Investigation of Changes in Biochemical Parameters in Some Diseases Occurring During the Transition Period in Simmental Cows

Safak, T.,[1,*] Yilmaz, O.[2] and Risvanli, A.[3,4]

[1] Kastamonu University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Kastamonu, Türkiye
[2] Siirt University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Siirt, Türkiye
[3] Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Bishkek, Kyrgyzstan
[4] Fırat University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Elazığ, Türkiye

* **Corresponding author:** Dr. Tarik, Kastamonu University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Kastamonu, Türkiye.
E-mail: tsafak@kastamonu.edu.tr

ABSTRACT

The transition period is important in cows. This study was conducted to evaluate the effects of retained fetal membranes (RFM), clinical mastitis, and metritis on biochemical and selected mineral levels in Simmental cows. Cows were divided into five groups; cows with RFM (n=17), clinical mastitis (n=25), metritis (n=21) and postpartum healthy cows (n=21) within 21 days postpartum, and also prepartum healthy cows (n=20) in the 15±5 days before the expected parturition date. The activity of alkaline phosphatase (ALP) (85.18±15.83 U/L), aspartate transaminase (AST) (123.02±19.15 U/L), and gamma-glutamyl transferase (GGT) (28.18±2.66 U/L) in the metritis group increased compared to the prepartum healthy cows. Moreover, GGT (41.83±14.61 U/L) and a myocardial band of creatine kinase (CK-MB) (155.25±27.85 U/L) activities were highest in the RFM group, while creatine kinase – N-acetyl-cysteine activity (CK-NAC) (540.45±157.67 U/L) and creatinine concentration (2.29±0.88 mg/dL) were observed in the metritis group. Total protein (6.39±0.38 g/dL) concentration was highest in the case of mastitis. Urea, on the other hand, was highest in the metritis group with a concentration of 61.40±17.38 mg/dL. Our results showed changes in the biochemical profile of cows with RFM, clinical mastitis, and metritis. Biomarker profiles were determined using receiver-operator characteristic (ROC) curves. It was determined that activities of ≥ 89 (U/L) AST, ≥ 24 (U/L) GGT, and ≥ 106 (U/L) CK-MB for metritis, ≥ 21(U/L) GGT for RFM, and ≥ 105 (U/L) CK-MB for mastitis can be used in the preliminary diagnosis. Also, further studies with a larger cow cohorts are recommended.

Key words: Cows; Mastitis; Metritis; Receiver Operating Characteristics; Transition Period.

INTRODUCTION

The transition period, defined as the period between three weeks before calving and three weeks after calving, is a demanding period for cows (1). Infectious disorders occur in the first weeks postpartum (2). Approximately 90% of cows are exposed to bacterial contamination during the two weeks postpartum. Bacteria are eliminated over time; nevertheless, in some cases, these bacteria remain in the lumen of the uterus in cows. This contributes to the occurrence and higher prevalence of postpartum diseases (3, 4).

Puerperal metritis is one of the most important diseases in the postpartum period in cattle (3). This disease is characterized by fetid watery red-brown uterine discharge and an abnormally enlarged uterus, as well as high fever (≥39.5˚C) and systemic disease symptoms, within 21 days after calving. Clinical metritis within 21 days postpartum is limited to

a uterus that has not completed involution. Purulent and foul-smelling symptoms from the vagina without signs of systemic disease are recognized (3). One of the diseases seen in this period is acute puerperal mastitis. Mastitis is a very common disease in dairy cows and one of the most costly diseases for the dairy industry (5).

Biochemical tests used in the early diagnosis and prevention of diseases that cause abundant postpartum economic losses in cows could be beneficial for the dairy industry. It may contribute to the development of protocols for the treatment and prevention of metritis, retained fetal membranes (RFM), and mastitis in the postpartum period. Ruprechter *et al.* (6) and Paiano *et al.* (7) demonstrated that the evaluation of some biochemical parameters in the prepartum period in Holstein cows could be used to predict the health of dairy cows in the postpartum period.

To the best of our knowledge, even though there are biochemical parameters and literature about peripartum diseases of Holstein cows, there is a dearth of information about the changes in the biochemical profile of mastitis, RFM, and metritis diseases in Simmental cows. Therefore, the purpose of this study was to characterize the relationships between changes in the biochemical profiles of peripartum Simmental cows and to screen biomarkers of disease status in Simmental cows with metritis, mastitis, and RFM.

