



Research Article

Determination of Serum Paraoxonase Activity, Total Sialic Acid Concentration, and Oxidative Status in Cattle with Clinical Mastitis

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Article History: Received: April 11, 2017 Revised: June 11, 2017 Accepted: June 18, 2017

ABSTRACT

The aim of this study is to determine serum paraoxonase activity (PON1), total sialic acid (TSA) concentration, total oxidant (TOS), antioxidant status (TAS), and oxidative stress index (OSI) in cows with clinical mastitis. Fifty Simmental cows, who were 3 and 5 years old and in early lactation period, were used in the study. Blood samples were taken from the groups (Clinical mastitis group, n=30 - Control group, n=20) and serum PON1, TSA concentration, and TOS and TAS levels were determined. PON1 activity, high density lipoprotein (HDL) and TAS levels were found to decrease inversely proportional to inflammation ($P<0.001$). In addition, TSA, TOS, and OSI levels were detected to increase due to clinical mastitis induced inflammation ($P<0.001$). In conclusion, it was seen that PON1 activity and TSA concentrations could be used for diagnostic purpose in cows with clinical mastitis. However, we are of the opinion that due to the fact that oxidant status increased but antioxidant status tended to decrease because of inflammation in mastitis, it could be useful to administer antioxidants in addition to treatment for such cases.

Key words: Clinical mastitis, Cow, Paraoxonase, Total sialic acid, Total oxidant and antioxidant status, Oxidative stress

INTRODUCTION

Response by mammary glands of udder tissue and ducts and cavities, which enable to store and excrete milk, towards any type of irritating or infectious factors is called as mastitis (Kuru and Oral, 2013; Vural *et al.*, 2016). Mastitis, today, is one of the important diseases that are common in dairy cattle and cause considerable economic loss. Mastitis induced economic loss is due to reasons such as decreased milk yield, unutilized milk, low quality milk, costs of treatment, decreased market price of animals in cases not responding to treatment and accordingly excluding from breeding status (Dingwell *et al.*, 2003; Biggs, 2009). The part of udder with mastitis has rash, swelling, rigidity and might be observed to be sensitive to palpation. Visible abnormalities occur in the mild, as well. Milk may have a purulent or thin consistency and may contain blood by showing a critical change (Philpot and Nickerson, 2000).

Serum paraoxonase (PON1) enzyme co-exists with high density lipoprotein (HDL) in the plasma and is considered to take part in preventing oxidation of plasma

lipoproteins. Because peroxidized lipids are metabolized by this enzyme, the accumulation of lipid peroxides in both HDL and low density lipoprotein (LDL) is inhibited (Aviram and Davies, 2004; Rousselot *et al.*, 1999; Kaya *et al.*, 2016). PON1 is known to be a negative acute phase protein and hepatic synthesis decreases in case of infection (James and Darkin, 2004). Previous studies indicated that the administration of inflammatory mediator (LPS) caused a significant decrease in PON1 activity (Feingold *et al.*, 1998).

Because sialic acids involve in most of biological and pathological events as they are in terminal position of macromolecules and cell membranes, they are one of the most crucial molecules of the life. Sialic acid is also localized in the terminal chain of several acute phase proteins (Haq *et al.*, 1993; Thougard *et al.*, 1998). Sialic acids generally occupy terminal positions of glycoconjugates on oligosaccharide chains and mostly mediate for inflammation and immune response. They also act as a ligand for various receptors (Malykhy *et al.*, 2001). Sialic acids frequently take place in critical cell surface communications and in cases of infection.

Cite This Article as: Deveci HA, Kuru M, Kükürt A, Makav M and Karapehlivan M, 2017. Determination of serum paraoxonase activity, total sialic acid concentration, and oxidative status in cattle with clinical mastitis. Inter J Vet Sci, 6(3): 136-140. www.ijvets.com (©2017 IJVS. All rights reserved)

Determination of total sialic acid (TSA) is a valuable indicator for diagnosis and prognosis of inflammatory disorders (Motoi, 1984).

Cells contain various antioxidants playing an important role in protection against excessive release of reactive oxygen species in blood and tissues (AbdEllaH *et al.*, 2009; Oral *et al.*, 2015). Mastitis may lead the increased formation of free radicals in the milk and the formation oxidative stress in cows during early lactation period (Sordillo *et al.*, 2007; Gu *et al.*, 2009). It was reported that release of free radicals, increase of total oxidant status (TOS), and decrease of total antioxidant status (TAS) may occur in both clinical mastitis and subclinical mastitis (Atakisi *et al.*, 2010).

The aim of this study is to determine serum paraoxonase activity, total sialic acid concentration, and total oxidant and antioxidant status in cows with clinical mastitis during early lactation period.

MATERIALS AND METHODS

Animal material and ration

This study was conducted in a private dairy cattle farm in the city center of Kars, Turkey. Fifty Simmental cows, which were aged between 3 and 5 years, had an increase of 0.25 in body condition score, varied between 2.75-3.25 according to 1-5 point system (Edmonson *et al.* 1989), produced 15-23 liters of milk, and were in early lactation period, were used in the study.

