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Research Article

Cardiac Troponin I Activity Compared with Other Cardiac Markers in the Dry Period, Early, and Peak Lactation in Dairy Cattle

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Abstract

The current study aimed to evaluate the effects of parturition and different stages of milk production on Cardiac Troponin I activity (CTnI) and other cardiac markers. A total of 42 healthy dairy cattle with an average body weight of 520±17 kg and an average parity of 2.4±0.7 were randomly selected from a commercial dairy farm. Through a clinical examination and ECG recording conducted by a veterinarian, blood samples were collected from all cattle's coccygeal vein one month before parturition (dry period), one week after parturition (early lactation), and 10 weeks after parturition (peak of the lactation). Dairy cows in the experimental group were observed closely before and during parturition and in the postpartum period. Biochemical parameters, including Cardiac Troponin I activity (CTnI), Creatine kinase (CK), Aspartate aminotransferase (AST), and Lactate dehydrogenase (LDH) were analyzed at three-time intervals. Regarding time intervals, there were no significant differences among the cattle in terms of CTnI, CK, LDH, AST, parturition, and different stages of milk production. It was found that the effect of late-stage gestation and different lactation periods on cardiac biomarkers in dairy cows was not significant.

Keywords: Creatine kinase, Dairy farm, Lactate dehydrogenase, Parturition

Introduction

Clinicians face significant challenges when dealing with cardiac diseases in cattle, specifically in the advanced stage. When the primary heart disease advances to the point in which compensatory mechanisms, including neural and hormonal responses, are no longer effective, heart failure occurs [1]. The early diagnosis of heart diseases in dairy cattle is crucial and can be life-saving. One effective method for diagnosing these conditions involves the evaluation of cardiac markers [2,3]. Early and prompt diagnosis of heart disease in cows can contribute to treating animals successfully. In addition, identifying and removing less productive cows from the herd can prevent further economic losses [4-6]. In the case of heart disease, many blood findings are nonspecific, which can challenge accurate diagnosis [7,8]. Troponin, creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are regarded as biomarkers for assessing cardiac injuries among various mammals [9]. Creatine kinase and lactate dehydrogenase are specific isoenzymes associated with cardiac function that exhibit elevated levels in a range of cardiac conditions. Nevertheless, in large animals, the sensitivity and specificity of these biomarkers are inferior to that of troponin [7]. Troponin is a globular protein that exists within the thin filaments of all striated muscles and has a crucial function in the contraction and relaxation of these muscles. It is a component of the myofibrillar proteins found in the cardiac contractile system, which regulates the interactions between actin and myosin in controlling the contractions of muscle cells [3,10]. These proteins exist in three forms, namely Cardiac Troponin C (TNNCI), Cardiac Troponin T (cTnT), and Cardiac Troponin I (cTnI). The cTnI and cTnT are specific isoforms uniquely

produced by cardiac myocytes, and they have proven to be valuable biomarkers for assessing myocardial damage. Notably, the amino acid sequence of cTnI exhibits over 96% homology between bovine and human species [2]. Studies conducted on laboratory animals and humans have confirmed that cardiac troponins, particularly cTnI, are typically found at very low concentrations or even below the detection limit in most measurements. However, when myocytes are damaged, these proteins are released into the bloodstream [10,11]. The presence of local or systemic hypoperfusion and myocardial lesions due to high metabolism and production in cows could potentially lead to an increase in blood concentrations of markers of hypoperfusion, such as L-lactate (LAC) and cardiac biomarkers, such as cTnI [2].

Consequently, the measurement of cardiac troponins is currently recognized and widely used as an available biochemical marker for diagnosing myocardial injuries in humans [12]. Some studies have demonstrated that the level of cardiac troponins in the blood is elevated in both primary and secondary cardiac disorders in dogs and cats [13]. The increased serum concentration is a valuable indicator of cardiac abnormalities in these animals [10,11].

