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Evaluation of fecal calprotectin levels in neonatal calves with diarrhea

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ABSTRACT

This study aimed to determine the levels of fecal calprotectin in calves that had diarrhea in the neonatal period. The study was carried out on a total of 52 calves, 42 with diarrhea (diarrhea group) brought to the Animal Hospital of the Faculty of Veterinary Medicine, Balikesir University, for diagnosis and treatment, and 10 healthy (control group). The diarrhea-afflicted calves ranged in age from 3 to 30 days and included a variety of races and sexes. For blood gases, hematological, and biochemical analyses, ten milliliters of blood were collected from each calf's jugular vein. To determine the levels of calprotectin, stool samples were gathered and stored in sterile containers. The heart rate and capillary refill time (CRT) of the calves with diarrhea were significantly ($P<0.05$) higher than the control group. The calves who had diarrhea also had increased potassium (K) and creatinine levels in addition to metabolic acidosis and hyponatremia. Furthermore, the serum albumin and oxygen saturation values of the diarrheal calves were found to be significantly lower than those of the control group. Fecal calprotectin levels were found to be significantly higher in the calves with diarrhea compared to the control group. When fecal calprotectin levels were examined in terms of etiological factors, no statistical difference was detected between the groups. Consequently, it was determined that fecal calprotectin levels in calves with diarrhea could be a potential biomarker for enteritis.

Key words: calf; diarrhea; calprotectin; etiology

Introduction

Calf diarrhea is one of the most important health problems in the neonatal period and is one of the most common causes of morbidity and mortality worldwide. It also causes very important economic losses (MEGANCK et al., 2014; MEGANCK et al., 2015). Diarrhea in newborn calves can cause potentially serious metabolic disturbances, including strong ion (metabolic) acidosis, hyper-D-lactatemia, hyper-L-lactatemia, azotemia, hypoglycemia, hyperkalemia, and hyponatremia (TREFZ et al., 2017). Neonatal calf diarrhea is a multifactorial disease, and factors, including nutrition, hygiene, care, and immunity, can affect how diarrhea develops. The most common pathogens are enterotoxigenic *Escherichia coli*, *Cryptosporidium parvum*, *rotavirus* and *coronavirus*. While these pathogens can cause diarrhea alone, mixed infections are also common in field conditions (CONSTABLE, 2009; FOSTER and SMITH, 2009; AYDOĞDU et al., 2018).

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Calprotectin is a 36 kDa calcium and zinc binding protein and constitutes approximately 60% of the neutrophil cytosol (LANGHORST et al., 2008; GISBERT and MCNICHOLL, 2009). Calprotectin can also be produced by bone marrow cells, keratinized and non-keratinized squamous epithelium, certain mucosal epithelial cells, microvascular endothelial cells, and fibroblasts. It can also be found to a small extent in monocytes and zinc-binding macrophages, usually as a result of activation (MONTALTO et al., 2013; VAOS et al., 2013). In cases of inflammation, calprotectin is released from that neutrophils that pass into the intestine, and its level increases in the stool. Fecal calprotectin is a non-invasive biomarker that can be used to diagnose intestinal diseases causing acute inflammation and to determine inflammation levels, as well as in the determination of tumoral formations in the intestines (JOHNE et al., 2001; LANGHORST et al., 2008; USLU et al., 2011). It is known that fecal calprotectin has immunomodulatory, antimicrobial and antiproliferative properties. There are studies showing that it is a simple, low-cost, sensitive, specific and non-invasive method that can be used in the detection and monitoring of intestinal inflammation in children (FAGERBERG et al., 2005; CANANI et al., 2006; CANANI et al., 2008; ASHORN et al., 2009). Fecal calprotectin has been assessed by several authors as the intestinal C-reactive protein (FAGERBERG et al., 2007).

It was hypothesized that fecal calprotectin levels, a biomarker of intestinal inflammation in humans, would also increase in calves with diarrhea and could be used in the detection of enteritis. The aim of this study was to determine the fecal calprotectin levels in neonatal calves with diarrhea and to reveal the differences according to the etiological pathogen.

