

Pyrex Journal of Nutrition and Metabolism
Vol 1 (2) pp. 006-013 May, 2015
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Original Research Article

The predictive value of Endothelin-1 and histopathological changes during acute and chronic myocardial infarction in human and rat

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Accepted 27th May, 2015

Our study used cryo-injury model to induce myocardial infarction in adult male rats by using liquid nitrogen (- 190 C) and investigate the physiological changes during acute and chronic M.I through checking serum blood biomarker Endothelin-1. 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 follows:

- 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 Endothelin-1. 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 Endothelin-1 (ET-1) 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 Endothelin-1.

In this result shows a strong relation between histopathological changes during acute and chronic M.I and serum level of Endothelin-1.

Keywords: 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 a part of the heart, causing heart

cell 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 c°) (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 Endothelin-1 which release by myocardial damage (Christenson et al., 2011). The pathophysiology alteration during myocardial infarction occurs in two stages: early changes at time of acute infarction and late changes during myocardial healing and remodeling (Leonard, 2007).

The focus on the role of the ET-1 as a predictive marker of endothelial dysfunction that may help in the diagnosis of AMI 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 was admitted to an 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 and chronic MI and each groups contains 5 patients depend on the following time (4/hr., 8/hr., 24/hr.) (7/day, 14/day, 28/day) respectively, 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 patient groups 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 sacrificed (4/hr., 8/hr., 24/hr) (7/day, 14/day, 28/day) respectively, all groups compared with 5 male adult rats healthy weighting 250-300 g 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 weighing 250 - 300g were anesthetized with diethyl ether 10 mg/100 g under aseptic condition. The rat placed a supine position in a temperature- control plate (37 c). Shaving the chest of hair and sterilized by antiseptic solution (Alcohol 70 %), the rat heart was exposed through a 1.5c.m left lateral thoractomy incision.

The Cryo -injury was produced by an aluminum or a metal probe (0.5c.m 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 penicillinG (1500 u/ ml) and procaine penicillinG (1500 u/ml) was 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 aliquotes and immediately frozen at (- 20) c° until used.

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 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).

Detection of Endothelin-1 (ET-1) by ELISA

Enzyme -linked immunosorbent assay for the quantitative determination of Endothelin-1 (ET-1), in serum human and rat serum has been carried out. The kit used was provided by Enzo life sciences- company- Switzerland.

Table 1: The concentrate levels of Endothelin-1 (ET-1) (pg/ml) in human serum of acute M.I and control

Study Groups	No.	The Concentrate Levels Of Endothelin-1(ET-1) (pg/ml)	t-test
		Mean \pm SD.	
Control	5	6.59 \pm 0.90	
4/ hr.	5	12.70 \pm 2.01	6.21**
8/hr.	5	14.39 \pm 1.24	11.38**
24/hr.	5	17.91 \pm 2.61	9.17**

** Highly significant at level $p < 0.01$

Table 2: The concentrate levels of Endothelin-1 (ET-1) (pg/ml) in human serum of Chronic M.I and control

Study Groups	No.	The Concentrate Levels Of Endothelin-1(ET-1)(pg/ml)	t-test
		Mean \pm SD.	
Control	5	6.59 \pm 0.90	
7/days	5	8.87 \pm 1.43	3.01*
14/days	5	9.81 0 \pm 2.38	2.84*
28/days	5	8.106 \pm 0.84	2.76*

* Significant at the level $p < 0.05$

Table 3: The concentrate levels of Endothelin-1 (ET-1) (pg/ml) in rat serum of acute M.I and control

Study Groups	No.	The Concentrate Levels Of Endothelin-1(ET-1) (pg/ml)	t-test
		Mean \pm SD.	
Control	5	5.49 \pm 0.98	
4/ hr.	5	9.06 \pm 1.46	18.53**
8/hr.	5	13.37 \pm 0.91	31.56**
24/hr.	5	15.83 \pm 1.57	25.73**

** Highly significant at level $p < 0.01$

Table 4: The concentrate levels of Endothelin-1 (ET-1) (pg/ml) in rat serum of Chronic M.I and control

Study Groups	No.	The Concentrate Levels Of Endothelin-1(ET-1)(pg/ml)	t-test
		Mean \pm SD.	
Control	5	5.49 \pm 0.98	
7/days	5	7.31 \pm 0.52	25.80**
14/days	5	8.14 \pm 0.81	23.00**
28/days	5	6.974 \pm 0.63	23.93**

** Highly significant at level $p < 0.01$

Statistical Analysis

All results were given as mean \pm 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

Results and Discussion

A- Statistic analysis in table (1) revealed highly increase in the mean concentration levels of Endothelin-1 (ET-1) of all human study groups in acute M.I (4/hr., 8/hr and 24/hr) (12.70 \pm 2.01),

(14.39 \pm 1.24) and (17.91 \pm 2.61) respectively compared with control group (6.59 \pm 0.90).