Furthermore, a rise in troponin serum concentration is proposed as a prognostic indicator of disease. In other words, the continued elevation of troponin levels in the blood may provide valuable information about the progression and severe condition [11]. Measuring cardiac troponins has proven to be highly sensitive and specific in detecting cardiac damage, and it is considered the gold standard for evaluating myocardial injury in animals [14]. While the troponin protein complex has been identified in animals, limited research has been conducted on its clinical utility. Most animal studies have focused on laboratory animals that mimic human heart infarction [15]. Consequently, limited information on the diagnostic application of cTnI in cattle is available.

Early detection of myocardial damage could be determined by evaluating myocardial markers in primary and secondary myocardial diseases. Utilizing cardiac troponin tests (cTnI and cTnT) has facilitated the early diagnosis of secondary myocardial degeneration from foot-and-mouth disease [16]. In a study by Mellanby et al. on cows with pericarditis, an increased level of cTnI was observed in 4 out of 5 cases of cows with traumatic pericarditis [17]. However, upon necropsy, none of these cows showed evidence of myocardial penetration by foreign bodies, indicating a predominance of chronic disease. Peek et al.[18] found that endotoxemia caused an elevation in cTnI concentration in calves, while the level of cTnT remained unchanged. Measurement of cTnI and cTnT serum levels could be used as a diagnostic test for assessing cardiac cell damage in traumatic pericarditis [19].

Cardiac troponin kits have been utilized to quantitatively assess myocardial cell damage resulting from traumatic reticuloperitonitis in cattle [19]. High-producing cows are particularly susceptible to various metabolic and infectious diseases during the peripartum period, especially at the production peak. These cows experience a negative energy balance, metabolic disturbances, and electrolyte deficiencies [3]. Furthermore, the proximity to calving makes them increasingly vulnerable to infectious diseases and other associated health issues. The combination of these factors increases the risk of diseases in high-producing cows [20].

The current study aimed to investigate the possibility of increasing CTnI and its association with other cardiac markers of dairy cows in different production periods.

Material and Methods

Ethical Statement

All procedures performed in studies involving animals were in accordance with the ethical standards of the School of Veterinary Medicine, Shiraz University, Shiraz, Iran with approval code number: 47317.

Animals, Sampling Process, and Study Design

The present study investigated a dairy cattle unit with 120 heads, operating within an industrial setting in Shiraz, Iran (spring 2012). For the research, 42 dairy Holstein cows were randomly selected, with an average body weight of 520±17 kg, an average parity of 2.4±0.7, and an average age of 3.2±0.6. The inclusion criteria were body weight, parity, and milk production in animals with healthy evaluated cardia and the exclusion criteria were animals that have diseases such as heart disease, laminitis, and metritis. Throughout the study, the cows were maintained under proper and consistent management and appeared to be in good health. The feeding regimen followed the NRC 2000 guidelines for dairy cows during parturition. The diet consisted of alfalfa, silage, straw, concentrate, mineral, and vitamin supplements. All cows underwent general clinical examinations. The average body temperature, respiratory rate, and heart rate were 37.4, 15, and 70 respectively. The specific cardiac evaluations, including heart auscultation, observation of vein condition, and ECG were recorded and all animals were in normal condition [9]. Considering the predicted calving time, a 10 mL blood sample was collected from the coccygeal vein in the tube without anticoagulant for all cows one month before parturition (dry period). Additional samples were collected one week after parturition (early lactation) and 10 weeks after parturition (peak of the lactation).

Study Parameters

Cardiac troponin levels were measured using the CTnI

AccuBind Elisa assay kit (Monobind Inc, USA) based on an immunoenzymometric test. The test had a sensitivity (detectable limit) of 0.05 ng/mL and a specificity of 25 ng/mL. Levels of AST were measured using a commercial biochemical kit (Zistshimi, Iran). The CK levels were measured using a commercial biochemical kit (Zistshimi, Iran). A commercial biochemical kit (Zistshimi, Iran) and the Caboud-Wroblewski calorimetric method were utilized to measure LDH levels.

