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Malondialdehyde and glucose-6-phosphate dehydrogenase levels in healthy and subclinical mastitic cows

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Abstract: This study was designed to determine the relationship between subclinical mastitis and oxidative stress by measuring blood and milk malondialdehyde (MDA), and blood glucose 6 phosphate dehydrogenase (G6PD) levels in normal and subclinical mastitic cows. Twenty one healthy and 29 subclinical mastitic cows were used. It was found that blood MDA concentrations of mastitic cows were significantly higher ($P<0.01$) than those of healthy cows. It was also found that other parameters such as, milk MDA in healthy, infected and uninfected quarters of mastitic cows and their blood G6PD level were not significantly different. We think that increased MDA level promotes an oxidative stress-induced damage in circulating fluid and cells. Thus, supplementation with antioxidant added rations and antioxidant drugs might be useful for prevention of healthy herds or for minimization of oxidative damage of the cows with subclinical mastitis.

Key Words: Subclinical mastitis, Malondialdehyde, G6PD, Antioxidants.

Sağlıklı ve subklinik mastitisli ineklerde malondialdehit ve glukoz-6-fosfat dehidrogenaz seviyeleri

Özet: Bu çalışma, subklinik mastitis olgularında malondialdehit (MDA) ve Glikoz .6. Fosfat Dehidrogenaz (G6PD)'ın süt ve kan sıvısındaki konsantrasyonu aracılığıyla ineklerdeki oksidatif durumunu belirlemek amacıyla gerçekleştirilmiştir. Araştırma kapsamında 21 sağlıklı 29 subklinik mastitisli inekten alınan kan ve süt örnekleri çalışılmıştır. Kan MDA düzeyi subklinik mastitisli hayvanlarda kontrollerden $P<0.01$ düzeyinde daha yüksek bulunmuştur. Süt MDA ve kan G6PD değerleri gibi diğer parametreler ise, istatistiksel önemde bir değişim göstermemiştir. Bu tablo kanda yoğun bir oksidatif stresin varlığını ortaya koymaktadır. Sonuç olarak; antioksidanlarca desteklenmiş rasyonlar ve antioksidan terapi uygulamaları sağlıklı sürülerin korunması ve subklinik mastitisli hayvanlarda oluşabilen oksidatif hasarın azaltılmasında koruyucu hekimlik açısından yararlı olabilir.

Anahtar Kelimeler: Subklinik mastitis, Malondialdehit, G6PD, Antioksidanlar.

INTRODUCTION

Clinical and subclinical mastitis is the most commonly reported and quite probably the costliest disease affecting dairy cattle (1). The economic costs of mastitis and disturbing effect of oxidative stress indicate that the urgent need for better understanding of the disease, free radicals and antioxidants. The acceptance of such an approach will bring in improvements in the scientific, medical and financial well-being of dairy cattle enterprises (1-3). In recent years, much attention has been paid to the role of radical oxygen metabolites and antioxidants in mastitis and milk production. This attention has largely focused on the increase in free radicals generated during the last days of prepartum and postpartum period and the inadequacy in antioxidants (4).

This is an observational study involving a single herd. The purpose of the study is to determine the effect of subclinical mastitis on blood and milk concentrations of malondialdehyde (MDA), an end product of lipid peroxidation, and on glucose 6 phosphate dehydrogenase (G6PD) which is a ubiquitous antioxidant enzyme and detected in all organisms from bacteria to higher animals, as well as in all cell type of multi cellular organisms (5).

MATERIALS AND METHODS

In this study, 21 healthy and 29 subclinical mastitic cows kept under standard dairy husbandry conditions were used. Each cow with subclinical mastitis was identified by microbiological production

control and California Mastitis Test. Blood samples were drawn from jugular vein in to vacutainer tubes containing EDTA and milk samples were collected by the same tubes from healthy animals and from healthy and mastitic quarters of subclinical mastitic cows. Milk samples were submitted for bacteriologic culture and bacteria are identified at laboratory of Microbiology Department, using standard protocols.

Blood and milk MDA and blood G6PD assays were performed within 2 hours of sample collection. MDA is estimated by the double heating method of Draper and Hadley (6). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml blood and to milk sample and placed in 90 °C water bath for 15 minutes. After cooling, the mixture was centrifuged at 1000 rpm for 10 minutes, and 1 ml of the supernatant was added to 0.5 ml of 6.7 g/l TBA solution in a test tube and placed in 90 °C water bath for 15 minutes again. The solution was cooled in water and its absorbance was measured by using the Shimadzu UV 1601 spectrophotometer at 532 nm. G6PD determination was made with commercial kits Randoks, Lot no: PD410 4-98 in fresh blood specimens by spectrophotometer.

