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Serological study of sub-clinical mastitis in local Cows/Basra-Iraq

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Abstract
The present study was conducted to evaluate the effect of subclinical mastitis, on biochemical and mineral profile in local breed of cows. For this reason, 91 milk and blood samples were collected from sub-clinically infected cattle, were aged from 3-7 years in Basra city/Iraq (between January 2015 to July 2015). The animal's were aged between three to seven years, the animals were healthy and without any clinical signs. The milk was collected from four quarters of each animal. The results of the present study revealed that there is a change in physical properties of subclinical mastitic milk such as color, odor, turbidity, consistency and increase in the pH of milk 7.9 ± 0.02. Blood samples were drawn to evaluate blood parameters like WBC and biochemical values. The study showed a significant increase (P<0.05) of serum aspartate aminotransferase (AST) 217.4 ± 1.8 l/l, alanine aminotransferase (ALT) 136.0 ± 0.2mmol/l,alkaline phosphatase (ALP) 120.5 ± 0.9mmol/l) and lactate dehydrogenase(LDH) 816. 0 ± 3.4.and increase the zinc level 5.9 ±0.4compared with control 1.26± 0.03. Also there is a decrease in monocyte count 3.52 ± 0.3 and 5.9 ± 0.1 from control 11.4 ± 0.3,the basophilic count reveal there is slight increase in stage 1 of subclinical mastitis 2.99 ± and stage 2 retained to normal 1.2 ±0.05,whereas decrease in macrophage count in both stage of subclinical mastitis 23.83 ± 0.4, 20.99 ± 0.1 respectively from control 47.3 ± 0.45.
The present study concluded subclinical mastitis in Cow increase the some biochemical, minerals and increases the somatic cell count, quantity of milk and alters the differential leukocyte count.

Keywords: Iraqi cows, biochemical, minerals and leukocyte

Introduction
Subclinical mastitis is the one that does not make obvious changes in the udder or milk, but decrease in milk production, and excess the number of somatic cells [1]. Subclinical mastitis is a disease by dairy farmers even though it causes economic loss of 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 mastitic animals the quantification of cells in milk or somatic cell count (SCC), is evaluated by using the direct microscopic study or by an indirect method of evaluating SCC by using the California mastitis test (CMT) [2]. The CMT is already used on-farm to detect subclinical mastitis indirectly (SCM) for dairy Cows, the specificity and sensitivity of the CMT reported in the literature is changeable [3]. In the cattle population, both clinical and sub-clinical mastitis can affect the composition and characteristics of milk [3, 4]. 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 [5-7]. The alkaline phosphatase (ALP) milk and lactate dehydrogenase (LDH) changes have been used as amarker of SCM in lactation cows [8]. The present study aimed to assess the relationship between enzymes, mineral concentrations, and leukocyte count with subclinical mastitis.

2. Materials and methods
Collection of milk and blood samples
A total of 91 milk and blood samples were collected from sub-clinically infected cattle during the period from January 2015 to July 2015.The animal aged between three to seven years old. All the cattle selected in this study were apparently healthy and without any clinical disease and palpable udder lesions. Before collection of milk the udder and teat ends of each animal were washed using sterile water and ethyl alcohol. After discarding 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. Serum was obtained by centrifugation of blood sample at 2000-2500 rpm for 15 minutes to determine enzymes by spectrophoto-metrically by 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 by using colorimetric method according the kits procedure. other 5 ml of blood samples with anticoagulant (EDTA) were collected for hematological examination, differential leukocyte count was determined as described by Coles [7].

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 Coles [7].

In the milk samples the California test was used, by the method in Schalm [8]. The CMT is used as a base on the amount of WBC in milk [9]. The reaction concerned in the CMT is the breakdown of leukocytes when reagent is mixed with the milk. According to the visible reactions, the results were classified in four scores: 0 =negative or trace, 1 = weak positive, 2 = distinct positive and 3 =strong positive

Statistical analysis

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

3. Results

The result of the Present study revealed that there is achange in physical properties in subclinical mastitic milk as change in color, odor, turbidity and consistency of milk. And there is an 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 Table (1).

Table 1: values of pH and physical examinations of subclinical mastitis

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

The study investigated the values of some enzymes, there is increase in level of Aspartate amino transferase (AST), in stage 1 and stage 2 of subclinical mastitis 200.7 ± 0.8,217.4 ± 1.8 compared with control 122.2 ± 1.1. Increase in (ALT) levels 107.1± 5.0 and 136.0 ±0.2 respectively compared with control 82.3± 2also highly increase in level of Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) in stage 1 and stage2of subclincal mastitis 527.6± 2.9,816.0 ± 3.4,102.9 ± 0.4 and 120.5 ±0.9 respectively compared with control 265.3±0.9 and 50.6 ± 1.0 respectively table (2).