MATERIALS AND METHODS

Animals and sampling

The study used 4-6 year old multiparous (2nd and 3rd lactation) Simmental cows (n=104) weighing 450-500 kg and was conducted on a livestock farm in Kastamonu (Türkiye). This study was conducted following approval by the Kastamonu University Local Ethics Committee of Animal Experimentation. The cows were diagnosed with metritis according to the criteria of Sheldon *et al.* (3), RFM according to the criteria of Beagley *et al.* (8), and mastitis according to the criteria of Cobirka *et al.* (9). Cows were excluded from the study when there was more than one disease evident. In the study, blood samples were collected from cows with RFM (n=17), clinical mastitis (n=25), metritis (n=21), and postpartum healthy cows (n=21) within 21 days postpartum, and also prepartum healthy cows (n=20) in the 15±5 days before the expected parturition date. The blood samples were collected from the jugular vein into tubes without anticoagulants. The blood samples were centrifuged at 6,000 rpm for 8 minutes, and sera were transferred to the eppendorf tubes. The sera were stored at -20°C until biochemical analysis.

Biochemical analysis

Serum albumin, direct bilirubin, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), aspartate transaminase (AST), the myocardial band of creatine kinase (CK-MB), creatine kinase – N-acetyl-cysteine (CK-NAC), unsaturated iron-binding capacity (UIBC), total protein, urea, creatinine, magnesium, phosphorus, calcium, and glucose concentrations were measured with an automatic biochemistry analyzer (Gesan Chem 200, Italy), in accordance with the manufacturer's guidelines.

Statistical methods

Statistical analyses were performed using SPSS Version 22.0 (Chicago, Illinois, USA). The conformity of the data of the parameters examined in the study to the normal distribution was examined visually (probability graphs and histogram) and using the *Shapiro-Wilk* test. It was determined whether the data showed normal distribution or not. According to the evaluation, it was determined that all the data did not meet the parametric test assumptions, that is, they did not show a normal distribution. Therefore, the Kruskal-Wallis test, which is a nonparametric test, was used for intergroup comparisons for all parameters. Mann-Whitney-U test with Bonferroni correction was also used in the *post-hoc* pairwise analysis ($P<0.05$).

Biomarker profiles were determined using receiver-operator characteristic (ROC) curves. The area under the curve (AUC)>0.90 was considered highly accurate, AUC between 0.70 and 0.90 was considered moderate accuracy, AUC between 0.5 and 0.7 was considered low accuracy, and AUC≤0.5 was considered a chance result. In the evaluation of the area under the curve, it was accepted that the diagnostic value was not statistically significant for parameters with $P>0.05$ (10).

RESULTS

Table 1 and Table 2 show the results of the biochemical profiles. The highest ALP activity was found in the metritis

Table 1: Concentration of biochemical parameters of groups I of Simmental cows

Groups (n)	ALT (U/L)	ALP (U/L)	AST (U/L)	GGT (U/L)	Albumin (g/dL)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)
Prepartum healthy cows (20)	23.50±10.36^{b}	63.75±10.36^{abc}	23.30±1.43^{a}	23.30±1.43^{a}	3.06±0.11	0.07±0.01^{a}	0.14±0.02^{a}
Clinical mastitis (25)	19.42±3.11^{ab}	53.46±7.59^{ab}	90.96±7.49^{ab}	28.86±2.92^{ab}	3.25±0.18	0.21±0.06^{b}	0.27±0.04^{ab}
RFM (17)	12.00±0.01^{a}	73.25±15.08^{bc}	129.25±36.64^{b}	41.83±14.61^{b}	2.88±0.05	0.14±0.02^{ab}	0.40±0.08^{a}
Clinical metritis (21)	18.22±2.07^{ab}	85.18±15.83^{c}	123.02±19.15^{b}	28.18±2.66^{ab}	2.94±0.21	0.16±0.02^{ab}	0.43±0.05^{b}
Postpartum healthy cows (21)	27.23±4.68^{b}	41.66±3.25^{a}	133.92±41.16^{b}	22.90±0.77^{a}	2.87±0.05	0.11±0.02^{ab}	0.30±0.07^{ab}
P	0.001	0.025	0.047	0.009	0.089	0.000	0.000

$^{a, b, c}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

Table 2: Concentration of biochemical parameters of groups II of Simmental cows

Groups (n)	Creatinine (mg/dL)	CK-MB (U/L)	CK-NAC (U/L)	Total protein (g/dL)	UIBC (µg/dL)	Urea (mg/dL)
Prepartum healthy cows (20)	1.38±0.10^{a}	100.15±11.34^{a}	198.10±15.27^{a}	6.39±0.38^{a}	185.35±9.78	37.25±0.91^{a}
Clinical mastitis (25)	1.17±0.05^{a}	112.50±3.17^{a}	148.71±16.01^{a}	7.55±0.17^{b}	183.00±19.21	28.85±3.83a
RFM (17)	1.28±0.048^{a}	155.25±27.85^{b}	457.08±80.91^{b}	6.55±0.11^{a}	214.00±16.48	27.00±1.23^{a}
Clinical metritis (21)	2.29±0.88^{b}	134.54±13.11^{ab}	540.45±157.67^{b}	7.22±0.29^{ab}	151.70±14.03	61.40±17.38^{b}
Postpartum healthy cows (21)	1.24±0.08^{a}	107.14±9.27^{a}	327.80±83.85^{ab}	6.81±0.18^{ab}	180.00±9.64	36.52±1.95^{a}
P	0.047	0.036	0.009	0.004	0.052	0.002

