International Journal of Microbiological Research 2 (1): 18-27, 2011
ISSN 2079-2093

Epidemiological and Microbiological Studies on Mastitis in She-Camels

[1]Amena M. Ibrahim, [2]A.A. AL-Humiany, [3]Ahmed M.A. Mansour and [2]Hoda M. Zaki

[1]Department of Medical Microbiology, Faculty of Applied Medical Science, Taraba, Taif University, KSA
[2]Department of medical Microbiology, Faculty of Applied Medical Science, Taraba, Taif University, KSA
[3]Department of Medical Record, Faculty of Applied Medical Science, Taraba, Taif University, KSA

Abstract: A total number of 180 milk samples was collected from she-camel at different Makkah districts and examined for mastitis using California Mastitis Test (CMT). The prevalence of mastitis was 20, 18.6 and 18.18% in Taif, Taraba and EL-Khorma, respectively. Many bacteria were isolated from milk samples including *E. coli*, *Srept. agalactiae*, *Staph. aureus*, *Pseudomonas aeruginosa, Staph. hyicus* and *Klebsiella pneumoniae*. The effect of different antimicrobial drugs was studied. In milk whey from mastitic, animals the level of antioxidant was low while LDH, lipid peroxidation and lysozyme level were high. The level of immunoglobulin increased in the presence of infection except in case of recent infection. The indirect ELISA was performed using the antigens prepared from the isolated strains to detect the presence of specific IgG and it revealed the presence of high level of IgG specific for isolated strains in the tested milk whey from different cases of she-camel. In conclusion, mastitis in she-camel is present with high incidences and accompanied with many microorganism infection either single or mixed. Clear changes were recorded in the antioxidant and immunological status in mastitis cases.

Key words: California mastitis test · Immune response · ELISA · Electrophoresis

INTRODUCTION

The dromedary camel (*Camelus dromedarius*) is the most important livestock animal in the arid and semi-arid areas [1]. The camel milk is one of the most valuable food resources for pastoral people in arid and semi-arid areas [2].

Mastitis is the outcome of a complex interaction between host, causative agents (micro-organisms) and environment and remains a major cause of both an extreme zoonotic and economic importance throughout the world [3]. *Arcanobacterium pyogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Strepococcus uberis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus*, *Pasterulla haemolytica* and *Pseudomonas aeruginosa* were isolated from mastitic mammary glands either in the pure or mixed form from infected cases [4].

Physiological stress of milking process is accompanied by a high energy demand and an increased oxygen requirement [5]. This increases in oxygen demand enhanced the production of reactive oxygen metabolites (ROS). Also, infectious diseases in farm animals as mastitis are thought to be associated with these ROS [6]. Increased lipid peroxidation as a result of changed intracellular ratio between ROS and antioxidant system has been suggested to be related with mastitis [7]. The free radicals can react with enzymes, cell membranes, and DNA causing cell damage or cell death. So the body has developed a sophisticated antioxidant system that relies on antioxidant nutrients [8].which might reduce cell damage.

Lysozyme is one of the minor milk proteins that have attracted increased attention recently due to its potent antimicrobial activity against a wide range of microorganisms [9]. Detection and evaluation of proteins or enzymes in milk during the course of mastitis are important to elucidate the pathogenic mechanism of mastitis. The changes of some major proteins including albumin, immunoglobulin, lactoalbumin and LDH isoenzymes in mastitic animals were also, recorded [10].

This work was designed to monitor the incidence of mastitis in she-camel in three governorates in Makkah district, isolate and identified the bacterial causes of mastitis and study the effect of different chemotherapeutic drugs on the isolated strain. Also, studying the immunologically related parameters and

Corresponding Author: Amena M. Ibrahim, Department of Medical Microbiology, Faculty of Applied Medical Science, Taraba, Taif University, KSA. E-mail : amenamaher@live.com.

alteration during the course of mastitis , include; total antioxidant activity, lipid peroxidation, LDH level, lysozyme, total protein, electrophoretic pattern of whey by SDS-PAGE and indirect ELISA for estimating immunoglobulin G content was another target of the current study.

MATERIALS AND METHODS

A total number of 180 lactating she-camels from three governorates in Makkah district, raised by nomadic tribe was examined during the period from April to July 2010.

