Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Clinical, hematological and serological study of sub-clinical mastitis in local cows in Basrah Province

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(Received 24/03/2019, Accepted 28/05/2019)

Abstract

The present study was conducted to investigate the effect of subclinical mastitis on biochemical, hematological and minerals profile in local cows. For this reason, 91 cows milk and blood samples were collected from sub-clinically infected cattle, were aged from 3-7 years in Basra/Iraq(between January 2015 to July 2015). All the cows selected in this study were healthy without any clinical signs. The result of present study revealed that there is change in physical properties of subclinical mastitis milk such as color, odor, turbidity, consistency and increase in the pH of milk, ranged from 7.07± 0.02 to 7.9 ± 0.02. The study showed a significant increase of serum aspartate aminotransferase (AST) 217.4 ± 1.8 l/l, alanine aminotransferase (ALT) 136.0 ± 0.2 mmol/l, alkaline phosphatase (ALP) 120.5± 0.9 mmol/l) and lactate dehydrogenase (LDH) 816. 0 ± 3.4.and increase the zinc level compared with control 1.26± 0.03. Also there was decrease in monocyte count 3.52 ± 0.3 and 5.9 ± 0.1 from control 11.4 ± 0.3,the basophile count reveal there was slight increase in stage 1 of subclinical mastitis 2.99 ± and stage 2 retained to normal 1.2 ±0.05. The present concluded that cows were causes change in physical properties of subclinical mastitis milk, changes in the biochemical, minerals and leukocyte count.

Keywords: Biochemical, Hematology, Mastitis, Minerals, Subclinical mastitis, Serological,

Introduction

Subclinical mastitis is the one that does not make obvious changes in the milk and/or the udder, but milk yield is reduced, and there are increases in the number of somatic cells, the pathogens are presents in the secretion, and the composition of milk is change (1). Subclinical mastitis is a disease by dairy farmers even though it causes an economic loss to the dairy industry. It cause disruption of the blood-milk barrier in addition to decreased production and secretion from udder epithelial cells which causes a change in milk composition, in mastitis cows the quantification of cells in milk or somatic cell count (SCC), is predestined and evaluate through using the direct microscopic analysis method or by an indirect method of evaluate and interpret the SCC using the California mastitis test (CMT) (2). The CMT is formerly used on-farm to diagnose indirectly subclinical mastitis (SCM) for dairy Cows. The sensitivity and specificity of the California Mastitis test reported in the manuscripts is variable (3). Both clinical and sub-clinical mastitis can affect the composition and characteristics of milk in the dairy cattle population, (4 and 5). Subclinical mastitis can be detected by monitoring some biochemical parameters such as Na, Cl, K, Ca, Mg, albumin and lactose in milk and identification of pathogenic factors and somatic cell count (6,7). The lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) changes have been used as an indicator of sub-clinical mastitis in dairy cows (2). The present study aimed to assess the relationship between action of

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some enzyme, mineral concentrations, and leukocyte count change with subclinical mastitis.

Materials and Methods

Ethical approval

The Ethical Committee of Veterinary Medicine College, University of Basrah, Iraq, has approved the present study.

Collection of milk and blood samples:

A total of 91 cows milk and blood samples were collected from sub-clinically infected cattle during the period from January 2015 to July 2015. The animal's aged between three to seven years old. All the cattle selected in this study were apparently healthy without any clinical illness and palpable udder lesions. Before collection of milk the udder and teat ends of each animal washed using sterile water and ethyl alcohol. After dismissing first few lacteal secretions about 40 ml milk samples were collected in plastic sterile tubes. The blood samples (10 ml) were collected from the jugular vein divided into two parts; the first part of blood without anticoagulant 5 ml immediately excreted the serum, serum was separated from blood of infected animals using the cold centrifuge. The serum only was transferred with a pipette to another test tube to determine enzymes by spectrophotometrically using commercially available kits were selected for estimation of enzymes (AST, ALT, LDH and ALP).Blood calcium, magnesium, total phosphorus, iron and zinc concentrations investigated by commercial kits (BIOMAGHRIB COMPANY®). Estimation of it was done using colorimetric method according the kits procedure. The other 5 ml of blood samples with anticoagulant (EDTA) were collected from healthy as a control and subclinical mastitis lactating cows for hematological examination, differential leukocyte count was determined as described by (8).

Physical examination

Milk pH was measured with the help of pH meter, while the physical examination (color, odor, foam consistency and turbidity) were done according to (8).

