

# Hematological Changes in an Ovine Model of Acute Myocardial Infarction

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## Abstract

To diagnose acute myocardial infarction, selected cardiac markers as well as hematological and biochemical indices have been studied in human being, however, rare investigations were done on the values of hematologic analysis in an ovine model of myocardial infarction. To observe the change of the values in hematologic parameters in sheep, acute myocardial infarction was induced by ligation of left anterior descending coronary artery. For this purpose, ten healthy sheep were randomly divided into two group (5 each), the control group (group I; thoracotomy without myocardial infarction) and the experimental group (group II; with myocardial infarction). Animal in each group subjected for the hematologic analysis 1 week post-myocardial infarction. In comparison of hematologic analysis between two groups, the

mean values of hemoglobin, white blood cell, red blood cell, platelets, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration showed a significant increase in myocardial infarction group ( $P < 0.05$ ). In conclusion, we could observe the alterations in early hematologic mean values in an ovine model of experimental acute myocardial infarction.

**Keywords:** Hematologic, Left anterior descending coronary artery, Myocardial infarction, Sheep model

## 1. Introduction

Today, acute myocardial infarction (MI) is the foremost cause of mortality in many countries around the world. When studying MI in large mammals, pigs or sheep are usually used. We chose sheep because, unlike pigs, whose cardiomyocytes have up to 32 nuclei, ovine cardiomyocytes have only 1–4 nuclei, thus being more similar to the human (Adler et al., 1996). In addition, pigs are more prone to develop irreversible ventricular fibrillation than sheep, this leading to higher mortality. A detailed guide with a practical, safe and reliable 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 (Kim et al., 2005; Rabbani et al., 2008). Many studies have been published for hematologic and biochemical analysis in relation to acute MI in human (Friedman et al., 1974; Jan et al., 1975; Zalokar et al., 1981; Tahnk-Johnson and Sharkey, 1993; Kobayashi et al., 2001), however, few studies for the observation of alterations in these indices from acute MI has been accomplished (Dodds et al., 1980; Nikolaidis et al., 2003; Aronson et al., 2007). The present study was designed to explore the relationship between the extent of myocardial injury following coronary ligation and the degree of hemodynamic changes in sheep within 1 week after LAD ligation.

## 2. Material and Methods

All procedures were approved by the Laboratory Animal Care and Use Committee of the University of Tehran, and performed in accordance with the Guide for Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 85-23, revised 1996). Ten healthy, non-obese, adult, male sheep weighing 30-35 kg were randomly divided into two groups ( $n=5$  each) including Group I (without MI or sham-operated control group) and Group II (with MI) with age- and sex-matched ones. During the study, the animals had free access to water and were fed with a mixed diet of hay and sheep pellets. All animals were housed for 1 week in the animal house so they would be adapted to the environment. They were examined by a veterinarian and a cardiologist, clinically, and some were excluded from the study if any serious morbidity was detected. Animals in group II were subjected to coronary artery ligation after lateral thoracotomy. Surgical procedures were performed under general anesthesia by intravenous injection of sodium pentobarbital (30 mg/kg) and electrocardiographic monitoring (Kim et al., 2005; Rabbani et al., 2008). Acute MI was induced by ligating the second diagonal branch of the LAD, as described previously by Rabbani et al. (2008). This method has been documented as a practical, reliable and safe ovine model of inducing MI in paraclinical investigations. After surgical preparation/drape, a 15- to 20-cm-long left lateral thoracotomy incision was carried out through the fourth intercostals

space. After the pericardium was opened, the coronary anatomy was inspected. The 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). The thoracotomy was closed (pericardium with 5-0 prolene, muscles and skin with 2-0 Vicryl sutures) and a chest tube was placed. For anti-arrhythmic prophylaxis, lidocaine was given as an intravenous bolus dose just before ligation of the diagonal branch (2 mg/kg) and 15–20 min there after (1 mg/kg). Cases stayed at animal ICU for 24h after surgery and then were discharged if there were no perioperative morbidities. All animals were studied one week after ligation. In group I, the thorax and pericardium were opened, but the LAD was not ligated; these animals comprise the control group referred to hereafter as sham-operated control group. Monitoring for cardiac function was assessed both clinically and echocardiographically 1-2 days after induction of MI (Fathi et al., 2013). Blood samples were collected from jugular vein 1 week after MI and hematological parameters were assessed from both groups. Laboratory data obtained were performed within 1 week of the MI using commercially available kits (Pars-Azmoon, IRAN).