Clinical Examination of Udder and Milk: The udder of animals under investigation was carefully examined and any udder abnormalities were recorded. Clinical mastitis was recognized by abnormal milk and, signs of udder inflammation. Subclinical mastitis was recognized by an increase in leukocyte counts as evidenced by CMT. However, in both cases, the condition was confirmed after detection of pathogens following bacteriological culture.

Milk Sample Collection: Before milk sampling, the teats were disinfected with cotton moistened with 70% alcohol. After discarding the first few squirts of milk about 20 ml were collected in sterile universal bottles and kept in an icebox, and transported as soon as possible to the laboratory for analysis.

California Mastitis Test: CMT was used according to the procedure described by Schalm *et al.* [11]. All milk samples of positive CMT results were bacteriologically examined.

Isolation and Identification of Bacteria: Bacteriological examinations were carried out following standard methods [12].

Milk Whey: was prepared from whole milk according to [13].

Total Antioxidant Activity: using colorimetric diagnostic kits (Bio-Diagnostic) according to EL-Hatmi *et al.* [14].

Lipid Peroxidation: (Malonedialdehyde) was assayed using colorimetric diagnostic kits (Bio-Diagnostic) according to Ohkawa *et al* [15].

LDH Leakage Assays: Was carried out as outlined in Tietz [16)] using chemical kit (Biosystems, S.A., Spain) and the manufacture instructions.

Serum Lysozyme Activity: Was measured by the lysoplate assay method according to Peters and Vantrapen [17].

ELISA: Antigens were prepared from isolated bacteria; *Streptococcus* according to Galan and Timoney [18], for *Staphylococcus aureus* according to Esmaily and Sharifi–Yazdi [19], for *E. coli* according to Dhasarathan *et al.* [20] and for *Klebsiella* and *Pseudomonas* according to Xu *et al.* [21].

The protein concentration of the prepared antigens was estimated by the method of Lowery *et al.* [22].

ELISA: Procedure was performed according to Maghraby and Bahgat [23].

Total Protein: Concentration was assayed by the method of Bradford [24], using bovine serum albumin (BSA) as standard.

Electrophoresis: Whey samples were separated [25] using with the aid of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of 1.1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol. Electrophoresis was performed at 4.9% staking and 15.4% resolving polyacrylamide gels, running in 0.125M Tris–HCl, pH 6.8 and 0.38M Tris–HCl, pH 8.8 buffers, respectively. Whey samples (20µl), (2mg protein/ml), were loaded in the gel. Proteins were stained for 30 min by 0.1% (w/v) in black amido in a mixture of 50% (v/v) ethanol and 10% (v/v) acetic acid. The following molecular mass standards (Sigma) were used: myosin (200.0kDa) β-galactosidase (116.2kDa), phosphorylase b (97.4kDa), bovine serum albumin (66.2kDa), ovalbumin (45.0kDa), carbonic anhydrase (31.0kDa), trypsin inhibitor (21.5kDa), lysozyme (14.4kDa), and aprotinin (6.5kDa).

Data were computed and expressed as Mean±MSE

RESULTS

Results obtained in table 1 showed that, out of 180 animals examined from different governorates 34 animals were mastitic (18.89%) including 13 animals showing clinical signs of mastitis (7.72%) meanwhile 21 were proved to be sub-clinical mastitis (11.67%).

The prevalence rate of single isolation was 72.97% in clinical cases and 81.40% in subclinical cases. The most predominant single isolates was *E. coli* 21 (26.25%), followed by *S. agalactiae* 16 (20%), *S. aureus* 14 (17.5%), *P. aeruginosa* 7 (8.75%) and *S. hyicus* 2 (5%).