California mastitis test (CMT)

It is easy and important test was used on all samples of milk (2), The CMT result is based on the number of leukocytes in the sample of milk (9). The reaction involved in the CMT is the decadence and degradation of leukocytes when sample of milk is mixed with the reagent. According to the visional reactions, the results were classified in three scores: 0 = negative or trace (termed as control), 1 = weak positive (termed as positive I), and 2 =strong positive (termed as positive II) as shown in the tables below.

Statistical analysis

All statistical analyses were performed using SPSS statistical software version 20 (IBM SPSS Statistics 20).

Results

The result of present study revealed that there is change in physical properties in subclinical mastitis milk as change in color, odor, turbidity and consistency of milk and there is increase in pH value of milk in subclinical mastitis in stage-1 and stage-2 subclinical mastitis 7.07± 0.02 and 7.9 ± 0.02 as in table (1). In the other hand, the study investigated the activity for some enzymes and the present study explained that there is increase in level of Aspartate amino transferees (AST) in stage-1 and stage-2 of subclinical mastitis 200.7 ± 0.8, 217.4 ± 1.8 when compare with control 122.2±1.1. Increase in (ALT) 107.1± 5.0 and 136.0 ±0.2 respectively when compere with control 82.3± 2, also highly increase in level of Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) in positive I and positive II of subclinical mastitis (527.6± 2.9, 816.0 ± 3.4) (102.9 ± 0.4 and 120.5±0.9) respectively compare with control 265.3±0.9 and 50.6±1.0 respectively as shown in table (2).

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Table (1): PH and physical examinations of subclinical mastitis.

Test name	Control	Positive I	Positive II
Milk PH	6.61 ± 0.1	7.07 ± 0.02	7.9 ± 0.02
Color	-	-	+
Odor	-	-	+
Foam	-	+	+
Turbidity	-	+	+
Consistency	-	+	+

Table (2): Enzymes level of subclinical mastitis and control.

Test/ IU/L	Control	Positive I	Positive II
AST	122.2 ± 1.1	200.7 ± 0.8	217.4 ± 1.8
ALT	82.3 ± 2	107.1 ± 5.0	136.0 ± 0.2
LDH	265.3 ± 0.9	527.6 ± 2.9	816. 0 ± 3.4
ALP	50.6 ± 1.0	102.9 ± 0.4	120.5 ± 0.9

***P<0.05**

Serum level of some mineral in animals with subclinical mastitis reveal that in positive I and positive II subclinical mastitis include decrease in calcium 6.48 ± 0.02 and 5.9 ± 0.01 compare to the control 7.91 ± 0.02, and decrease in magnesium 0.8 ± 0.01 and 0.61±0.02 whereas in control 1.01±0.01. Also there is decrease in phosphorus 2.5±0.09 and 2.11±0.01 compare to these in control 3.1± 0.02 and decrease in Iron concentration 12.05 ± 0.6 and 9.9±0.8 from control 18.6±1.4. Whereas there is increased level of zinc in positive I subclinical mastitis 5.9 ±0.4 and in positive II 2.11±0.05 compare to that in control 1.26± 0.03 as appear in table (3).

Table (3): Some mineral value in serum of subclinical mastitis

Parameter	Control	Positive I	Positive II
Calcium	7.91± 0.02	6.48 ± 0.02	5.9 ± 0.01
Magnesium	1.01 ±0.01	0.8 ± 0.01	0.61 ± 0.02
Phosphorus	3.1 ± 0.02	2.5 ± 0.09	2.11 ± 0.01
Zinc	1.26 ± 0.03	5.9 ± 0.4	2.11 ± 0.015
Iron	18.6 ± 1.4	12.05 ± 0.6	9.9 ± 0.8

***P<0.05**

Table (4) showed the result of deferential leukocyte count, there is an increase in neutrophil count 56.9±21 in positive I of subclinical mastitis and in positive II 59.88±1.8 compare with control 21.1±0.3 and there is a decrease in lymphocyte, 13.1±0.2 and 12.2±0.2 from control 18.5±0.4. Also there is a decrease in monocyte count 3.52± 0.3 and 5.9 ± 0.1 from control 11.4 ± 0.3.Result of basophiles count reveal there is slight increase in positive I of subclinical mastitis 2.99 ± and positive II groups 1.2±0.05 retained to normal control group 1.1±0.07, whereas increase in eosinophil count in both positive I and positive II of subclinical mastitis 0.2 ± 0.01 and 1.2 ± 0.05 respectively compare to the values of the control 0.1±0.05.

