RESEARCH ARTICLE

Effect of Subclinical Mastitis on Fibrinogen, Orotic Acid and Some Enzymes in the Milk of Dairy Cows

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Abstract

The present study was undertaken to investigate the effect of subclinical mastitis on some indicators of inflammation in milk, and their correlation with somatic cell count (SCC) in cattle. A total of 20 lactating cows positive for subclinical mastitis and equal number of cows negative for subclinical mastitis by both modified California mastitis test (MCMT) and somatic cell count (SCC) were examined for the levels of fibrinogen, orotic acid and some of milk enzymes. Cows having subclinical mastitis were found to have significantly higher levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and aspartate amino transferase (AST) activity in the milk in comparison to the control cows. Fibrinogen and orotic acid levels in subclinical mastitis (SCM) milk were also significantly higher than that of healthy cows. Milk somatic cell count (SCC) showed highest correlation with ALP ($r = 0.83$, $P < 0.01$) followed by LDH ($r = 0.71$, $P < 0.01$) and AST ($r = 0.70$, $P < 0.01$). Fibrinogen, an acute phase protein ($r = 0.79$, $P < 0.01$) and orotic acid ($r = 0.88$, $P < 0.01$), an intermediate metabolite also had significant positive correlation with SCC. Thus, among all 5 indirect indicators of inflammation, ALP could be considered as most economical and sensitive marker of subclinical mastitis. Therefore, it could be used for early detection and control of SCM in dairy herds.

Keywords: Cattle, Enzymes, Fibrinogen, Milk, Orotic acid, Subclinical mastitis.

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Introduction

Mastitis is one of the most important diseases affecting lactating animals leading to huge economic loss to the dairy industry (Aghamohammadi *et al.,* 2018). In India, the annual economic loss due to mastitis has been estimated to be ₹7165.51 crores. Subclinical mastitis has been estimated to account for 57.93 % (4151.16 crore) of the total economic loss due to mastitis (PDADMAS, 2011). Subclinical mastitis continues to be a major problem in many dairy herds despite proper application of proven control methods of teat dipping and dry cow therapy.

Clinical mastitis is easy to detect, but in subclinical mastitis there is no apparent changes in mammary gland and in the milk. For detection of subclinical mastitis, bacteriological sampling is not feasible as a routine test. Therefore, it is necessary to identify quarter with intramammary infection (IMI) through monitoring of inflammatory mediators like fibrinogen, orotic acid and some enzymes in the milk of dairy animals. So, keeping this fact in mind, the present work was undertaken to study the effect of subclinical mastitis on the levels of fibrinogen, orotic acid and some enzymes in the milk of dairy animals.

Materials and Methods

A total of 100 crossbred cows (HF/Jersey) in early stage of lactation belonged to private dairy farms of Durg district were included. Animals were screened for detection of subclinical mastitis (SCM) by modified California mastitis test (MCMT) and somatic cell count (SCC). Out of 100 crossbred cows tested, 28 and 35 animals were positive for subclinical mastitis by MCMT and SCC, respectively. A total of 20 lactating cows positive for subclinical mastitis by both MCMT and SCC were included to study the effect of subclinical mastitis on some indicators of inflammation, *viz.* enzymes, fibrinogen, an acute phase protein (APP) and orotic acid in milk. A total of 20 healthy cows in early stage of lactation negative for SCM by MCMT and SCC were used for comparison.

Enzymes, viz., aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated in milk sera/whey following standard method using diagnostic kit on semi-autoanalyzer (Chemistry analyzer, RA-50) and expressed as U/Liter. Fibrinogen

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concentration in milk sera/whey was estimated by heat precipitation method with slight modification. The amount of fibrinogen in the milk sera was calculated by a ratio of fibrinogen deposit length (AB) to fibrinogen plus milk sera length (AC) multiplied by 100, thus giving results as ml/100 ml (Millar *et al*, 1971). The fibrinogen level in milk sera was expressed as mg/dl.