The animals used in the study were fed with corn silage, dry meadow grass, wheat straw, and concentrated feed (barley, corn, bran, sunflower seed meal, calcium carbonate, salt, vitamin-mineral premix) twice a day and water was provided *ad libitum*.

Study Groups

Group 1 (Clinical Mastitis Group, n=30): This group included 30 Simmental cows which were in early lactation period and had no postpartum uterus infection (such as metritis, endometritis, retentio secundinarum) and no clinical problem except for mastitis. Cases associated with the changes occurring in the milk as well as local symptom such as sensitivity in udder tissue, swelling, increased temperature were accepted as clinical mastitis. Changes in milk such as becoming thin, color changes, involving clot or flakon, and blood were checked by using strip cup test (Vural *et al.*, 2016).

Group 2 (Control Group, n=20): Twenty Simmental cows, which were in early lactation period, had no postpartum uterus infection (such as metritis, endometritis, retentio secundinarum) and were clinically healthy, were used in this group.

Blood samples

Blood was drawn into 8.5 mL vacuum tubes with gel containing no anticoagulant (BD Vacutainer®, Becton, Dickinson and Company New Jersey, USA) via sterile holder needles through *vena coccygea* of all cows from Group 1 following the diagnosis of clinical mastitis and all cows from Group 2. Serums were separated by centrifuging blood samples at 3500 rpm for 10 min (Hettich Universal 320®, Hettich, Germany). Serums were kept at -18°C until the measurements.

Biochemical analysis

PON1 activity was measured according to methods of Eckerson *et al.*, (1983) and Gülcü and Gürsu, (2003). PON1 activity was determined by spectrophotometric measurement of absorbance at 25°C and 412 nanometer by colored compound yielded from 4-nitrophenol, the enzymatic hydrolysis product of paraoxone (Sigma®, London, UK) used as substrate. For PON1 activity, enzyme activity of enzyme in 1 mL serum transforming 1 nmol paraoxone into 4-nitrophenole within 1 min was described as unit and results were given in U/L.

HDL was studied in autoanalyzer (Hitachi 917®, Roche, Germany) using a commercial kit (Biotrol®, Biotrol Diagnostic, Fransa) and results were given in mg/dL.

TSA was measured according to colorimetric method by Sydow, (1985) using spectrophotometer (PowerWave XS, BioTek®, USA) and results were given in mg/dL.

TAS was determined by using the automatic measurement method based on decolorization of characteristic color created by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical via antioxidants in sample that was added into medium (Erel, 2004). Results were given in mmol Trolox equivalent/L.

TOS was measured by using the automatic measurement method (Erel, 2005). Oxidants in sample transform ferrous ion complex into ferric ion. Ferric ion (Fe³⁺) occurring by oxidation of iron (Fe²⁺) into its more stable form (Fe₂O₃) creates color reaction with xylenol orange in acidic medium. Intensity of color measured spectrophotometrically is associated with total amount of oxidant molecules found in the sample. Measurement was calibrated with hydrogen peroxide (H₂O₂) and results were given in micromolar H₂O₂ equivalent per liter (µmol H₂O₂ equiv./L).

TOS to TAS ratio was accepted as OSI. Resultant TAS unit was converted into µmol/L and OSI value was calculated according to the formula below: OSI (arbitrary unit) = TOS (µmol H₂O₂ equiv./L) / TAS (µmol Trolox equiv./L).

Statistical analysis

Statistical analysis of the results was carried out by using SPSS® software program (SPSS 18.0, Chicago, IL, USA), Distribution of data in the groups was assessed using Shapiro-Wilk test. Because the data showed parametric distribution, groups were compared by using Independent-Samples T-Test. Correlations between variables were determined by using Pearson's correlation test. The obtained results were expressed in mean ± standard error of mean. P<0.05 was accepted as statistically significant in statistical evaluation.

RESULTS

Measurements showed that PON1 activity and TAS level were affected by clinical mastitis. Both PON1 activity and HDL and TAS levels were determined to decrease inversely proportional to inflammation (P<0.001). However, TSA, TOS, and OSI levels were found to increase because of mastitis induced inflammation (P<0.001). In addition, there were a strong negative correlation (r = -0.721, P<0.01) between OSI and

TAS and a strong positive correlation between OSI and TOS ($r = 0.816$, $P < 0.01$) in mastitis group. Tables 1 and 2 show changes of PON1 activity, HDL, TSA, TAS, TOS and OSI levels in clinical mastitis and control group and correlations between them.

DISCUSSION

Mastitis is one of the primary big problems in dairy cows. Dairy cattle facilities make numerous attempts to prevent economic losses caused by mastitis (Kuru and Oral, 2013; Vural *et al.*, 2016). In recent years, several studies have been conducted in order to reveal diagnostic use of biochemical markers or occurrence of inflammation in mastitis (Deveci and Güven, 2008; Nazifi *et al.*, 2011). This study was also conducted to determine the diagnostic use of serum paraoxonase activity, total sialic acid concentration, total oxidant and antioxidant status in cows with clinical mastitis.

