Assiut Vet. Med. J. Vol. 49 No. 98, July 2003

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STUDIES ON SOME BIOCHEMICAL AND HEMATOLOGICAL CHANGES IN CATTLE MASTITIS

(With 5 Tables)

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(Received at 24/6/2003)

دراسات عن بعض التغيرات البيوكيميائية والدموية في حالات التهاب الضرع في الماشية

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أجريت هذه الدراسة على ١٥ بقرة حلوب من السلالات المصرية البلدية. ثماني بقرات منهن كن في حالة صحية جيدة وكانت نتيجة اختبار كاليفورنيا لالتهاب الضرع والذي اجري على عينات ألبان مأخوذة من جميع أرباع كل ضرع لكل بقرة من هذه الأبقار الثماني سلبية، استخدمت هذه الحيوانات كمجموعه قياسية ضابطة. باقي الحيوانات (سبع بقرات) عانت من التهابات في الضرع، شملت الالتهابات الربعين الخلفيين في ثلاث بقرات وجميع أرباع الضرع في أربع بقرات. استهدفت الدراسة معرفة بعض التغيرات البيوكيميائية والدموية المصاحبة لالتهاب الضرع في الأبقار ، وكذلك معرفة تأثير التهاب الضرع على العوامل المؤكسدة لخلايا الدم الحمراء بالجسم وذلك من خلال قياس إنزيم الجلوكوز ٦ فوسفات ديهيدروجينيز وكذلك إنزيم الجلوتثيون بيروكسيديز المتواجدين داخل خلايا الدم الحمراء لهذه الحيوانات. تم أيضا قياس مستويات البروتين الكلى والالبيومين والبليروبين وإنزيمات الاسبرتيت امينوترانسفيريز وإلجاماجلوتميل ترانسفيريز والجلوتامات ديهيدروجينيز والادينوزين دى امينيز في أمصال دم هذه الأبقار. أوضحت الدراسة وجود انخفاض معنوي في العدد الكلى لخلايا الدم الحمراء وكذلك إنزيم الجلوتثيون بيروكسيديز المتواجد بها، كما انخفض أيضا مستوى الهيموجلوبين في الأبقار التي عانت من التهابات الضرع. يرجع هذا الانخفاض إلى أن إصابة نسيج الضرع وحدوث التهابات به، قد أدى إلي انطلاق كميات هائلة من العوامل المؤكسدة التي تسببت في تكسير الغشاء البلازمى لخلايا الدم الحمراء وكذلك تكسير الهيموجلوبين بها، مما ترتب علية حدوث نقص في العدد الكلي لخلايا الدم الحمراء وكذلك مستوى الهيموجلوبين وحدوث الأنيميا (فقر الدم). أوضحت الدراسة أيضا وجود زيادة معنوية في مستويات البروتين الكلى والجلوبيولين والبليروبين وكذلك مستوى أنزيم الاسبرتيت امنيوترانسفيريز في أمصال دم الأبقار اللاتي عانت من التهاب الضرع ، كما أوضحت أن المواد المؤكسدة ازدادت داخل خلايا الدم الحمراء لهذه الحيوانات، مما ترتب عليه حدوث نقص في مستوى الجلوتثيون المضاد للعوامل المؤكسدة بهذه الخلايا.

SUMMARY

A total number of 15 cows (Egyptian balady native breed cows) were subjected to this study. 8 cows of them were clinically healthy, of good condition and California mastitis test applied on milk samples collected from each quarter of the udder of each one of these cows revealed negative results for mastitis. These cows were kept as control. The rest (7 cows) were found suffer from mastitis (two hind quarters were inflamed in the udder of 3 cows however the four quarters were inflamed in the other 4 cows). The study aimed to investigate the effect of mastitis on erythrocyte oxidative status through measuring the erythrocytic glucose 6-phosphate dehydrogenase (G6PD) and glutathione peroxidase (GSH-Px) activities. Also, studying the hematological changes associating cattle mastitis as well as the changes in serum levels of total protein, its fractions, total bilirubin and the changes in the activities of aspartate amino transferase (AST), gamma glutamyl transferase (γ-GT), adenosine deaminase (ADA) and glutamate dehydrogenase (GLDH). A significant decreases in the erythrocyte GSH-Px activity; total erythrocyte counts and hemoglobin concentration were found in cows suffering mastitis. The infection of udder tissues and its inflammation lead to release of voluminous amounts of oxyradicals (free radical and oxygen reactive species). Large amounts of GSH-Px and other antioxidants were consumed to neutralize these released free radicals and this may result in lowering their level in the body. The released oxyradicals resulted in denaturation of hemoglobin and disruption of red blood cell membrane, and the development of anemia. The results also showed significant increase in the blood serum levels of total protein, globulin, total bilirubin and AST activity.

