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Serum Homocysteine Levels in Calves with Foot and Mouth Disease

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Abstract: The association between serum Homocysteine (Hcy) and myocarditis in calves with Foot and Mouth Disease (FMD) are not well defined. To investigate the relationship between serum Hcy concentrations and myocarditis in healthy calves and calves with FMD. A total of 9 healthy calves (control) and 15 calves with FMD. In the study, all animals underwent a comprehensive clinical and laboratuar findings study to document cardiac health or presence of FMD. Blood samples were collected via jugular venipuncture. Serum Hcy, Nitric Oxide (NO), cardiac Troponin I (cTnI) concentrations, serum levels of activity Creatine Kinase (CK), Lactac Dehydrogenase (LDH) and cardiac origin Creatine Kinase (CK-MB) were measured. Serum Hcy, NO, cTnI, CK, CK-MB and LDH values in calves with FMD significantly higher compared to control ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$ and $p<0.05$, respectively). The results suggest that determination of serum Hcy concentrations in calves with clinical signs compatible with FMD might prove useful as a guide to quantify cardiac remodeling associated with heart damage.

Key words: Homocysteine, calf, foot and mouth disease, myocarditis, serum

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral infection of wild and domestic cloven-hoofed (Brown *et al*., 1992). FMD is one of the most important economic losses in cattle industry (Tunca *et al*., 2008; Ryan *et al*., 2008). FMD is world wide in its and is also endemic in the Van Province of Turkey.

Foot and mouth disease in calves causes significant mortality. Young calves are mostly cattle, depending on the characteristic vesicular lesions in the development of severe acute myocardial injury suddenly die. Young animals myocarditis hyaline degeneration, necrosis of muscle fibers and the main dense infiltration of lymphocytes acutely (Aslani *et al*., 2013). High mortality due to FMD in young animals is associated to acute myocarditis (Aslani *et al*., 2013; Alexandersen *et al*., 2003; Tunca *et al*., 2008).

Homocysteine is an intermediate amino acid in the methionine metabolism which is not present in proteins. Hyperhomocysteinemia is an independent risk factor for cardiovascular diseases (Brattstrom and Wilcken, 2000; O'Grady *et al*., 2002). It has been considered as a risk factor for various arterial thrombosis and may have a role in generating oxidative damage (Pooya *et al*., 2010). It is generally accepted that the accumulation of homocysteine in plasma can damage the endothelium (Rezaei and Dalir-Naghadeh, 2009). The same researches reported that in the selenium-deficient lambs, the levels of plasma and heart tissue homocysteine were higher in the cardiac form and lower in the skeletal form of the disease (Rezaei and Dalir-Naghadeh, 2009).

Elevation of cardiac troponin I which regulates the calcium interaction between actin and myosin is a sensitive and highly specific marker for cardiac injury (Tunca *et al*., 2009). It has been reported that serum cardiac troponin concentration is an earlier marker of myocardial damage after virus infection was the histologic finding of inflammation (Gunes *et al*., 2008; Tunca *et al*., 2009). Following myocardial damage in humans, cardiac troponins leaks rapidly from myocytes and appears in blood after 2-4 h and persists up to 10-21 days (Aslani *et al*., 2013).

Nitric Oxide (NO) is an important gas molecule that plays a crucial role in physiology and pathology in various systems (Chen *et al*., 2007). Nitric oxide in the body endogenously is synthesized from L-arginine by the action of enzyme inducible nitric oxide synthase in injury (Tunca *et al*., 2009). NO has functions such as antimicrobial, antienflamatuar, protect cell and dieding cell (Gokce *et al*., 2004; Yarim *et al*., 2006).

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Blood serum biochemical analysis in animals is very useful to get detailed information about the health and metabolic status. During the diagnostic method, the values of sick animals is very important to compare the values of healthy animals (Jezek *et al*., 2006). Activities of CKCK-MB and LDH can help the muscle damage and prognosis of the disease heart muscle in animals (O'Brien*et al*., 1997).

The current study was designed to investigate the practical value of using biochemical, histopathological assessment and serum Hcy concentrations to detect myocardial degeneration in calves with FMD.

MATERIALS AND METHODS

In present study consisted of 15 calves with foot and mouth disease between 2-4 months ages and 9 healthy calves. Calves with foot and mouth disease were used as FMD group and healthy calves were used as control group. Three calves died due to foot and mouth disease were submitted to the Department of Pathology, Faculty of Veterinary Medicine, University of Yuzuncu Yil for pathological examination. Samples collected from cases were also submitted to the Foot and Mouth Disease Institute (Ankara, Turkey) for disease confirmation and serotyping.

