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Research Article
Relationship Between Serum Biomarkers and Oxidative Stress in Dairy Cattle and Buffaloes with Clinical and Sub-clinical Mastitis

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Abstract

Background and Objective: Mastitis causes significant economic losses in dairy industry globally. The present study evaluated levels of Blood Urea Nitrogen (BUN) and lipid peroxidation (LPO) along with enzymic activities of lactate dehydrogenase (LDH), alkaline phosphatase (ALP)and glutathione peroxidase (GPx) in the serum samples, lipid peroxidation (LPO) and activities of glutathione peroxidase (GPx) in skimmedmilk of dairy animals (cows and buffaloes) showing sub-clinical mastitis (SCM) and clinical mastitis. **Methodology:** A total of 100 lactating animals were divided into two groups i.e., cattle and buffaloes, each group contain 50 animals. Each group is further divided into three subgroups healthy (10), sub-clinical mastitis (20) and clinical mastitis (20). Blood serum and defatted milk were used for enzyme activity estimations. **Results:** The LDH and ALP activities along with LPO levels were significantly higher ($p<0.05$) in SCM and CM the milk as compared to healthy milk from udders. Non significant differences were observed in BUN values. The mean activities of GPx were significantly reduced ($p<0.05$) in SCM and CM milk than in healthy milk. Increased lipid peroxidation in serum and milk indicated direct correlation between oxidative stress and tissue damage in clinical and sub-clinical mastitis in dairy animals. **Conclusion:** From the present study, it may be suggested that optimum antioxidant intake carry sufficient potential in affording protection against sub-clinical and clinical mastitis in the dairy animals.

Key words: Alkaline phosphatase, glutathione peroxidase, lipid peroxidation, oxidative stress, mastitis, sub-clinical mastitis, dairy animals, lactate dehydrogenase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mastitis refers to inflammation of mammary gland and leads to significant economic losses in dairy industry globally[1-3] owing to decrease in milk yield, cost incurred in treatment and care of affected animals, milk with holding after treatment and premature culling[4-7]. Depending upon the climatic conditions, animal species and animal husbandry practices, the etiological agents vary place to place and case to case. As a result, the largest numbers of pathogens (approximately 135) have been reported from a single disease condition[1,8]. Thus, the control and prevention of mastitis is a challenge and despite all efforts, it continues to be a major cause behind the severe economic losses to dairy industry[9].

Production of free radicals is a normal phenomenon in each living tissue. Under conditions of homeostasis, the ROS are effectively neutralized by cellular defense mechanisms (enzymes) or non-enzymatic antioxidants[10]. Oxidative stress causes damage to biological macromolecules and affects the normal metabolism and physiology leading to ill health in animals[11-13]. The oxidative stress is the primary factor leading to dysfunction of immune system, impairing the response to inflammatory conditions, which ultimately lead to various inflammatory manifestations in animals and above all, the inflammation of mammary tissues[14,15]. The reduced resistance to the pathogenic microorganisms leads to infection and further release of inflammatory substances causing inflammation of mammary glands i.e., mastitis.

The first and foremost markers of oxidative stress are alterations in the lipid peroxidation and glutathione in the biological fluids. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) are used as general indicators of the maintenance and severity of tissue damage. The present study was carried out to assess the possible oxidative stress and biochemical alterations involved during clinical and sub-clinical mastitis and to assess if any role of antioxidants is feasible in the control of mastitis in dairy animals.

MATERIALS AND METHODS

Study design and animals: The study was conducted on 100 clinical bovines (cattle and buffaloes) presented to the Teaching Veterinary Clinical Complex (TVCC), U.P. Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, India. Animals in late pregnancy or early lactation were excluded from the study. Animals were divided into two broad groups i.e., cattle and buffaloes, each group contain 50 animals. Each group was further divided into three subgroups; healthy (10), sub-clinical mastitis (20) and clinical mastitis (20).

Collection of samples: Teats for each quarter was scrubbed thoroughly using cotton soaked in 70% ethyl alcohol. Lacteal secretions were collected in sterile test tubes (15 mL) after discarding the first few strips of milk. All samples were kept cool (4°C) during transportation and were processed within 4 h of collection. Additionally, 3 mL of blood were collected by jugular venepuncture and serum was recovered.

Biochemical analysis: Blood serum and defatted milk were used for enzyme activity estimations. The milk samples were skimmed of butter fat by centrifugation at at 5000 g for 15 min at 4°C. The extent of lipid peroxidation (LPO) was estimated in milk and serum samples as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA)[16]. The values of lipid peroxidation were expressed as nano moles (nm) of MDA produced per milliliter. Milk and serum glutathione peroxidase (GPx) activity was determined by diagnostic kit (Sigma-Aldrich, USA) as per the manufacturer's protocol and expressed as U mL^{-1}. The enzyme LDH, ALP and Blood Urea Nitrogen (BUN) were determined by the diagnostic kits (Span diagnostics) as per the manufacturer's protocol.

