

Cardiac biomarkers and biochemical changes in cattle with traumatic pericarditis*

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Summary

Traumatic pericarditis (TP) emerges as a result of foreign bodies pricking the heart, and these animals cannot be treated. Traumatic pericarditis is one of the most important cardiac diseases that may result in a decrease in milk yield, animal death, and fetal deaths in affected pregnant animals and lead to economic losses. Cardiac markers, which have been used in human medicine for years and have recently started to be used in veterinary medicine, can detect mild myocardial damage that may occur in the early stages of heart-related diseases. In this study it was aimed to determine the cardiac damage in cattle with TP by cardiac biomarkers. In the study, cTn-I, ANP, NT-proBNP, homocysteine, CRP, CK-MB, LDH, AST, and ALT levels were determined to be statistically higher in cattle with TP than in healthy animals ($P < 0.01$). NT-proBNP increased approximately 11 times, ANP about 10 times, cTn-I and Homocysteine increased 3 times, and CRP and CK-MB increased 2 times in cattle with TP. In conclusion, some cardiac markers were examined in the early detection of cardiac damage and diagnosis of cattle with TP, and it was concluded that the use of these markers would be beneficial. Moreover, it was concluded that ANP, Nt-proBNP, and homocysteine, which were the first data on cattle with TP, could be used in the detection of the severity of cardiac damage.

Keywords: atrial natriuretic peptide, homocysteine, N-terminal pro-brain natriuretic peptide, traumatic pericarditis, troponin

Metal objects such as wires and nails, which are thrown into the environment, particularly in the regions of range animal husbandry, come into the reticulum after being swallowed by cattle and usually puncture the reticulum wall and prick the peritonitis or organs (liver, kidney, spleen, lung, and heart), causing inflammation to various degrees. Traumatic pericarditis (TP) emerges as a result of foreign bodies pricking the heart, and these animals cannot be treated (4, 7). TP is one of the most important cardiac diseases that leads to great economic losses due to a sudden/sharp decrease in milk yield and animal losses (1, 36). Pica creates a predisposition for TP, which is usually seen in high-yielding dairy cattle (7).

Although the first clinical sign of traumatic pericarditis is tachycardia, it is not specific as it can be found in many physiological/pathological conditions, and

cardiac dysfunction is also seen in advanced stages (1). Even though bilateral swelling of the jugular veins and neck edema are characteristics of the disease, they are not sufficient for the diagnosis (20, 24). Early diagnosis in TP cases is quite difficult due to nonspecific clinical findings and the late onset of symptoms (1). Therefore, additional diagnostic methods in addition to anamnesis, clinical examination, and ultrasound are required for the diagnosis of the disease.

Various biomarkers [lactate dehydrogenase (LDH), aspartate aminotransferase (AST), creatine kinase-myocardial band (CK-MB), cardiac troponins (T and I), natriuretic peptides, etc.] are used to detect cardiac dysfunctions (2, 3, 6, 22). It was reported that the serum activities of some enzymes, such as cardiac troponins (T and I), CK-MB, LDH, and AST, were elevated during myocardial damage (2, 19).

The plasma atrial natriuretic peptide (ANP) concentration reflects the clinical stages of myxomatous

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mitral valve disease and is used as a cardiac marker in patients with myxomatous mitral valve disease (28). N-terminal pro-atrial natriuretic peptide (NT-proANP), N-terminal pro-brain natriuretic peptide (NT-proBNP), and ANP concentrations are elevated with a left atrial enlargement (14). NT-proBNP is released into the blood circulation, mainly in response to the stretch of ventricular myocytes (18). In particular, the plasma NT-proBNP concentration is high in patients with cardiac disease regardless of myocyte damage (28, 29).

Homocysteine is a sulfur-containing amino acid that is formed during methionine metabolism. Hyperhomocysteine is an independent risk factor for chronic cardiac diseases (17, 34). C-reactive protein (CRP) is an acute-phase protein with a half-life of 19 hours that is usually used as a general inflammation and infection biomarker (25). Acute phase proteins are plasma proteins whose production and serum concentrations increase in response to inflammation, infection, cardiovascular disease, cancer, and tissue damage (8).

This study aimed to determine the cardiac damage with levels of cardiac biomarkers (cTn-I, ANP, NT-proBNP, homocysteine, CRP) and some biochemical (CK-MB, LDH, AST, ALT) parameters of cattle with TP that lead to great economic losses. Furthermore, this is the first study to determine the levels of ANP, NT-proBNP, and homocysteine in animals with TP.

Material and methods

Ethical approval. This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2021-019).

