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RESEARCH ARTICLE

Investigation of Milk and Blood Serum Biochemical Profile as an Indicator of Sub-Clinical Mastitis in Cholistani Cattle

Abdul Qayyum[1,2], Jawaria Ali Khan[1], Riaz Hussain[2*], Muhammad Avais[1], Nisar Ahmad[3] and Muhammad Sarwar Khan[1]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences Lahore, Pakistan
[2]University College of Veterinary and Animal Science, The Islamia University of Bahawalpur-63100, Pakistan
[3]Department of Parasitology, University of Veterinary and Animal Sciences Lahore, Pakistan
*Corresponding author: driazhussain@yahoo.com

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ABSTRACT

Bovine mastitis causes severe economic losses in terms of poor milk production and culling of chronically infected animals. In present study milk samples were collected from Cholistani cattle and examined using California mastitis test (CMT). Different milk and blood serum biochemical parameters were investigated. The results indicated that milk pH, milk electrical conductivity, malondialdehyde concentration and total dissolved solids were significantly ($P<0.0001$) increased with increase in CMT score. The values of milk fat, protein, lactose and solids not fat were significantly reduced in milk samples of sub-clinically infected cattle. The results showed that the values of total milk leukocyte and neutrophil counts were significantly higher in infected cattle, while the percentages of monocytes, macrophages and lymphocytes were lower. The levels of potassium, calcium, magnesium, phosphorous, iron and zinc were significantly lower in milk and blood serum of infected than normal cattle. The enzymes such as aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase were higher in milk and blood serum of infected animals. Similarly, malondialdehyde and nitric oxide were significantly higher in infected animals. The results showed that mastitis poses deleterious effects on milk and blood of infected animals.

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INTRODUCTION

About 8.43 million human people in Pakistan are involved in rearing buffaloes (29.9 million) and cattle (33.0 million) which share more than 95% of the total milk produced in country (Government of Pakistan, 2007–2008). Huge efforts regarding expansion in the field of dairy animal breeding, animal nutrition, husbandry practices and welfare has played important role in increasing global milk production over the last few decades to meet the increasing demand for milk, milk products and meat (Tiwari *et al.,* 2013). Mastitis is the most substantial disease of dairy animals, hugely effects the farm economics by decrease in production of milk and increase in treatment costs (Mohammadian, 2011). Mammary gland infection is multifactor and a complex disorder of milk secretory tissues of lactating animals which requires the understanding of exact mechanism of its development (Khan *et al.,* 2013). Inflammation of mammary parenchyma results in release of various harmful toxins in the udder which lead to increased milk somatic cells count and severe tissues changes (Yousaf *et al.,* 2010; Ibrahim *et al.,* 2011). Milk leukocyte consists of different types such as neutrophils, lymphocytes, macrophages and small percentage of epithelial cells (Abera *et al.,* 2010). The activities of newly recruited and resident milk somatic cell in udder at early period of lactation play crucial role in establishment of intra-mammary infection and ultimately results in tissue changes. The tissue lesions in udder can vary from no visible changes in milk to increased vascular permeability, increased milk leukocytes counts and development of fibrosis (Hussain *et al.,* 2012a). During udder infection, the inflammatory leukocyte and damaged epithelial cells release different products such as hydrolytic and non-lysosomal enzymes including lactate dehydrogenase or *β*-

galactosidase lysosomal enzyme which lowers the milk quality. Mastitis causes the decrease level of milk casein, fat, lactose and various important micro and macro minerals in animals (Hussain *et al.,* 2012b; Hamadani, 2013). Different milk enzymes, like lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) are originated from disintegrated blood leukocytes, interstitial cells and damaged epithelial cells of the mammary parenchyma. These enzymes in milk and blood of infected animals are considered as the best biomarkers of udder health as their level is increased during inflammation in mastitic animals (Kalantari *et al.,* 2013). Various biochemical changes in milk including increased concentration of malondialdehyde, sodium and decreased levels of potassium are useful tools to monitor the udder health in animals. Previously, it has been reported that milk electrical conductivity, milk pH and potassium are usually the most important and sensitive diagnostic tool to determine the sub-clinical mastitis in bovine (Eshratkhah *et al.,* 2012). Therefore, the current study was conducted to determine the biochemical changes in milk and blood serum of sub-clinically infected Cholistani cattle.

