Serum cardiac troponin I concentrations in ewes diagnosed with parturient paresis: correlation with blood ionized calcium and conventional cardiac enzymes

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SUMMARY

Calcium is an essential mineral for cardiac muscle excitation-contraction coupling and relaxation, and hypocalcemia results in a decrease in myocardial contractility force and an increase in cardiac cell permeability. Therefore, myocardial damage can occur in ewes with parturient paresis, a metabolic disease caused by decreased blood ionized calcium (iCa) concentrations. This study aimed to investigate the occurrence of myocardial damage in ewes diagnosed with parturient paresis by measuring serum cardiac troponin I (cTnI) concentrations as well as to determine whether a correlation exists among cTnI, iCa, and conventional enzymes, namely, myocardial band of creatine kinase (CK-MB), creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). Twelve ewes diagnosed with parturient paresis (iCa 1.0 mmol/L) and ten healthy control ewes (iCa ≥ 1 mmol/L) were used in this study. To analyze the blood iCa, beta-hydroxybutyrate (β-OHB), and serum cTnI, CK-MB, CK, AST, and LDH concentrations, we collected venous blood samples from the vena jugularis of the ewes. Serum cTnI concentrations were significantly increased in the patient group (1.11 ± 0.62 ng/mL) compared with those in the control group (0.02 ± 0.01 ng/mL). The serum concentrations of CK-MB, CK, AST, and LDH were also higher in the patient group than in the control group. Moreover, cTnI was positively correlated with AST (r = 0.639; P = 0.001), LDH (r = 0.553; P = 0.008), and CK (r = 0.598; P = 0.003). However, a significant negative correlation was detected between cTnI and iCa (r = −0.867; P = 0.001). The results showed that myocardial damage occurred in ewes with parturient paresis.

KEY WORDS

Cardiac troponin I, ewe, ionized hypocalcemia, parturient paresis.

INTRODUCTION

Parturient paresis is an acute-onset metabolic disturbance in pregnant and lactating ewes and is characterized by tetany, ataxia, incoordination, recumbency, and coma.[1] This condition is mainly caused by a decrease in blood ionized calcium (iCa) concentrations due to increased calcium (Ca) demands for fetal skeleton mineralization during late gestation.[2]

Ca is necessary in many physiological functions in the body. For instance, Ca plays a role in muscle contractions.[3] Apart from its function in skeletal muscle, Ca plays a critical role in cardiac muscle excitation-contraction coupling and relaxation. In cardiac muscle, action potential induces the opening of L-type channels, allowing the flow of extracellular Ca into the myocytes . This phenomenon results in the Ca-induced release of Ca from the sarcoplasmic reticulum and in the increased intracellular Ca concentration. Subsequently, Ca binds to the troponin-tropomyosin complex and activates the formation of actin-myosin cross-bridges.[4,5] Regardless of its cause, hypocalcemia reduces the myocardial contractility force.[6]

The occurrence of hypocalcemia-related myocardial dysfunction is well recognized in human patients, and many of the cases are associated with chronic hypocalcemia.[7] However, a closer look on the literature on hypocalcemia-related myocardial damage in veterinary science revealed the limited number of studies conducted on ruminants. In these studies, the presence of myocardial damage in cattle with hypocalcemia was evaluated by using cardiac markers or by examining macroscopic and microscopic changes in the myocardium.[8-10] To our knowledge, no previous research has investigated the presence of myocardial damage in relation to parturient paresis in ewes. Thus, this study aimed to investigate the presence of myocardial damage in ewes diagnosed with parturient paresis by measuring serum cardiac troponin I (cTnI) concentrations and to determine whether a correlation exists among cTnI, iCa, and conventional enzymes, namely, myocardial band of creatine kinase (CK-MB), creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH).