B- Table (2) shows the mean of ET-1 concentration in all human study groups in chronic M.I (7/days, 14/days and 28/days) (8.87 \pm 1.43), (9.81 \pm 2.38) and (8.11 \pm 0.84) respectively compared with control group (6.59 \pm 0.90).

A- Statistic analysis in table (3) revealed highly increase in the mean concentration levels of Endothelin-1(ET-1) of all rat study groups in acute M.I (4/hr., 8/hr and 24/hr) (9.06 \pm 1.46), (13.37 \pm 0.91) and (15.83 \pm 1.57) respectively compared with control group (5.49 \pm 0.98).

B- Table (4) shows the highest increase in the mean of ET-1 concentration in all rats study groups in chronic M.I (7/days, 14/days and 28/days) (7.31 ± 0.52), (8.14 ± 0.81) and (6.97 ± 0.63) respectively compared with control group (5.49 ± 0.98).

Histopathological Results

Acute M.I

During A.M.I the rat heart muscle (myocardium) show different histopathological changes during the serial period of time (4/hr, 8/hr. and 24/hr.) which can be summarized as follows:

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 cell infiltration, [Picture 3].

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

Chronic M.I

On the day 7 of M.I the cross section of ventricle showed the beginning of necrosis cardiac muscle fibers and the 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].

The present study revealed highly significant increased in concentration mean levels of serum endothelin -1 (ET-1) in acute and chronic M.I in clinical and experimental study versus control groups (table 1, 2), (table 3, 4) respectively.

These results agree with other authors like (LLi et al., 2012; ANNA, 2002) who reported the concentration of ET-1 was significantly higher in patients with A.M.I compared with the healthy control, and in patients with A.M.I who had the macro vascular disease versus those without macro vascular disease. The clinical study, showed highly significant increased concentration levels of serum endothelin -1 (ET-1) in acute M.I, especially at 4/hours and 24/hours and continuous the elevation in chronic M.I at 7/days and 14/days then after that the concentration of ET-1 begin declining, that finding agreement with a prior study submitted by Spieker et al., (2001) who found in patients with uncomplicated A.M.I, ET-1 plasma levels rise within hours, peak at 6/hours and return to normal with 24/hours. In patients with M.I complicated by pulmonary

edema or cardiogenic shock ET-1 levels remain elevated for a longer period.

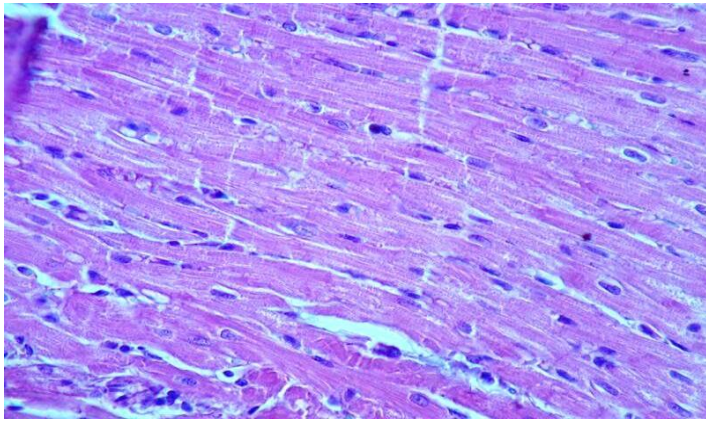
Moreover, the results of our study (clinical) show also significant increased in concentration levels in serum endothelin -1 in chronic M.I only at 7/days and 14/days, this may be related to various risk factors for atherosclerosis, such as diabetes mellitus, smoking and hypercholesterolemia, enhance ET-1 secretion in addition, angiotension II activated in A.M.I, is a potent stimulator of ET-1. On the other hand, the oxidized low density lipoprotein cholesterol (LDL) induces the production of ET-1 by human macrophages and enhances the release of ET-1 by endothelial cells (Yildiz et al., 1996; Boulanger et al., 1992).

The present experimental study, shows highly significant increase in concentration levels in serum endothelin -1 in acute and chronic M.I as general, but we show high concentrate at 8/hours and 24/hours that finding agrees with (Xavier et al., 2011) in animal models he shows ET-1 increase at 3–4 hours after the onset of STEMI, peak within the first 24/hours and remain elevated after 48/hours.

