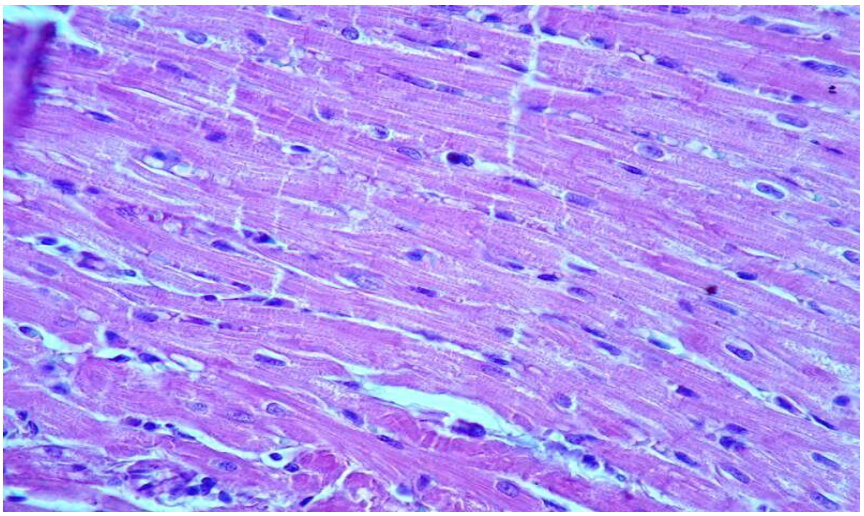
At 7 days there is persist necrosis cardiac muscle fibers, beginning the formation of fibrosis, present fibroblast cells and appear collagen fibers. On the other hand, the optimal cardiac repair requires containment of the inflammation in the infracted area, extension of the inflammation in to the non infracted area could results in expansion of the neutrophil cells infiltration and worsening of the remodeling (Jakob, 2004). The present histological study revealed present fibroblast cells at day 7 and day 14 of M.I.

This finding agrees with some reported revealed proliferation of fibroblast cells was not evident until the day 7, when a spindle-shape cell with plump to elongated nuclei appeared at the edge of the necrotic muscle, all infarcts had fibroblastic proliferation along with deposition of collagen, from the seven days until the formation of scar was complete (Nathan, 2002; Michael et al., 1990). Moreover, during day 14 of chronic M.I showed continuous cardiac muscle fiber necrosis, infiltration of monocyte predominant macrophage cells.

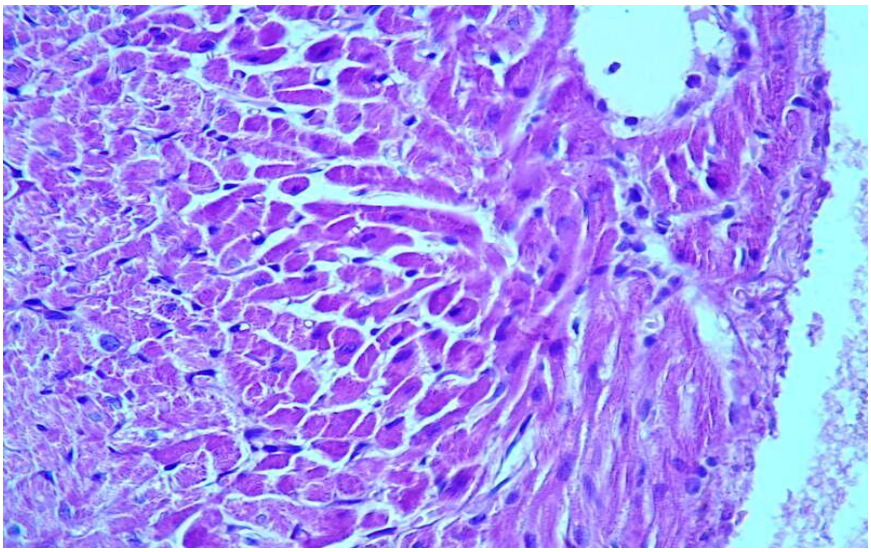
Irreversible injured myocytes do not regenerated, rather, the cell is remove and replaced by fibrous tissue. Macrophage cell invade the inflamed myocardium shortly after nutrophil cells infiltration and remove necrotic tissue (Leonard, 2007).

At 28 days the myocardium section shows establish fibrotic area.

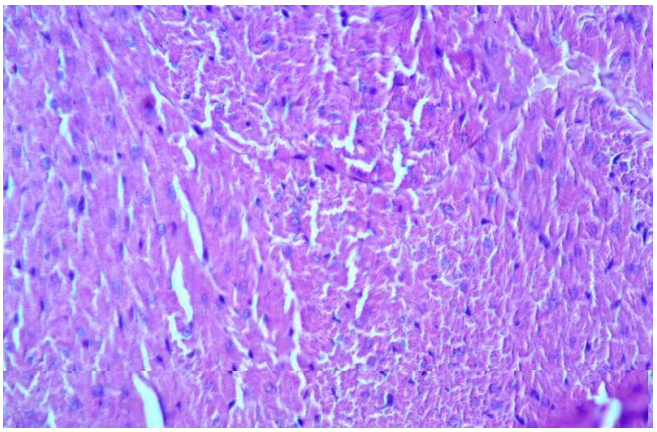
This change may be related to cytokines release such as Transforming Growth Factor (TGF-ß) is a multifunctional cytokine that control proliferation and cellular differentiation in most cell (Michael et al., 1990). On the other hand, reduced myocardium perfusion might lead to activation of fibroblasts cells in myocardium and consequently lead to excessive collagen deposition and fibrosis (Deuk and Moo, 2009)



