

Clinicopathological study in an ovine model of experimental acute myocardial infarction

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Summary

To evaluate the prognostic significance of clinicopathological measurement in the diagnosis of acute myocardial infarction (MI) in sheep, acute MI was induced by ligation of left anterior descending coronary artery (LAD). For this purpose, twenty four healthy sheep were divided into the sham-operated or control group (group I, thoracotomy without MI) and the experimental group (group II, with MI) (n=12 in each), then all animals were subjected to the echocardiographic and clinicopathological analysis 2 days post-MI. Echocardiography revealed significant differences in left ventricular end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV ejection fraction (LVEF) and LV fractional shortening (LVFS) between groups (P<0.05). In biochemical analysis, the mean values of troponin (Trop), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) increased in the MI group compared with the control group (P<0.05). In conclusion, alterations in echocardiographic and early clinopathological mean values were observed in an ovine model of the experimental acute MI, which could aid researchers in interpreting their results when using this model for MI studies.

Key words: Clinopathological study, Acute myocardial infarction, Sheep

Introduction

Despite the drawbacks in the use of large animal models for cardiovascular research, they have recently become an issue of interest because of their necessity for substantial housing resources and care. To induce myocardial infarction (MI) in animal model, coronary artery ligation is now considered a widely used and attractive method for experimental research because of its clinical relevance (Millner *et al.*, 1993; Mihaylov *et al.*, 2000; Rademaker *et al.*, 2000; Kim *et al.*, 2001). However, there are only a few published studies describing the procedure in detail (Ikram *et al.*, 1997; Kim *et al.*, 2003; Rabbani *et al.*, 2008). A detailed guide with a practical, safe and reliable procedure for induction of MI in ovine models by ligating the main diagonal branch of the left anterior descending (LAD) coronary artery has been reported previously

(Ikram *et al.*, 1997; Charles *et al.*, 2000; Rabbani *et al.*, 2008). Changes that might occur in the clinicopathological values following the induction of MI have not been previously reported in an ovine model. Selected cardiac markers have been used in human being (Rosalki *et al.*, 2004), however, the investigation of an ovine model of MI has rarely been performed. The present study was designed to explore the relationship between the extent of myocardial injury following coronary ligation and the degree of alteration in cardiac markers. Many studies have been published for hematologic and biochemical analysis in relation to acute MI in humans (Zalokar *et al.*, 1981; Tahnk-Johnson and Sharkey, 1993; Kobayashi *et al.*, 2001); however, few studies have been accomplished on the observation of alterations in these indices about acute MI (Nikolaidis *et al.*, 2003; Aronson *et al.*,

2007). The aim of this study was to evaluate the prognostic significance of clinicopathological measurement in the diagnosis of acute MI in an ovine model.

Materials and Methods

This study was performed in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23, revised 1996). The study protocol was approved by the Animal Care Committee of Tehran Heart Center (north-central part of Iran with an altitude of 1200 meters above sea level).

A total of 24 clinically healthy 10-month-old male Zandi sheep weighing 28-32 kg (Tehran University Farm, Clinical Researches Ward, Iran) were divided into two groups (n=12 in each) including group I (without MI or sham-operated control group) and Group II (with MI). During the study, all animals were housed in an air-conditioned light-controlled room, had free access to water, and were fed with a mixed diet of hay and sheep pellets. Animals in group II were subjected to coronary artery ligation after lateral thoracotomy. Surgical procedures were performed under general anesthesia and electrocardiographic monitoring (Ikram *et al.*, 1997; Charles *et al.*, 2000; Kim *et al.*, 2005; Rabbani *et al.*, 2008). Echocardiography was performed using a Toshiba SSA-380A echocardiography system (Toshiba Corporation, Tochigi-ken, Japan) provided with a 3.5-MHz linear ultrasound transducer. The sheep were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg). The animals underwent left thoracotomy and pericardiotomy under general anesthesia and aseptic conditions. A 15-20 cm long left lateral thoracotomy incision was performed through the 4th intercostal space. The main (i.e., second) diagonal branch of LAD coronary artery was ligated using a curved round needle and 6-0 Prolene™ suture at a point approximately 40% distant from its base. Occlusion of the coronary artery was confirmed by the cyanotic appearance of the ischemic area (Fig. 1), and ventricular hypokinesia plus ST-segment changes on electrocardiography (ECG). When hemodynamic and rhythm