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MATERIALS AND METHODS

Animals

This study was approved by the Fırat University Local Ethics Committee on Animal Experimentation (14.05.2020, 2020/7). The ewes whose owners signed a consent form were enrolled in this study. Twelve pregnant and lactating ewes that were admitted to the Fırat University Veterinary Teaching Hospital and were diagnosed with parturient paresis comprised the patient group. Of the 12 ewes, three were in their first week of lactation and the rest were in their last month of pregnancy. The ewe breeds in this group were Akkaraman (n = 10) and Awassi (n = 2). The ewes in this group met the following criteria: a blood iCa concentration of less than 1.0 mmol/L[11], a blood beta-hydroxybutyrate (β-OHB) concentration of less than 0.8 mmol/L[12], and being in their late pregnancy or early lactation stage. The control group consisted of 10 ewes that were clinically healthy and had a blood iCa concentration above 1.0 mmol/L and a blood β-OHB concentration below 0.8 mmol/L. Of the 10 ewes, 5 ewes were in their last month of pregnancy and 5 were in their early lactation stage. The ewe breeds in the control group were Akkaraman (n = 5) and Awassi (n = 5). Ewes with anemia, systemic inflammation, and any pathological conditions associated with pregnancy based on the clinical and hematological findings were excluded from this study.

Hematological, biochemical, and venous blood gas-electrolyte analysis

Blood samples for the hematological, biochemical, blood β-OHB, and venous blood gas-electrolyte analysis were extracted from the vena jugularis of all the ewes and placed into tubes containing EDTA (BD Vacutainer, Plymouth, UK), into serum tubes with clot activator (BD Vacutainer), and into an injector containing electrolyte-balanced dry heparin (Pico 50, Radiometer, Copenhagen, Denmark), respectively. Packed cell volume (PCV) was measured using the spun method, and the total white blood cell (WBC) counts were determined by a hemocytometer. Venous pH and sodium, potassium, chloride (Cl), and iCa concentrations were analyzed using a bench-top blood gas analyzer (ABL80 Flex Basic, Radiometer). A point-of-care analyzer (Freestyle Optium NeoH, Abbott, Alameda, CA, USA) was used to measure the whole blood β-OHB concentration. Serum CK-MB, CK, AST, LDH, magnesium (Mg), and phosphorus (P) concentrations were determined with an automatic chemiluminescence immunoassay system (Advia Centaur XP, Siemens Healthcare Diagnostics, Malvern, PA, USA). A human-based cTnI analyzer (Advia Centaur TnI-Ultra, Siemens Healthcare Diagnostics) was used to measure serum cTnI concentrations.

Statistical analysis

Statistical differences between groups were assessed using SPSS21 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was performed to determine whether the variables were normally distributed. Data are presented as mean ± standard deviation. An independent sample t-test was used to determine the statistical difference between groups. Moreover, correlation was tested using Pearson's correlation coefficient test. A P-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Clinical and hematological findings

The results are shown in Table 1. The mean ages for the patient group and control group were 4.75 ± 0.89 and 3.50 ± 1.19, respectively. The ewes in the patient group showed a different clinical presentation, as follows: they displayed flaccid paralysis, lateral recumbency, and absence of pupillary light reflex (n = 2); they were able to stand when supported, and they displayed sternal recumbency and absence of pupillary light reflex (n = 6); they were able to stand when supported but showed severe muscle tremors (n = 2); and they were able to stand when supported but showed stiff gait (n = 2). Rectal temperature did not differ between the groups (P = 0.549). However, the heart rate increased significantly (P = 0.013) in the patient group compared with that in the control group. No statistical differences in PCV and WBC counts were observed between the groups.

Venous blood gas and electrolyte results

The results are shown in Table 2. No significant differences were observed between the groups in terms of venous blood pH and partial CO_2 pressure (pCO_2). In the patient group, blood Cl concentrations were significantly decreased (P = 0.001) compared with those in the control group. The mean blood iCa con-

Table 1 - Mean, standard deviation (S.D), median, minimum and maximum values of the rectal temperature, heart rate, PCV, and WBC counts in the patient group (n=12) and control group (n=10).

Variables	Unit	Groups	Descriptive Statistics					P
			Mean	S.D	Median	Minimum	Maximum	
Rectal Temperature	˚C	Control	38.81	0.33	38.85	38.20	39.30	0.549
		Patient	38.62	0.90	39.95	36.70	39.60	
Heart Rate	beats/min.	Control	98.00	7.65	97.00	88	110	0.013
		Patient	12.08	26.85	112.50	94	180	
PCV	%	Control	33.40	3.16	33	28	39	0.234
		Patient	31.58	3.67	30.50	28	39	
WBC	× 10^3/μL	Control	7.08	1.50	7.05	5.2	9.7	0.393
		Patient	7.64	1.49	7.50	5.9	10.5	

PCV: packed cell volume; WBC: white blood cell.