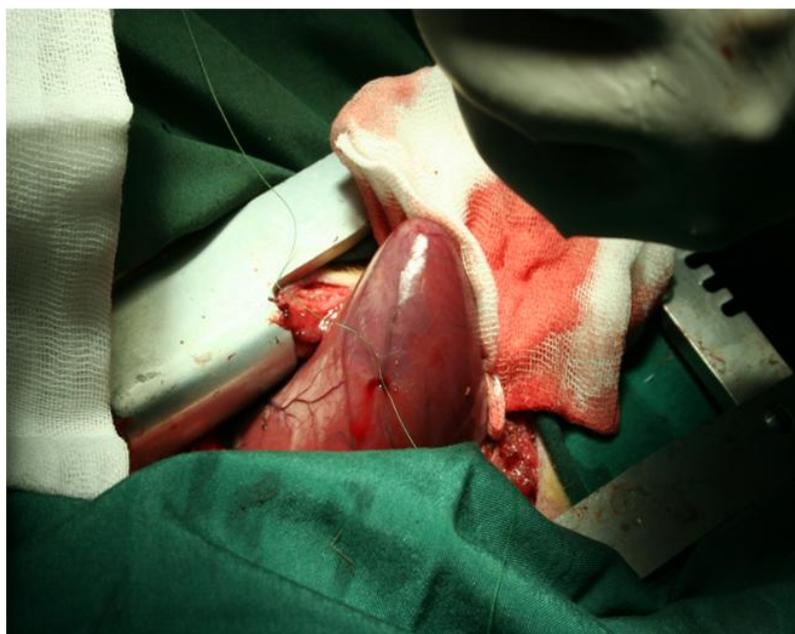


Figure 1. Ischemic bluish discoloration (Hypokinesia) of the infarct area indicated induction of MI.

The data related to hematological indices of packed cell volume (PCV), hemoglobin (Hgb), number of white blood cells (WBCs) and red blood cells (RBCs), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) as well as some biochemical parameters; albumin (Alb), total protein (Tp), fibrinogen (Fb), Tp/Fb ratio and bilirubin (Bili) were obtained for each group. Hematological parameters were measured in an automatic hematological analyzer (Vet Hema-screen 18, Hospitex Diagnostics, Italy). Fb was determined quantitatively by heat precipitation method at the day of sampling on centrifuged ethylenediaminetetraacetic acid

(EDTA) plasma. For determination of Alb and Tp serum was separated following centrifugation for 15 min at 750 g. Serum samples were stored at -20 °C until analysed. Alb concentrations were determined with bromocresol green (BCG) method. Tp concentrations were determined using a standard biuret method. Echocardiography was performed using a Toshiba SSA-380A echocardiography system (Toshiba Corporation, Tochigi-ken, Japan) provided with a 3.5-MHz linear ultrasound transducer.

### 2.1 Statistical Analysis

Statistical analysis was performed using SPSS software (Ver. 16, IBM Corporation, USA). Data were reported as mean  $\pm$  standard deviation at significance level of  $p < 0.05$ . Outliers were rejected during data processing using the T procedure (Bolton et al., 2009). Differences between groups were analyzed using two independent sample t-test and considered as statistically significant when the p value was less than 0.05.

## 3. Results

No inflammatory reaction was detected in the infarcted area indicated by normal plasma Fb or absence of abnormal swelling at the surgical area. Among hematological values except PCV and Hgb, a significant changes were observed between the two groups ( $P < 0.05$ ; Table 1). Among biochemical values there was only a significant differences in serum Bili between the two groups ( $P < 0.05$ ; Table 2). Echocardiography revealed significant differences between groups (Fathi et al., 2013).

Table 1. Comparison of hematological indices in case (group II) and control (group I) 1 weeks post-MI (Mean  $\pm$  SD).

Groups Parameters	PCV %	Hgb g/dl	WBC $\times 10^3 / \mu\text{l}$	RBC $\times 10^6 / \mu\text{l}$	PLT $\times 10^3 / \mu\text{l}$	MCV fL	MCH pg	MCHC %
I	40.82 $\pm$ 9.27	8.84 $\pm$ 1.16	7.06 $\pm$ 2.89	7.70 $\pm$ 1.14	474 $\pm$ 40	34.05 $\pm$ 8.83	9.99 $\pm$ 2.56	24.25 $\pm$ 6.64
II	41.19 $\pm$ 1.99	11.87 $\pm$ 2.72	17.01 $\pm$ 5.10*	12.10 $\pm$ 1.78*	859 $\pm$ 213*	54.13 $\pm$ 2.73*	11.50 $\pm$ 1.47	28.91 $\pm$ 1.43*
P-value	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	< 0.05

\* Statistically significant

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

Groups Parameters	Alb (g/dl)	Tp (g/dl)	Fb (mg/dl)	Tp/Fb ratio	Bili (mg/dl)
I	3.1 $\pm$ 0.06	6.80 $\pm$ 0.97	545.83 $\pm$ 9.21	12.62 $\pm$ .41	1.5 $\pm$ 0.21
II	3.5 $\pm$ 0.13	7.10 $\pm$ 0.82	575.50 $\pm$ 7.28	12.46 $\pm$ 1.92	2.5 $\pm$ 0.18*
P-value	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05

\* Statistically significant

#### 4. Discussion

In various animal models such as mice, rat, sheep, dog, goat and pig coronary artery occlusion and hence inducing MI have been reported (Schaper et al., 1970; Pfeffer et al., 1979; Mannion et al., 1996; Shou et al., 1997; Park and Lucchesi, 1999; Wang et al., 2006; Rabbani et al., 2008; Bani Ismail et al., 2009). However, a few studies describing the procedure of coronary artery ligation to induce MI in sheep model (Kim et al., 2003; Rabbani et al., 2008). 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).