stability were assured, the thoracotomy was closed in layers using standard techniques (pericardium with 5-0 Prolene™, muscles and skin with 2-0 Vicryl™ sutures) and a chest tube was inserted (Rabbani *et al.*, 2008). The pericardium was not closed. The animals were allowed to recover and were returned to the animal house when able to ambulate. For antiarrhythmic prophylaxis, lidocaine was given as an intravenous bolus dose just before ligation of the diagonal branch (2 mg/kg). Post-operative analgesia was provided by 50 mg pethidine given intramuscularly. Cases were kept at animal ICU for 24 h after the surgery and then were discharged if there were no perioperative morbidities. All animals were studied on the second day after ligation. In group I, the thorax and pericardium were opened, but the LAD were not dissected or ligated. These animals, which comprise the control group, are referred to as “sham-operated” control group hereafter. Monitoring for cardiac function was assessed both clinically (heart rate measurement, typically expressed as beats per minute) and echocardiographically within 2 days after induction of MI. Blood samples were collected from jugular vein and clinicopathological parameters were assessed in both groups.



Fig. 1: Cyanotic appearance of area shown by arrows indicated induction of MI

The data related to biochemical parameters of total protein (Tp), fibrinogen (Fb), the ratio of Tp to Fb, troponin (Trop),

aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase MB isoenzyme (CK-MB) were obtained from each group. Fb was determined quantitatively by heat precipitation method on the day of sampling on centrifuged ethylenediaminetetraacetic acid (EDTA) plasma. Other laboratory data were obtained 2 days post-MI using commercially available kits (Pars-Azmoon, Iran). For determination of Tp, Trop, AST, LDH, and CK-MB serum were separated following centrifugation for 15 min at $750 \times g$. Serum samples were stored at -20°C until analysed. Tp concentrations were determined using a standard biuret method (Merckotest Manufacture for the determination of quantitative electrophoretic fractions). Trop, AST, LDH, and CK-MB concentrations were determined using an automated analyser (Eppendorf EPOS Analyser 5060, Germany). Left ventricular (LV), dimensions and function such as LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV ejection fraction (LVEF) and LV fractional shortening (LVFS) were measured by echocardiography. The experimental results were calculated as the mean \pm SD in both groups. Statistical analyses were done by SPSS software version 16, using two

independent samples t-test and p-value <0.05 was considered significant.

Results

There was no apparent surgical site infection or inflammation indicated by the absence of abnormal swelling or discharge. There were significant differences between the groups among biochemical parameters of Trop, AST and LDH ($P < 0.05$, Table 1). Echocardiography revealed significant differences ($P < 0.05$) of LVEDD, LVESD, EF and FS values between groups (Table 2, Figs. 2a-d).

Discussion

In this study, to evaluate the prognostic significance of clinicopathological measurement in the diagnosis of MI in an ovine model, the acute MI was conducted using ligation of the LAD. Also, this study was carried out to establish echocardiographic parameters of LVEDD, LVESD, LVEF and LVFS in two experimental groups of sheep (control: without MI and case: with MI, $n=12$ in each), and compare them with standardized values for normal human adults to find out whether this animal model

Table 1: Comparison of biochemical indices in case (group II) and control (group I) 2 days post-MI (Mean \pm SD)

Groups	Parameters						
	Tp (g/dl)	Fb (mg/dl)	Tp/Fb (g/dl)	Trop* (IU/l)	AST* (IU/l)	LDH* (IU/l)	CK-MB (IU/l)
I	6.80 \pm 0.97	545.83 \pm 69.21	12.62 \pm 2.41	0.0039 \pm 0.003	142.66 \pm 63.91	1274.41 \pm 99.73	1.11 \pm 0.137
II	7.10 \pm 0.82	575.50 \pm 77.28	12.46 \pm 1.92	1.57 \pm 0.471	339.83 \pm 86.37	2504.25 \pm 275.59	1.16 \pm 0.182
P-value	0.425	0.333	0.857	0.00	0.00	0.00	0.576

Tp: Total protein, Fb: Fibrinogen, Alb: Albumin, Tp/Fb: Total protein to fibrinogen ratio, AST: Aspartate aminotransferase, Trop: Troponin, LDH: Lactate dehydrogenase, and CK-MB: Creatin kinase MB isoenzyme. * Statistically significant

Table 2: Results of the echocardiographic analyses between groups measured by 2-dimensional echocardiography following MI (Mean \pm SD)

Groups	Parameters			
	EF*	FS*	LVEDD* (mm)	LVESD* (mm)
I	0.71 \pm 0.06	0.39 \pm 0.06	30.85 \pm 5.05	21.4 \pm 5.16
II	0.47 \pm 0.02	0.23 \pm 0.00043	43.85 \pm 10.76	30.9 \pm 5.52
P-value	0.00	0.001	0.007	0.012