Statistical analysis: All statistical analyses were performed using SPSS statistical software version 19 (IBM SPSS Statistics 19). One-way ANOVA was used to compare the mean activity of ALP, LDH, Urea, LPO and GPx between the normal milk and samples with sub-clinical and clinical mastitis[17]. The difference was considered statistically significant at p-value of <0.05.

RESULT

Changes in the oxidative stress and antioxidant status in the different clinical groups has been presented in Table 1.

The LDH and ALP activities along with LPO levels were significantly higher ($p<0.05$) in SCM and CM the milk as compared to healthy milk from udders. Non significant differences were observed in BUN values. Glutathione peroxidise (GPx) activity showed significant variations ($p<0.05$) in blood and milk samples (Table 1). The Glutathione peroxidise activity in blood samples from healthy cows has an average of 0.94 ± 0.04 U mL^{-1} while in samples taken from cows diagnosed with sub-clinical mastitis value was 0.70 ± 0.01 U mL^{-1} and mastitis cows 0.49 ± 0.02 U mL^{-1}. Comparative analysis of GPx activity in milk revealed significant differences ($p<0.05$), the mean of this parameter

Table 1: Mean±SEM activities of ALP, LDH, urea, LPO and GPx in serum and milk from normal cows and those with sub-clinical mastitis and clinical mastitis

	Cows			Buffaloes		
Parameters	Healthy (n = 10)	Sub-clinical mastitis (n = 20)	Clinical mastitis (n = 20)	Healthy (n = 10)	Sub-clinical mastitis (n=20)	Clinical mastitis (n = 20)
Serum						
Alkaline phosphatase (IU L^{-1})	49.60±1.00[a]	79.60±5.52[c]	143.42±3.52[c]	51.12±0.80[a]	81.13±2.92[b]	137.64±2.72[c]
Lactate dehydrogenase (IU L^{-1})	129.70±3.44[a]	334.69±26.8[b]	644.62±19.51[c]	139.87±2.90[a]	366.07±17.68[b]	705.76±17.16[c]
Urea (md dL^{-1})	36.20±2.16	39.90±0.97	37.30±1.48	37.80±1.44	39.80±1.46	37.30±1.48
Lipid peroxidation (nM MDA mL^{-1})	14.15±0.69[a]	19.61±0.30[b]	22.10±0.43[c]	23.15±0.56[a]	20.07±0.52[b]	22.10±0.43[c]
Glutathione peroxidase (U mL^{-1})	0.94±0.04[c]	0.70±0.01[b]	0.49±0.02[a]	0.47±0.18[c]	0.73±0.02[b]	0.49±0.02[a]
Milk						
Lipid peroxidation (nM MDA mL^{-1})	23.15±1.06[a]	47.48±0.67[b]	53.54±0.68[c]	24.51±1.06[a]	46.94±0.84[b]	53.69±0.68[c]
Glutathione peroxidase (U mL^{-1})	34.19±1.13[c]	25.78±0.56[b]	17.57±0.63[a]	37.78±0.88[c]	26.46±0.61[b]	19.71±0.39[c]

being lower for mastitis than for normal milks. Increased lipid peroxidation in serum and milk indicated direct correlation between oxidative stress and tissue damage in clinical and sub-clinical mastitis in dairy animals.

DISCUSSION

The health of mammary gland is assessed by the quantity and quality of milk produced. Healthy udder produces milk with low Somatic Cell Count (SCC) and no abnormal appearance such as clots[18]. The somatic cells are the milk-secreting epithelial cells that have been shed from the lining of the mammary glands, while leukocytes are due to injury or infection in mammary glands[15]. The usual changes observed in mastitic milk are the consequences of the secretions of these cells. Lipid oxidation is an autocatalytic process that occurs in food and biological membranes[19]. Malondialdehyde (MDA), lipid peroxidation end product is considered as common and reliable indicator of oxidative stress[20]. Mastitis as well as normal milk production involves active participation of free radicals. Milk and its different fractions bear good antioxidant properties which keep in check the level of oxidative stress that develops during the lactation, least it over rules the defense system and leads to the initiation of mastitic changes. Casein, the milk protein inhibits the formation of peroxide and whey inhibits the formation of copper-catalysed peroxides and oxygen uptake. Lactoferrin present in the milk bind iron and inhibit Fe-induced lipid peroxidation. Even the hydrolysates from milk or fermented milk have been found to be antioxidative and a few of them have been patented. As soon as the free radical outburst goes uncontrolled, the infiltration of polymorphonuclear (PMN) leukocytes and macrophages serve as the first body defences against the initiation of sub-clinical mastitis, leading to production of a reparative inflammatory response. The inflammatory and damaged epithelial cells of the mammary glands secrete hydrolytic enzymes such as LDH and/or β-galactosidase[21] which are indicators of damage in the cell structure. Several earlier studies have evaluated milk LDH and ALP activities to diagnose udder infections in dairy cattle[22,23] and buffaloes[24].

