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RESEARCH ARTICLE

Milk Somatic Cell Counts and Some Hemato-Biochemical Changes in Sub-Clinical Mastitic Dromedary She-Camels (*Camelus dromedarius*)

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ABSTRACT

The dromedary camels are considered as the best livestock animals in arid, semiarid and desert areas and camel milk is known as the valuable food source in these areas. The present study was aimed to investigate milk somatic cell counts and some biochemical changes in milk due to sub-clinical mastitis in camels. For this purpose milk samples were collected from 33 lactating animals and examined for sub clinical mastitis using California Mastitis Test. The chi-square and frequency analysis did not show any significant association with age, lactation stage, parity and quarter involved. The results indicated significant ($P<0.01$) increase in milk electrical conductivity and milk pH while significantly lower values for milk proteins, lactose and fat contents were recorded. The results revealed that the total milk somatic cell and neutrophil counts were significantly increased while the lymphocytes and macrophages were decreased in infected animals. Moreover, milk enzymes; aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase were significantly increased in mastitic animals as compared to the non-infected animals. The results indicated that milk electrical conductivity and some milk enzymes can be screened to investigate the sub-clinical mastitis in Camelus dromedaries.

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INTRODUCTION

In Pakistan about 43.56 million tons of milk is produced annually which places the country at 4th position among milk producing countries in the world (Hussain *et al.,* 2012). In Pakistan nearly 8.43 million people is involved in dairy animal breeding and rearing, particularly cattle (33.0 million) and buffaloes (29.9 million) which produce more than 95% milk in country (Hussain *et al.,* 2013). In spite of huge efforts regarding the expansion of dairy sector in terms of husbandry practices, animal breeding and animal nutrition the demand for milk is increased throughout the world. The expansion in dairy industry over the last few decades to meet the requirements of milk products had played prominent role in increasing global milk production to meet the increasing demand (Tiwari *et al.,* 2013). Mastitis is the most complex interaction between hosts and the etiological agents (micro-organisms) and remains the major threat to milk producing animals. The disease has an extreme economic and zoonotic importance and hugely impacts the efficiency of animals by lowering milk production, increase in treatment costs, and culling of animals due to poor milk production (Mohammadian, 2011). Mastitis is a multifactorial problem of milk producing animals. Understanding its pathogenesis and early diagnosis is of vital importance in mastitis (Khan *et al.,* 2013). The dromedary camels are considered as important dairy animals in arid and semi-arid regions of the world. The camel milk is the major and valuable source of food for the pastoral people in these areas. Determination of changes in some milk biochemical parameters such as milk proteins, albumin, lacto albumin, immunoglobulin and various isoenzymes elucidate the mechanisms of development of udder infection.

The mammary gland infection causes the release of different harmful toxins that ultimately results in

increased milk leukocyte counts along with tissues changes (Ibrahim *et al.,* 2011; Hussain *et al.,* 2012). Milk somatic cells include lymphocytes, neutrophils, macrophages and minute quantity of epithelial cells (Abera *et al.,* 2010; Hussain *et al.,* 2012). The actions of these newly recruited inflammatory and resident milk leukocytes in mammary parenchyma during the early stage of lactation play significant role to establish the intra-mammary infections leading to tissue degeneration. The severity of udder infection can vary from non-visible alterations in milk to severe vascular permeability, increased leukocytes and development of fibrosis (Hussain *et al.,* 2012). During mammary gland infection various products including non-lysosomal and hydrolytic enzymes such as lactate dehydrogenase, *β*-galactosidase lysosomal enzymes are released from the inflammatory cells and the degenerate epithelial cells, which results in reduction in milk quality. The mammary gland infection causes lower milk fat, casein, lactose and different micro and macro elements (Hussain *et al.,* 2012; Hamadani, 2013). The different enzymes both in blood and milk of the infected animals are considered to be the best biomarkers to determine the udder health (Kalantari *et al.,* 2013). Various physical and biochemical alterations in infected milk like increased electrical conductivity and pH, increased level of malondialdehyde are useful tools to monitor the udder health (Yang *et al.,* 2011; Eshratkhah *et al.,* 2012). Therefore, the present study was carried out to investigate the biochemical alterations in milk and blood samples collected from sub clinically infected camels.