MATERIALS AND METHODS

Collection of milk and blood samples: A total of 320 milk and blood samples were collected from sub-clinically infected Cholistani cattle kept in and around district Bahawalpur during November 2013 to December 2014. All the cattle selected in this study were apparently healthy and free of any clinical disease and palpable udder lesions. Prior to collection of milk for diagnosis the udder and teat end of each animal was thoroughly washed using sterile water. After discarding first few lacteal secretions about 50 ml milk samples were collected in plastic sterile tubes. The blood samples (10 ml) were collected from the jugular vein of infected cattle.

California mastitis test (CMT) and milk biochemical changes: The California mastitis test (CMT) was used to detect the subclinical mastitis on all the collected milk samples (Schalm *et al.,* 1971). The severity of infection was measured on the basis of CMT results as 0 to 3 (0=No reaction or trace, 1=Weak positive, 2=Moderate positive; 3=Strong positive).

Milk pH was measured with the help of pH meter. Electrical conductivity and milk temperature was measured by electrical conductivity meter. Milk protein, fat, lactose and solids not fat were determined as described by Hussain *et al.* (2012b). The milk total and differential leukocyte count was determined as previously described (Gargouri *et al.,* 2008). Various trace minerals and milk electrolytes were investigated through wet digestion. Sodium and potassium concentrations in milk were investigated by flame photometer. Estimation of total phosphorus was done by using colorimetric method at a wavelength of 720 nm against standard and blank. The calcium, copper, magnesium, iron and zinc were investigated by using atomic absorption spectrophotometry (Hussain *et al.,* 2012b).

Enzyme and oxidative parameters: Equal numbers of defatted milk and blood serum samples of healthy and mastitic animals were selected for estimation of enzymes. Briefly, all the infected quarter milk samples were centrifuged for 5 min to remove butter fat at 0^{o}C and 5000g. Blood samples without anticoagulant (EDTA) were collected from healthy and mastitic lactating cows for serum analysis. Serum was removed from blood of infected animals by placing the blood samples on ice. The activities of different enzymes in milk and blood samples were determined spectrophoto-metrically by using commercially available kits. The activity of AST (Human, Catalog # 12011), LDH (Randox, Catalog # 259500) and ALP (Analyticon, Catalog # 954) was estimated on a spectrophotometer at wavelengths of 340, 405 and 340 nm respectively (Babaei *et al.,* 2007). Malondialdehyde (MDA) and nitric oxide (NO) oxidative stress parameters both in milk and blood samples were determined by using spectrophotometer (Nisebet *et al.,* 2007).

Statistical analyses: The data collected in this study were analyzed by using one way of ANOVA and means were compared by t-test. $P<0.05$ was considered as significance level.

RESULTS

The results indicated that the temperature of milk obtained from infected and healthy cattle did not reveal significant statistical difference. The pH, milk electrical conductivity, malondialdehyde concentration and total dissolved solids were significantly ($P<0.0001$) higher in milk samples collected from infected cattle when compared to healthy animal. However, the values of milk fat, protein, lactose and solids not fat were significantly lower in milk of sub- clinically infected cows (Table 1).

Table1: CMT score based milk biochemical changes (Mean±SE) in healthy and sub clinically mastitis cattle

Parameters	CMT score			
	0	+	++	+++
Milk Temp (˚C)	37.3±0.1	37.56±0.10	37.8±0.08	37.7±0.10
Milk pH	6.74±0.01	6.96±0.01	7.04±0.02	7.89±0.03*
Electrical Conductivity (mS/cm)	4.71±0.05	5.35±0.07*	5.97±0.12*	5.97±0.04*
Fat (%)	4.85±0.03	3.34±0.01*	3.25±0.042*	3.21±0.03*
Protein (%)	3.69±0.03	2.91±0.02*	2.83±0.03*	2.64±0.04*
Lactose (%)	4.45±0.03	3.69±0.01*	3.60±0.05*	3.39±0.05*
Solid not fat (%)	10.8±0.15	7.93±0.07*	7.66±0.09*	7.31±0.10*
Total solid (mg/L)	1405.3±12.1	1634.9±26.3*	1748.3±30.5*	1927.5±16.3*

Asterisk values differed significantly in rows. CMT results were categorized on the basis of severity into severe (+++), moderate (++), mild (+) and negative (0).