Key words: *Mastitis, GSH-Px, G6PD.*

INTRODUCTION

Mastitis is one of the most significant health problems of dairy herds, together with lameness and fertility problems (Kossaibati and Esslemont, 1997). Public interest in the welfare of animal production and the recognition of mastitis as a major source of pain and stress for affected cows give added focus to mastitis concerns (Fitzpatrick *et al.*, 1998). Reactive oxygen metabolites [(Hydoxyl (OH^-), hydrogen peroxide

(H_2O_2), hypochlorus acid (HOCL)] are generated during normal metabolism and metabolism stimulated by xenobiotics. These oxyradicals can enter into reactions that, when uncontrolled, can impair performance of dairy cows. Direct effects include peroxidative changes in membranes and other cellular components. It was reported that oxidative stress are involved in the etiologies of certain disorders of dairy cattle as mastitis, retained placenta and udder edema (Miller *et al.*, 1993).

Glutathione peroxidase (GSH-Px) and glucose 6-phosphate dehydrogenase (G6PD) are the key enzymes involved in protection of the cells from oxidative damage. GSH-Px prevents cellular injury by removing highly reactive peroxides produced in various oxidative processes (Khan *et al.*, 1987). Significant negative correlation were found between the prevalence of intramammary infection with major pathogens and mean herd activity of GSH-Px (Erskine *et al.*, 1987). It was reported that a decrease in GSH-Px activity is caused by an increase in the levels of the reactive oxygen species (Atroshi *et al.*, 1989 and Atroshi *et al.*, 1996).

The present study aimed to investigate the effect of cow's mastitis on erythrocyte oxidative status through measuring the activities of erythrocytic glucose 6-phosphate dehydrogenase (G6PD) and glutathione peroxidase (GSH-Px). Also, studying the hematological changes associating cattle mastitis, as well as the changes in blood serum total protein and its fractions, total bilirubin and the changes in the activities of aspartate amino transferase (AST), gamma glutamyl transferase (γ-GT), adenosine deaminase (ADA) and glutamate dehydrogenase (GLDH).

MATERIALS and METHODS

1- Animals:

A total number of 15 cows (Egyptian balady native breed) were used in this study. 8 cows were clinically healthy, their body conditions were very good and California mastitis test applied on milk samples of each quarter of their udders revealed negative results. These cows were kept as control. The other 7 cows were suffering from mastitis. 2 hindquarters of the udder in 3 cows and the 4 quarters of the udder in 4 cows were enlarged (swollen), hot, reddish and painful (clinical mastitis).

2- Samples and adopted methods:

A) Whole blood samples (about 8 ml), with anticoagulant (disodium salts of EDTA) were collected from each animal in dry clean tubes and divided into two parts.

1. About 2 ml whole blood was used for complete blood picture according to Coles (1986).
2. About 6 ml whole blood samples were kept directly in cold container containing some ice pieces and were used for making the hemolysate used for the estimation of erythrocyte GSH-Px and G6PD according to the following steps:
3. Blood samples were centrifuged at 3500 rpm for 15 min at 4 C.
4. Directly after centrifugation, the plasma and buffy coat were drawn off.
5. The packed cells were washed one with ten volumes of cold saline.
6. The packed erythrocytes were divided into two parts in sterile ependorff tubes.
7. One part for determination of erythrocyte GSH-Px activity; the RBCs were hemolysed by adding 4 volumes of cold deionized water.
8. The second part was used for determination of the activity of erythrocytes G6PD (RBCs were hemolysed by mixing 0.05 ml of the washed cell suspension with 0.5 ml of lysing solution (0.02 % digitonin containing NADP, 15 µmol/l; dissolve 16 mg digitonin in 80 ml deionized water, filter (Whatman # 1), then addition of 1 mg NADP) and then the hemolysates were stored at –70 ;C till subjected to analysis.