MATERIALS AND METHODS

Mastitis detection and sample collection: A total of 33 lactating she camels kept at desert environment of Cholistan were screened for udder infection. Data about age, parity and stage of lactation were obtained from the attendants. Clinically the udders of all milking camels were palpated to examine any gross abnormalities. The milk was observed for any visible changes such as color, odor and clots prior to detection of hidden infection. All the milking udders were washed with sterile water prior to milk collection. About 20 ml of milk sample was collected from each apparently healthy quarter after removal of first few lacteal secretions. All of the milk samples were tested using California mastitis test (Schalm *et al.,* 1971). The severity of infection was categorized as 3=strong reaction, 2=moderate reaction, 1=weak and 0=no reaction on the basis of formation of gel. The blood samples were also collected from all California mastitis test (CMT) positive camels by venipuncture.

Milk biochemical analysis: Milk electrical conductivity and pH were recorded by electrical conductivity meter and pH meter respectively. Milk lactose, protein, fat and solids-not-fat percentages were detected as previously described (Hussain *et al.,* 2012). Total milk somatic and differential somatic cell counts were also determined (Hussain *et al.,* 2012).

Enzyme and oxidative parameters in milk and blood: For milk enzymes peroxidation product equal numbers of milk samples both from mastitic and healthy camels were centrifuged (5000 rpm) for 10 minutes to remove the milk fat. The concentration of different enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and malondialdehyde were determined according to the previously described methods (Nisbet *et al.,* 2007; Hussain *et al.,* 2012). Blood samples from equal number of infected and healthy camels were also collected. The total leukocyte, neutrophil population and packed cell volume was determined. Serum was separated on ice, and then serum total proteins, albumin, and haemoglobin were measured. The values of various enzymes such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were recorded (Babaei *et al.,* 2007; Javed *et al.,* 2015; Mikail and Keskin, 2015).

Statistical analyses: The data were analyzed by analysis of variance (ANOVA). The means were calculated and compared by t-test. $P \leq 0.05$ was considered significant, and where appropriate 95% CL was also determined.

RESULTS

The results of present study indicated an overall prevalence (24.24%) of sub-clinical mastitis. The prevalence of sub-clinical was non-significantly different on the basis of age, parity, stage of lactation and the quarter involved (Table 1 and 2). The results showed that the pH of infected milk significantly increased in relation to the severity of CMT reaction. The electrical conductivity of mastitic milk was significantly higher while fat, proteins; lactose and solid-not-fat % were significantly lower depending upon the score of CMT (Table 3). The milk malondialdehyde concentration and different enzymes such as aspartate aminotransferase, alanine amino-transferase and alkaline phosphatase were significantly increased depending upon the CMT scores (Table 3). The results indicated that the total and differential milk somatic cell counts were significantly increased in milk samples obtained from infected udder as compared to healthy udder (Table 4). Total milk somatic cell and neutrophil counts were significantly higher while the numbers of lymphocytes and macrophages decreased on the basis of the severity of infection. The results showed that the values of total leukocyte counts in blood collected from mastitic camels were increased significantly when compared to the healthy camels. The neutrophil population was significantly higher in blood of the infected camels. The values of blood monocytes and lymphocyte were significantly decreased in mastitic camels. The hemoglobin, serum total proteins and albumin levels were significantly reduced in the infected camels. Serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels were significantly increased on the basis of the severity of CMT score (Table 5).

DISCUSSION

Camels are considered as multipurpose animals in most parts of the world and also kept for milk purpose in spite of being seen on the way (Ibrahim *et al.,* 2011). The reports have indicated that sub-clinical infection in udder in dromedary causes lower milk yield, changes milk properties and impairs the processing and preservation of milk (Saleh and Faye, 2011). The clinical mastitis in

animals can be detected by visual examination of udder and milk. However, for detection of sub-clinical in udder a number of tests are used to detect changes in milk (Batavani *et al*., 2007; Hussain *et al*., 2012). However, the relation of CMT score, presence of different inflammatory cells and the microorganisms indicate that the she-camel milk is similar to that of cattle (Djabri *et al*., 2008). Scanty information is available about the etiological agents of sub-clinical infections in Pakistan in she-camels (Ahmad *et al*., 2012). However, no report is available about the changes in milk composition of mastitis she-camels.