Table 2: CMT score based milk total somatic cell and differential count in healthy and mastitic cattle

Parameters	CMT score			
	0	+	++	+++
Total SCC (x10^5/ml)	2.17±0.05	9.57±0.25*	33.9±1.05*	79.4±1.15*
Neutrophils (%)	18.6±0.42	27.8±0.35*	53.5±0.40*	60.5±0.34*
Macrophages (%)	46.4±0.38	31.7±0.21*	21.8±0.21*	19.6±0.32*
Monocytes (%)	13.6±0.24	7.55±0.13*	5.53±0.11*	5.45±0.15*
Lymphocytes (%)	21.3±0.55	14.0±0.31*	14.3±0.39*	13.4±0.35*

Values (Mean±SE) bearing asterisk in a row differ significantly. CMT results were categorized on the basis of severity into severe (+++), moderate (++), mild (+) and negative (0).

Table 3: CMT score based various minerals in milk and blood of healthy and mastitic cattle

Parameters	CMT Score			
	0	+	++	+++
Serum milk minerals (mg/dl)				
Sodium	50.67±0.43	67.47±0.54*	84.31±0.39*	90.91±0.24*
Potassium	181.82±0.71	171.49±0.55*	153.72±0.60*	143.93±0.39*
Calcium	118.59±0.52	111.19±0.40*	100.27±0.20*	94.15±0.59*
Magnesium	9.84±0.15	8.81±0.091*	8.40±0.031*	8.004±0.01*
Phosphorous	34.72±0.21	29.19±0.37*	24.41±0.14*	21.18±0.20*
Iron	0.07±0.04	0.04±0.03*	0.04±0.00*	0.03±0.03*
Zinc	0.05±0.04	0.04±0.03	0.03±0.00*	0.03±0.00*
Copper	0.01±0.04	0.01±0.02	0.01±0.02	0.01±0.00
Serum blood minerals (mg/dl)				
Calcium	7.95±0.01	6.57±0.03*	6.2±0.01*	6.0±0.01*
Phosphorus	4.88±0.09	4.19±0.01*	3.97±0.09*	3.49±0.03*
Zinc	1.06±0.06	0.98±0.05*	0.88±0.04*	0.83±0.04*

Values (Mean±SE) bearing asterisk in a row differ significantly. CMT results were categorized on the basis of severity into severe (+++), moderate (++), mild (+) and negative (0).

Table 4: CMT score based milk and blood serum enzymes concentrations in healthy and sub clinically mastitic cattle

Parameters	CMT Score			
	0	+	++	+++
Milk serum enzymes (IU/L)				
Alkaline phosphatase	60.79±0.25	70.8±0.32*	99.9±0.31*	109.86±0.34*
Aspartate aminotransferase	150.17±1.34	160.33±0.44*	184.69±1.14*	191.32±1.29*
Lactate dehydrogenase	167.86±1.51	264.44±5.85*	477.85±5.52*	777.69±13.29*
Blood serum enzymes (IU/L)				
Alkaline phosphatase	49.7±0.50	64.1±0.21*	77.9±0.22*	81.7±0.28*
Aspartate aminotransferase	111.1±1.01	130.9±0.29*	139.6±0.39*	147.6±0.25*
Lactate dehydrogenase	376.4±2.66	514.2±2.75*	556.8±2.0006*	637.6±2.72*

Values (Mean±SE) bearing asterisk in a row differ significantly. CMT results were categorized on the basis of severity into severe (+++), moderate (++), mild (+) and negative (0).

Table 5: Lipid peroxidation products Malondialdehyde and Nitric oxide in milk and blood serum (Mean ± SE) of healthy and sub clinically infected cattle

Parameters	CMT score			
	0	+	++	+++
Malondialdehyde levels in milk (nmol/ml)	5.31±0.07	7.63±0.09*	8.59±0.08*	10.5±0.16*
Malondialdehyde levels in blood (nmol/ml)	1.62±0.01	2.18±0.08*	2.56±0.05*	2.78±0.06*
Nitric oxide levels in blood (µmol/L)	29.8±0.26	36.4±0.15*	44.9±0.93*	59.9±0.22*

Values (Mean±SE) bearing asterisk in a row differ significantly. CMT results were categorized on the basis of severity into severe (+++), moderate (++), mild (+) and negative (0).

The results showed that the values of total milk leukocytes and differential leukocyte counts (Table 2) were significantly different in infected and normal cattle. The values of total milk leukocyte counts along with neutrophil percentage were significantly higher in infected cattle as compared to healthy animals. The values of monocytes, macrophages and lymphocytes were significantly lower in milk samples collected from infected animals than the normal cattle. The mean±SE values of milk macro and micro mineral profile is presented in Table 3. The results indicated that the level of potassium, calcium, magnesium, phosphorous, iron and zinc was significantly lower in milk and blood serum of infected cattle as compared to normal animals. However, the values of sodium were significantly increased in mastitic cattle.