In the present study, there is significant increase in erythrocytic MDA production in the clinically mastitic cows as compared to healthy control which was in agreement with previous studies[25,26], while sub-clinical cases revealed an intermediate value. This might be due to the excessive reactive oxygen species production such as hydroxyl radicals by activated neutrophils from the clinically inflamed mammary gland causing peroxidative damage to membranes[27]. Mastitic milk has high number of polymorphonu clear cells, indicating the oxidative reactions[28] and clinical status of mastitis has been found to be positively associated with malondialdehyde level in milk[29]. The significant increase of lipid peroxidation as revealed by elevated blood and milk LPO/MDA levels in present study clearly indicated the involvement of oxidative stress and the possible oxidative tissue damage in both sub-clinical and clinical mastitis cases in animals.

Glutathione peroxidase (GPx) is enzyme and acts as antioxidant by reducing the lipid hydroperoxides to their corresponding alcohols and also free hydrogen peroxide to water. The GPx protects against the oxidative changes in the milk and a decrease in GPx value indicates a shift of the udder towards the clinical mastitis[30]. While LPO was highest in clinical mastitis cases, intermediate in sub-clinical cases and minimal in healthy animals, GPx was highest in sub-clinical cases in both cows and buffaloes. This might be attributed to the body defenses which were attempting to maintain the antioxidant environment locally in udder as well as systemically. In cows with mastitis, increased serum lipid peroxidation levels and decreased blood glutathione peroxidase levels in comparison to healthy cows was observed, which was in perfect agreement with previous studies[31], indicating an exhausted antioxidant defense system

of the udder. In contrast with findings, an increase in erythrocytic GPx activity in ewes with gangrenous mastitis, which might be attributed to increased requirement of this enzyme to boost the defensive mechanism of the animal against oxidation[32]. Comparatively lower levels of GPx in milk as compared to serum might be due to increased cellular damage in the udder.

The ideal treatment for any clinical condition depends upon pathogen type and severity of clinical signs, as the antibiotic selection differs for gram-positive and gram-negative bacterial mastitis pathogens. Serum urea nitrogen is considered as the only serum biochemical parameter, which is associated with the type of pathogen in mastitis cases. Higher level of serum urea nitrogen level was reported from cows suffering with gram-negative mastitis than in those with gram-positive mastitis[33]. In the present study, the sub-clinical cases showed higher urea level as compared to the clinical mastitis cases, perhaps due to more inclination of gram-negative bacteria to produce lower level of inflammation, slowly releasing their endotoxins in the local milieu in comparison to gram-positive bacteria which mainly possess exotoxins. Gram-negative infection cases have been previously studied to produce more severe milk loss compared with gram-positive bacteria[34]. The mean LDH and ALP activities were significantly higher in sub-clinical mastitis as compared to clinical mastitis and healthy animals and in accordance with the previous studies[22-24,35]. The LDH has been considered as a sensitive indicator of alterations in mammary gland function, while ALP is considered reliable in early sub-clinical mastitis[23,36]. Data on LDH activity due to different pathogens are also scarce. Differences in both severity of mastitis and mastitis pathogens might be associated with differences of oxidative products in infected udders. The presence of this enzyme in the blood serum at levels above normal suggests an increase rate of systemic tissue destruction or remodulation, perhaps including demineralization of bone as mastitis is usually associated with reduced serum calcium levels.

ALP is an enzyme that is naturally found in biological tissues and fluids. The elevated serum levels of this enzyme are suggestive of increased level of inflammatory mediators circulating in the animal body resulting in increased oxidative stress and predisposing the animal to diseases. The increase in ALP concentration in mastitis animals may also be linked with the degree of tissue damage occurring in mammary tissue[35].

CONCLUSION

In conclusion, the present study showed significance increase in level of LPO and activity of GPx enzyme in milk and blood serum of cows and buffaloes with mastitis compared to the healthy control animals indicating severe oxidative stress in the clinical animals. Higher ALP and LDH activities observed from SCM and CM animals also indicate tissue damage. The study offers a view that reducing oxidative stress in dairy animals or increasing dietary antioxidants might serve as a useful prophylactic and therapeutic measure to minimize the productivity losses taking place owing to mastitis.

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