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Significance of homocysteine and cardiac markers in cattle with hypocalcemia

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Abstract: This study aimed to determine the levels of homocysteine and cardiac markers in cows with hypocalcemia. This research was conducted with a total of 20 cows, including 10 with hypocalcemia and 10 healthy controls. Hypocalcemia was diagnosed by determining low blood calcium (Ca) levels, and a standard hypocalcemia treatment was initiated in those cows with the disease. Blood samples were taken before the treatment and again 12 h after the treatment, and there was no significant difference in the homocysteine levels between the groups. Ca, ionized calcium (ICa), magnesium (Mg), and phosphorus (P) levels were lower, while cardiac troponin I (cTnI), parathormone (PTH), creatine kinase (CK), and creatine kinase-MB (CK-MB) levels were higher before treatment as compared with the control group. Increases in the Ca, Mg, ICa, and P levels, and a decrease in the PTH levels were detected after treatment when compared with those levels before treatment. The CK, CK-MB, and Mg levels also increased, while the ICa level decreased in the after-treatment group as compared with the control group. Therefore, the cTnI, CK, and CK-MB in cows with hypocalcemia were determined to be important diagnostically, but insignificant for prognosis. Homocysteine was not important for either diagnosis or prognosis.

Key words: Hypocalcemia, homocysteine, cardiac biomarkers, troponin I

1. Introduction

Hypocalcemia is an acute disease of the metabolism characterized by paresis due to a decrease in the blood ionized calcium (ICa) level, general weakness of the muscles, a collapse in circulation, depression, and sensory loss within a few days following birth in cows (1–3). A sudden and acute decrease in the blood ICa levels causes the unresponsiveness of motor muscular fibers to nerve impulses and poor muscle tone (4). Hypocalcemia or parturient paresis is significant in milk cattle for both health and economic reasons (3,4).

Particularly in cows with a high milk yield, hypocalcemia develops during birth or during the first 72-h postnatal period, as well as during the later prenatal period. Rarely, it can develop during the first postnatal month as well (5).

The plasma Ca is found in two forms: soluble and nonsoluble (bonded to proteins), while 55% of the soluble calcium is ICa, which functions actively. The plasma Ca and P concentrations are regulated by parathormone (PTH), 1,25-dihydroxyvitamin D_3 cholecalciferol ($VitD_3$), and nutritional factors (6,7).

Calcium plays an important role in various metabolic events, one of them being the contraction of the heart muscle. Ca is needed for heart muscle contraction and for metabolism regulation. Since the intracellular sarcoplasmic reticulum cannot maintain sufficient Ca levels to start myocardial contraction, plasma ICa levels are quite significant. Hypocalcemia causes a decrease in myocardial contractions, left ventricle systolic dysfunction, and thus systolic heart failure (8,9).

Homocysteine is an amino acid found in the blood and produced in methionine metabolism; it contains sulfur. It has been reported that an increase in blood homocysteine levels could be a signal for coronary heart disease, paralysis, peripheral vascular disease, and intravascular thrombosis (10,11).

The use of heart-based troponins and myoglobin, and biochemical parameters including muscle-based enzymes are important in the determination of the existence and degree of myocardial damage. The lack of sufficient sensitivity and specificity of creatine kinase (CK), lactic dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) used for this purpose has directed researchers (12,13) to develop new methods. Heart-based creatine kinase-MB (CK-MB) is one of the tests with the highest diagnostic value; however, because the CK-MB level increase in serum lasts for a short period

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and exists in exocardial tissue, its diagnostic value is rather limited. Although cardiac troponins are reagents with a high sensitivity for myocardial necrosis (12), there is no troponin complex to regulate the contraction of smooth muscle cells (14). Thus, cardiac troponin I (cTnI) is used predominantly in the determination of cardiac damage and indication of myocardial cases (12,13,15).

Our literature review revealed no studies on homocysteine and cTnI levels in cows with hypocalcemia. Thus, this study aimed to determine the diagnostic and prognostic significance of homocysteine and cTnI, in addition to routine cardiac parameters, in cows with hypocalcemia.