EF: Ejection fraction, FS: Fractional shortening, LVEDD: Left ventricular end diastolic diameter, and LVESD: Left ventricular end systolic diameter. * Statistically significant

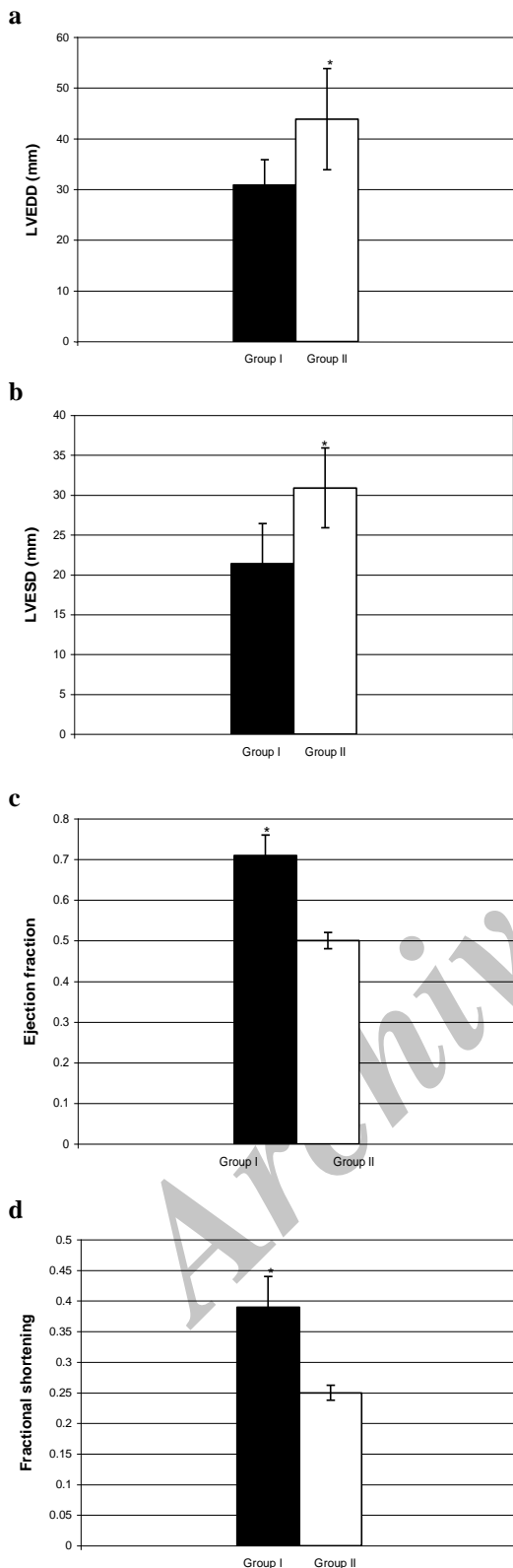


Fig. 2: The left ventricular end diastolic diameter (a), left ventricular end systolic diameter (b), ejection fraction (c), and fractional shortening (D) were assessed by 2-dimensional echocardiography. * Statistically significant: P-value: a=0.007, b=0.012, c= 0.00, d=0.001

supports human MI. Coronary artery occlusion and hence inducing MI have been reported in various animal models such as mice (Wang *et al.*, 2006), rats (Pfeffer *et al.*, 1979; Bani Ismail *et al.*, 2009), sheep (Ikram *et al.*, 1997; Charles *et al.*, 2000; Rabbani *et al.*, 2008), dogs (Schaper *et al.*, 1970; Shou *et al.*, 1997), goats (Mannion *et al.*, 1996), and pigs (Park and Lucchesi, 1999). Inducing MI in large animal models is a practical method for examining novel therapeutic protocols in cardiovascular research. However, these animals, such as sheep, lack good coronary collateral circulation which may lead to a remarkable incidence of fatal arrhythmias as a result of myocardial ischemia during such procedures (Nevalainen, 1994; Kim *et al.*, 2001). For this and other reasons (docility, slow growth rate, easy housing for protocols requiring long term follow up), ovine models of ischemic heart disease and heart failure are increasingly used (Dixon and Spinale, 2009). In previous studies, the LAD artery was ligated at a point approximately 40% of the distance from the apex to the base of the heart, with simultaneous ligation of the diagonal vessel at a point that was nearly in line with the point at which the LAD artery was ligated (Millner *et al.*, 1993; Rademaker *et al.*, 2000). Kim *et al.* (2001, 2003) carried out a modified method in canine and ovine models with sequential ligation of the LAD artery and its diagonal branch, i.e., they ligated the LAD artery first and then its diagonal branch 1 h later. In this study, ligation of the major diagonal branch of the LAD artery proved to be practical for inducing MI. Ischemic bluish discoloration and hypokinesia in the cardiac tissue in an area were easily speculated immediately after coronary artery ligation (Fig. 1). This method has been documented as a practical, reliable and yet safe ovine model of inducing MI by Ikram *et al.* (1997), Charles *et al.* (2000) and Rabbani *et al.* (2008) in paraclinical investigations, previously. In this study, serum biochemical values of twelve sheep without MI are considered as baseline data and placed at sham-operated control group. In recent years, there have been a few reports relating hemostatic factors to the incidence of heart disease (Zalokar *et al.*, 1981; Wilhelmsen *et al.*,