The orotic acid level in the milk sera/whey was determined by high performance liquid chromatography (HPLC) (Cecil 1100, UK) with a C18 column (25 cm, 3.9 mm ID, 0.25 inch OD, Techopak) and UV detector and expressed as mg/dl. Milk sera of each of the solutions were subjected to HPLC and the area under the peak of orotic acid was recorded. The amount of Orotic acid was calculated in the test material using the regression equation (Fig. 1).

To see the effect of subclinical mastitis on some indicators of inflammation (such as Enzyme, APP, orotic acid) in the milk and control group, 't' test was applied as per method described by Snedecor and Cochran (1994). To see the correlation of SCC with other indicators (such as Enzyme, APP, orotic acid), Karl Pierson's correlation coefficient was estimated as per standard procedure.

Results and Discussion

Effect of SCM on indicators of inflammation

The effects of subclinical mastitis on some enzymes, fibrinogen and orotic acid in milk of dairy cows are presented in Table 1. The mean serum aspartate amino transferase (AST) activity in milk of subclinical mastitis cows was significantly ($P < 0.01$) higher (174.87 ± 10.31 U/L) than that of healthy control (71.84 ± 1.30 U/L). There was more than four fold increase in ALP activity (163.91 ± 10.30 U/L) in milk of cows with subclinical mastitis in comparison to healthy control (34.51±1.47 U/L). The mean LDH activity in milk was also significantly ($P < 0.01$) higher in subclinical mastitis affected animals than healthy control (276.46 ± 9.46 vs. 143.26 ± 4.16 U/L).

The present findings in respect to higher levels of enzymes in the milk were in agreement with the earlier reports (Hiss *et al*., 2007; Zaki *et al.,* 2008; Katsoulos *et al.,* 2010; Mohammadian, 2011). Chagunda *et al*. (2005) developed a statistical model for the detection of mastitis based on LDH activity, which showed 76.5% sensitivity and 97.7% specificity for diagnosing clinical mastitis. The increase in LDH activity in SCM milk might be due to the presence of leukocytes and epithelium cells from the udder. LDH in milk was a sensitive indicator of epithelial cell damage and subsequently LDH originated mainly from the damaged udder epithelial cells and also from the elevated numbers of leucocytes. Significantly increased level of milk ALP in subclinical mastitis was attributed to mammary epithelium damage and breach in blood-milk barrier selectively damaged by bacterial toxins (Katsoulos *et al.,* 2010). The highly significant increase in AST values recorded in milk sera was attributed to stressful condition (Zaki *et al*., 2008). On the other hand, Babei *et al.* (2007) and Gera and Guha (2009) could not record significant alteration in AST activity.

The mean fibrinogen level in the milk of affected quarter was significantly higher (28.01±1.46 mg/dl) than that of normal milk (10.71±0.73 mg/dl). It was in agreement with the report of Tabrizi *et al.* (2008), who also reported significantly increased level of fibrinogen in both subclinical and clinical mastitis milk. Fibrinogen is the coagulation factor I, acute phase protein, and is produced more rapidly than degradation during inflammation. Another important function of fibrinogen is the formation of matrix that enables the movement of fibroblast and other cells and stimulates

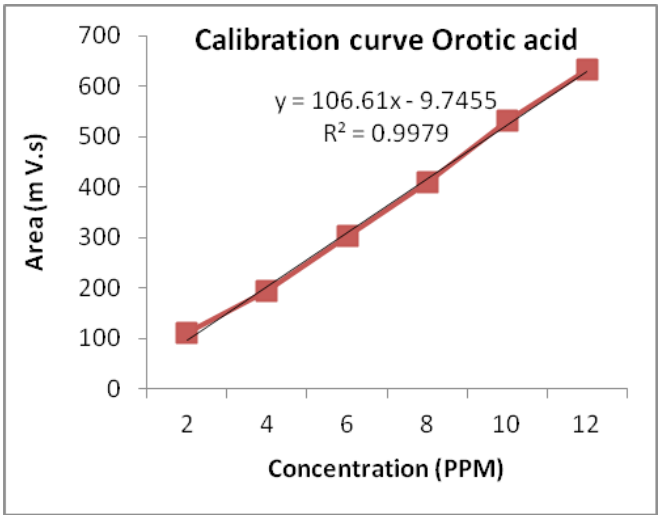

Fig 1: Regression equation for calculating orotic acid concentration in milk

Table 1: Somatic cell count and other indicators of inflammation in normal and SCM milk (Mean ± SE)