2. Materials and methods

2.1. Animal material

The materials used for this research included 10 cows diagnosed with hypocalcemia after birth in Van Province in Turkey, and 10 healthy cows (a total of 20 cows). Cows with a blood serum Ca ranging between 9.7 and 12.4 mg/dL, according to the reference values (8), and declared healthy after physical examination and according to laboratory findings, were included in the healthy control group. Hypocalcemia was diagnosed based on blood Ca levels in the presence of consistent clinical symptoms. Serum Ca levels below 8.5 mg/dL were reported to define the onset of hypocalcemia (16); values in the 4.1–6.0 mg/dL range were considered to indicate medium, and those below 4 mg/dL were considered to indicate severe hypocalcemia (17,18). In the present study, cows with a Ca level below 6.0 mg/dL were considered to be suffering from hypocalcemia. This research was approved (21/05/2015 and 2015/07) by the Animal Research Ethics Committee of Yüzüncü Yıl University in Van, Turkey.

2.2. Blood samples

Blood samples were taken once from the healthy cows, and twice from the cows with hypocalcemia, before and 12 h after treatment. The samples were placed in tubes without anticoagulant for the biochemical tests and in tubes with anticoagulant for the plasma homocysteine measurement.

2.3. Treatment

The animals with hypocalcemia were treated for hypocalcemia. For the treatment procedure, the animals were initially administered 500 mL of sterile solution containing 3.1 g of calcium gluconate, 42.9 g of calcium borogluconate, 1.32 g of calcium hydroxide, and 6.50 g of magnesium chloride per 100 mL of solution intravenously (IV). The animals that had difficulties standing were rested for 15 min, and then another 500 mL of the same solution was administered via IV and they were allowed to stand up. Just before the commencement of the treatment, a 5–15 mg/kg dose of caffeine SC was administered. During the treatment, the heart rates of the animals were monitored for the determination of bradycardia. Serum was collected, and the animals were allowed to rest, following which the treatment was recommenced.

2.4. Laboratory analyses

The plasma homocysteine levels were analyzed using commercial ELISA test kits (Homocysteine AXIS), and the serum cTnI levels were analyzed with ELISA equipment (ELISA reader, DAS) using commercial troponin I test kits (Troponin I Kit, DRG Diagnostic). The biochemical parameters of PTH, CK, CK-MB, LDH, AST, alkaline phosphatase (ALP), ALT, total bilirubin, direct bilirubin, indirect bilirubin, glucose, total protein, albumin, globulin, Ca, P, Mg, Na, K, and Cl levels were measured using the Integrated Immunoassay and Clinical Chemistry Systems (ARCHITECT Plus ci16200), while the ICa levels were measured with veterinary blood gas equipment (VetStat Electrolyte and Blood Gas Analyzer-Idexx).

2.5. Statistical analyses

The compliance of the continuous variables with the normal distribution was tested using the Shapiro–Wilk and Kolmogorov–Smirnov tests prior to the statistical analyses. In the comparison of the control group with the other test groups, the t-test or Mann–Whitney U test was used in the independent groups, depending on the features of the distribution. In the comparison of the pretreatment and posttreatment clinical parameters, the paired t-test was utilized. In the analysis of the relationships between the clinical parameters, the Pearson or Spearman's correlation coefficients were considered based on the distribution of the data. For all analyses, the level of significance was set at $P < 0.05$. For the data entry and statistical analyses, SPSS-15 was used (19,20).

3. Results

3.1. Clinical findings

Loss of appetite, lack of defecation and urination, prostration, ruminal atonia, mild tympani, paralysis, dilatation and loss of reflex in the eye pupils, mild increase in heart frequency, and slight decrease in body temperature were observed between the 2nd and the 5th day following birth in the cows with hypocalcemia.

3.2. Laboratory findings

Homocysteine, cardiac indicators, and biochemical parameters in the cows with hypocalcemia and in the control group are displayed in Table 1. The correlations of certain selected data for the cows with hypocalcemia are provided in Table 2.

A significant increase in the levels of cTnI, PTH ($P < 0.01$), CK, and CK-MB ($P < 0.05$), and a significant decrease in the levels of Ca, ICa, Mg ($P < 0.001$), and P ($P < 0.01$) were found in the control group as compared with those in the pretreatment (Pre-T) group. Additionally, a significant

Table 1. Homocysteine, cardiac indicators, and biochemical parameters in the cows with hypocalcemia and in the control group (mean ± SEM).