Clinical chemistry analysis: For biochemical analyses, blood samples collected into test tubes without anticoagulant were centrifuged (Rotofix 32®-Hettich) at 3000 rpm for 10 min then the serum were collected and kept at -20°C until analysis. Serum homocysteine levels were measured by using the homocysteine commercial test kit (Axis® Homocysteine EIA-UK) as described in the procedure. Serum levels of CK, CK-MB and LDH concentration were determined using auto analyzer device (BS-800M®-Mindray). Serum cTnI concentration was determined using ELISA device (ELISA reader®-LiaiSon). Serum nitric oxide level were determined by Griess Reagent Method which is a Commercial Colorimetric Method test kit (nitrate/nitrite colorimetric assay kit, Cayman Chemical Company, Catalog No.: 780001) according to the kits procedure using ELISA device (ELISA reader®-DAS).

Histopathological analysis: Necropsy was performed on the three calves died by FMD. Tissue samples were taken from the calves with yellowish disseminated necrosis center on their myocards. The tissue samples were embedded to the parafine blocks after being fixed in 10% formaline for 24 h and were cut by microtome with the size of 5 μm. The incisions were stained by Hematoxylin-Eosin (H&E) protocol and examined under microscope.

Statistical analysis: All the datas were expressed as mean±SD. Student's t-test used to compare the datas obtained from animals in the FMD group and the control group.

RESULTS AND DISCUSSION

Samples collected from cases were tested positif for FMDV type Asia I. As characteristic clinical signs; blister, vesicles, erosion and ulcer were observed in the mouth, tongue and gums. Fever and lameness were also observed in the some calves in FMD group. Three young calves in FMD is usually characterized by high mortality without appearance of classic signs of the disease, vesicular and ulcerative lesions in the oral cavity. High mortality due to FMD in no healing young calves are associated to acute myocarditis.

The values of Hcy, NO, cTnI, CK, CK-MB and LDH are given in Table 1. In the present study, Hcy, NO, cTnI, CK, CK-MB and LDH levels were found to be significantly higher in FMD group compared to those of control group (Table 1).

As macroscopic findings, necrotic myocarditis with pale foci having hyperemia on the peripheral was found on the ventricular and papillar muscles in the heart. As microscopic findings; hyalin degeneration, necrosis and lymphohistiocytic infiltration were observed on the muscle fibers (Fig. 1).

FMDV has high affinity for cardiac tissue of young animals including calves and infection frequently causes death due to multifocal myocardial necrosis and cardiac failure (Aslani *et al*., 2013; Tunca *et al*., 2008; Bozukluhan *et al*., 2013; Brown *et al*., 1992). In this study, the probable diagnosis of myocarditis and FMD in the calves was confirmed by histologic examination and serological analysis, respectively although, the calves had no vesicular or erosive lesions typical of the FMD. Histopathological findings of this study supports researchers's findings (Aslani *et al*., 2013; Tunca *et al*., 2008; Bozukluhan *et al*., 2013; Brown *et al*., 1992).

Hyperhomocysteinemia is a significant and independent risk factor for cardiovascular disease (O'Grady *et al*., 2002). It has been reported that lowering plasma Homocysteine (Hcy) levels improved endothelial dysfunction and reduced the incidence of major adverse events after percutaneous coronary intervention (Wang *et al*., 2005). Although, there are a lot of researches relationship between homocysteine levels and cardia diseases in human medicine. Veterinary medicine is very limited studies have been reported in the field (Uren *et al*., 2009; McMichael *et al*., 2000; Kozat *et al*., 2011; Kilickap and Kozat, 2013). Studies in the field of

Table 1: Biochemical parameters in FMD group and control group

Parameters	FMD group Mean±SD	Control group Mean±SD	Statistical significance
Hcy (μmol L^{-1})	8.69±3.41	4.49±1.23	p<0.01
NO (μM)	3.96±1.96	0.84±0.45	p<0.001
cTnI (ng L^{-1})	0.12±0.01	0.02±0.01	p<0.01
CK (IU L^{-1})	1351.67±1180.34	328.99±112.83	p<0.001
CK-MB (IU L^{-1})	1105.87±1130.11	163.04±49.55	p<0.001
LDH (IU L^{-1})	1091.47±452.98	753.67±117.07	p<0.05

Different letters in the same line indicate significant differences between the groups