Statistical Analysis

All statistical analyses were performed using SPSS version 24 software (USA). The variables were examined for normality using the Kolmograph-Smirto test. The one-way repeated measure ANOVA was used to analyze the variables in this longitudinal study. The Bonferroni test was used as a confident interval adjustment to compare the main effects in three sampling times. In all cases, $P<0.05$ was considered statistically significant. All data is presented as mean ± standard deviation (SD).

Results

The obtained results related to the biochemical parameters of dairy cows' blood serum in different periods, including one month before parturition, one week after calving, and 10 weeks after calving are shown in *Fig 1*, *Fig 2*,

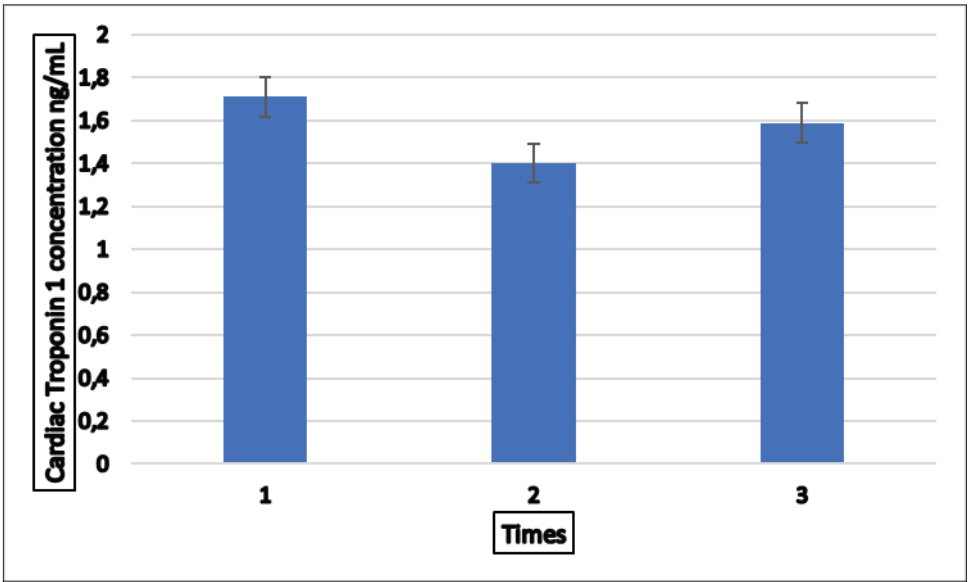

Fig 1. The troponin 1 activity in dairy Holstein cows in three sampling times, including one month before parturition, one week after parturition, and 10 weeks after parturition