1984; Grimm *et al.*, 1985; Stone and Thorp, 1985; Kannel *et al.*, 1987; Tahnk-Johnson and Sharkey, 1993). Anemia is associated with adaptive hemodynamic changes that may have deleterious effects on myocardial remodeling (Levin *et al.*, 1999; O’Riordan and Foley, 2000; Anand *et al.*, 2004). In this research, the presence or the development of anemia during acute MI may impose hemodynamic load during a period of active LV remodeling and contribute to the development of heart disease. Biochemical parameters such as bilirubin, AST, LDH, CK, and CM-MB are usually abnormal in severe heart failure due to hepatic congestion (Granger, 1999; Park and Lucchesi, 1999). In one previous study in a rat model, there was no significant change in plasma total protein, albumin, glucose, urea, creatinine, total bilirubin, AST, ALP, GGT, and LDH following experimental induction of acute MI at any sampling time, however, statistically significant differences were noted in plasma concentrations of CK and CK-MB (Bani Ismail *et al.*, 2009). In another study reported by Lee *et al.* (2008) the mean values of total CK, CK-MB, and LDH were increased in all MI groups compared with the control groups. In human beings, like animals, Trop which is the most sensitive and specific test for myocardial damage, is a superior marker for myocardial injury (Ikram *et al.*, 1997; Rosalki *et al.*, 2004). In this study, significant differences were seen in Trop values between the two groups (group I: 0.0039 ± 0.003 , group II: 1.57 ± 0.471) ($P=0.00$). Serum biochemical alterations which occur following induction of MI have not been previously reported in the sheep model, however, in our study, there was a significant increase among biochemical parameters of Trop, AST and LDH when compared to the control group ($P<0.05$).

In terms of LV dimensions and function, few studies have reported normal reference values from animal population (Avizeh *et al.*, 2010; Locatelli *et al.*, 2011a, b; Janavel *et al.*, 2012). The echocardiographic values reported by authors are based on significantly smaller number of sheep. In one study, LVEDD, LVESD, LVEF and LVFS of sheep in case and control groups were much lower than those of our sheep

(Moainie *et al.*, 2002). This may be due to the fact that Moainie’s sheep were under general anesthesia (1 to 2% isoflurane in oxygen), while ours were under sodium pentobarbital anesthesia (30 mg/kg). In another study, the baseline LVEF calculated in different experimental groups comprising 5 to 10 animals per group ranged from $37.9 \pm 2\%$ to $43.7 \pm 1.7\%$ (Hamamoto *et al.*, 2009). The sedation used was not reported, but given the similarity with the preceding values, it is logical to assume that the sheep were undergoing general anesthesia. The echocardiographic values in case and control groups closer to those of the present study were reported by Janavel *et al.* (2012). In other studies reported by some researchers, the values were lower than our studies (Ikeda *et al.*, 2001; Ghanta *et al.*, 2007; Locatelli *et al.*, 2011a). Ikeda’s and Ghanta’s sheep were under isoflurane anesthesia and Locatelli’s sheep were sedated with diazepam, while ours were under sodium pentobarbital anesthesia. It should be noticed that displaying similar LV dimensions and function parameters does not mean that the ovine model would behave in a similar manner to humans in the setting of disease or physiological perturbations. It just provides a solid ground to propose echocardiographic LV performance variables as reliable end points in these kinds of studies.

In conclusion, echocardiographic parameters of LV performance in two experimental groups of young, adult sheep with and without MI, can be reliably extrapolated to adult humans, thus supporting the use of ovine models of human heart disease in translational research. Therefore, this difference should not challenge the validity of the sheep as a model of human LV dimensions and function. These data will aid scientists and researchers in the interpretation and better application of their results when using ovine model for acute MI studies.

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