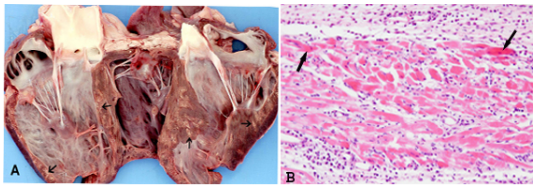

Fig. 1: A) Myocarditis caused by foot and mouth disease disseminated necrotic foci on myocardium (arrows); B) Microscopically, dark eosinophilic coagulation necrosis in the myocardium musclular fibers (arrows). Hematoxylin and eosin (Bar = 200 mm)

veterinary medicine, healthy cats with chronic renal failure in cats is higher than the levels of homocysteine (Uren *et al*., 2009). Another study, depending on the selenium deficiency in lambs with miyocarditis forms was higher than the serum levels of Hcy in healthy lamb (Kozat *et al*., 2011) and homocysteine levels in healthy cows culture have been studied (Kilickap and Kozat, 2013). Homocysteine levels in healthy cats was 7.6±4.1 mmol L^{-1}, cardiomyopathy in cats was 10.1±6.10 mmol L^{-1} and cats with tromboembolism was 8.0±4.1 mmol L^{-1} were determined (McMichael *et al*., 2000). According to the data obtained by the statistical, homocysteine values in cats with cardiomyopathy were higher than cats healthy (p<0.05) (McMichael *et al*., 2000). Significant increases in the levels of oxidant capacity, decreases in levels of antioxidant are the reported levels increased in studies regarding to FMD (Bozukluhan *et al*., 2013). It reported that increased plazma and heart tissue homocysteine concentrations could be considered as a risk factor in myocardium damage in conditions associated with oxidative stress (Rezaei and Dalir-Naghadeh, 2009).

The study showed that increased serum homocysteine concentrations could be considered as risk factor in myocardium damage in conditions associated with oxidative stress in FMD. In the present study, a significantly high level of homocysteine was calculated in serum samples obtained from FMD group. The results of the present study indicate that FMD induce production of total oxidant capacity.

Cardiac troponin I has proven to be a highly specific and sensitive marker for myocardial cellular damage in many mammalian species (Sleeper *et al*., 2001). Cardiac troponin I concentrations in cows with idiopathic pericarditis is reported to be higher than in healthy cows (Gunes *et al*., 2008). cTnI concentration in calves with foot and mouth disease is significantly increased than healthy calves (Tunca *et al*., 2008). In the performed study, increased level of cTnI is reported in cattle and calves. Increases of cTnI levels in the blood indicate useful bio-markers for myocardial damages (Peek *et al*., 2008). Besides cTn I is used as histological bio-marker in humans and dogs (Sleeper *et al*., 2001). Increased cTnI levels is reported in humans with acute viral pericarditis and in cattle with traumatic pericarditis (Braun, 2009). Serum cTn I concentration is around 0-0.04 ng mL^{-1} in clinically healthy ruminants is increased to 0.89 ng mL^{-1} in ruminants with idiopathic pericarditis and serum cTn I reference value is 0-2 ng mL^{-1} for other large animal species (Gunes *et al*., 2008). In the current study, the mean cTnI concentration was 0.12±0.01 ng L^{-1} in FMD calves; the mean cTnI concentration was 0.02±0.01 ng L^{-1} in healthy calves. The mean cTnI concentration in FMD group is higher than compared to healthy group's the mean cTnI concentration (p<0.01). Data in this study are similar to the data and supports researchers (Braun, 2009; Gunes *et al*., 2008; Tunca *et al*., 2008).

Nitric oxide is a cytotoxic factor released by a variety of cells (Bozukluhan *et al*., 2013), leukocytes especially macrophages (Yarim *et al*., 2006). It is generated from the terminal guanidine nitrogen atom of L-arginine by NO synthase (Bozukluhan *et al*., 2013). NO-producing leukocytes determined that in septic shock, peripherial blood in malaria disease, fluid bronco alveolar in tuberculosis disease. It supported that NO is produced by inducible leukocytes. It reported that serum NO levels in sheep with foot and mouth disease were significantly higher compared with healthy sheep (Yarim *et al*., 2006). Another study, serum NO concentration in cattle with foot and mouth disease was reported as 15±1.53 mmol L^{-1} and values of healthy cattle as 4.23±0.75 (Bozukluhan *et al*., 2013). In current study, the serum NO levels in group are similiar to researhers (Yarim *et al*., 2006; Bozukluhan *et al*., 2013).

They reported that considerable increases activities of CK, LDH and AST in calf and can be used as indicator of cardiac damage but they are not specific and can also increase following skeletal muscle damage (Aslani *et al*., 2013). Serum AST activities is also elevated during hepatic damage (Aslani *et al*., 2013; Gunes *et al*., 2005).

But increases in LDH together with AST are indicative of myocarditis (Gunes *et al*., 2005). In current present study, elevation of serum CK, CK-MB and LDH activities in parallel with high levels of serum Hcy, cTnI and NO can supported the myocardial injury caused by FMD. These biochemical findings were also supported by pathological findings of myocardial cell degeneration and necrosis. Similar results have been reported in calves with FMD (Tunca *et al*., 2008; Karapinar *et al*., 2010).

CONCLUSION

Elevated Hcy is strongly implicated in FMD but a cause and effect relationship remains to be proven. In this study, between high levels of serum Hcy and increased levels of cTnI to be paralleled, concluded thata useful parameter for determining the treatment and prognosis in heart damages.

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