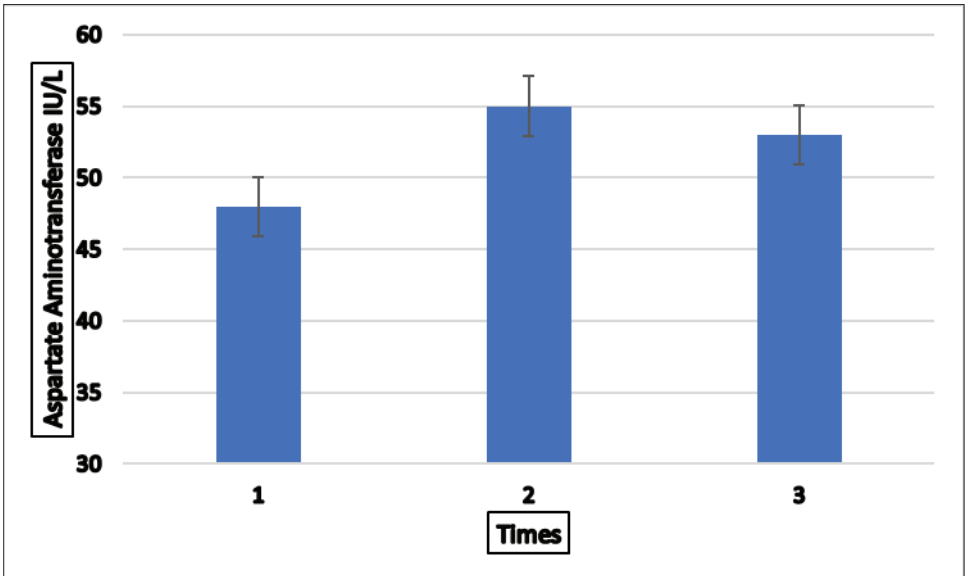

Fig 2. The Aspartate aminotransferase activity in dairy Holstein cows in three sampling times, including one month before parturition, one week after parturition, and 10 weeks after parturition

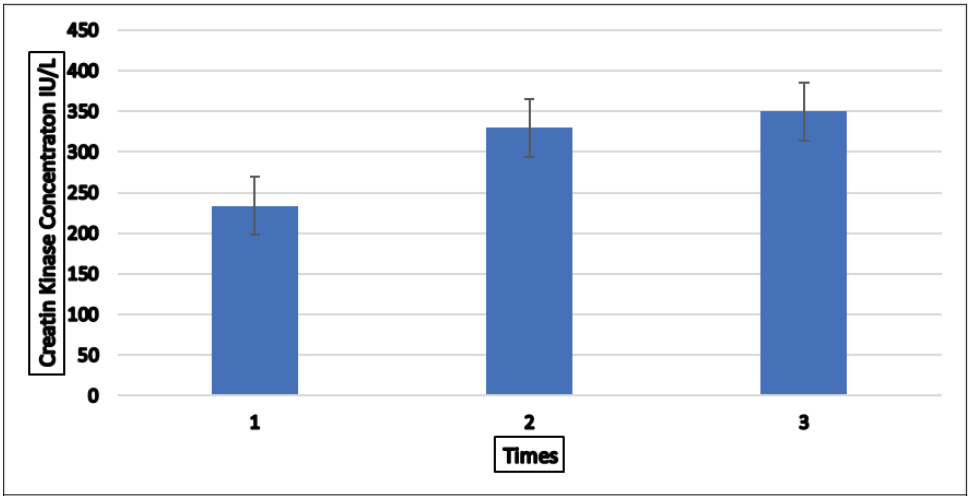

Fig 3. The creatine kinase activity in dairy Holstein cows in three sampling times, including one month before parturition, one week after parturition, and 10 weeks after parturition

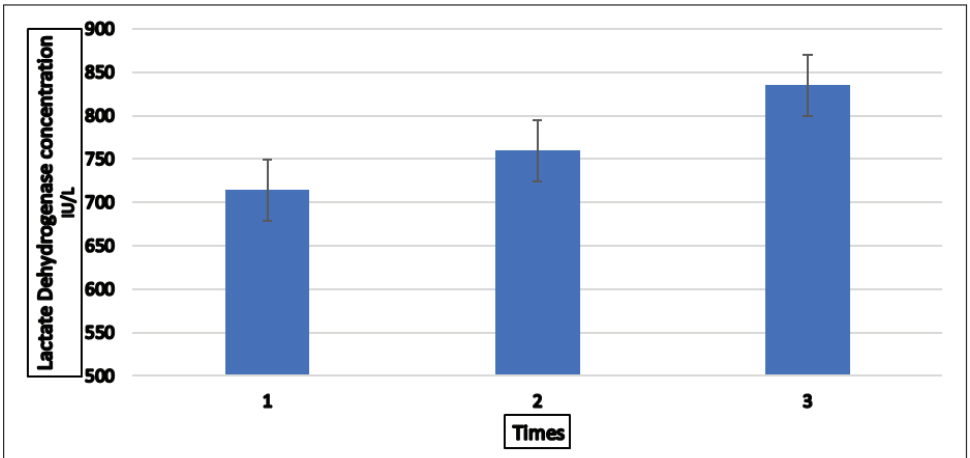

Fig 4. The Lactate dehydrogenase activity in dairy Holstein cows in three sampling times, including one month before parturition, one week after parturition, and 10 weeks after parturition