Table (4): Leukocyte count in subclinical mastitis

Parameter	Control	Positive I	Positive II
TLC	9200	9310	9330
Neutrophil	21.1 ± 0.3	56.9 ± 21	59.88 ± 1.8
Lymphocyte	18.5 ± 0.4	13.1 ± 0.2	12.2 ± 0.2
Monocyte	11.4 ± 0.3	3.52 ± 0.3	5.9 ± 0.1
Eosinophil	0.1±0.05	0.2 ± 0.01	0.2 ± 0.04
Basophile	1.1± 0.07	2.99 ± 0.02	1.2 ± 0.05

***P<0.05**

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Discussion

Many events lead to major alterations of milk compositions secreted from cells. Therefore CMT is a suitable measure for use on large scale monitoring subclinical mastitis. The California mastitis test (CMT) has been standardized for cow's milk and only reacts with liberated nuclear DNA (10), moreover the pH of sub-clinical mastitis milk was higher than that of normal milk, which is proportionate with the results of previous studies which show the same. (11, 12, 13). The study of (10) indicted that milk from quarters with subclinical mastitis demonstrated an elevated pH (6.69 vs 6.59) these alteration in pH of quarters show the presence of tissue damage induced by sub-clinical mastitis. However the pH testing represent a guide to detect the subclinical mastitis beside it is economical, easy and rapid. Regard as cow's defiance mechanism, the new mammary infection is quickly followed by an inflow of leucocytes into the milk and an increase of the milk somatic cell counts. (14). The result of present study agree with many studies as (15) indicated that there is highly significant increases detected in ALT levels, AST values were increased in sub-clinical mastitis infected cows compared to healthy cows, and many studies have evaluated the enzymes (LDH, ALP and AST) changes to diagnose mammary gland infections in dairy cows (2,11, 10), this agree with the result reports by (2) and (16) which show the elevated of these enzyme could be due to stressful conditions and changes in enzyme activities in blood can be a consequence of cell structural damage (16). Other studies done by (17) show that the mean level of activities of LDH and ALP were significantly higher in sub-clinical mastitis milk than in normal milk. Some biochemical investigations were carried out by (18) with blood serum manifested subclinical mastitis. It was established that they were at a higher level of alkaline phosphatase. Other study show different result indicted that milk from quarters with subclinical mastitis showed no alterations were seen in blood serum LDH activity (10). On the other hand (2) studded the activity of enzyme in milk has been considered as a sensitive indicator of changing udder function due to disease and the ALP activities test was authoritative in the early diagnosis of subclinical mastitis. Infiltration of polymorph nuclear leukocytes into mammary glands is one of the major and main defense mechanisms against clinical and subclinical mastitis. During the inflammatory process, the polymorphonuclear leukocytes and damaged cells of the udder's epithelial and interstitial cells secrete products that contain hydrolytic enzymes. Some of these enzymes, such as lactate dehydrogenase (LDH) are among the non-lysosomal enzymes and other enzymes are lysosomal ones (12) so that the LDH is a cytoplasmic enzyme that has been proposed as a biomarker for udder health (1, 6, 12, 14). The results of (18) study showed that decrease in Calcium level found in infected cows whereas Magnesium level of subclinical mastitis affected cow slower than normal cows, and result of (19) indicated that Ca levels in cows with subclinical mastitis were low compared to healthy cows and no significant changes in serum levels of Mg, Zn and Fe. While the study which done by (20) observed a reduction in the Ca levels in the infected animals which similar to our findings, while variation in magnesium level was statistically insignificant ($P>0.05$) this agree with the present study. Study (15) showed that estimation of some minerals revealed significantly higher average values of Ca, P, and that no significant changes was observed in Mg. Also other study revealed that magnesium level of the plasma samples showed no significant effect (21), moreover study reported by (22) and (23) also revealed that there are no significant differences in the plasma magnesium levels in mastitis and healthy animals, also there was an increase in plasma phosphorus level in infected cows. The present study

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accordance with (24) and (25) whom reported an increase in total leukocytes count(TLC) in affected animals along with a higher monocyte, neutrophil and eosinophil count. Hematology of animals carry significantly increase in total leukocytes count ($P<0.05$) this result agree with (21). Differential leukocyte count revealed neutrophilia and lymphopenia in sub-clinical mastitis in addition to study of (15) which showed that lymphocyte count showed significant decrease. In enzymes; (26) indicated that the median and mean LDH activities in cows with subclinical mastitis were significantly higher than for control cows, while the mean LDH and ALP activities in the serum samples of the affected cows had no significant difference compared to those of healthy cows. The present study gives the proof for the relationship between action of some enzyme, mineral concentrations, and leukocyte count change with subclinical mastitis.

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