Animal material

Sick group. The study consisted of forty female cattle at the ages of 1-7 years who were clinically diagnosed with TP [tachycardia, bilateral swelling of the jugular veins, neck edema, pericardial rub/friction sound, pulse ≥ 100 , body temperature at 39.5-40°C, Rückengriff test, Kalchschmidt's zone test, Götze's stick test, and Diernhofer's punch test were used to detect the presence of pain, radiographic and metal detector examination, and positive glutaraldehyde (GLA) test] and were brought to Veterinary Hospital of the Kafkas University Faculty of Veterinary Medicine ranging from 2-10 days after clinical symptoms were shown. Pasture farming is carried out in and around Kars province, and the animals used in the study are from similar small family businesses under the same maintenance and feeding conditions. In the pregnancy examination performed on the cattle used in the study, 28 of them were 6 months and older pregnant. It was determined that 8 of them were in the early pregnancy period (3-6 months). Pregnancy was not found in 4 animals at the age of 1 year.

Healthy group. Twenty female cattle at the age of 1-7 years which were found to be healthy according to the clinical and haematological examination were used. The control group in the study was selected from animals that were 14 pregnant at 6 months and above, 4 were pregnant between

3-6 months, and 2 were not pregnant so that there was no hormonal difference.

Blood collection. Blood samples were taken from cattle, which were determined to have traumatic pericarditis and to be healthy, into gel serum tubes (BD Vacutainer, BD, Franklin Lakes, NJ). After the samples were centrifuged at $3000 \times g$ for 15 minutes, their sera were separated and retained at -20°C until the levels of cTn-I, ANP, NT-proBNP, homocysteine, CRP, CK-MB, LDH, AST, and ALT were measured. Although troponin concentrations could remain constant at -70/-80°C for a long time, it was reported that they remained constant at -20°C for a maximum of 3 months (23). Therefore, the serum samples used in this study were retained for a maximum of 90 days.

Measurement of cardiac markers. Serum (cTn-I, ANP, NT-proBNP, homocysteine, CRP, and CK-MB) levels were determined utilizing an ELISA device (ELISA Reader®-DAS Italy and ELISA Reader-Elecsys® 2010-Roche) and calculated utilizing a commercial test kit (ELK Biotechnology, P.R.C.) as prescribed by the manufacturer (ng/mL, pg/mL, pg/mL, $\mu\text{mol/L}$, $\mu\text{g/mL}$, U/L, respectively).

Measurement of Cardiac Enzymes. LDH was determined with commercial spectrophotometric kits (TML, Türkiye). Values of serum AST and ALT were measured with a Cobas C501 autoanalyzer (Roche-Cobes, Switzerland).

Statistical analysis. Statistical comparison of data was performed using the SPSS® software program (SPSS 22.0, Chicago, IL, USA). Cattle were grouped as healthy and sick. Then, healthy patients and patients who died were compared using an independent sample T-test. As the distribution of data was normal, data are expressed as the mean $\pm$ standard deviation (SD). The statistical significance was set at P value < 0.05 .

Results and discussion

The study was conducted in Kars province and its districts. In the relevant region, feeding is carried out in the pasture for about 7 months of the year. For this reason, wires, nails, etc. are thrown into the environment. It causes the formation of PT. In addition, magnets are only used in sick animals in the relevant region, and magnets are not used within the scope of preventive medicine.

Radiographic examination and metal detector examination were performed to detect the presence of a metal foreign body in all animals used in the study. In the metal detector examination, positivity was detected in 36 animals. In addition, radiographic examination revealed that the foreign body had passed through the diaphragm and penetrated the heart in 30 animals, and the foreign body was at the diaphragm border in 7 animals.

In the study, the levels of cardiac biomarkers and some biochemical parameters of a total of 60 female cattle, 20 of which were healthy and 40 of which were diagnosed with TP, are given in Table 1. Table 1 shows that the levels of cardiac biomarkers (cTn-I, ANP, NT-proBNP, homocysteine, CRP) and some biochemical (CK-MB, LDH, AST, ALT) parameters