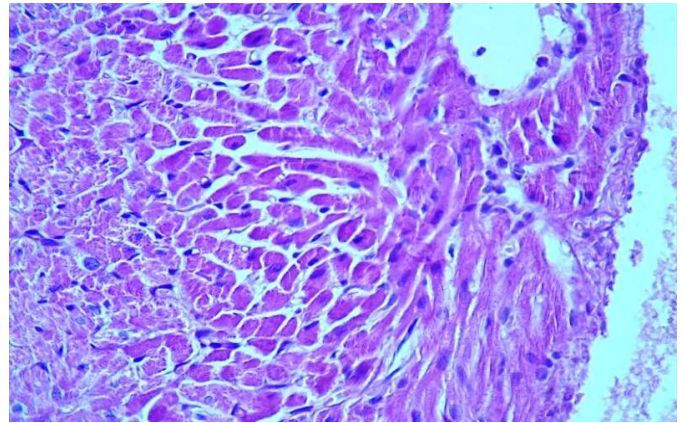
Moreover, ET-1 has very potent vasoconstrictor properties. It causes stenosis of atherosclerosis, coronary arteries and induces smooth cell proliferation, stimulates adhesion of neutrophils to the endothelium and platelet aggregation, thus contributing to the pathophysiology of A.M.I, also it was found that the active coronary atherosclerosis plaques contain significant amount of ET-1.

Recent studies suggested ET-1 plays an important role in causing M.I, post-infarct scar formation, left ventricular remodeling and the reflow phenomenon (Khan, 2005; Spieker et al., 2001). Khan, (2005) shows endothelin-1 is much higher in the infarcted area than in healthy myocardium, suggesting that ET-1 contributes to the stabilization of scarring. Giampaolo et al., (2006) found ET-1 plasma levels have been found to be elevated in the first hours after A.M.I unstable plaques display a high content of ET-1.

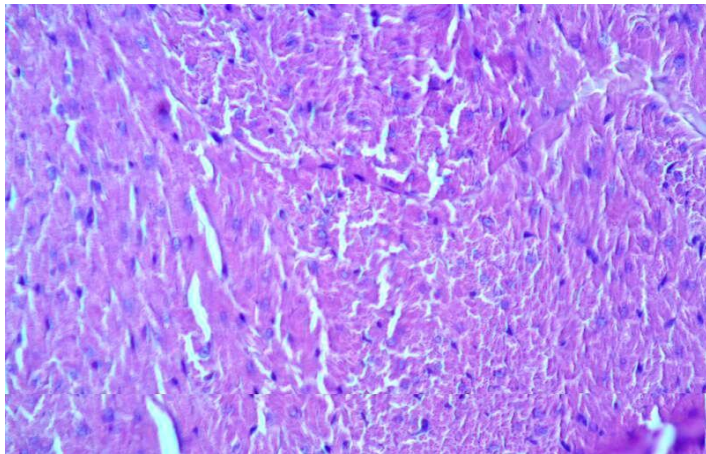
Furthermore, elevated systemic ET-1 levels have recently been reported to predict a poor prognosis in patients admitted for A.M.I treated by primary The elevated plasma levels of ET-1 observed during A.M.I are the results of stimulated both cardiac and extracardiac productions of ET-1 (Tomoda, 1993). The increased release of ET-1 mediated vasoconstriction and smooth muscle cell proliferation via ET-A receptors, acts as a chemoattractant for circulating monocyte and induces neutrophil adhesion, platelet aggregation and adhesion molecule expression. Thereby, ET-1 is involved in atherogenesis and promotes lesion growth and coronary thrombosis (Alexei and William, 2002). Yip et al., (2005) mention elevated systemic ET-1 levels have recently been reported to predict a poor prognosis in patients admitted for A.M.I treated by primary percutaneous coronary intervention (PCI) (Yip et al., 2005).



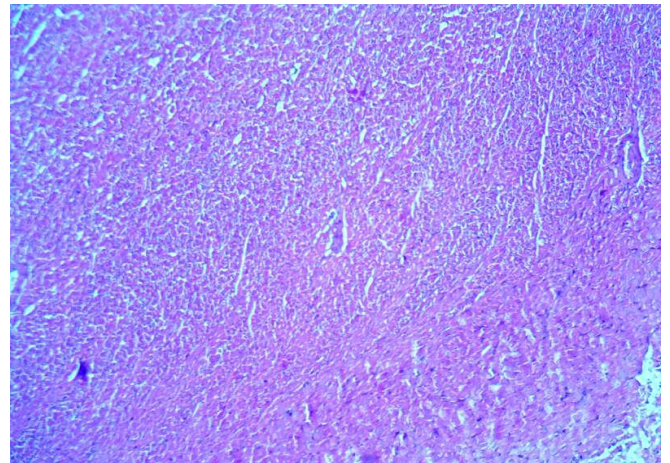
Picture (1): Control negative. H. and E. x25