Parameters	Control (n = 10)	Pretreatment (n = 10)	Posttreatment (n = 10)
Homocysteine (µmol/L)	15.89 ± 2.13	4.75 ± 0.89	5.82 ± 1.51
cTnI (ng/mL)	0.33 ± 0.09	9.93 ± 4.83**	9.42 ± 3.88**
PTH (pg/mL)	44.54 ± 6.03	411.34 ± 191.52**	63.42 ± 33.41[a]
CK (U/L)	231.79 ± 24.86	1041.81 ± 359.83*	1500.42 ± 587.54*
CK-MB (U/L)	344.87 ± 29.66	823.68 ± 101.72*	380.62 ± 115.75*
ICa (mg/dL)	3.98 ± 0.13	1.09 ± 0.09***	2.94 ± 0.26*[b]
Ca (mg/dL)	10.35 ± 0.30	3.87 ± 0.44***	13.48 ± 1.68[c]
P (mg/dL)	5.14 ± 0.54	2.14 ± 0.72**	3.59 ± 0.63[b]
Mg (mg/dL)	3.98 ± 0.41	1.78 ± 0.08***	5.75 ± 0.62***[c]
AST (U/L)	91.20 ± 10.64	99.77 ± 13.73	121.55 ± 30.03
ALT (U/L)	38.00 ± 9.10	27.66 ± 2.26	30.55 ± 2.79
ALP (U/L)	173.03 ± 40.78	44.00 ± 11.14*	61.88 ± 19.27*
LDH (U/L)	3022.80 ± 142.94	2157.44 ± 141.80**	2516.55 ± 264.66
Total bilirubin (mg/dL)	0.10 ± 0.00	0.38 ± 0.98**	0.28 ± 0.08**[b]
Direct bilirubin (mg/dL)	0.02 ± 0.00	0.05 ± 0.03	0.06 ± 0.04
Indirect bilirubin (mg/dL)	0.07 ± 0.00	0.32 ± 0.07**	0.22 ± 0.05**[b]
Glucose (mg/dL)	59.60 ± 4.82	56.88 ± 10.85	78.55 ± 10.23*[a]
Total protein (g/dL)	8.22 ± 0.36	6.51 ± 0.43**	6.51 ± 0.43**
Albumin (g/dL)	3.36 ± 0.11	2.92 ± 0.18	2.93 ± 0.20
Globulin (g/dL)	4.86 ± 0.32	3.59 ± 0.27**	3.57 ± 0.26**
Na (mmol/L)	140.60 ± 0.47	131.66 ± 3.09**	132.11 ± 2.61**
K (mmol/L)	4.29 ± 0.11	3.76 ± 0.33	4.21 ± 0.27
Cl (mmol/L)	103.00 ± 1.21	97.11 ± 2.03*	96.55 ± 1.29**

Statistical significance of the Pre-T and Post-T parameters on the same line with respect to the control group: (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$. Statistical significance of the Post-T parameter with respect to the Pre-T: (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$.

increase was observed in the cTnI ($P < 0.01$), CK, CK-MB, ICa ($P < 0.05$), and Mg ($P < 0.001$) levels in the control group as compared with those in the posttreatment (Post-T) group. In the Pre-T group PTH significantly ($P < 0.05$) decreased, and ICa, P ($P < 0.01$), Ca, and Mg ($P < 0.001$) significantly increased as compared with those in the Post-T group.

There was a significant ($P < 0.01$) increase in the total bilirubin and indirect bilirubin levels, and a decrease in the LDH, total protein, globulin, Na ($P < 0.01$), ALP, and Cl ($P < 0.05$) levels of the control group when compared with those in the Pre-T group. Additionally, there was a significant increase in the total bilirubin, indirect bilirubin, and glucose ($P < 0.05$) levels, and a decrease in the ALP ($P < 0.05$), total protein, globulin, Na, and Cl ($P < 0.01$) levels in the control group as compared with those in the Post-T group.

In the Post-T group, there was a significant decrease in the total bilirubin and indirect bilirubin ($P < 0.01$) levels, and an increase in the glucose ($P < 0.05$) level when compared with those in the Pre-T group.

A significant negative correlation was found between the PTH and cTnI values ($P < 0.05$), while there was a significant positive correlation between Ca and ICa, Mg,

Table 2. Correlations of certain selected data for the cows with hypocalcemia.