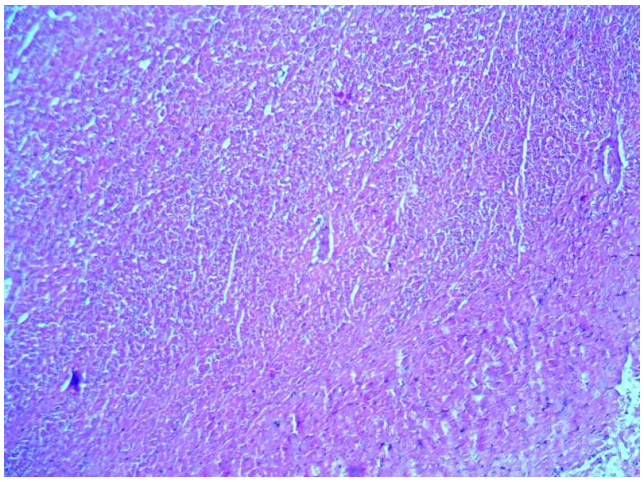
Picture 1: Control negative. H. and E. ×25



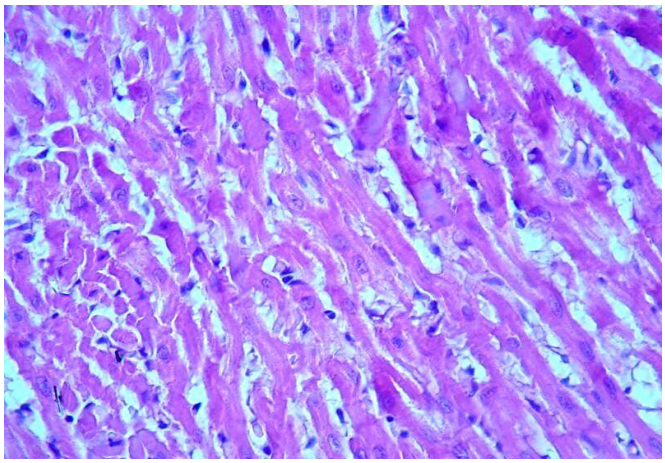
Picture 2: H and E.×25



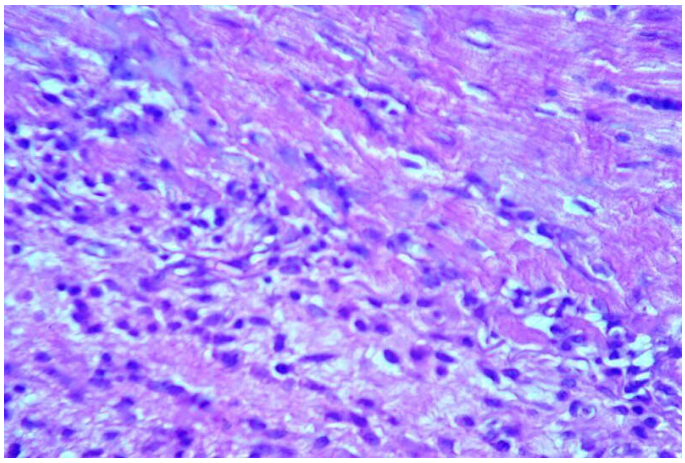
Picture 3: H and E.×25



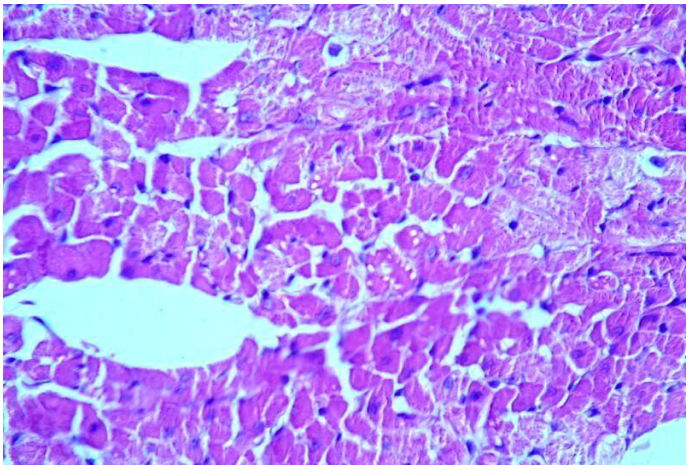
Picture 4: H and E.×10



Picture 5: H and E.×25



Picture 6: H and E.×25



Picture 7: H and E.×25

Conclusion

This study revealed a highly significant increase in the concentration of serum levels of MPO at AM.I and persist elevated till 28/days during chronic M.I. This result means the importance of MPO as an independent marker of plaque instability that may help in the diagnosis of AM.I and predict the high risk patients to develop major adverse cardiac events. 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 and related with duration of infarction.

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