Table 1: Prevalence of mastitis among she-camels regarding to examined governorates using CMT

Examined governorate	No. of exami animals	Types of mastitis: Clinical No.	Clinical %	Subclinical No.	Subclinical %	Total No. of mastitic she-camels No.	Total No. of mastitic she-camels %
EL-Taif	50	3	6	7	14	10	20
Taraba	75	6	8	8	10.67	14	18.6
EL-Khorma	55	4	7.2	6	10.9	10	18.18
Total	180	13	7.22	21	11.67	34	18.89

Table 2: Prevalence of single and mixed bacteria from mastitic she-camels

Types of isolates	Clinical (37) No.	Clinical (37) %	Subclinical (43) No.	Subclinical (43) %	Total (90) No.	Total (90) %
Single infection						
E. coli	9	24.32	12	27.9	21	26.25
S. agalactiae	8	21.62	8	18.6	16	20.00
S. aureus	5	13.51	9	20.93	14	17.50
P. aeruginosa	3	8.1	4	9.3	7	8.75
S. hyicus	2	5.4	2	4.65	4	5.00
Total	27	72.97	35	81.4	62	77.50
Mixed infection						
S. aureus + *E. coli*	3	8.1	3	6.98	6	7.5
S. hyicus + *K. pneumoniae*	2	5.4	2	4.65	4	5.00
S. agalactiae + *P. aeruginosa*	2	5.4	1	2.32	3	3.75
Total	7	18.91	6	13.95	13	16.25
Negative for bacterial isolation	3	8.1	2	4.65	5	6.25

Percentage calculated according to total number of examined positive quarter milk samples.

Table 3: Efficacy of various antimicrobial agents on bacterial isolation of milk samples of mastitic she-camels

Bacterial isolates	No. of isolates	Sensitivity	Antimicrobial agents used: C	E	G	Ne	N	P	S	TE	V
E. coli	27	S	20	27	27	27	16	0	22	9	10
		%	74.1	100	100	100	59.3	0	81.5	33.3	37.1
		I	7	0	0	0	2	0	5	4	7
		%	25.9	0	0	0	7.4	0	18.5	14.8	25.9
		R	0	0	0	0	9	27	0	14	10
		%	0	0	0	0	33.3	100	0	51.9	37.1
S. auerus	20	S	12	18	18	5	20	12	16	20	0
		%	60	90	90	25	0	60	80	100	0
		I	2	2	2	15	0	4	4	0	0
		%	10	10	10	75	0	20	20	0	0
		R	8	0	0	0	0	4	0	0	20
		%	40	0	0	0	0	40	0	0	100
S. agalactiae	19	S	11	0	19	19	0	5	0	19	0
		%	57. 9	0	100	100	0	26.3	0	100	0
		I	4	0	0	0	0	3	0	0	0
		%	21.1	0	0	0	0	15.8	0	0	0
		R	4	19	0	0	19	11	19	0	19
		%	21.1	100	0	0	100	57.9	100	0	100

Continued Table 3: Efficacy of various antimicrobial agents on bacterial isolation of milk samples of mastitic she-camels

			Antimicrobial agents used								
Bacterial isolates	No. of isolates	Sensitivity	C	E	G	Ne	N	P	S	TE	V
P. aeruginosa	10	S	3	0	10	2	3	0	0	4	2
		%	30	0	100	20	30	0	0	40	20
		I	6	1	0	7	6	0	0	2	7
		%	60	10	0	70	60	0	0	20	70
		R	1	9	0	1	1	10	0	4	1
		%	10	90	0	10	10	100	100	40	10
S. hyicus	8	S	6	8	6	8	4	0	4	8	0
		%	75	100	75	100	50	0	50	100	0
		I	2	0	0	0	2	0	0	0	0
		%	25	0	0	0	25	0	0	0	0
		R	0	0	2	0	2	8	4	0	8
		%	0	0	0	0	25	100	50	0	100
K. pneumoniae	4	S	3	4	4	1	1	0	4	4	0
		%	75	100	100	25	25	0	100	100	0
		I	1	0	0	2	2	0	0	0	0
		%	25	0	0	50	50	0	0	0	0
		R	0	0	0	1	1	4	0	0	0
		%	0	0	0	25	25	100	0	0	100

C= Chloramphenicol. E= erythromycin. G= gentamicin. Ne= neomycin

N= nitrofuraltadon. P= polymyxin. S= streptomycin. Te= tetracycline.

V= vancomycin. S= sensitive. I= intermediate. R= resistant.