$^{a, b}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

group (85.18±15.83 U/L) compared to postpartum healthy cows (41.66±3.2 U/L) (P<0.05). Compared to the prepartum healthy cows, mastitis, metritis, and RFM groups had higher AST and GGT activities (P<0.05). Alanine aminotransferase activity was the lowest in the RFM group (12.00±0.01 U/L) (P=0.001). The highest direct bilirubin concentration was found in the mastitis group (0.21±0.06 mg/dL) (P=0.000), while total bilirubin was found in the metritis group (0.43±0.05 mg/dL) (P=0.000). Creatine concentration (2.29±0.88 mg/dL) and CK-NAC (540.45±157.6 U/L) activity were found to be higher in the metritis group, while CK-MB (155.25±27.85 U/L) activity was the highest in the RFM group (P<0.05). While the total protein concentration is at its lowest level in the prepartum period (6.39±0.38 g/dL), it increased when mastitis (7.55±0.17 g/dL) occurred (P<0.05). In terms of the urea concentration, it seemed that the metritis group (61.40±17.38 mg/dL) had increased when compared with the other groups (P<0.05). There was no difference between the groups with regard to albumin (P=0.089) and UIBC (P=0.052) concentrations.

Table 3 shows blood mineral constituents and glucose concentrations. Blood calcium concentrations were higher in prepartum (9.83±0.10 mg/dL) and postpartum healthy cows (10.33±0.10 mg/dL), yet mastitis (9.61±0.24 mg/dL), metritis (9.00±0.32 mg/dL), and RFM (9.75±0.21 mg/dL) groups were lower (P<0.05 for all conditions mentioned above). Magnesium (1.86±0.11 mg/dL) and phosphorus (3.81±0.4 mg/dL) concentrations were the lowest in the RFM group, while glucose concentration was the lowest in the postpartum healthy cows (38.95±5.17 mg/dL) (P<0.05).

Receiver-operator characteristic curve analysis results for AST, GGT, and CK-MB activities for metritis, GGT activity for RFM, and CK-MB activity for mastitis are given in Table 4. Receiver-operator characteristic curve analysis values were determined for AST, GGT, and CK-MB activities with P<0.05.

Table 3: Blood mineral substance and glucose concentrations in groups of Simmental cows

Groups (n)	Phosphorus (mg/dL)	Calcium (mg/dL)	Magnesium (mg/dL)	Glucose (mg/dL)
Prepartum healthy cows (20)	5.36±0.21^{c}	9.83±0.10^{bc}	3.08±0.37^{ab}	57.70±5.33^{b}
Clinical mastitis (25)	5.10±0.31^{bc}	9.61±0.24^{b}	2.03±0.29^{a}	64.73±5.29^{b}
RFM (17)	3.81±0.43^{a}	9.75±0.21^{b}	1.86±0.11^{a}	59.16±7.24^{b}
Clinical metritis (21)	5.06±0.55^{bc}	9.00±0.32^{a}	2.85±0.44^{ab}	72.81.± 7.21^{b}
Postpartum healthy cows (21)	4.22±0.20^{ab}	10.33±0.10^{c}	3.95±0.54^{b}	38.95±5.17^{a}
P	0.005	0.001	0.031	0.000

$^{a, b, c}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

Table 4: ROC analysis results of AST, GGT, and CK-MB activities in groups of Simmental cows for metritis, mastitis, and RFM

	Item	Optimized Cutoff	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P
Metritis	AST (U/L)	≥ 89	82	75	0.786	0.009
	GGT (U/L)	≥ 24	73	70	0.736	0.032
	CK-MB (U/L)	≥ 106	100	75	0.786	0.009
RFM	GGT (U/L)	≥ 21	91	55	0.750	0.023
Mastitis	CK-MB (U/L)	≥ 105	93,3	75	0.740	0.016