Fig 3, and *Fig 4*. *Fig 1*, *Fig 2*, *Fig 3*, and *Fig 4* includes data after measuring the serum enzymes CK, LDH, AST, and troponin. Regarding the CTnI parameter, the results indicated insignificant differences in the amount of this enzyme in the whole trial *(Fig 1*, P>0.01).

The analysis of AST showed no significant statistical difference in the amount of this enzyme in the study animals *(Fig 2*, P>0.01). No significant changes were observed during the study for LDH *(Fig 3*, P>0.01). In addition, CK concentrations in dairy cows were insignificant in three sampling time intervals *(Fig 4*, P>0.01).

Discussion

High-productive cows are prone to various metabolic and infectious diseases around the calving time, which is more evident at the production peak [21,22]. The present study aimed to examine potential variations in the levels of CK, LDH, CTn-I, and AST in dairy cows across different lactation stages, and to compare these levels with those observed during the dry period of these cows. It is widely recognized that high-yielding dairy cows may experience varying degrees of negative energy balance, compared to low-yielding cows [21]. Negative energy balance may occur in three situations, including at the end of pregnancy, in the first weeks of lactation, and during diseases [5,23].

Metabolic diseases predispose animals to various infectious diseases around parturition; therefore, the priority in milk production should be prevented. Heart damage, including endocarditis, pericarditis, and myocarditis, is one of the diseases with a high incidence rate in this period [9,22]. The timely diagnosis of a disease can lead to effective treatment of valuable cows to prevent economic losses [6]. The obtained results indicated no significant difference in cardiac biomarkers in the dry period, early, and peak lactation in dairy cattle in this study. Most blood findings in the case of heart disease are nonspecific [7,24]. However, cardiac troponin has been used in animals. A few studies are addressing its clinical use, partly due

to the lack of sufficient information on their natural values. Animal studies, which mostly include laboratory animals, are designed to diagnose myocardial infarction in humans [15,25]. Insufficient information is available regarding the diagnostic use of CTnI in cattle. Early diagnosis of myocardial damage may be useful in treating primary and secondary myocardial diseases. Using tests to measure cardiac troponin enables veterinarians to make an early diagnosis of myocardial degeneration caused by foot and mouth diseases [16]. This is particularly crucial as animals in such cases may be killed before sudden death, making a cardiac index, such as troponin important for diagnosing animal myocarditis [16]. Hajimohammadi et al.[26] pointed out that secondary heart disease caused by salinomycin poisoning could increase CTnI levels in the bloodstream. In all the poisoned groups with different doses of salinomycin, there was a statistically significant difference in the CTnI levels, compared to the control group. Mellanby et al. conducted a study on cows with pericarditis [17]. They observed that a small number of cows with pericarditis had CTnI concentration within the source range. In the necropsy study, the foreign body did not penetrate any of these cows, and most had chronic diseases. The findings of the present study demonstrated that the animals did not exhibit any significant changes in cardiac markers, during the different production stages in this study.

The assessment of the blood concentration of cTnI in healthy cows was below 0.02 ng/mL in a cohort of 30 cows representing various breeds, specifically Jersey and Holstein, in differing physiological conditions, which included 20 lactating cows and 10 dry cows [2]. Additionally, in a group of 28 healthy Holstein calves that were 2.5 months old, the measured values varied from 0.00 to 0.04 ng/mL, although no reference interval was provided for comparison [18].