Table 2 - Mean, standard deviation (S.D), median, minimum and maximum values of the venous blood gas and electrolytes in the patient group (n=12) and control group (n=10).

Variables	Unit	Groups	Descriptive Statistics					P
			Mean	S.D	Median	Minimum	Maximum	
Venous pH	-	Control	7.43	0.33	7.43	7.39	7.49	0.188
		Patient	7.47	0.68	7.47	7.37	7.58	
pCO_2	mmHg	Control	37.56	2.72	37.95	32.00	41.30	0.336
		Patient	35.40	6.42	33.75	30.00	53.90	
Sodium	mmol/L	Control	148.60	3.83	147.50	145	156	0.360
		Patient	146.58	5.82	147.00	133	155	
Potassium	mmol/L	Control	4.23	0.41	4.29	3.25	4.86	0.049
		Patient	3.65	0.78	3.59	2.64	5.30	
Ionized Calcium	mmol/L	Control	1.22	0.07	1.24	1.10	1.32	0.001
		Patient	0.69	0.18	0.67	0.43	0.98	
Chloride	mmol/L	Control	108.60	0.96	108.50	107.00	110.00	0.003
		Patient	102.75	5.32	102.00	95.00	115.00	
Phosphorus	mg/dL	Control	4.32	1.24	4.20	2.41	7.00	0.009
		Patient	7.10	2.82	7.05	3.10	14.10	
Magnesium	mg/dL	Control	2.36	0.41	2.26	1.80	3.27	0.948
		Patient	2.35	0.36	2.34	1.71	2.95	

Table 3 - Mean, standard deviation (S.D), median, minimum and maximum values of the serum cTnI, CKMB, CK, AST, LDH, and blood β-OHB concentrations in the patient group (n=12) and control group (n=10).

Variables	Unit	Groups	Descriptive Statistics					P
			Mean	S.D	Median	Minimum	Maximum	
cTnI	ng/mL	Control	0.02	0.01	0.02	0.01	0.04	0.001
		Patient	1.11	0.62	1.07	0.14	2.27	
CKMB	U/L	Control	151.69	45.00	153.44	73.07	215.42	0.041
		Patient	239.85	120.21	202.03	104.31	509.85	
CK	U/L	Control	172.20	144.43	144.50	54	648	0.001
		Patient	400.17	103.81	398.50	223	648	
AST	U/L	Control	87.30	15.49	86.50	67	115	0.001
		Patient	169.17	42.66	170.00	97	253	
LDH	U/L	Control	451.40	52.52	449.50	371	571	0.001
		Patient	809.23	266.94	800	443	1292	
β-OHB	mmol/L	Control	0.23	0.13	0.20	0.10	0.50	0.157
		Patient	0.34	0.20	0.30	0.10	0.70	

centration in the patient group was 0.69 ± 0.18 mmol/L whereas that in the control group was 1.22 ± 0.07 mmol/L. Also, serum P concentration was significantly higher (P = 0.009) in the patient group than in the control group.

Biochemical results

The results are shown in Table 3. The mean serum cTnI concentration was significantly increased (P = 0.001) in the patient group relative to that in the control group. Also, the mean concentrations of the other cardiac markers were increased in the patient group. iCa concentrations were significantly negatively correlated with CK-MB (r = −0.458; P = 0.032), CK (r = −0.694; P = 0.001), AST (r = −0.750; P = 0.001), and LDH (r = −0.677; P = 0.001). The most evident negative correlation was detected between iCa and serum cTnI concentrations (r = −0.867; P = 0.001). By contrast, serum cTnI concentrations were positively correlated with serum AST (r = 0.639; P = 0.001), LDH (r = 0.553; P = 0.008), and CK (r = 0.598; P = 0.003) concentrations, but no correlation was observed between cTnI and CK-MB. In terms of mean serum β-OHB concentration, no significant difference was found between the groups.