Table 2: The AST, ALT, LDH and ALP enzyme 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

The Serum of some mineral level with subclinical mastitic animals reveals that in stage 1 and stage 2subclinical mastitis include decrease in calcium 6.48 ± 0.02 and 5.9 ± 0.01 from control 7.91 ± 0.02, and decrease in magnesium 0.8 ± 0.01 and 0.61 ± 0.02 compared with control 1.01±0.01also there is decrease in phosphorus 2.5 ±0.09 and 2.11±0.01 compared with control 3.1 ± 0.02 and decrease in Iron concentration 12.05 ± 0.6 and 9.9 ±0.8 compared with control 18.6 ±1.4. Whereas there is increase level of zinc in stage 1 subclinical mastitis 5.9 ±0.4 and in stage 2.11± 0.05 compared with control 1.26± 0.03.Table (3).

Table 3: The mineral values 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

Table (4) showed the result of differential leukocyte count, there is increase in Neutrophil count 56.9±21 in stage 1 of subclincal mastitis and in stage 59.88±1.8 compared with control 21.1±0.3, and there is decrease in lymphocyte, 13.1±0.2 and 12.2±0.2 compared with control 18.5±0.4. Also there is decrease in monocyte count 3.52 ± 0.3 and 5.9 ± 0.1 compared with control 11.4 ± 0.3,the result of eosinophil count there slight increase in stage 1 and stage 2 of sub clinical mastitis is 2..99 ± 0.02 and 0.2 ± 0.04 compared with control 0.1±0.05,Result of basophilic count reveal there is slight increase in stage 1 of subclinical mastitis 2.99 ± and stage 2 retained to normal 1.2 ±0.05,whereasdecrease in macrophage count in both stage of subclinical mastitis 23.83 ± 0.4, 20.99 ± 0.1 respectively from control 47.3 ± 0.45.

Table 4: The leukocyte count of subclinical mastitis

parameter	Control	Positive I	Positive II
Neutrophyle	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
macrophage	47.3 ± 0.45	23.83 ± 0.4	20.99 ± 0.1

4. Discussion

The major alterations of milk compositions in mastitic animals without changes in udder. Therefore CMT is a suitable measure for use on large scale monitoring subclinical mastitis. The California mastitis test (CMT) has been standardized for lactating cows and only reacts with activist nuclear DNA [10]. The pH of SCM milk was higher than that of normal milk, which is consistent with the results of previous report [11-13]. The study of Kitchen [11] indicted that milk from quarters with subclinical mastitis showed elevated pH (6.69 to6.59), these changes in pH of quarters show the presence of tissue damage provoked by SCM. The pH testing can be considered as a guide to detect the subclinical mastitis as this is economical, easy and rapid. As part of the cow's defence mechanism, the new intra mammary infection is quickly followed by an influx of leucocytes into the milk and an increase of the milk somatic cell counts [14]. The Result of Present study agrees with many studies of Sarvesha [15]. There is highly significant increases detected in ALT, AST values were increased in SCM infected compared to healthy animals, and numerous studies have astimated milk AST, ALP LDH,

and activities changes to diagnose udder infections in dairy cows the study agree with whom [16, 11, 10, 2]. The present results also agree with reports of Chandrasekaran [17], the elevated of these enzyme could be due to stressful conditions. and Changes in enzyme actions in blood can be a result of damage of cell structural [16, 18], show that the mean level of activities of LDH 724.49±34.91 and ALP724.49±34.91 were significantly high in SCM milk than in normal milk ($P < 0.01$). Some biochemical investigations were carried out by Symons [19], with blood serum manifested subclinical mastitis. It was established that were at a higher level of alkaline phosphates [10] indicated that milk from quarters with subclinical mastitis showed no changes were seen in blood serum LDH activity.

On the other hand agree with Symons and Babaei, *et al* [20, 2], the early diagnosis of subclinical mastitis depend upon the ALP test. During the inflammatory process, these cells and damaged cells of the udder's epithelial and interstitial cells, secrete products that contain hydrolytic enzymes. Some of these enzymes, such as lactatedehydrogenase (LDH) are among the non lysosomal enzymes and other enzymes are lysosomal ones [2]. LDH is a cytoplasmic enzyme that has been proposed as a biomarker for udder health check [2, 5, 12, 16].

The results agree with the findings of Siddiqe [21] and Hamit [22] these study showed that decrease in Calcium and Magnesium level than normal in infected cows with subclinical mastitis and no significant changes in Serum levels of Mg, Zn and Fe. Study of Zaki [23].

The Study of Sarvesha [15] showed that estimation of some minerals revealed significantly ($P< 0.05$) higher average values of Ca, P, and that no significant ($P> 0.05$) in Mg level.

The present study agree with Yildz, Bruckmaier [25, 14], no significant variation in the plasma level of Mg in mastitic animals. Also increase in phosphorus level accordance with Zaki, Khan [23, 26]. Differential leukocyte count revealed to high level of monocyte, neutrophil and eosinophil count disagree with Singh. [24]. neutrophilia and lymphopenia in clinical as well as sub-clinical mastitis agree with the Study of Sarvesha [15] showed that Macrophage and lymphocyte count showed significant decrease. In contrast Study of Shahabeddin [27] indecated that the LDH activities in cows with subclinical mastitis were significantly ($P< 0.001$) higher than healthy cows agree with our study.

5. Conclusion

The present study concluded that the cows in subclinical mastitis showed a significant increase of AST, ALT ALP, and LDH enzymes and increase of the zinc level. Also there is decrease in monocyte count and the basophile count.

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