Tab. 1. Cardiac biomarker levels by the group with TP and healthy groups

Parameters	Group	N	Mean $\pm$ SD	T/P
cTn-I (ng/mL)	Healthy	20	0.07 $\pm$ 0.02	9.735 P < 0.01
	TP	40	0.25 $\pm$ 0.08	
ANP (pg/mL)	Healthy	20	27.52 $\pm$ 6.29	17.813 P < 0.01
	TP	40	265.10 $\pm$ 59.23	
NTproBNP (pg/mL)	Healthy	20	66.16 $\pm$ 9.07	25.103 P < 0.01
	TP	40	713.97 $\pm$ 114.74	
Homocysteine (μ mol/L)	Healthy	20	15.45 $\pm$ 1.42	13.835 P < 0.01
	TP	40	49.48 $\pm$ 10.91	
CRP (μ g/mL)	Healthy	20	45.16 $\pm$ 4.94	17.565 P < 0.01
	TP	40	76.32 $\pm$ 7.11	
CK-MB (U/L)	Healthy	20	346.47 $\pm$ 17.68	23.364 P < 0.01
	TP	40	768.86 $\pm$ 79.55	
LDH (U/L)	Healthy	20	266.59 $\pm$ 46.89	16.944 P < 0.01
	TP	40	616.28 $\pm$ 85.87	
AST (U/L)	Healthy	20	310.94 $\pm$ 45.18	17.185 P < 0.01
	TP	40	711.95 $\pm$ 99.01	
ALT (U/L)	Healthy	20	7.54 $\pm$ 2.26	11.928 P < 0.01
	TP	40	15.93 $\pm$ 2.71	

Explanations: cTn-I – cardiac troponin I; ANP – atrial natriuretic peptide; NT-proBNP – N-terminal pro-brain natriuretic peptide; CRP – C-reactive protein; CK-MB – creatin kinase myocardial band; LDH – lactate dehydrogenase; AST – aspartate aminotransferase; ALT – alanine aminotransferase; N – total number of samples; SD – standard deviation; P – significance level

were very high in cattle with TP compared to healthy cattle, and the difference was statistically significant (P < 0.01).

The values of cardiac biomarkers and some biochemical parameters in healthy female cattle and cattle with TP by the breeds of animals in the study are given in Table 2. Accordingly, it is seen that cardiac biomarkers and some biochemical parameter values in healthy cattle and cattle with TP do not show a significant change by breed, and the difference is statistically insignificant (P > 0.05).

The values of cardiac biomarkers and some biochemical parameters by the ages of cattle are given in Table 3. In Table 3, it is seen that cardiac biomarkers and some biochemical parameter values of 3-year-old cattle and younger, as well as of 4-year-old cattle and older, do not show any significant change and the difference is statistically insignificant (P > 0.05).

Traumatic pericarditis is one of the most important cardiac diseases that may result in a decrease in milk yield, animal death, and fetal deaths in affected pregnant animals and lead to economic losses (1, 33, 36). The incidence of the disease is higher in female animals. This can be attributed to the fact that the foreign body is pushed forward during pregnancy, causing pericardial penetration and labor effort (1). In our study, 36 of 40 cattle with a diagnosis of TP were

Tab. 2. Cardiac biomarker and some biochemical parameter values by breed (Mean $\pm$ SD)

Group	Breed	N	Parameters								P	
			cTn-I (ng/mL)	ANP (pg/mL)	NT-proBNP (pg/mL)	Homocysteine (μmol/L)	CRP (μg/mL)	CK-MB (U/L)	LDH (U/L)	AST (U/L)		ALT (U/L)
Healthy	Simmental	14	0.06 ± 0.02	29.22 ± 6.30	67.18 ± 9.88	15.48 ± 0.79	45.28 ± 5.52	346.47 ± 18.58	251.26 ± 47.30	299.06 ± 48.97	7.65 ± 2.47	P > 0.05
	Montofon	6	0.07 ± 0.03	23.53 ± 4.46	63.77 ± 7.02	15.37 ± 2.45	44.87 ± 6.29	346.50 ± 17.07	302.37 ± 18.58	338.67 ± 14.27	7.27 ± 1.85	
PT	Simmental	30	0.24 ± 0.08	263.75 ± 57.56	708.99 ± 120.71	48.11 ± 11.02	77.83 ± 7.20	780.07 ± 82.95	623.92 ± 93.44	728.78 ± 88.79	15.37 ± 1.96	P > 0.05
	Montofon	10	0.25 ± 0.08	269.18 ± 67.08	728.91 ± 98.85	53.61 ± 9.97	71.81 ± 4.63	735.24 ± 59.79	593.37 ± 55.18	661.48 ± 111.33	17.64 ± 3.88	

pregnant, in line with this literature. However, the disease can also be seen at young ages in areas where pasture breeding is carried out because there are a lot of metal foreign bodies. Due to cattle not having good olfactory and being gluttonous TP is seen in all ages (37). In the study, four 1-year-old cattle with PT were not pregnant.