Table 4: Total antioxidant, lipid peroxidation and malonedialdehyde in camel whey milk from different animals cases (Mean±SE)

	Total antioxidant (mM)	MLD (nmol)	LDH (U/L)
Normal	0.266 ± 0.042^{A}	1.093 ± 0.174^{A}	1619.0 ± 0.00^{A}
Sub-clinical	0.184 ± 0.017^{B}	2.784 ± 0.203^{B}	3355.3 ± 295.8^{B}
Clinical	0.074 ± 0.017^{B}	8.418 ± 0.423^{C}	7285.5 ± 809.5^{C}

Means in the same column with different superscripts are significantly different at least at ($P<0.05$).

Table 5: Value of total protein and lysozyme from she-camel milk whey with different cases (Mean±SE)

	Total protein (g/dl)	Lysozyme (µg/ml)
Normal	1.723 ± 0.088^{A}	7.78 ± 0.24^{A}
Subclinical	2.918 ± 0.135^{B}	8.65 ± 0.28^{A}
Clinical	3.453 ± 0.407^{B}	11.63 ± 1.03^{B}

Means in the same column with different superscripts are significantly different at least at ($P<0.05$).

Table 6: ELISA optical density for different isolated micro-organisms antigen in different cases of milk samples (Mean±SE)

	Cases		
Isolates	Healthy	Sub-clinical	Clinical
E. coli	0.07014 ± 0.0014^{A}	0.18563 ± 0.0175^{B}	0.65644 ± 0.0136^{C}
P. aeroginosa	0.06471 ± 0.0008^{A}	0.16900 ± 0.0090^{B}	0.60063 ± 0.0214^{C}
K. pneumonaie	0.07000 ± 0.0018^{A}	0.18675 ± 0.0086^{B}	0.66400 ± 0.0185^{C}
S. agalactia	0.05957 ± 0.0007^{A}	0.21488 ± 0.0699^{B}	0.60738 ± 0.0222^{C}
S. aureus	0.06186 ± 0.0017^{A}	0.19125 ± 0.0116^{B}	0.37063 ± 0.0162^{C}

Means in the same row with different superscripts are significantly different at least at $P<0.05$

Table 7: Electrophoretic patterns of camel whey protein from different cases

lane	Total protein (g/l)	Percentage of IgG volume	IgG (g/l)	CMT
1	3.54	6.446	0.228	Clinical
2	2.54	6.774	0.180	Sub-clinical
3	2.57	2.353	0.061	Positive***
4	2.62	12.615	0.331	Clinical
5	2	0.996	0.020	Negative
6	2.48	6.950	0.172	Sub-clinical
7	2.58	9.491	0.245	Sub-clinical
8	3	9.646	0.289	Sub-clinical
9	2.19	8.206	0.180	Sub-clinical
10	4.03	15.175	0.621	Positive
11	3.55	14.353	0.508	Clinical
12	2.52	6.4	0.161	Negative
13	1.35	0.35	0.005	Negative
14	1.55	1.08	0.017	Negative
15	1.61	1.035	0.017	Negative
16	1.52	1.223	0.019	Positive***
17	2.1	1.184	0.025	Negative
18	1.84	3.463	0.064	Positive***

Concerning mixed infection *S. aureus* + *E. coli* was the most common (7.5%), followed by *S. hyicus* + *K. pneumoniae* (5%) and *S. agalactiae* + *P. aeruginosa* (3.75%).

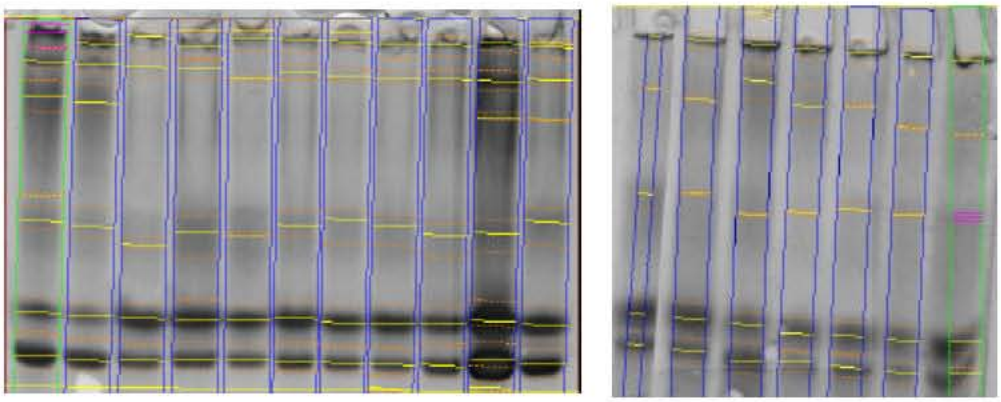
Results in table 2 indicated that, *E. coli* isolates were the most prevalent bacteria in single and mixed infection and isolated from clinical mastitis with an incidence of (33.33 and 21.42%), respectively followed by *S. aureus*, *S. agalactiae*, *P. aeruginosa, S. hyicus* with an incidence of 18.51 & 21.42, 29.36 & 14.39, 11.11 & 14.29 and 7.40 & 14.29%, respectively. The prevalence rate of mixed isolation was 18.91% in clinical and 13.95% in subclinical cases.