Table 1: Bivariate frequency analysis of different parameters in mastitic and healthy camels

Parameters	Positive		Negative	95% CI	MH Chi-sq P value
	n	%			
Age (Years)					
4	2	33.33	4	6.02-73.81	P = 0.380
5-6	3	30.00	7	8.26-61.99	
7-8	2	25.00	6	4.43-61.17	
9-12	1	11.11	8		
Overall	8	24.24	25	11.94-40.89	-
Parity (n)					
1-2	3	23.07	10	6.23-50.86	P = 0.736
3-4	3	25.00	9	6.79-54.12	
5	2	22.22	7	3.91-56.21	
Lactation stage (Months)					
1-2	3	21.42	11	5.76-47.95	P = 0.886
3-4	3	18.18	9	3.17-48.27	
Above 5	2	25.00	6	4.43-61.17	

Table 2: Quarter based prevalence of sub-clinical mastitis in Cholistani camels

Quarter	No. Positive (CMT 1+ to CMT 3+)	Total examined	% prevalence	95% CI
RR	7	8	87.50	51.97-99.37
LR	6	8	75.00	38.83-95.57
LF	6	8	75.00	38.83-95.57
RF	5	8	62.50	27.80-89.44
Overall	24	32	75.00	57.99-87.66

LR=Left Rear, LF=Left Front, RR=Right Rear and RF=Right Front.

The results of present study indicate that the prevalence of sub-clinical mastitis cases in camels is not significantly different on the basis of age, parity, stage of lactation and quarter involved. Increased prevalence of mastitis with increase in age, lactation, parity and hind quarters in cattle and buffaloes has been reported (Hussain *et al*., 2012; Hussain *et al*., 2013). In this study, the results indicate that milk electrical conductivity and pH is significantly higher in subclinical infection when compared to non-infected animals. The increased in electrical conductivity and milk pH in relation to severity of CMT score shows the severity and tissue changes in udder. The milk increased electrical conductivity and pH could also be due to increased milk somatic cells (Kasici *et al.,* 2012; Yarabbi *et al.,* 2014). In addition, it has been reported that increased electrical conductivity and milk pH is due to increased leakage of various ions and salts as a result of increased permeability of vascular membranes following inflammatory reactions (Hussain *et al.,* 2012). The milk biochemical parameters such as milk proteins, fat, lactose and solid-not-fat were significantly decreased in relation to CMT scores indicating inflammatory changes in udder. These changes might be due to poor and impaired efficiency of mammary gland due to adverse impacts of pathogens in mammary parenchyma. Previously similar reports are available in sub-clinically infected buffaloes and cattle (Hussain *et al.,* 2012; Calderon-Rangel *et al.,* 2014: Yarabbi *et al.,* 2014). In infected cases, the milk total somatic cells and neutrophil number significantly increased in this study. Different studies have indicated that increased values of total somatic cells and neutrophil leucocytes in milk results due to inflammatory response in udder (Guliye *et al.,* 2011; Haron *et al.,* 2014; Kamal *et al.,* 2014). The values of lymphocytes and macrophages were reduced significantly in the present study which also has been reported in infected milk of cattle and buffaloes (Bhutto *et al.,* 2012; Jin-bo *et al.,* 2012). The level of malondialdehyde, a lipid peroxidation product, was significantly increased in milk of infected she-camels. Previously similar results have also been reported in cattle (Yang *et al.,* 2011).

The milk and blood alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were detected to be higher in mastitic camels when compared to healthy animals. The increased concentration of enzymes in the present study could be due to the tissue damage in udder. Previously, it has been reported that the increased activity of alkaline phosphatase enzymes is an indication of infection and plays significant role in development of infection (Hussain *et al.,* 2012). In contrast to our findings decreased concentration of lactate dehydrogenase enzyme has been reported (Mohammadian, 2011; Kalantari *et al.,* 2013). Different studies have shown the increased concentration of milk alkaline phosphatase and aspartate aminotransferase enzymes in mastitic animals (Hussain *et al.,* 2012; Kalantari *et al.,* 2013). The increased concentration of various enzymes and lipid peroxidation product in this study can be linked with increased microcircular permeability induced by free radicals (Yang *et al.,* 2011; Hussain *et al.,* 2012). From the results of this study it can be concluded that the mammary gland infection causes adverse impacts on the milk composition and hemato-biochemical changes in sub-clinical mastitic dromedary she-camels.