Changes that might occur in the hematological values following induction of myocardial infarction have not been reported previously in an ovine model, however, hematological and biochemical indices have been published in human being (Tahnk-Johnson and Sharkey, 1993; Kobayashi et al., 2001; Nikolaidis et al., 2003; Aronson et al., 2007). The results of present study were similar to findings in human beings, as well as rat model with acute MI (Lee et al., 2008; Bani Ismail et al., 2009), that is, except PCV and Hgb, significant changes were seen in WBC, RBC, PLT, MCV and MCHC as a result of acute MI. Reports relating hemostatic factors to incidence of heart disease showed WBCs count was associated with risk of the disease (Friedman et al., 1974; Zalokar et al., 1981; Wilhelmsen et al., 1984; Grimm et al., 1985; Stone and Thorp, 1985; Meade et al., 1986; Kannel et al., 1987).

Anemia can develop during MI even in the absence of overt bleeding (Jan et al., 1975; Tahnk-Johnson and Sharkey, 1993). Therefore, many patients who survive the acute event are discharged with hemoglobin levels that are lower than admission values. The prognostic consequences of the changes in hemoglobin concentrations in patients with acute MI have not been studied (Dodds et al., 1980). 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). 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. High PCV value is associated with an increase in blood viscosity, which may lead to vascular occlusion (Burch and DePasquale, 1965; Lowe et al., 1980). It has also been reported that the PCV level and the severity of the coronary artery disorders are positively associated in IHD patients (Lowe et al., 1980). Among the subjects in one study, PCV exceeded 46.0% in 19 out of 22 cases (86.4%) compared to 63 out of 110 controls (57.3%) (Kobayashi et al., 2001). In a report from the Netherlands, PCV was shown to be an independent IHD risk factor; the mean PCV value among the cases was also greater than 46.0% (Knottnerus et al., 1988). In fact, increased PCV and Hgb are considered to be possible risk factors for IHD (Wannamethee et al., 1994; Erikssen et al., 1993; Goubali et al., 1995), but several studies have not supported this (Knottnerus et al., 1988; Sorlie et al., 1981; Carter et al., 1983; Yano et al., 1984; Abu-Zeid and Chapman, 1976). In this study, there was an increase in PCV value in MI group and a reduction in Hgb value in control group but no significant differences were seen in comparison with the same mutual group. The explanation appeared to be the opposing influences of a rise in plasma Fb and a fall in PCV (Dodds et al., 1980). A

reduction in PCV will lead to a reduction in blood viscosity whereas a rise in plasma Fb concentration will increase red cell aggregation, and thus increase blood viscosity measured at low shear rates (Lowe et al., 1980). After MI, whole blood viscosity, plasma viscosity, and plasma Fb have been shown to be raised (Jan et al., 1975; Fulton and Duckett, 1976; Dodds et al., 1980; Fathi et al., 2013). In our study, plasma Fb in MI group increased but it was not significant compared to the control group.

In one study, Alb and Tp were significantly lower than those in control group (Dodds et al., 1980). In this survey, although Alb and Tp were increased in group II, but significant differences were not observed in comparison with sham-operated control group. The same results were seen in our previous paraclinical investigations carried out in two experimental groups of sheep (control: without MI and case: with MI, n=12 each), 2 days following MI (Fathi et al., 2013). These results are not in agreement with what had been reported by Kuller et al. (1999) and Nusier et al. (2004) who found that there were significant inverse relation between serum albumin level and risk of coronary heart disease.

High serum total Bili level in patients with MI may suggest that there is a direct correlation between serum total Bili and MI. Our results are agreement with those reported by Olusi et al. (1999) and Nusier et al. (2004). Thus high Bili level might be considered as a risk factor for MI. Biochemical parameters such as Bili, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB are usually abnormal in severe heart failure due to hepatic congestion (Park et al., 1999; Lee et al., 2008; Granger, 1999). In one previous study in a rat model, there was no significant change in plasma Tp, Alb, glucose, urea, creatinine, total Bili, AST, Alkaline phosphatase (ALP), Gama-glutamyl transferase (GGT), and LDH following experimental induction of acute MI at any sampling time, however, a statistically significant differences was noted in plasma concentrations of CK and CK-MB. Serum biochemical and hematological alterations occur following induction of MI have not been reported previously in the sheep model, however, the present study showed that there were not significant changes among biochemical parameters of Alb, Tp and Fb when compared to control group ( $P>0.05$ ).

## **5. Conclusion**

In conclusion, experimental MI in the sheep model achieved by occlusion of LAD results in significant changes in some hematological and biochemical profile 1 week following acute MI.

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