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Assessment of antioxidant profile in subclinical and clinical mastitis in dairy cattle

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Abstract

Mastitis is a major threat to dairy industry which causes significant economic losses globally. The compromised antioxidant defense system is the primary factor responsible for various diseases in cattle including mastitis. The present study was designed to study the lipid peroxidation (LPO), blood urea nitrogen along with the enzymatic activities of Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) in the plasma samples from subclinical and clinical mastitis affected dairy cows. A total of 200 animals was selected for the study, 50 were suffering from subclinical mastitis, 30 from clinical mastitis and 120 were normal healthy cows. Blood plasma was used for biochemical and enzymatic estimation. Our study shows that ALP, LDH and LPO concentration were found significantly higher in subclinical and clinical mastitis than normal healthy cows. There was no significant difference observed in BUN levels. The mean activities of SOD, CAT and GPx were significantly reduced in subclinical and clinical mastitis than normal healthy cows. The study demonstrated that there is a significant reduction in the activities of SOD, CAT, GPx and marked increase in ALP and LDH concentration in both subclinical and clinical mastitis.

Keywords: clinical, subclinical, GPx, CAT, LDH

Introduction

Mastitis is the inflammation of the mammary gland characterized by physical and chemical alteration in the quality of milk and causing significant economic losses in dairy industry all over the world [1]. It not only deteriorates the quality of milk but also leads to the reduction in milk yield, the heavy cost of treatment to the affected animal and premature culling [2]. The etiological agents of mastitis vary from place to place and animal to animal resulting tremendous diversity in causative agents of mastitis [3]. The heavy losses caused by this economic disease in the dairy industry are a major cause of concern [4]. Living cells are continuously exposed to the free radicals and the beauty of the natural antioxidant defense system is that these free radicals, reactive oxygen species (ROS) are neutralized [5]. The imbalance in production and neutralization of ROS leads to severe oxidative stress which in turn affects the cellular metabolism leading to deteriorated health in animals [6]. The oxidative stress is the primary factor leading to immune dysfunction, impairing the inflammatory response which in turn is reflected in inflammatory manifestations particularly the inflammation of the udder [7]. The suppression of immune resistance due to oxidative stress leading to the establishment of different microbial pathogens in mammary gland leading to infection and release of inflammatory substances. The most common markers used for oxidative stress in biological fluids is lipid peroxidation glutathione activity whereas ALP, and LDH is used as general stress markers in the severity of tissue damage. The present study was designed to evaluate the possible oxidative stress and alterations in biochemical profile during clinical and sub clinical mastitis and to investigate if the possible role of antioxidants is feasible to control this major economic disease of dairy animals.

Material and Methods

Experimental Design: The blood (10ml) and milk (5ml) was collected from the jugular vein in 200 animals of different areas of district Rajouri (J&K). Out of these, 50 were diagnosed for subclinical mastitis, 30 for clinical mastitis and 120 were normal healthy on the basis of

physical tests (CMT, PH, electrical conductivity) microscopic tests (somatic cell count) and culture test.

Sample Processing

Anticoagulant containing blood was subjected to centrifugation at 3000g for 10 min at 4°C and plasma buffy coat was removed to harvest the red blood cells (RBC). RBC pellet was diluted by using chilled distilled water (1:10) for the preparation of hemolysate which was used for the estimation of LPO, glutathione peroxidase, catalase activity and SOD activity.

Antioxidant Assay

SOD and CAT activities were measured as per Madesh and Balasubramanian and Bergmeyer respectively [8].

Lipid peroxidation was measured in terms malondialdehyde (MDA) production as per Placer *et al.*, 1966 [9].

Biochemical Assay

The enzyme LDH, ALP and Blood Urea Nitrogen (BUN) were determined by the diagnostic kits (Span diagnostics) as per the manufacturer's protocols.

Statistical Analysis: The data from individual groups are presented as the mean \pm standard error of the mean (SEM). Difference between groups were analysed by using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test and minimum criteria for statistical significance was set at $p \leq 0.05$ for all.

Table 1: Mean \pm SEM activities of LPO, SOD, CAT and GPx in plasma from healthy, subclinical and clinical mastitis.

Parameters	Healthy	Subclinical	Clinical
Lipid peroxidation (nmole MDA/ml)	13.15 \pm 0.32 ^a	18.35 \pm 0.41 ^b	23.27 \pm 0.79 ^c
Superoxide dismutase (μ mol MTT formazan/mg Hb)	0.62 \pm 0.008 ^a	0.49 \pm 0.005 ^b	0.32 \pm 0.002 ^b
Catalase (μ mol H ₂ O decomposed/min/mg Hb)	175 \pm 6.35 ^a	82 \pm 4.41 ^b	65 \pm 2.17 ^c
Glutathione peroxidase (U/ml)	32.17 \pm 2.13 ^c	24.27 \pm 1.65 ^b	14.35 \pm 0.98 ^a

Values with superscripts a, b and c differ significantly ($P < 0.05$) in a row

Table 2: Mean \pm SEM activities of ALP, LDH and urea in plasma from healthy, subclinical and clinical mastitis.