Table 3: CMT score based milk biochemical changes in healthy and sub clinically mastitic camel

Parameters	CMT Score			
	0	+	++	+++
Milk PH	6.37±0.01	6.93±0.15	6.87±0.01	7.48±0.05*
Electrical conductivity (mS/cm)	6.21±0.04	7.37±0.06*	7.77±0.04*	7.93±0.01*
Fat (%)	4.12±0.07	3.52±0.03*	3.36±0.04*	3.28±0.02*
Protein (%)	3.87±0.02	2.90±0.02*	2.64±0.02*	2.49±0.02*
Lactose (%)	4.15±0.10	3.46±0.03*	3.3±0.02*	3.21±0.02*
Solid not fat (%)	9.53±0.07	7.23±0.10*	6.68±0.03*	6.33±0.04*
Malondialdehyde concentration	1.41±0.01	2.51±0.03*	2.65±0.03*	2.70±0.02*
Aspartate aminotransferase (U/L)	8.74±0.12	9.86±0.20*	11.6±0.24*	12.39±0.22*
Alanine aminotransferase (U/L)	10.80±0.22	14.17±0.28*	16.75±0.23*	18.62±0.21*
Alkaline phosphatase (U/L)	18.11±0.36	23.67±0.36*	27.8±0.56*	31.81±0.37*

Values with asterisk differed significantly in rows as compared to negative (0) reaction. The results were categorized on the basis of CMT reaction negative (0), mild (+), moderate (++) and severe (+++).

Table 4: CMT score based milk total and differential somatic cell count in healthy and sub clinically mastitic camel

Parameters	CMT score			
	0	+	++	+++
Total SCC (x10^5/ml)	0.97±0.04	2.68±0.07*	3.61±0.06*	7.20±0.17*
Neutrophil (%)	24.51±1.02	37.98±1.44*	45.41±1.43*	50.31±0.85*
Lymphocyte (%)	20.08±0.77	14.64±0.27*	12.64±0.3*	11.5±0.27*
Macrophage (%)	43.37±1.39	30.97±1.78*	27.68±0.92*	24.54±0.42*

Values with asterisk differed significantly in rows as compared to negative (0) reaction. The results were categorized on the basis of CMT reaction negative (0), mild (+), moderate (++) and severe (+++).

Table 5: CMT score based hemato-biochemical changes in healthy and mastitic camels

Parameters	CMT score			
	0	+	++	+++
Total leukocyte count	12.37±0.34	15.78±0.43	18.22±0.37*	19.18±0.54*
Pack cell volume (%)	41.12±0.59	26.92±0.19*	24.72±0.19*	23.9±0.49*
Neutrophils (%)	30.81±0.53	40.87±1.14*	42.05±0.52*	43.88±0.72*
Monocytes (%)	2.62±0.01	3.65±0.09*	3.95±0.04*	4.21±0.06*
Lymphocytes (%)	57.9±0.65	42.44±0.82*	40.15±1.02*	37.61±0.36*
Hemoglobin (g/ld)	12.65±0.15	8.04±0.15*	7.82±0.04*	7.54±0.03*
Serum total protein (g/ld)	7.31±0.11	5.51±0.09*	4.94±0.05*	4.75±0.05*
Serum albumin (g/ld)	3.86±0.02	2.75±0.01*	2.56±0.04*	2.42±0.03*
Alanine aminotransferase (U/L)	12.99±0.12	20.04±1.17*	31.4±0.92*	41.55±0.99*
Aspartate aminotransferase (U/L)	33.08±0.94	41.22±0.71	45.31±0.79	51.71±0.31
Alkaline phosphatase (U/L)	92.6±1.54	105.77±1.95*	115.77±1.22*	110.15±2.50*

Values with asterisk differed significantly in rows as compared to negative (0) reaction. The results were categorized on the basis of CMT reaction negative (0), mild (+), moderate (++) and severe (+++).

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