Concerning to subclinical mastitis, *E. coli* was isolated within an incidence of 34.29 & 25% followed by *S. aureus*, *S. agalactiae*, *P. aeruginosa*, *S. hyicus* with incidence of 25.71 & 25.0, 22.86 & 8.33, 11.43 & 8.33, 5.71 & 16.67, respectively from single and mixed infection. While *K. pneumoniae* was isolated from mixed infection only in both clinical and subclinical mastitic animals with an incidence of 14.29 & 16.67. There were five mastitic milk samples negative for bacterial isolation.

Table 3 shows the sensitivity of tests of the organisms isolated to antibiotics. The *in vitro* susceptibility testing of 27 *E. coli* isolates showed that, the most effective drugs were erythromycin, gentamycin and neomycin. Twenty *Staphylococcus* isolates showed 100% sensitivity to neomycin and tetracycline, while 90% were sensitive to erythromycin. While 19 *S. agalactiae* isolates showed 100% sensitivity to gentamycin, neomycin and tetracycline. Ten isolates of *P. aeruginosa* were full sensitive to gentamycin. Regarding to 8 *S. hyicus* isolates showed 100% sensitivity to chloramphenicol, erythromycin, neomycin and tetracycline, while 75% were sensitive. All *K. pneumoniae* isolates were sensitive to chloramphenicol, erythromycin, gentamycin, streptomycin and tetracycline. Results illustrated in table 4 reveal that total antioxidant levels in clinical and subclinical mastitis was significantly ($P<0.05$) decreased than healthy cases without any significant ($P<0.09$) variation between the subclinical and clinical cases. The rest of the results of antioxidant status in table 4 indicated that the amount of DLH and malonedialdehyde (MDA) in milk serum there are significantly ($P<0.05$) increase in subclinical and mastitic camel than the healthy one.

Table 5 shows the total protein percentage which increased significantly ($P<0.05$) in both subclinical and clinical cases than healthy one, in addition it showing an increase in total protein from subclinical to clinical cases but didn't reach to the level of significantly. Also, the lysozyme level in the milk whey of she-camel showing absence of difference between healthy and subclinical cases while the clinical cases showing a significant ($P<0.05$) increase in the lysozyme level.

Table 6 shows the results from interaction between isolated strains prepared antigens and the immunoglobulin inside the milk whey from different she-camel cases. Concerning all isolated strains the clinical cases giving the highest optical density in comparing with both subclinical and healthy one and the differences between such cases are significant ($P<0.05$) indicating the presence of special IgG toward the isolated strains.



Photoes 1: (left samples from 1 to 11) and 2 (right samples from12 to 18): Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of proteins from camel's milk whey. Volumes of 20 µL of each sample (at 2 mg/ml) were loaded in the gel. Revelation of proteins was performed with amido black. Lane one started from the left in the upper photo till eleven then lane twelve started from left in the lower photo.

Data in table 7 show the amount of protein in milk whey from different cases in addition to the amount of the immunoglobulin andit showed an increased total protein and immunoglobulin in all positive case except three cases which showing no increase in both parameters.

DISCUSSION

Camels are still multipurpose animal increasingly kept for milk, but specialization may be on the way [26]. Reports of mastitis in traditionally managed camels are on the rise and are likely to increase further as the milk production per individual camel is increased.

Bacterial infections are considered the primary causes of mastitis in domestic animals, clinical mastitis in the camel can be detected by examination of the udder, the milk, or both. Detection of subclinical mastitis is, however, difficult and depends on various test procedures aimed at detecting the cause or products of inflammation in milk [27]. However, the relation among CMT, the presence of inflamed udders and the bacteriological findings indicated that she-camel milk is like that the cows [28].