Parameters (n = 30)	Homocysteine (μmol/L)	PTH (pg/mL)	cTnI (ng/mL)	Ca (mg/dL)	Ica (mg/dL)	Mg (mg/dL)	P (mg/dL)	CK (mg/dL)
PTH (pg/mL)	0.289							
cTnI (ng/mL)	–0.301	–0.381(*)						
Ca (mg/dL)	0.038	–0.139	0.091					
ICa (mg/dL)	0.345	–0.322	0.241	0.815(**)				
Mg (mg/dL)	0.172	–0.168	0.283	0.625(**)	0.527(*)			
P (mg/dL)	0.076	–0.103	–0.2	0.564(**)	0.699(*)	0.517(**)		
CK (mg/dL)	–0.122	0.128	0.233	–0.113	–0.091	0.290	–0.219	
CKMB (mg/dL)	–0.066	–0.174	–0.158	0.138	–0.027	–0.099	0.292	–0.12

Statistical significance of the parameters in the same column based on the parameter in the line: (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.

and P values ($P < 0.01$). Additionally, a significant positive correlation between ICa and Ca ($P < 0.01$), Mg, and P values ($P < 0.05$) was found. Finally, there was a significant positive correlation between Mg and Ca, P ($P < 0.01$), and ICa values ($P < 0.05$).

4. Discussion

In hypocalcemia, due to the sudden abnormal decrease in plasma ICa levels, clinical symptoms such as ruminal atonia, tympani, constipation, a decrease in body temperature, prostration, and paralysis are observed (1,4,5,21). In addition, hypocalcemia causes a decrease in the heart sound volume, mild tachycardia with arrhythmia, and a decrease in the heart frequency (6,22,23). In the present study, the observed clinical symptoms specific to hypocalcemia were similar to those reported in other studies (1,4,6,21).

It is known that blood homocysteine levels increase in heart and coronary diseases, myocardial infarction, peripheral vascular diseases, and intravascular thrombosis (11). In the present study, it was determined that homocysteine levels decreased in both the Pre-T (4.75 ± 0.89 μmol/L) and Post-T (5.82 ± 1.51 μmol/L) groups, when compared with those in the control group, but this decrease was insignificant (Table 1). The relative decrease in homocysteine in the Pre-T and Post-T groups could be due to the fact that 70%–80% of the homocysteine in the blood is bonded to plasma proteins, and especially to albumin (10,11). This is because the total protein and albumin levels in both the Pre-T and Post-T groups were lower than those in the control group in the present study (Table 1).

One heart-based cardiac troponin with clinical significance is cTnI (12,13); it has high sensitivity to myocardial necrosis (12,13,15). In the present study the cTnI levels were higher in both the Pre-T (9.93 ± 8.83 ng/mL) and Post-T (9.42 ± 7.88 ng/mL) groups than in the control group; however, the difference between the Pre-T and Post-T was not significant (Table 1). In the cows with hypocalcemia, the increases found in the cTnI corroborate the results reported by Gunes et al. (12), where a cTnI level of 9–11 ng/mL indicated heart damage in cattle with traumatic reticuloperitonitis. Thus, the increase in cTnI observed in this study could be a reflection of the development of cardiac damage in hypocalcemia.

To regulate the reduced serum Ca level in hypocalcemia, PTH increases to higher than its normal levels (22,24). In the present study, the PTH level was significantly higher in the Pre-T (411.34 ± 191.52 pg/mL) cows with hypocalcemia as compared with that in the control group (0.33 ± 0.09 pg/mL). The increased PTH levels in the cows with hypocalcemia corroborate the findings reported in other studies (1,21,22,24) and are a reflection of the regulation of reduced calcium levels. Similar to the findings reported by Basoglu and Sevinc (7), we also found that the PTH levels decreased ($P < 0.05$) in parallel to the increasing Ca levels in the Post-T group, as a response to the standard hypocalcemia treatment.

The CK and isoenzymes found in the skeletal muscles, heart muscles, and brain are organ-specific enzymes used in clinical studies (22). In the present study it was observed that the CK and CK-MB levels (Table 1) displayed a significant increase in both the Pre-T and Post-T groups when compared with those in the control group ($P <$

0.05). This finding corroborates the values reported for hypocalcemia in other studies (22,25). It was considered that this fact was due to the effects of an ongoing increase in Post-T CK activity as a result of muscular myopathy that develops in hypocalcemia.

In the diagnosis of myocardial diseases, enzymes with a muscular origin, such as AST, ALP, ALT, and LDH, are utilized as a fortiori data (6,8,22). In the present study, it was observed that the AST, ALT, ALP, and LDH levels (Table 1) increased, in accordance with the statements of previous authors; however, only the increase in ALP was significant ($P < 0.05$), similar to the findings reported by Çitil (1). The fact that these enzymes are also secreted by different organs and tissue systems, and the fact that they do not possess sufficient sensitivity and specificity for the heart muscle, limit their cardiac diagnostic significance (6,8,22).