Parameters	*Healthy animals (n=20)*	*SCM infected animals (n=20)*
SCC ($\times 10^5$)	1.10 ± 0.11	6.18 ± 0.66**
AST(U/L)	71.84 ± 1.30	174.87 ± 10.31**
ALP (U/L)	34.51 ± 1.47	163.91 ± 10.30**
LDH (U/L)	143.26 ± 4.16	276.46 ± 9.46**
Fibrinogen (mg/dl)	10.71 ± 0.73	28.01 ± 1.46**
Orotic acid (µg/ml)	44.35 ± 2.17	136.90 ± 9.38**

** $P < 0.01$ between healthy and infected animals

Table 2: Correlation Coefficient of milk SCC with other indicators of intramammary infection

Indirect indicators	*Correlation coefficient*
AST (U/L)	r = 0.70**
ALP (U/L)	r = 0.82**
LDH (U/L)	r = 0.71**
Fibrinogen (mg/dl)	r = 0.88**
Orotic acid (µg/ml)	r = 0.79**

n=20, df=18, **P < 0.01,

their production during healing of damaged tissue (Bakes and Illek, 2006). Fibrinogen specially binds to CD11/CD18 integrins on the cell surface of migrated phagocytes, thereby triggering a cascade of intracellular signals that lead to enhancement of degranulation, phagocytosis, antibody-dependent cellular cytotoxicity and delay of apoptosis. So, there is considerable potential for the use of a biological marker, such as an acute phase protein, which is present in milk and can be used routinely and reliably, for the objective and early diagnosis of mastitis. Such a marker could be particularly important for the continued development of robotic milking systems in which the manual examination of milk and cows is not practicable. It might also provide more accurate earlier diagnosis of intramammary infection, reducing the time to treatment and thus possibly reducing the adverse effect of mastitis in both economic and welfare terms.

The mean orotic acid level in subclinical mastitis milk was 136.90±9.38 µg/ml and that of normal milk was 44.35±2.17 µg/ml. Orotic acid (an intermediate metabolic product of pyrimidine biosynthesis pathway) formerly designated as vitamin B13, is a normal constituent of bovine milk and other biological fluids. It plays an important role on stimulation of cell growth, protein biosynthesis, tissue regeneration and wound healing. Furthermore, orotic acid is a carrier for a lot of minerals in the cells and has a strong influence on regeneration of cells as well.

Correlations of SCC with other indicators of inflammation

Correlations of SCC with enzymes, fibrinogen, and orotic acid have been shown in the Table 2. In the present study, SCC showed highest correlation with ALP (r = 0.83, P < 0.01) followed by LDH (r = 0.71, P < 0.01) and AST (r = 0.70, P < 0.01). Similarly SCC showed highly positive correlation with orotic acid (r = 0.88, P < 0.01) and fibrinogen (r = 0.79, P < 0.01) of sub clinical mastitis milk.

Among all 3 enzymes, alkaline phosphatase was found to be the most important marker for detection of subclinical mastitis followed by LDH and AST as reflected by highest correlation with SCC in milk. Present finding of LDH enzyme activity corroborated to earlier report of Chagunda *et al*. (2005), Hiss *et al.* (2007) in respect to correlation with SCC. The present finding was in agreement with the report of Ying *et al*. (2009), who also reported a highly positive correlation between SCC and ALP activity. However, definite comparison regarding correlation of SCC with orotic acid and fibrinogen could not be made due to paucity of literature.

Conclusion

Among all 5 indirect indicators of inflammation, ALP could be considered as most economical and sensitive marker of subclinical mastitis. Therefore, it could be used for early detection and control of SCM in dairy herds.

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