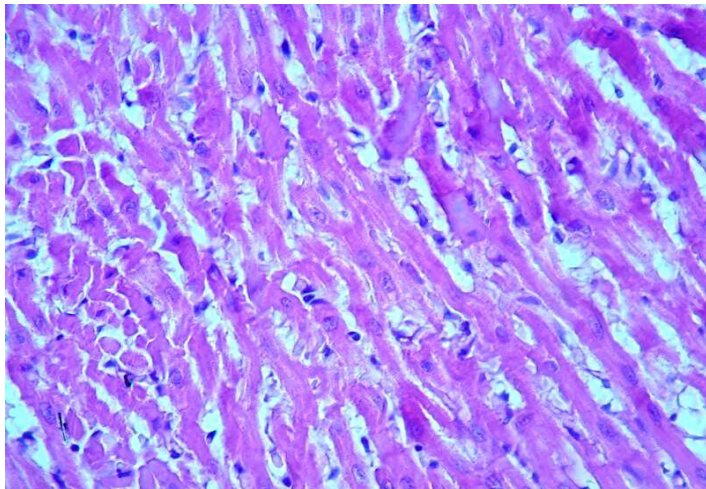
Picture (2): H and E.x25



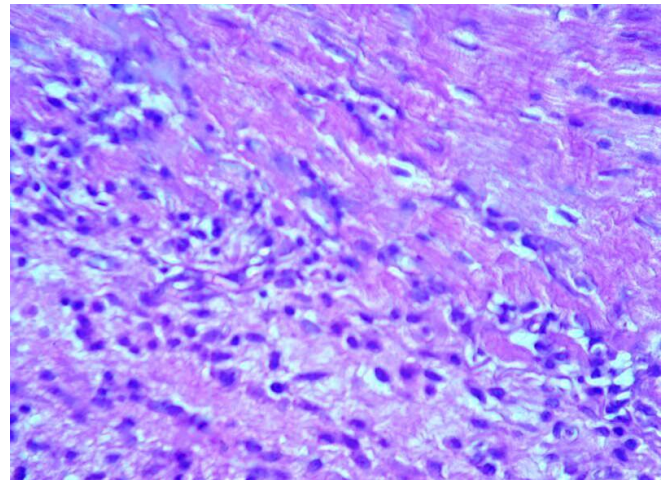
Picture (3): H and E.x25



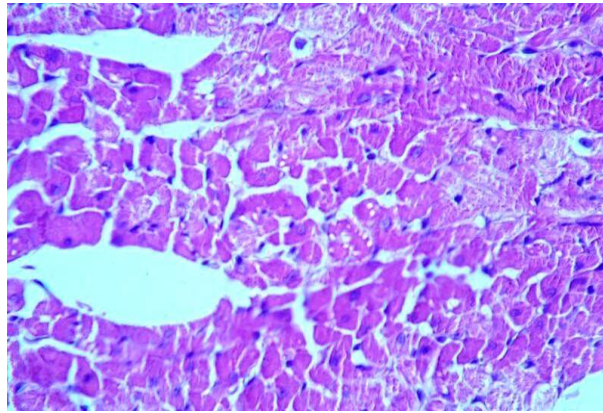
Picture (4): H and E.x10



Picture (5): H and E.x25



Picture (6): H and E.x25



Picture (7): H and E.x25

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 et al., 2006; Cotran et al., 1994). This study revealed necrosis of myocardial cells with loss of nuclei and striations and increase of neutrophils cells to interstitium at 24 hours of A.M.I.

This finding agrees with Jakob (Jakob, 2004) showed that neutrophils cells activated during inflammation, these cells respond to intracellular signals. In the 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 infarcted area, extension of the inflammation in the non infarcted area could result in the expansion of the neutrophil cell 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 fibers necrosis, infiltration of monocyte predominant macrophage cells. Irreversible injured myocytes do not regenerate, rather, the cell is removed and replaced by fibrous tissue. Macrophage cells invade the inflamed myocardium shortly after neutrophil cell 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- β) 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).

Conclusion

This study revealed a highly significant increase in the concentration of serum levels of ET-1 at AM.I and persist elevated till 28/days during chronic M.I. This result means the importance of ET-1 as a predictive marker of endothelial dysfunction that may help in the diagnosis and follow up of Iraqi patients.

And the histopathological changes during acute and chronic M.I it is closely related to physiological changes and occurs in cardiac biomarker concentration and appear early in blood and related to duration of infarction.

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