Materials and methods

The study was carried out on a total of 52 calves, 42 with diarrhea brought to the Animal Hospital of the Faculty of Veterinary Medicine, Balikesir University, for diagnosis and treatment, and 10 healthy calves from local farms in Balikesir province. There were 28 Holstein, 11 Simmental, 2 Charolea, and 1 Montafon calves among the diarrheal ones, while all the calves in the control group were of the Holstein breed. The average age of calves with diarrhea was 13.45±1.00 day (3-30 day) and the control group was 23.00±1.94 day (16-30 day). In the control group, 5 calves were female and 5 males, whereas 18 of the calves with diarrhea were male and 22 were female. The gender record of 2 calves was not obtained. The study comprised cases with acute diarrhea that had not received medical treatment. Following a routine clinical examination and record of every calf, 10 mL of blood was taken from the jugular vein for analysis of blood gases, hematological, and biochemical parameters. The blood samples, taken into gel tubes without anticoagulant, were kept at room temperature and then centrifuged at 2500 g for 5 minutes and the serum was removed. For determining the amounts of calprotectin and the etiological agents, stool samples were collected and placed into sterile containers. Stool samples were examined for *rotavirus*, *coronavirus*, *Cryptosporidium spp.*, *E. coli* and *Cl. perfringens,* using immunochromatographic rapid test kits (Vetima 313/5, Turkey). Serum and stool samples were stored at -20°C until analysis. Blood gas analyses were performed using a blood gas analyzer (Epoc, Canada), and hematological analyses were performed with a complete blood count analyzer (Abacus Junior Vet5, Diatron MI Ltd., Hungary) from blood collected in K3EDTA tubes. Serum total protein (TP) levels were analyzed using a refractometer (Loyka, Turkey), and albumin levels were analyzed in an autoanalyzer (Monaco, Randox, England) using commercial kits. Calprotectin levels from stool samples were determined with bovine-specific ELISA kits (Bovine Calprotectin, MBS020907, MyBioSource®, San Diego, USA) according to the manufacturer's instructions. For this purpose, 10 mg of stool samples were taken and 100µl of phosphate buffer solution was added and vortexed. Afterwards, the homogenate was centrifuged, and the supernatant was collected and analyzed. Optical density was determined using a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany).

Statistical Analysis. Data were presented as mean and standard error of mean (Mean±SEM). The Shapiro-Wilk test was used to determine whether the data had a normal distribution. The difference between the normally distributed parameters was determined by the Student's t-test, and the difference between the non-normally distributed parameters was determined by the Mann-Whitney U test. The Kruskal–Wallis test was used to determine the differences in fecal calprotectin according to etiological factors. Tamhane's T2 test was used for post-hoc group comparisons. Pearson's coefficient was used to assess the correlation between fecal calprotectin levels and the blood parameters and clinical findings. The statistical significance level was determined as $P<0.05$.

Results

Clinical findings, such as loss of appetite, increased heart and respiratory frequency, hypothermia, dehydration and prolonged CRT were observed in calves with diarrhea. The heart rate and CRT of the calves with diarrhea were significantly ($P<0.05$) higher compared to the control group (Table 1).

The calves who had diarrhea also had increased potassium (K) and creatinine levels in addition to metabolic acidosis and hyponatremia. Additionally, oxygen saturation and serum albumin levels were significantly ($P<0.05$) lower in the calves with diarrhea compared to the control group (Table 2).

Fecal calprotectin levels were significantly higher ($P<0.05$) in the calves with diarrhea compared to the control group (Table 3). Although it was observed that all groups were mathematically higher than the control group, no statistical difference ($P>0.05$) was detected when etiological factors were examined (Table 4).

When the relationship between fecal calprotectin levels and clinical findings and blood parameters was examined, a positive correlation was found between fecal calprotectin and heart rate and CRT, and a negative correlation was found with temperature and lactate level (Table 5).

Table 1. Clinical findings of the calves with diarrhea and the control group (Mean±SEM)

Parameters	Control n=10	Diarrhea n=42	P
Temperature (°C)	38.78±0.16	37.69±0.33	0.058
Heart rate (min)	88.70±3.10	116.28±4.72	0.001
Respiratory rate (min)	23.80±1.53	34.12±3.33	0.085
CRT (sec)	1.90±0.10	3.32±0.26	0.001

CRT: capillary refill time

Table 2. Blood gases, hematological and serum biochemical analysis results of the calves with diarrhea and the control group (Mean±SEM)