Cardiac markers (cTn-I, ANP, NT-proBNP, homocysteine, CRP, and CK-MB) are significant biomarkers that help to diagnose diseases by elevating serum concentrations even before the onset of symptoms of cardiac diseases (1, 12, 21, 26-28). Moreover, cardiac biomarkers are accepted as beneficial for the early diagnosis of TP (24). It is not possible all the time to see the foreign body with ultrasound or radiography in cattle with PT. However, cardiac markers, which have been used in human medicine for years and have recently started to be used in veterinary medicine, can detect mild myocardial damage that may occur in the early stages of heart-related diseases. In this study it was aimed to determine the cardiac damage in cattle with TP by serum cardiac biomarkers. Thus, it was thought that economic losses arising from drug/veterinary costs due to mismatched treatment practices could be prevented.

Troponins are structural proteins that are a part of the contractile apparatus of skeletal and cardiac muscles and are responsible for regulating the interaction of actin and myosin (23). When heart damage emerges, cTn-I reaches its highest level in blood within 6-12 hours and remains high for 14-21 days (11, 13, 35). In a study it was reported that the cTn-I value significantly increased in cattle with TP (16, 36). Similar results were obtained in our study.

Plasma ANP concentration reflects the clinical stages of mitral valve disease (32). ANP has a short (1-4 minute) half-life and is not stable (28). NT-proBNP is released into the blood circulation, principally in response to the stretch of ventricular myocytes, and has a long half-life in the blood (18). In the literature review, no previous data on ANP and NT-proBNP in cattle with TP could be found. Therefore, this study provides the first data in this field. In our study, it was determined that the levels of ANP and NT-proBNP significantly increased in cattle with TP compared to healthy cattle.

Elevated homocysteine, which is an amino acid formed during methionine metabolism, is an independent risk factor for chronic heart disease (17). In the literature review, no previous data on homocysteine in cattle with TP could be found. Therefore, this study has been the first data in this field. In our study, it was determined that the level of homocysteine increased significantly in cattle with TP compared to healthy cattle.

C-reactive protein (CRP) is an acute-phase protein that is usually used as a general inflammation and infection biomarker (25). In a study conducted on buffaloes

with TP, it was reported that the CRP level increased significantly compared to healthy buffaloes (12). In our study, the results parallel to the study of El-Ashker et al. were obtained, and significant elevations were determined at the CRP level.

It was reported that the serum activities of some enzymes, such as lactate dehydrogenase and AST, increased with heart damage (2, 19). In different studies, it was reported that LDH, AST, and ALT levels were significantly elevated in animals with TP (9, 15, 36). In our study, elevated LDH, AST, and ALT levels were detected similarly to the findings of the foregoing researchers. It was thought that increased LDH, AST, and ALT levels in animals with PT were due to cardiac damage. In healthy animals in the control group, the AST value was determined above the reference limit. We have no data about the existence of difficult birth or perinatal period that may cause this situation.

When homocysteine, CK-MB, LDH, AST, and ALT levels in healthy cattle and cattle with different diseases were evaluated in terms of breed differences, it was reported that they were not statistically significant (5, 10, 13). Similar to the investigations conducted in our study, no difference was determined in terms of breed characteristics.

In human medicine, slightly elevated cTn-I levels were reported in elderly patients due to possible cardiomyocyte loss (31). In a study conducted on dogs, it was reported that there was a significant correlation between age and cTn-I level (30). However, although ANP, CRP, CK-MB, LDH, and ALT levels were found to be numerically higher in 4-year-old cattle and older and cTn-I, NT-proBNP, and AST levels were found to be higher in 3-year-old cattle and younger in our study, no statistically significant difference was found between the groups in the analysis. It can be said that NTproBNP is a better marker because the half-life of ANP is between 1-4 minutes and it is not stable.

The limitation of this study: the studies conducted so far show that the initial clinical symptoms of TP are many times difficult to recognize, and often cases minor abnormalities are observed for weeks or months, therefore it is not always possible to determine how long the disease process lasted. The sensitivity and specificity of the tests performed have not been definitively established.

In conclusion, some cardiac markers were examined in the early detection of cardiac damage and diagnosis of cattle with TP, and it was concluded that the use of these markers would be beneficial. Moreover, it was concluded that ANP, NT-proBNP, and homocysteine, which were the first data on cattle with TP, could be used in the detection of the severity of cardiac damage. In the study it was determined that NTproBNP increased approximately 11 times, ANP about 10 times, cTn-I and Homocysteine increased 3 times, and CRP and CK-MB increased 2 times in cattle with TP. NTproBNP seems to be a better marker than ANP.

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