Student's t test is used for statistical analyses.

RESULTS

Some characteristics of the animals are shown in Table 1. Statistical values of MDA and G6PD in milk and in venous blood of normal and subclinical mastitic cows are shown in Table 2. Blood G6PD activity of normal cows was lower, but not significantly different from blood of subclinical mastitic animals. However, the MDA level in blood of subclinical mastitic cows was significantly higher ($P<0.001$) from that of normal cows. Milk MDA concentration did not change significantly between normal and subclinical mastitic cows; between affected and normal quarter milk samples of same animals; and between milk of normal cows and bacteria identified samples of subclinical mastitic cattle.

Table 1. Some characteristics of the study material.

Criteria	Characteristic of subjects
Animal number	21 normal, 29 subclinical mastitic cows
Bacteriological status	Microbiological production was seen in 9 of 29 cows
CMT results	4 quarters (+), 10 quarters (++), 15 quarters (+++)
Housing	Single herd, standard dairy husbandry conditions

Table 2. MDA, G6PD concentrations in milk and venous blood of normal and subclinical mastitic cows. Data is expressed as mean ± standard deviation.

Groups	MDA (nmol/ml)	G6PD (μU/ml)
Healthy cows blood (control)	3.797 ± 0.385	0.730 ± 0.089
Mastitic cows blood	6.694 ± 0.595*	0.765 ± 0.098
Healthy cows milk (control)	4.198 ± 0.556	-
Mastitic cows milk	4.082 ± 0.469	-
Mastitic cows (normal quarter)	4.313 ± 0.510	-
Mastitic cows (mastitic quarter)	4.204 ± 0.708	-
Quarters with bacteriologic production	3.948 ± 0.301	-
Mastitic quarters no bacteriologic production	4.170 ± 0.744	-

* Value is significantly different ($P < 0.001$)

DISCUSSION AND CONCLUSION

Subclinical infections of bovine udder are usually chronic because they do not show any clinical signals (7). Neutrophils try to kill bacteria commonly by oxidative method. When fagositic cells is stimulated, there is a coincident increase in oxygen consumption and production of radical oxygen metabolites such as superoxide resulting from activation of NADPH oxidase which forms an electron transport chain converting molecular O_2 to superoxide (8). Increasing blood MDA observed in this study can be an indicator of oxidative stress in subclinical mastitis.

Inflammation of the mammary gland results in a dramatic reduction in the O_2 concentration of milk to a level < 10 % of that in normal milk. This fall in O_2 level is probably caused by an increased demand for O_2 in the udder owing to O_2 utilization by the large numbers of neutrophils (9). In this study, milk MDA levels in normal and mastitic cows are similar and at normal stages. This result supports the mentioned idea of Mayer et all. It implies that mastitis induced reactive oxygen metabolites increases blood lipid peroxidation because of the increase in O_2 concentration. Investigation of the mastitis induced oxidative stress by measuring milk samples may not help to learn the real status.

G6PD is an important cytoplasmic antioxidant enzyme that effects the production of the reduced form of extramitochondrial nicotinamide-adenosine dinucleotide phosphate coenzyme (NADPH) by controlling the step from glucose 6 phosphate to 6 phosphogluconate in the pentose phosphate pathway. In blood cells, antioxidant defense against oxidative

damage is heavily dependent on G6PD activity (10). Activity of the NADPH also causes a reduction in oxygen radical production in human and bovine (11). In this study, G6PD level of normal cows was lower from mastitic cattle, but this difference was not statistically important. To obtain more remarkable results about antioxidant enzyme status in subclinical mastitis, similar studies can be done.

In conclusion, prevention of mastitis is paramount. Oxidative stress markers and antioxidants like MDA and G6PD, easily measured parameters, are important factors to monitor and to overcome the disease. It was thought that increased MDA level promotes oxidative stress induced damage in circulating fluid and cells. If so, then augmentation of antioxidant defense system through dietary and therapeutically supplementation might be useful for protection of healthy cows or for minimization of oxidative damage in mastitic cows.

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