Parameters	Control n=10	Diarrhea n=42	P
pH	7.41±0.01	7.19±0.04	<0.001
pCO2 (mmHg)	43.60±1.82	46.99±2.56	0.387
pO2 (mmHg)	29.10±2.07	27.67±2.49	0.711
Bicarbonate (mmol/L)	27.76±0.91	20.39±2.16	0.075
Base excess (mmol/L)	2.80±1.16	-7.81±2.84	0.025
O_2 saturation (%)	54.60±4.51	38.11±4.24	0.022
Sodium (mmol/L)	142.00±0.61	133.79±1.61	0.001
Potassium (mmol/L)	4.32±0.11	5.34±0.21	0.001
TCO2 (mmHg)	29.11±0.96	21.87±2.21	0.099
Lactate (mmol/L)	0.87±0.21	2.69±0.86	0.068
Creatinine (mg/dL)	1.38±0.08	2.85±0.49	0.019
Leukocyte (10^3/mm^3)	12.13±0.40	11.84±1.16	0.234
Erythrocyte (10^6/mm^3)	10.00±0.45	8.84±0.36	0.055
Hemoglobin (g/dl)	10.47±0.33	9.52±0.37	0.064
Hematocrit (%)	31.55±1.65	29.43±13	0.303
MCV (fl)	31.40±0.43	33.61±0.62	0.040
MCHC (g/dl)	33.66±1.70	32.47±0.60	0.428
Platelet(10^3/mm^3)	475.80±39.01	733.02±245.15	0.601
Total protein (g/dL)	5.10±0.08	4.77±0.15	0.166
Albumin (g/dL)	3.09±0.05	2.87±0.07	0.012
Globulin (g/dL)	2.01±0.11	1.90±0.13	0.528

pCO2: partial pressure of carbon dioxide; pO2: partial pressure of oxygen; TCO2: total carbon dioxide; MCV: mean erythrocyte volume; MCHC: mean erythrocyte hemoglobin concentration

Table 3. Fecal calprotectin levels of the calves with diarrhea and the control group (Mean±SEM)

	Control n=10	Diarrhea n=42	P
Fecal calprotectin (ng/mL)	6.75±0.86	16.30±1.46	<0.001

Table 4. Fecal calprotectin levels according to etiological pathogens (Mean±SEM)

Etiological pathogens	Fecal calprotectin (ng/mL)
Control (n=10)	6.75±0.86
Cryptosporidium spp. (n=11)	13.51±2.11
Mix (n=7)	14.20±1.73
Cl. perfringens (n=6)	14.49±2.46
Negative (n=12)	17.37±3.24
Rotavirus (n=4)	18.46±4.14
E.coli (n=2)	33.69±15.69

Table 5. Level of correlation between fecal calprotectin levels and clinical findings and blood parameters

	Temperature	Heart rate	CRT	Lactate
Fecal calprotectin	-0.481**	0.406*	0.403*	-0.608**

CRT: capillary refill time; *P < 0.05; **P < 0.01

Discussion

In the present study, fecal calprotectin was demonstrated to be an effective biomarker for intestinal inflammation in calves with enteritis.

Dehydration due to loss of water and electrolytes in the stool is a common finding in calf diarrhea. Decreased milk consumption also contributes to dehydration. Metabolic acidosis, electrolyte disorders (hyponatremia, hypochloremia and hyperkalemia), an increase in lactate level, and azotemia occur due to fluid and electrolyte loss (SMITH and BERCHTOLD, 2014; CONSTABLE et al., 2017; LEE et al., 2020; AYDOĞDU et al., 2023). COŞKUN et al. (2020) reported that the most common electrolyte disturbances in 126 newborn calves with diarrhea are hyponatremia, hypochloremia and hyperkalemia. In the present study, as reported in previous studies, metabolic acidosis, hyponatremia and an increase in potassium levels were detected in calves with diarrhea. AYDOĞDU et al. (2023) in a recent study reported that azotemia is common in newborn calves with diarrhea and therefore it would be beneficial to monitor renal functions during the treatment of neonatal calves with diarrhea. The present study determined that azotemia was common and that the calves with diarrhea had significantly higher creatinine levels than the control group.

Albumin is a negative acute phase protein, and its concentration decreases more than 25% during the inflammatory response (JAIN et al., 2011; SCHMIDT and ECKERSALL, 2015). AYDOGDU and YURDAKUL (2020) reported that the serum albumin level in calves with local and systemic inflammation was significantly lower than in the control group. In the present study, it was found that the serum albumin levels in the calves with diarrhea were significantly lower than those in the control group. This result shows that a decrease in albumin level, a negative acute phase protein, was observed as a result of enteritis-related inflammation.