Intracellular erythrocyte G6PD (U/g Hb.) activity was measured spectrophotometerically according to the method described by Deutsch (1983). Intracellular erythrocyte GSH-Px (U/ g Hb.) activity were determined by using test kits supplied by sigma - Aldrich, according to the method described by Paglia and Valentine (1967)

B) Whole blood samples without anticoagulants (about 5 ml), were collected for obtaining blood serum samples for the determination of blood serum levels f total protein, albumin, total bilirubin levels and serum activities of aspartate amino transferase (AST), adenosine deaminase (ADA), glutamate dehydrogenase (GLDH) and γ-Glutamyl transferase (γ-GT)) by using test kits supplied by Boehringer Manheim GmBH diagnostica.

Statistical analysis:

Statistical analysis of the obtained data were done by means of Software computer program (SPSSWIN, 1995).

RESULTS

Hematological results showed insignificant decrease in total leucocytes counts and significant decrease in total red blood cell (RBCs, T/l), hemoglobin concentration (Hb, g/l) and PCV percent. Also there were an insignificant change in the MCV, MCH and MCHC (Tables 1 and 2).

Table 1: Mean and standard deviation values of total leucocytes counts (WBC, G/l), total erythrocyte counts (RBC, T/l) and hemoglobin concentration (Hb., g/l) in control and diseased cattle.

Animal group	WBC, G/l	RBC, T/l	Hb., g/l
Control (N = 8)	8.3 ± 2.47	7.94 ± 1.01	12.02 ± 1.09
Mastitis (N = 7)	5.96 ± 1.51 ^{NS}	5.33 ± 0.80**	8.96 ± 1.02**

N: Number of investigated animals. NS: Non-significant.
*: Significant (P< 0.05) **: Highly significant (P< 0.01).

Table 2: Mean and standard deviation values of PCV, MCV, MCH and MCHC in control and diseased cattle.

Animal group	PCV, %	MCV, pg	MC, fl	MCHC, %
Control (N = 8)	39.34 ± 2.42	50.11 ± 3.55	15.24 ± 1.64	30.58 ± 2.43
Mastitis (N = 7)	29.22 ± 2.77**	54.98 ± 3.92 ^{NS}	16.92 ± 1.52 ^{NS}	30.62 ± 1.15 ^{NS}

N: Number of investigated animals. NS: Non-significant.
*: Significant (P< 0.05) **: Highly significant (P< 0.01).

Biochemical investigations revealed significant increases in blood serum levels of total protein, albumin and globulin in mastitic cattle (Table 3). Serum enzymes activities showed significant increase in AST activity without significant alteration in γ-GT, GLDH and ADA activity as shown in Table (4).

Erythrocyte's GSH-Px activity showed significant decreases in cases of mastitic cattle however erythrocyte's G6PD activity revealed insignificant changes (Table 5).

Table 3: Mean and standard deviation values of blood serum levels of total protein and its fractions in control and diseased cattle.

Animal group	Total Protein (g/l)	Albumin (g/l)	Globulin (g/l)	A/G ratio (%)
Control (N = 8)	64.30 ± 3.80	39.10 ± 4.90	25.20 ± 4.90	1.6 ± 0.5
Mastitis (N = 7)	7 2.88 ± 4.49**	29.84 ± 3.61**	43.04 ± 7.43**	0.72 ± 0.21**

N: Number of investigated animals. NS: Non-significant.
*: Significant (P< 0.05) **: Highly significant (P< 0.01).

Table 4: Mean and standard deviation values of blood serum AST, γ-GT, GLDH and ADA activities in control and diseased cattle.

Animal group	AST (U/L)	γ-GT (U/L)	GLDH (U/L)	ADA (U/L)
Control (N = 8)	59.56 ± 13.98	23.74 ± 5.37	16.12 ± 8.44	4.25 ± 1.79
Mastitis (N = 7)	94.2 ± 24.69**	21.84 ± 7.21 NS	8.75 ± 2.45 NS	5.4 ± 2.16 NS

N: Number of investigated animals. NS: Non-significant.
*: Significant (P< 0.05) **: Highly significant (P< 0.01).