In the present study, the prevalence of mastitis in different governorate using CMT revealed 13 (7.22%) clinical cases of mastitis and 21 (11.67%) sub-clinical cases of mastitis which subsequently subjected to cultural isolation. These results agree with that of Sargant *et al*. [29] who suggested that CMT can be used to detect sub-clinically infected udders of camels and Barbour *et al*. [30] who indicated that, CMT is a useful screening test in the detection of mastitis in camels and may serve to segregate udder infected with major pathogens in sub-clinical form.

Bacteriological examination of milk samples collected from both clinical and subclinical mastitic cases revealed isolation of six types of bacteria of which *E. coli* isolates was the most predominant bacteria isolated from the present mastitic cases. These results nearly coincide with that reported by Wilesmith *et al*. [31] and Smith and Hogan [32] who found that *E. coli* was the most common cause of mastitis. Also, Erskine *et al*. [33] reported that *E. coli* are generally the predominant cause of intramammary infection.

In the current study, *S. aureus* and *S. agalactiae* were the most important causative organism of she-camel mastitis, whereas the incidence of their isolation were 22.73 and 21.59%, respectively. These finding are similar to that reported by Woubit *et al*. [34] and Abdurahman [35] who suggested that *S. aureus* and *S. agalactiae* seem to be also the major causative agent of mastitis in camels. On the other hand *P. aeruginosa*, *S. hyicus* and *K. pneumoniae* were isolated from mastitic she-camels within an incidence of 11.39, 9.09 and 4.55%, respectively. The present results are in agreement with Hawari and Hassawi [36] who isolated these bacteria from mastitic camels in various incidences. Hogan *et al*. [37] reported that *K. pneumoniae* intramammary infection do become chronic and can be present for several months from one lactation to next while *P. aeruginosa* tend to be of long duration than mastitis caused by *E. coli*.

In this study mixed infection of bacteria had been observed among clinical and subclinical mastitic camels. These finding nearly similar to that reported by Konte *et al*. [38], and Kapur *et al*. [39] who detected mixed infection in clinical and subclinical mastitic animals.

Most cases of mastitis of economic importance are caused by coliform and staphylococcal infections and because the clinical signs range from mild visibly undetectable local reaction of an infected quarter to life-threating systemic reaction, several approaches for therapy are required [40]. Selection of an effective drug with adequate concentration for adequate time is very important for correct treatment of mastitis [41].

The antibiogram presented in this study shows that, erythromycin, gentamycin or neomycin were the most effective drug for *E. coli* mastitis and neomycin, tetracycline followed by erythromycin and gentamycin were the drug of choice for staphylococcal mastitis [42]. Gentamycin, neomycin and tetracycline were most effective against *S. agalactiae* while the drug of choice for mastitis caused by *P. aeruginosa* was gentamycin. All *S. hyicus* isolates were sensitive to erythromycin, neomycin or tetracycline and K. pneumonia isolates were full sensitive to erythromycin, gentamycin, streptomycin or tetracycline [36].

In the present study malonedialdehyde (MDA) increase significantly after infection even when that infection is subclinical this increase is a marker of lipid peroxidation which considered the most widely used methods for determination of the oxidative stress [43]. A positive correlation was determined between MDA concentrations and CMT scores in the mastitic groups (CM and SCM), these results agree with Nyskaa [43] and it may be due to the oxidative stress which is a secondary aggravating factor in most diseases [44]. Also, the total antioxidant decrease significantly in the clinical and subclinical mastitis as compared to healthy camels and come in in agreement with previous investigations [45-47]. In the same time we observed a negative correlation between total antioxidant concentrations and CMT scores in the mastitic groups (CM and SCM) this decrease in the total antioxidant may be attributed to the exhaustion of that total antioxidant owing to increase in oxidation process in case of mastitis or due to the decrease in antioxidant in the nutrient supplement in spite of increasing demand, which act as predispose factor to the mastitis in the camel. Our results come in the same line obtained by Castillo *et al.* [47] who stated that, optimum antioxidant intake in the feed may enhance the resistance against mastitis by augmenting the immune system. Our findings showed that tissue disturbances of the mammary gland in mastitis were accompanied by marked increase of Lactate dehydrogenase isoenzymes (LDH) activity in the secretions. Higher LDH activity in milk serum of inflamed udders has been previously reported in cows [48] and in sheep [49], the condition was probably librated from disintegrated leukocytes and the parenchymal cells of the inflamed udder [10].