DISCUSSION

In this study, we evaluated the presence of myocardial damage in ewes with parturient paresis by measuring the serum concentrations of cTnI and of the conventional enzymes CK-MB, CK, AST, and LDH. The results indicated the occurrence of myocardial damage in ewes with parturient paresis. Studies have

shown the presence of myocardial lesions and the increased cardiac markers in cattle with clinical hypocalcemia.[8-10] However, to our knowledge, no prior studies have investigated myocardial damage in ewes with parturient paresis.

The clinical manifestation of parturient paresis may vary and is strongly associated with both the circulating Ca concentration and the stage of the disease.[1,13,14] Additionally, ewes that have multiple fetuses are more prone to develop parturient paresis during late gestation.[14] Moreover, studies have reported various clinical manifestations of parturient paresis, including depression, incoordination, muscular weakness, muscular tremor, recumbency, flaccid paralysis, twisted or extended head, increased respiration, increased heart rate, ruminal tympany, and decreased reflexes.[15,16] In the present study, the ewes in the patient group showed clinical signs similar to those previously reported.

Ca, Mg, and P metabolisms are closely related. When blood iCa concentration decreases, the parathyroid glands are stimulated to secrete parathyroid hormone (PTH). PTH secretion results in increased reabsorption of Ca from kidneys, intestines, and bones. While PTH secretion increases the reabsorption of Ca, it also increases P excretion via the kidney and saliva and raises the renal threshold of Mg. Consequently, hypocalcemic animals tend to be hypophosphatemic and hypermagnesemic.[3,6] There have been numerous studies investigating the serum Mg and P concentrations in sheep with parturient paresis. Woldemeskel et al. reported no difference in serum concentrations of Mg and P between hypocalcemic and normocalcemic pregnant ewes.[16] In another study, the researchers reported a normal serum P concentration and a decreased serum magnesium concentration in hypocalcemic sheep.[15] Contrary to the previous reports, we found hyperphosphatemia and normal serum Mg concentration in our patient group. The possible explanations for the increased serum P concentration are that the PTH and calcitriol might have increased the serum P concentration as they stimulated P absorption from bones and intestines and that the high dietary P concentration might have contributed to the increased serum P concentration.[6]

Troponins are regulatory and structural proteins found in both cardiac and skeletal muscles. A troponin complex consists of three subunits, namely, troponin T, troponin C, and troponin I (TnI). TnI has three isoforms; the cardiac isoform of TnI (cTnI) differs from the two other TnI isoforms found in skeletal muscle based on the additional 32 amino acids at its N-terminal region. This feature of cTnI renders it as a gold standard in the determination of myocardial damage.[17,18] In veterinary medicine, circulating cTnI concentrations are evaluated in different diseases that lead to primary or secondary myocardial damage.[19-24] Increased cTnI concentrations were also reported in sheep with pregnancy toxemia, acute ruminal lactic acidosis, babesiosis, experimental salinomycin toxication, white muscle disease, and foot and mouth disease.[12,25-29] In our study, we found a significantly higher serum cTnI concentration in the patient group than in the control group, consistent with the previous findings. In addition to measuring serum cTnI concentration, we also measured other cardiac biomarkers, namely, CK, CK-MB, AST, and LDH. Although these markers are commonly used in research studies, their use in clinical practice is limited in both human and veterinary medicine because of their lack of sensitivity and specificity for cardiac tissue.[30] The results for CK-MB, CK, AST, and LDH concentrations indicated an obvious muscle damage in the patient group. However, it is improper to attribute the increase of these enzymes to cardiac muscle damage because these enzymes demonstrate a high activity in skeletal muscle.[31] Also, parturient paresis causes neuromuscular dysfunction, and it is highly possible that the increase in these enzymes is due to recumbency.[32]

Although the role of Ca during muscle excitation-contraction coupling and relaxation is well recognized, the pathophysiological mechanism of myocardial damage during hypocalcemia has not yet been fully understood. Some researchers have reported that myocardial damage is reversible during hypocalcemia and that circulating cTnI concentration decreased and cardiac contractility improved after an appropriate treatment.[33,34] Also, it has been shown that hypocalcemia increases cellular permeability by keeping the cellular membrane pore gates open.[35] One of the possible mechanisms of troponin release from cardiac myocytes into the circulation is the increased cellular permeability.[23] In the present study, the possible cause of increased serum cTnI concentration in ewes with parturient paresis is the increased permeability of the cell membrane of cardiac myocytes.