Table 5: Mean and standard deviation values of; the activities of erythrocyte's G6PD and GSH-Px as well as the serum concentration level of total bilirubin in control and diseased cattle.

Animal group	G6PDH (U/g Hb)	GSH-Px (U/g Hb)	Total bilirubin (µmol/L)
Control (N = 8)	7.58 ± 1.28	172.48 ± 30.66	4.25 ± 1.79
Mastitis (N = 7)	6.51 ± 1.82 NS	117.14 ± 35.06**	6.39 ± 0.67*

N: Number of investigated animals. NS: Non-significant.
*: Significant (P< 0.05) **: Highly significant (P< 0.01).

DISCUSSION

Mastitis in dairy cattle is considered one of the potent infectious stress factors that are associated with the release of voluminous amounts of oxyradicals. The incidence and severity of both clinical and sub clinical mastitis in dairy herds are greatly influenced by the level of antioxidants in the body specially selenium and or vitamin E (Smith *et al.*, 1984, Ivandijia, 1987 and Jerry, 1994). Selenium is essential component of the selenium dependent GSH-Px (Koller *et al.*, 1984). Each molecule of GSH-Px contains four atoms of selenium (Willson and Judson, 1976). In addition, there is a positive correlation between blood

GSH-Px activity and selenium level, indicating that its activity can be used to asses blood selenium level in cattle (Koller *et al.*, 1984, Richard and Lawrence, 1979).

In the present study the significant decrease in erythrocyte GSH-Px activity may be attributed either to the deficiency of selenium or to increased oxyradicals (free radicals and reactive oxygen species) levels. Previous studies reported insignificant change in the blood selenium level associated with significant decrease in the erythrocyte GSH-Px activity in mastitic cattle (Erskine *et al.*, 1987 and Parantainen *et al.*, 1987). Another study reported that nutritional selenium deficiency is related to increased incidence and severity of mastitis (Hogan *et al.*, 1993). It was reported that the significant decrease in erythrocyte GSH-Px activity might be due to increase the reactive oxygen species in the erythrocytes (Atroshi *et al.*, 1989 and Atroshi *et al.*, 1996) and also, that there were significant negative correlations between the prevalence of intramammary infection and mean herd activity of blood GSH-Px activity (Erskine *et al.*, 1987). This finding is in agreement with previous studies regarding the decreased GSH-Px activity in cases of cattle mastitis. GSH-Px prevents cellular injury by removing highly reactive peroxides produced in various oxidative processes (Khan *et al.*, 1987).

The occurring anemia in mastitic cows in the present study may be attributed to the increased reactive oxygen species especially H_2O_2, which resulted in accumulation of hydrogen peroxide and caused oxidation of the sulfhydryl groups of the globulin chains. The erythrocyte cell membrane may be damaged resulting in the removal of the erythrocyte from circulation (Robbins and Kumar, 1994). Furthermore the significant increase in the total bilirubin levels may be attributed to the decreased life spans of the erythrocytes.

The biochemical investigations in the present study revealed also significant elevation in the blood serum levels of total protein, albumin, globulin and the activities of asparate aminotransferase in cows suffering mastitis, on the other hand the activities of γ-GT, GLDH and ADA were not altered in these diseased cases. It was reported that globulin levels significantly increased as a response to inflammatory state (Joan, 1982). This increase resulted in significant increase in the total protein concentration. Previous studies reported a significant decrease in albumin levels, a significant increase in AST activity and insignificant changes in γ-GT activity in cases of mastitic cow (Kathlom and Anderson, 1992).

It could be concluded that; the decrease in GSH-Px activity in the erythrocyte in mastitic cattle may lead to an increase in the reactive oxygen species levels and decreased antioxidant status that resulted in denaturation of hemoglobin and disruption of red cell membrane, which resulted in decreased life span of the erythrocytes and a state of anemia. Because the reactive oxygen species increased in the erythrocytes in cases of mastitis, it is essential to supply mastitic cattle and high producing dairy cattle with sufficient quantity of antioxidants.

ACKNOWLEDGMENT

Great thanks to Dr. M.M. Anour, Assistant professor of physiology, Faculty of Med., Assiut University, for his help and giving me the facilities to use the laboratories of the department of physiology.

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