Lipid peroxidation particularly occurring due to oxidative stress may take place in lipids of HDL and LDL, as well. PON1 was reported to protect both LDL and HDL against oxidation. It is suggested that PON1 contributes to antioxidant effect via HDL (Hahn and Subbiah, 1994; La Du 1996). Especially in early lactation period, epithelial cells of udder show high metabolic activity. Conditions like exposure to infectious factors in addition to high metabolic activity may cause a considerable increase in production of free radicals which are released as by-products during many normal cellular reactions (Nispert *et al.*, 2007; Jin *et al.*, 2014; Ozcan and Ogun 2015; Deveci *et al.*, 2017). PON1 and HDL level were observed to decrease in clinical mastitis, which starts with a local inflammation but may transform into a systemic infection, in the present study; this result is compatible with the literature. This indicates that PON1 activity may be a

mediator with antioxidant effect in inflammation induced by mastitis.

Sialic acid in cell membranes has receptor-like functions and potential roles in intercellular interactions. What is the most interesting pathologically is the involvement of sialic acid into bindings sites of bacteria, virus and other infectious agents. Under normal conditions, sialic acid is found in cattle milk. It can be detected at high levels particularly in colostrum and milk of dry period transition. Concentrations of sialic acid were reported to increase in milk of cattle during mastitis (Atroshi *et al.*, 1986). Pathophysiological roles of sialic acid and its relations with other markers in mastitis have not been known exactly (Atroshi *et al.*, 1986; Nazifi *et al.*, 2011). In the present study, serum TSA concentration was found to increase in cattle with clinical mastitis compared to control group ($P < 0.001$). The fact that increased TSA level especially in cases of mastitis which may be induced by infectious agents is an indicator for that it might take part in inflammations. In addition, changes of serum TSA level occurring in clinical mastitis might also be the sign for general systemic symptoms to occur.

Antibacterial activity of polymorphonuclear cells is relatively mediated by reactive oxygen species (ROS). This species shows correlation in the absence of optimal amount of antioxidant and may cause oxidative stress. Some peripartum disorders (udder edema, mastitis, retentio secundinarum) or metabolic disorders may lead development of oxidative stress in cattle (Andrei *et al.*, 2016). It was determined in this study that TAS level was lower and TOS level was higher compared to healthy cows ($P < 0.001$). These results showed that an inflammation originating from udder caused oxidative stress; therefore it could influence serum TAS and TOS levels. Changes of serum TAS and TOS levels in clinical mastitis starting as a local inflammation and correlations

Table 1: Change of PON1 activity, HDL, TSA, TAS, TOS, and OSI levels in cows with clinical mastitis.

Parameters	Group 1 (n=30)	Group 2 (n=20)	P value
PON1 (U/L)	56.71±0.93	104.51±2.49	<0.001
HDL (mg/dL)	45.03±1.04	68.05±1.56	<0.001
TSA (mg/dL)	67.60±0.95	49.35±1.24	<0.001
TAS (mmolTrolox eq/L)	1.24±0.02	1.71±0.03	<0.001
TOS (µmol H ₂ O ₂ eq/L)	9.31±0.17	7.41±0.19	<0.001
OSI (AU)	0.74±0.03	0.44±0.02	<0.001

Group 1: Group with clinical mastitis, Group 2: Control group, PON1: Paraoxonase, HDL: High density lipoprotein, TSA: Total sialic acid, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, AU: Arbitrary unit.

Table 2: Pearson correlation coefficients between variables of clinical mastitis and control groups.

Groups	Parameters	PON	HDL	TSA	TAS	TOS
Group 1 (n=30)	PON1	-	-	-	-	-
	HDL	-0.182	-	-	-	-
	TSA	0.018	-0.024	-	-	-
	TAS	-0.065	-0.091	-0.090	-	-
	TOS	0.097	0.099	0.227	-0.362*	-
	OSI	0.059	0.013	0.236	-0.721**	0.816**
Group 2 (n=20)	PON1	-	-	-	-	-
	HDL	0.026	-	-	-	-
	TSA	-0.300	-0.235	-	-	-
	TAS	-0.296	-0.213	0.102	-	-
	TOS	0.300	0.268	-0.129	-0.981**	-
	OSI	0.321	0.236	-0.101	-0.993**	0.991**

Group 1: Group with clinical mastitis, Group 2: Control group, PON1: Paraoxonase, HDL: High density lipoprotein, TSA: Total sialic acid, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index. *: Correlation is significant at the 0.05 level, **: Correlation is significant at the 0.01 level.

between OSI level and TAS and TOS might also be the indicator for a general systemic response by body against mastitis. In their study, Ibrahim *et al.*, (2016) determined that malondialdehyde (MDA) concentration increased and TAS level decreased in cows with clinical mastitis. They also reported that increased OSI reflecting oxidative stress status indirectly showed all of the free radical activity.

In conclusion, it was observed that PON1 activity and TSA concentrations in cows with clinical mastitis in early lactation period could be used for diagnostic purposes to determine inflammation. It was determined that oxidant status showed an increasing trend and antioxidant status showed a decreasing trend due to inflammation in mastitis. Accordingly, we are of the opinion that applying antioxidant substances in addition to therapy would be useful in mastitis treatment.

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