Calprotectin is a biomarker that is an indicator of neutrophil migration, and it increases in many inflammatory events (LANGHORST et al., 2008). In the case of inflammation in the intestines, calprotectin is released from the neutrophils that pass into the intestine, and its level increases in the stool. Fecal calprotectin can be used to diagnose intestinal diseases that cause acute inflammation and to determine inflammation levels. It is also reported that it can be used in the determination of tumoral formations in the intestines (JOHNE et al., 2001; USLU et al., 2011). The levels of calprotectin have been found to be proportional to the degree of inflammation in different body fluids. Under normal conditions, its concentration in stools is six times higher than in plasma (ROSETH et al., 1992). This suggests that it can act as a reliable indicator of intestinal inflammation (RICCIUTO and GRIFFITHS, 2019). According to AYDEMIR et al. (2012), infants with necrotizing enterocolitis have higher fecal calprotectin levels than healthy infants. In a study of dogs with chronic diarrhea it was found that they had fecal calprotectin levels 3.2 times greater than those in the healthy control group (GRELLET et al., 2013). AYDIN et al. (2022) determined that serum calprotectin levels were significantly higher in calves with diarrhea compared to healthy calves. In the present study, fecal calprotectin levels were found to be significantly higher (2.4 times) in the calves with diarrhea compared to the control group (Table 3). This result shows that fecal calprotectin is also an important biomarker for inflammation in calves with diarrhea.

In addition, it has been reported that fecal calprotectin may be useful in the differential diagnosis of pediatric diarrhea in human medicine. It has been reported that fecal calprotectin levels are higher in infectious diarrhea than in non-infectious diarrhea (irritable bowel syndrome) and its levels are proportional to the clinical severity of the disease (VAOS et al., 2013). In a study, it was reported that children with acute gastroenteritis with *Salmonella* or *Campylobacter* infection, had higher fecal calprotectin levels compared to those with rotavirus, norovirus or adenovirus infection (CHEN et al., 2012). Although there was no statistically significant difference detected, a mathematical increase in fecal calprotectin levels was seen when analyzed in relation to etiological pathogens in this study, perhaps because those groups had fewer cases than the control group.

When the correlations between fecal calprotectin levels and clinical findings and blood parameters were examined, a positive correlation was detected between fecal calprotectin and heart rate and CRT, and a negative correlation with temperature and lactate. These results suggested that when the disease becomes more severe, there may be an increase in fecal calprotectin levels.

The study's limitations include being unable to evaluate survivors and non-survivors by following calves that had diarrhea, and the lack of any grouping depending on the severity of the diarrhea. Furthermore, our study was limited by the fact that there were only two *E. coli* cases when fecal calprotectin was evaluated on the basis of the etiological factors.

In conclusion, it can be stated that the increase in fecal calprotectin levels in the calves with diarrhea is promising as an important biomarker for enteritis. It was concluded that studies involving more cases are needed to determine the etiology of enteritis.

Ethics approval

This research was approved by Balikesir University Animal Experiments Local Ethics Committee (HADYEK) (Decision no: 2019/5-5).

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Declaration of competing interest

No potential conflict of interest was reported by the authors.

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SAŽETAK

Cilj je istraživanja bio odrediti razine fekalnog kalprotektina u neonatalne teladi s proljevom. Istraživanje je provedeno na ukupno 52 jedinke teladi, među kojima su 42 jedinke imale proljev (skupina bolesne teladi) zbog čega su sa svrhom uspostavljanja dijagnoze i liječenja dovezene u bolnicu Fakulteta veterinarske medicine, Sveučilišta Belikesir. U kontrolnu skupinu uključeno je 10 zdravih jedinki teladi. Telad oboljela od proljeva bila je različitih pasmina i spola, dobi od 3 do 30 dana. Od svake jedinke je prikupljeno 10 mL krvi iz jugularne vene za pretragu plinova u krvi, hematološke i biokemijske analize. Za određivanje razine kalprotektina, prikupljeni su uzorci stolice i pohranjeni u sterilnim posudama. Frekvencija otkucaja srca i vrijeme punjenja kapilara (CRT) u teladi s proljevom bilo je znakovito povećano ($P<0,05$) u odnosu na kontrolnu skupinu. Telad s proljevom imala je i povišenu razinu kalija (K) i kreatinina, uz metaboličku acidozu i hiponatrijemiju. Osim toga, vrijednosti serumskog albumina i zasićenost kisikom u teladi s proljevom bile su znakovito manje u odnosu na kontrolnu skupinu. Vrijednosti fekalnog kalprotektina bile su znakovito veće u teladi s proljevom u usporedbi s kontrolnom skupinom. Analizom etioloških čimbenika vezanih uz razine kalprotektina u izmetu nije otkrivena statistički znakovita razlika među skupinama. Zaključeno je da bi razine fekalnog kalprotektina u teladi s proljevom mogle biti potencijalni biomarker za otkrivanje enteritisa.

Ključne riječi: tele; proljev; kalprotektin; etiologija