The different milk serum enzymes such as aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase were statistically increased in milk and blood serum of infected animals. These increased values of milk enzymes were dependent on the severity of CMT scores (Table 4). The values of lipid peroxidation products malondialdehyde and nitric oxide were also significantly increased in milk and blood of infected as compared to non-infected cattle (Table 5).

DISCUSSION

Literature related to biochemical and hematological changes due to sub clinical mastitis in Cholistani cattle in Pakistan is very little. However different studies have been conducted in various other breeds of cattle and buffaloes to assess only the biochemical changes in mastitic milk. In present study the results showed that the mean values of milk pH and electrical conductivity were significantly higher on the basis of CMT score in mastitic

milk as compared to healthy animals. The increased values of these parameters were associated with the severity of CMT score. Previously it has been reported that CMT test is accurate and reliable tool for cow's milk. The higher milk pH might be due to increased milk leukocytes (Hassan, 2013; Yarabbi *et al.,* 2014) while the increased milk electrical conductivity can be associated with alterations in mineral contents of infected milk (Kasikci *et al.,* 2012). The increased milk pH and electrical conductivity could be due to the increased leakage of salts and different ions as results of increased permeability of membrane following inflammatory reactions (Hussain *et al.,* 2012a). Decreased concentrations of milk fat, proteins, lactose and solid not fat in present study might be due to the inflammatory changes in mammary parenchyma. The effects on various component of milk in this study probability could be due to the impaired synthetic activity of mammary gland or due to damaging effects of pathogens ultimately reducing the secretory activity of milk producing cells in mammary glands. These results are similar to different previous studies indicating decreased milk fat, proteins, lactose and solid not fat in milk of mastitic cattle (Yarabbi *et al.,* 2014; Calderon-Rangel *et al.,* 2014). In present study results revealed that total milk leukocyte counts along with neutrophil percentage increased significantly in infected cattle. It is reported that influx of milk total and neutrophil cell population occurred due to the inflammatory reactions in mammary parenchyma. Previously different studies have shown that predominant neutrophil population in milk is the best indication of mammary infection (Kamal *et al.,* 2014). The percentage of monocytes, macrophages and lymphocytes was decreased significantly in infected milk. Previously, lower values of these cells in mastitic milk have also been reported (Bhutto *et al.,* 2012; Jin-bo *et al.,* 2012).

The mean±SE values of some macro and micro minerals such as potassium, calcium, magnesium, phosphorous in milk of infected cattle were significantly reduced. Moreover, in addition to these minerals the values of zinc in blood of infected cattle were also reduced significantly. However, the values of sodium were significantly higher in mastitic cattle. The values of milk iron, copper and zinc were non-significantly different between infected and healthy cattle. Previously decreased levels of potassium (Haron *et al.,* 2014), phosphorous (Batavani *et al*. 2007), calcium (Yildiz and Gusuzoglu, 2005), magnesium (Hussain *et al.,* 2012b), while increased level of sodium (Haron *et al.,* 2014) have been reported.

The different milk and blood serum enzymes including aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase were significantly higher on the basis of severity of CMT scores. The increased levels of these enzymes in milk and blood serum can be linked with tissue damage in mammary parenchyma. Moreover, the increased concentration of these enzymes in milk can be attributed to damaged leukocytes and secretory epithelial cells in the udder. Previously increased levels of lactate dehydrogenase in milk (Zaki *et al.,* 2010) while decreased in blood serum (Mohammadian, 2011; Kalantari *et al.,* 2013) have been reported in mastitic animals. Similarly, different studies have indicated the higher values of milk aspartate aminotransferase (Hussain *et al.,* 2012b) and non-significant different activity of aspartate aminotransferase in infected animals (Matei *et al.,* 2010). The higher level of serum alkaline phosphatase is suggestive of infection and plays important role in the pathogenesis of disease in infected animals (Hussain *et al.,* 2012b). Previously various studies have shown the higher levels of alkaline phosphatase in infected cattle (Kalantari *et al.,* 2013). The values of lipid peroxidation products malondialdehyde and nitric oxide were also significantly higher in milk and blood of infected cattle. The increased values of serum enzymes and lipid peroxidation products such as malondialdehyde concentration and nitric oxide in present study might be due to the increased permeability of microcirculatory vessels and oxidative stress due to free radical injury. Previously similar results have also been reported in cattle (Akerstedt et al. 2011).

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