CONCLUSION

Although parturient paresis is not as common in ewes as in cattle, it may occur in ewes during late pregnancy and during early lactation. The presence of myocardial damage in the hypocalcemic ewes may affect the prognosis of the disease. Future research should consider the potential prognostic value of serum cTnI changes in hypocalcemic ewes.

References

1. Sykes A.R. (2007). Deficiency of mineral macro-elements. In: Diseases of Sheep, Ed. Aitken I.D.,4th ed., 363-377, Blackwell, Oxford, UK.
2. Oetzel G.R., Goff J.P. (2009). Milk fever (Parturient Paresis) in cows, ewes and doe goats. In: Current Veterinary Theraphy in Food Animal Practice, Ed. Anderson D., Rings M., 5th ed., 132-134, Saunders, St. Louis, MO, USA.
3. Schenck P.A., Chew D.J., Nagode L.A., Rosol T.J. (2012). Disorders of calcium: Hypercalcemia and hypocalcemia. In: Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice, Ed. DiBartola S.P., 4th ed., 120-194, Saunders, St. Louis, MO, USA.
4. Bers D.M. (2002). Cardiac excitation-contraction coupling. Nature, 415: 198-205.
5. Eisner D.A., Caldwell J.L., Kistamás K., Trafford A.W. (2017). Calcium and excitation-contraction coupling in the heart. Circ Res, 121 (2): 181-195.
6. Goff J.P. (2015). Minerals. In: Dukes' Physiology of Domestic Animals, Ed. Reece W.O., 568-586, Blackwell, Iowa.
7. Newman D.B., Fidahussein S.S., Kashiwagi D.T.,Kennel K.A. Kashani K.B, Wang Z., Altayar O., Murad M.H. (2014). Reversible cardiac dysfunction associated with hypocalcemia: a systematic review and meta-analysis of individual patient data. Heart Fail Rev, 19 (2): 199-205.
8. Basbuğan Y., Yüksek N., Altuğ N. (2015). Significance of homocysteine and cardiac markers in cattle with hypocalcemia. Turk J Vet Anim Sci, 39 (6): 699-704.
9. Yamagishi N., Naito Y. (1997). Calcium metabolism in hypocalcemic cows with myocardial lesion. J Vet Med Sci, 59 (1): 71-73.
10. Yamagishi N., Ogawa K., Naito Y. (1999). Pathological changes in the myocardium of hypocalcaemic parturient cows. Vet Rec, 144 (3): 67-72.
11. Scott P. (2013). Hypocalcaemia in ewes. Livestock, 18 (1): 37-40.
12. Souza L.M., Mendonça C.L., Assis R.N., Oliveira Filho E.F., Gonçalves D.N.A., Souto R.J.C., Soares P.C., Afonso J.A.B. (2019). Cardiac biomarkers troponin I and CK-MB in ewes affected by pregnancy toxemia. Small Rumin Res, 177: 97-102.