Zebeli et al. (26) determined the Ca level in cows with hypocalcemia to be 7.18 mg/dL, the Mg level to be 2.20 mg/dL, and the P level to be 3.09 mg/dL. Sevinç and Aslan (25) reported the Pre-T total Ca, ICa, and P levels to be 4.44 mg/dL (1.11 mmol/L), 1.76 mg/dL (0.44 mmol/L), and 2.38 mg/dL (0.77 mmol/L), respectively, and the Post-T values of those to be 15.48 mg/dL (3.87 mmol/L), 8.4 mg/dL (2.10 mmol/L), and 4.52 mg/dL (1.46 mmol/L), respectively, in cows with hypocalcemia. It was argued that the most accurate results were determined by normalized calcium or ICa levels in cows suspected to have hypocalcemia, and the ICa level in cows with hypocalcemia was found to be 1.76 mg/dL (22). The Ca and ICa findings of the present study corroborate the values reported by Sevinç and Aslan (25). However, it was observed that, despite the increase in the Ca levels Post-T, the ICa levels were still lower when compared with those in the control group ($P < 0.05$). This could be a result of the utilization of ICa in metabolic activities.

The changes that the Mg, Ca, and P ions go through in the blood affect each other. Many studies (1,21,27) reported that Mg levels decrease in hypocalcemia, while Turgut (22) stated that the Mg levels could increase or stay at normal levels. The findings of the present study indicate that the Pre-T Mg levels in the cows with hypocalcemia were lower ($P < 0.001$), which is in agreement with the findings of other studies (1,21,27); however, the Mg levels increased Post-T when compared to Pre-T and the control group. It was reported that, usually, the P levels decrease with the Ca levels in hypocalcemia (7). Our findings also demonstrated that the serum P level was reduced ($P < 0.01$) (Table 1).

The total bilirubin, direct bilirubin, and indirect bilirubin levels are significant findings in the evaluation of erythrocyte, liver, and gall bladder functions (7,22). Additionally, it was reported that increases can occur in the CK, ALP, liver enzymes, and bilirubin levels in animals with hypocalcemia when compared with those in healthy animals (1). In the present study, it was determined that there was a significant increase in the Pre-T and Post-T indirect bilirubin levels in cows with hypocalcemia as compared with those in the control group ($P < 0.01$). The reasons for this could be explained by indigestion induced by hypocalcemia, decreased P levels (Table 1), and the shortening of erythrocyte life due to the deterioration in energy metabolism, as reported in previous studies (1,22,28). It was also observed that the total protein and globulin levels were significantly lower in the Pre-T and Post-T cows with hypocalcemia when compared with the control group (Table 1). This fact could be due to undernourishment and unbalanced nutrition, indigestion, enteropathy, and hunger in animals with hypocalcemia, as previously reported (3,22,25).

In the current study, it was observed that the Na ($P < 0.01$), K ($P > 0.05$), and Cl ($P < 0.05$) values were lower both Pre-T and Post-T, when compared with those in the control group (Table 1); this was assumed to be due to gastrointestinal loss (22).

The evaluation of the relationship between homocysteine and cardiac indicators in the present study demonstrated that there was no correlation between homocysteine and the other parameters in cows with hypocalcemia. However, it was determined that there was a negative correlation between PTH and cTnI ($P < 0.05$). This was assumed to be due to a decrease in the PTH levels as a result of increasing blood Ca levels induced by the Ca preparations applied during the treatment (7), while the half-life of the cTnI remained high and longer than the PTH in the blood (7,29). In the hypocalcemia cases, the ICa, P, and Mg levels decreased in parallel to the Ca level (2,21,22,27). A significant positive correlation was observed in the current study between the Ca values and the ICa, P, and Mg, similar to findings of other studies (Table 2).

In the present study, the homocysteine and cTnI levels were determined in cows with hypocalcemia for the first time. As a result, it was concluded that there was a negative correlation between PTH and cTnI, and these values could be a reference for future studies. The cTnI was significant diagnostically, in addition to the routine cardiac parameters of CK and CK-MB in the diagnosis of the disease, but insignificant in its prognosis. Finally, the homocysteine levels were insignificant in both the diagnosis and prognosis.

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