AUC: Area under the curve

DISCUSSION

Intense septicemia caused by intrauterine infection may damage the liver tissue, causing an increase in the activity of liver enzymes and thus deterioration of liver function (11). Risk factors for uterine infections and mastitis include hypocalcemia, hypomagnesemia, and hepatocellular damage (i.e. increased GGT, ALT, ALP, and AST) (12, 13). Based on the liver function results, it was possible to detect an increase in GGT and AST activities in the metritis group, indicating damage to the liver tissue in the group before the clinical diagnosis of metritis (7). In this study, it was determined that ALP, AST, and GGT activities increased when the disease developed compared to prepartum healthy cows, but ALT activity was lowest in the metritis and RFM groups. Dervishi *et al.* (14), found the activities of GGT and AST to be higher in the metritis group than in the healthy cows. Furthermore, it was stated that ALP, AST, and ALT activities increased in the mastitis group compared to healthy cows. In the same study, it was determined that total protein concentration increased in mastitis cases (15). When mastitis and metritis groups were compared with postpartum healthy cows, it was also found in our study that total protein increased. In another study, it was stated that there was no statistical difference in total protein concentration between the prepartum and during lactation. Since the disease did not develop during late pregnancy, early lactation, and mid-lactation, it was thought that there was no change in total protein concentration (16).

Serum AST concentration showed a significant difference between the groups and was higher in cows with RFM at day 10±4 and 3±1 day relative to calving (P<0.05), but there was no statistical difference at 10±4 and 30±4 days postpartum. Also, it was noted that there were no differences in ALP and ALT activities, glucose, urea, albumin, and phosphorus concentrations between cows with and without RFM (17). Furthermore, in another study, there was no difference in GGT activity and total protein concentration in pre/postpartum blood samples for cows with the RFM (18). Although there is no difference between groups in albumin concentration according to our study, studies have shown that it may be at a lower level in cases where RFM and metritis are present (19, 20). Contrary to our study, it was stated that albumin concentration increased in cows with mastitis (21). The prepartum detection of this increase brought to mind that mastitis can be used for pre-detection (6).

Our study showed that urea and creatine concentrations

increased in the metritis group, but decreased in cows with RFM and mastitis. Paiano *et al.* (7) and Senosy *et al.* (22) found that urea and creatine concentrations decreased in cows with metritis in their studies. On the other hand, when the GGT activity, total protein, creatine, and urea concentrations in the prepartum period are compared with the postpartum period, it is stated that there was no difference (18). It has been found that cows with vaginal discharge in the postpartum period had lower urea and albümin concentrations, while they showed higher serum GGT activity compared to the control group (23).

As for serum calcium concentrations, the findings of the present study showed lower calcium concentrations than postpartum healthy cows when RFM, metritis, or mastitis were present in cows, as determined in previous studies (24, 25). Akar and Yildiz (26) found the calcium level to be low in cows with RFM in their study, but there was no difference in magnesium levels compared to the control group. On the other hand, it was also stated that there was no difference in calcium levels between cows that displayed RFM and those which did not (27). In the present study, phosphorus, calcium, and magnesium levels were found to be the lowest in the RFM group. In addition, a decrease in the level of these minerals was observed in cows with mastitis. In another study conducted on cows with mastitis, it was determined that the above-mentioned minerals decreased (28).

Patbandha *et al.* (29) demonstrated higher sensitivity (75%), specificity (66.67%), and accuracy than Ospina *et al.* (30) for metritis diagnosis using a prepartum blood non-esterified fatty acids threshold value. They reported 37% sensitivity and 80% specificity, respectively. Milk lactose could detect the distinction between infected and non-infected udder quarters in cows with an 81% accuracy (31) and in buffaloes with 83.76% accuracy (32). Also, Pyorala (33) stated that milk lactose could be used to identify the distinction between mastitis and healthy quarters with an accuracy of 73.9 to 77.1%. This study, it is considered that activities of ≥ 89 (U/L) AST, ≥ 24 (U/L) GGT, and ≥ 106 (U/L) CK-MB for metritis, ≥ 21 (U/L) GGT for RFM, and ≥ 105 (U/L) CK-MB for mastitis can be used in the preliminary diagnosis of these diseases in Simmental cows. The overall test performance of these findings is moderate and their practical use for diagnosing these diseases is uncertain. Considering the association of elevated CK-MB of Simmental cows with metritis, CK-MB remains an important tool to test research hypotheses, especially when a more objective parameter for the metritis is needed.

CONCLUSIONS

In conclusion, our results show that there are biochemical changes in Simmental cows with metritis, mastitis, and RFM in the transition period. Regular monitoring of the specified parameters in the postpartum period in dairy cows may be useful for veterinarians and dairy farm owners to predict uterine health and mastitis. To better understand the role of the aforementioned parameters in the pathogenesis of RFM, mastitis, and metritis, further studies with a larger cohort of cows should be conducted with periodic blood samples taken before the onset of these disease symptoms and at different times in Simmental cows.

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