13. Cockcroft P.D., Whiteley P. (1999). Hypocalcaemia in 23 ataxic/recumbent ewes: clinical signs and likelihood ratios. Vet Rec, 144 (19): 529-532.
14. Elias E., Shainkin-Kestenbaum R. (1990). Hypocalcaemia and serum levels of inorganic phosphorus, magnesium parathyroid and calcitonin hormones in the last month of pregnancy in Awassi fat-tail ewes. Reprod Nutr Dev, 30 (6): 693-699.
15. El-Khodery S., El-Boshy M., Gaafar K., Elmashad A. (2008). Hypocalcaemia in Ossimi sheep associated with feeding on beet tops (Beta vulgaris). Turk J Vet Anim Sci, 32 (3): 199-205.
16. Woldemeskel M., Eneyew M., Kassa T. (2000). Study on ovine hypocalcemia in ewes in central Ethiopia. Rev Med Vet, 151 (4): 345-350.
17. Wells S., Sleeper M. (2008). Cardiac troponins. J Vet Emerg Crit Car (San Antonio), 18 (3): 235-245.
18. Sarko J., Pollack C.V. (2002). Cardiac troponins. J Emerg Med, 23 (1): 57-65.
19. Fartashvand M., Nadalian M.G., Sakha M., Safi S. (2013). Elevated serum cardiac troponin I in cattle with theileriosis. J Vet Intern Med, 27 (1): 194-199.
20. Karapınar T., Tümer K.Ç., Çalışkan M., Yılmaz Ö. (2019). Case report: serum cardiac troponin I concentration in three calves with doxycycline overdose. Rev Med Vet, 170 (1-3): 43-45.
21. Varga A., Angelos J.A., Graham T.W., Chigerwe M. (2013). Preliminary investigation of cardiac troponin I concentration in cows with common production diseases. Journal of Veterinary Internal Medicine 2013; 27 (6): 1613-1621.
22. Varga A, Schober K, Holloman C, Stromberg P, Lakritz J et al. Correlation of serum cardiac troponin I and myocardial damage in cattle with monensin toxicosis. J Vet Intern Med, 23 (5): 1108-1116.
23. Langhorn R., Willesen J.L. (2016). Cardiac troponins in dogs and cats. J Vet Intern Med, 30 (1): 36-50.
24. Karapinar T., Dabak D.O., Kuloglu T., Bulut H. (2010). High cardiac troponin I plasma concentration in a calf with myocarditis. Can Vet J, 51 (4): 397-399.
25. Hajimohammadi A., Rajaian H., Khaliji E., Nazifi S., Ansari-Lari M. (2014). Serum cardiac troponin I as a biomarker in cardiac degeneration following experimental salinomycin toxicosis in sheep. Vet Arh, 81 (4): 41-51.
26. Kırbaş A., Baydar E., Kandemir F.M., Dorman E., Kızıl O., Yıldırım B.A. (2014). Evaluation of serum cardiac troponin I concentration in sheep with acute ruminal lactic acidosis. Vet Arh, 84 (4): 355-364.
27. Karapınar T., Eröksüz Y., Beytut E., Sözdutmaz I., Eröksüz H., Dabak M. (2012). Increased plasma cardiac troponin I concentration in lambs with myocarditis. Ve Clin Pathol, 41 (3): 375-381.
28. Kılınç Orunç Ö., Göz Y., Yüksek N., Başbuğan Y., Yılmaz A.B., Ata A.D. (2015). Determination of serum cardiac biomarkers and plasma D-dimer levels in anemic sheep with babesiosis. Turk J Vet Anim Sci, 39: 606-610.
29. Tunca R., Erdoğan H.M., Sözmen M., Çitil M., Devrim A.K., Erginsoy S., Uzlu E. (2009). Evaluation of cardiac troponin I and inducible nitric oxide synthase expressions in lambs with white muscle disease. Turk J Vet Anim Sci, 33 (1): 53-59.
30. O'Brien P.J. (2008). Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. Toxicology, 245 (3): 206-218.
31. Braun J.P., Trumel C., Bézille P. (2010). Clinical biochemistry in sheep: A selected review. Small Rumin Res, 92 (1-3): 10-18.
32. Oetzel G.R. (1988). Parturient paresis and hypocalcemia in ruminant livestock. Vet Clin North Am Food Anim Pract, 4 (2): 351-364.
33. Aguiara P., Cruz D., Rodrigues R.F., Peixoto L., Araújo F., Soares J.L.D. (2013). Hypocalcemic cardiomyopathy. Rev Port Cardiol, 32 (4): 331-335.
34. Gupta P., Tomar M., Radhakrishnan S., Shrivastava S. (2011). Hypocalcemic cardiomyopathy presenting as cardiogenic shock. Ann Pediatr Cardiol, 4 (2): 152-155.
35. Tripp A. (1976). Hyper and Hypocalcemia. Am J Nurs, 76 (7): 1142-1145.