Parameters	Healthy	Subclinical	Clinical
Alkaline phosphatase	45.36 \pm 2.13 ^a	82.74 \pm 3.76 ^b	157.16 \pm 6.38 ^b
Lactate dehydrogenase	122.47 \pm 4.28 ^a	367.89 \pm 7.95 ^b	689.24 \pm 13.25 ^c
Urea	34.36 \pm 2.76	41.75 \pm 4.17	38.93 \pm 3.23

Values with superscripts a, b and c differ significantly ($P < 0.05$) in a row.

Results

Mean CMT point score and SCC recorded in milk of healthy lactating cows were 0.00 \pm 0.00 and 1.78 \pm 0.04 x 10 per ml respectively. Mean CMT point score and SCC recorded in the milk of subclinical mastitic cows were 2.67 \pm 0.13 and 74.12 \pm 1.17 x 10⁵ per ml and mean CMT point score and SCC recorded in milk of clinical mastitic cows were 3.89 \pm 0.12 and 89.12 \pm 2.34 x 10⁵ per ml respectively. Out of 80 cases of subclinical and clinical mastitis, 30 were found positive for *staphylococcus aureus* 20 for *E.coli* and 10 for *Streptococcus agalactiae* and 20 showed no bacterial growth on culture media. The blood antioxidant profile and lipid peroxides in dairy cows with subclinical and clinical mastitis are depicted in table 1. There was significant ($P < 0.05$) decrease in blood SOD, CAT and GPX activities and along with a significant ($P < 0.05$) increase in erythrocytic lipid peroxides in terms of malondialdehyde production in subclinical and clinical mastitic cows. The ALP and L Dactivities were found significantly higher in subclinical and clinical mastitis but there was no significant alteration in urea levels in subclinical and clinical mastitis (table 2).

Discussion

The mammary gland health is determined by assessing the SSC and mean CMT point score. Normal healthy udder milk is having low SSC and CMT point score and showing no abnormal constituents [10]. The somatic cells in milk consist of mammary epithelial cells and leukocytes resulting from injury or infections of the mammary gland. The present study revealed that *Staph aureus* infection is the predominant cause of both subclinical and clinical mastitis. Superoxide dismutase a copper-zinc containing antioxidant enzyme is localized in cytoplasm and leads to dismutation of superoxide to hydrogen

peroxides which in turn is scavenged by catalase and glutathione peroxidases [11]. The current study showed that there was significant reduction in SOD activity in both subclinical and clinical mastitis which might be attributed to the low levels of zinc concentration in the blood [12]. There was a significant decrease in catalase activity in both subclinical and clinical mastitis and our results are as per previous findings of Sharma *et al.*, 2010 [13] in staph aureus infected mastitis. Catalase has exerted protective effects in bovine neutrophil induced model of mammary cellular damage. Lipid peroxidation is the index used to measure cellular membrane damage induced by oxidative stress [12]. Malondialdehyde the intermediate product of lipid peroxidation is being considered as a reliable indicator of oxidative stress [14]. In normal milk there is active participation of free radicals but certain biochemical antioxidant constituents in milk like caseins, lactoferrin which exert their antioxidant effects and checks the oxidative stress. In the present study there was a significant increase in MDA production in clinical mastitis which was in consistent with previous findings [15, 16]. There was an intermediate increase in MDA concentration in subclinical mastitis. The increase in MDA levels in both subclinical and clinical mastitis may be due to excess free radical production from neutrophils in the infected mammary gland which in turn causes peroxidative damage to mammary cells [17]. The significant increase in blood MDA levels in the current study is indicative of involvement mammary cellular damage due to oxidative stress in both clinical and subclinical mastitis. Glutathione peroxidase (GPx) is an antioxidant enzyme which acts by reducing the lipid hydroperoxides and converting free hydrogen peroxide to water. The GPx offers protection against the oxidative alterations in the milk and a reduction in

GPx activity indicates a major shift of the udder health towards the clinical mastitis^[18]. As the LPO was highest in clinical mastitis but the GPx was found higher in subclinical mastitis. This may be attributed to the defense system of the body which are attempting to maintain antioxidant environment constantly in udder. Our findings are in agreement with the previous reports^[19]. The treatment of any clinical condition depends upon the type of pathogens, severity of clinical manifestations, as the antibiotic therapy in mastitis differs for gram gram-positive and gram-negative bacterial pathogens. Blood urea nitrogen is the only reliable biochemical marker used which is positively correlated with the type of bacteria present in mastitis. Cows suffering from gram negative infected mastitis have higher level of blood urea nitrogen as compared to gram positive infected cows^[20]. In the present study higher levels of urea in the blood suggests that prolonged infection by gram negative bacteria realizing endotoxins produce low grade of inflammation in comparison to the gram positive bacteria which mainly releases exotoxins. It has been reported that gram-negative bacteria causes more milk losses as compared with gram positive bacteria^[21]. The LDH is an ideal marker in mammary in determining the mammary gland functions while ALP are being considered as reliable biomarkers in early subclinical mastitis. In the present study the LDH and ALP activities were significantly higher in subclinical mastitis as compared to clinical mastitis and normal healthy cows, our results are in agreement with the previous findings^[22, 23].

Conclusion

The current study revealed that there is a major compromise in antioxidant defense system of the animals suffering from both clinical and subclinical mastitis due to the enormous rise in concentration of reactive oxygen species and it is imperative to supplement antioxidants along with the conventional antibiotic therapy in treating clinical and subclinical mastitis.

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