For many years, the increased blood concentration of CK indicated muscle damage [9]. The CK is presented in both skeletal tissue and cardiac muscles. Changes in the blood CK concentration are associated with acute necrosis of the heart muscle following very intense activity or diseases. In the present study, no statistically significant difference was observed in the amount of CK enzyme. Cozzi et al. indicated that the serum concentration of CK changes significantly in different stages of lactation [27]. The CTnI remains in the blood longer than CK (4-14 days vs. 24-36 hours), making it a valuable tool for diagnosing myocarditis a few days after its onset [28]. This is while serum cardiac troponin is the first biochemical indicator during myocardial injury [29,30]. The increase in CTnI concentration is related to the severity of histopathological lesions and myocardial necrosis. According to Lim et al.[31], after myocardial damage following viral infection, serum cardiac troponin concentration changes earlier than the histological findings of inflammation. In the immunohistochemistry study conducted by Tunca et al. on calves suffering from foot and mouth disease, some points had a clear reduction and absence of CTnI levels, indicating myocardial cell degeneration [32]. In a study by Jesty et al.[33], the concentration of serum CTnI in a cow with pericarditis of unknown origin was 0.49 ng/mL, while the CTnI concentration in a healthy cow was 0.04 ng/mL. In another study by Tunca et al.[32], serum CTnI concentration in calves with foot and mouth disease was 11.7-16.4 ng/mL, and the mean of CTnI in healthy calves was 0.24 ng/mL. A study indicated that the measurement of serum troponin I levels is beneficial for diagnosing traumatic pericarditis and traumatic reticuloperitonitis in cattle [34]. Normal values of CTnI in healthy dairy cows have been reported to be less or equal to 0.03 ng/mL in all studies [17,18,33]. In the present study, the mean serum CTnI for cows in the middle of the dry period was 2.16±1.29 ng/mL, one week after calving was 1.55±0.87 ng/mL, and at the peak of production was 1.33±041/ng/mL.

Aspartate aminotransferase exists in almost all tissues of the body. The muscles and liver are the main sources of this enzyme activity [35]. Regarding the AST enzyme, the present study revealed no significant statistical difference in the amount of this enzyme in cows during three sampling times.

In the current study, the mean serum LDH was 684.36±355.50 U/L in the middle of the dry period and one week after calving was 806.18±501.33 U/L, and at the peak of production was 853.5±276.84 U/L.

Lactate dehydrogenase is a widely distributed enzyme in the body, with high activity in the heart, liver, skeletal muscle, kidney, and erythrocytes. In contrast, a lower amount is found in the lung, smooth muscle, and brain [36]. The average serum LDH concentration was 825.35±434.22 U/L 10 weeks after calving, U/L 330.69±712 one week after calving, and 391.49±49 in the middle of the dry period according to this study.

In conclusion, there is a need to continuously evaluate the mentioned parameters in the production period to investigate animals' health. It is vital to have normal values of these parameters in different stages of production, especially CTnI, which has recently been proposed as a useful side test to diagnose myocardial diseases. More studies are suggested to evaluate the diagnostic benefits of CTnI analysis in cows that naturally suffer from heart diseases.

Declarations

Availability of Data and Materials: The data that support the findings ofthis study are available on request from the corresponding author (D.B.).

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Ethical Statement

All procedures performed in studies involving animals were in accordance with the ethical standards of the School of Veterinary Medicine, Shiraz University, Shiraz, Iran with approval code number: 47317.

Competing Interests: The authors declare that there is no competing of interest regarding the publication of this article.

Declaration of Generative Artificial Intelligence: The authors of the current study declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Authors' Contributions: Ali HAJIMOHAMMADI conceived and designed research. Mojtaba BARMAKI conducted experiments. Saeed NAZIFI contributed to new reagents and analytical tools. Seyed Amin RAZAVI and Mojtaba DANESHI analyzed the data. Seyed Amin RAZAVI and Mojtaba BARMAKI prepared the draft of the manuscript. Daryoush BABAZADEH revised the final draft of the manuscript. All authors read and approved the final version of the manuscript and also agreed to the submission of the article to this journal.

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