The present study, the total protein was increase significantly in both clinical and subclinical mastitis in compare with healthy she camels which agree and explained by Kato *et al.* [50] who reported that, the level of protein are increased in mastitis camel milk which may be due to proteins derived from granulocytes, infiltration from serum proteins owing to inflammation, protein leakage from mammary gland cells, produced by neutrophil or bacterial proteases and also the changes of the respective protein synthesis.

All protein types of whey from mastitic camel milk undergo dramatic changes. In our data, the most important protein from the immunological point of view is the immunoglobulin which increases in whey from mastitic milk in comparing with whey milk from healthy ones. The increase of immunoglobulin levels associated with inflammatory responses of the udder as documented by Coulon *et al.* [51]. Immunoglobulin in mammary secretions are serum–derived or produced in the udder, the concentrations of the immunoglobulin in normal milk are low and depend on the degree of vascular permeability of the udder tissues. When this permeability barrier is broken during inflammation, immunoglobulin concentrations increase in the secretions from the infected glands to prevent bacterial adherence to epithelial membranes, inhibit multiplication, agglutinate bacteria and neutralize toxins. In addition, a major function of immunoglobulin is opsonization of microorganisms for phagocytosis to reducing severity of mastitis [52, 27]. Moreover, in this study, immunoglobulin increase in case of clinical and subclinical mastitis except the sample number 3, 11 and 15 in which the level of immunoglobulin did not increase. Those results may be due to recent infection so the B cells not activated yet to secret immunoglobulin.

In this study indirect ELISA was used to detect the specific antibodies against various isolated bacterial strains, generally the antibodies levels measuring with optical density significantly increased in clinical and subclinical mastitis comparing with healthy animals these results with all isolates. In our results the subclinical cases yield low optical density with ELISA indicating low amount of IgG while the causative micro-organism isolated. Those results could be explained by data cited by Nentwich *et al.* [53] who stated that, the IgG antibodies are stimulated only with high amount of antigens. ELISA technique used to estimated

the presence of IgG against prepared antigens of different isolated micro-organisms which give results resembling previous reported data for *E. coli* [54, 55], *Staphylococcus aureus* [56], *Streptococcus agalactiae* [57], *Pseudomonas aeruginosa* [58].

The antibacterial activity of milk lysozyme is a part of the unspecific innate defense mechanism which acts either independently by lysing sensitive bacteria or as a component of complex immunological reactions to enhance the phagocytosis of bacteria by macrophages [9]. The milk of all mammals contains lysozyme either as a free soluble protein or within leucocytes [59]. In this study, the lysozyme present in the serum milk of camel and significant increase during clinical mastitis. Conflicting data concerning the level, presence or absence of lysozyme in camel milk are available in many literatures. In an early study [60], the presence of lysozyme in the camel milk which decreases linearly throughout the lactation period. Subsequent studies [61, 62] confirmed the presence of lysozyme in camel milk but at significantly lower concentrations than other ruminants. Furthermore, the enzyme was reported to be purified successfully from camel milk and characterized at the structural level [63, 64]. In contrast, Kappeler *et al.* [65] did not detect the enzyme in mature camel milk (middle to late-lactation period) by using molecular biology techniques specific to the c-type lysozyme. Therefore, further research is needed to provide a sound evidence for the presence or absence of lysozyme in camel milk. Studies on the genetic characterization of putative encoding gene(s), sequence organization and gene location.

In conclusion, mastitis in she-camel is present with high incidences and accompanied with many microorganism infection either single or mixed. Antioxidants levels was lower in the clinical and subclinical cases in compare with normal cases with the increasing in the level of LDH, lipid peroxidation and lysozyme. The level of immunoglobulin increased with the presence of infection except in recent case infection. Specific IgG prepared from the isolated strains revealed the presence of high level in the milk whey from mastitic she-camel. Clear changes were recorded in the antioxidant and immunological status in mastitis cases.

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