A comparison of milk protein status of healthy and mastitic cows under denaturing conditions

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Key Words:
SDS-PAGE; mastitis; normal cow; milk protein.

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Received 7 February 2009,
Accepted 14 December 2009

Abstract

Mastitis is one of the most important diseases of dairy cattle in the world. The identification and characterization of the constituent proteins in milk can be useful for studying the biochemistry and pathogenesis of mastitis. In this study, the electrophoretic patterns of milk from 10 healthy and 30 mastitic cows were studied. All of the latter milk samples were California Mastitis Test (CMT) positive, and these were cultured to isolate the infective agents. The electrophoretic patterns of these samples and those of healthy cows (negative CMT and cultures) were studied with the SDS-PAGE technique. The approximate molecular weight of protein bands were categorized by their different flow rates (Rf), and these ranged between 18.5 - 220 KDa in mastitis samples of milk. The electrophoretogram showed that higher molecular weight bands appeared in the milk of mastitic cows60-220KDa and many were in the range of 176-208 kDa.The major band for the healthy samples was 220 KDa. In this respect, the mastitis samples had a minimum of two bands and a maximum of five bands, while milks from healthy cows did not show any bands in this range. On the basis of the different result between the electrophoretic patterns of milk from healthy and mastitic cows, it can be concluded that SDS-PAGE is a suitable method for the diagnosis of cows withsub sub clincal mastitis.

Introduction

The exact components of raw milk vary between species of cow, but it always contains significant amounts of saturated fat, protein, calcium and vitamins (Bowen *et al*., 2005). The major proteins in milk are caseins, α-lactalbumin, ß-lactoglobulin, immunoglobulins, lactoferrin, serum albumin, N-acetyl-β-glucosaminidase, and α-antitrypsin. In this respect, the components of milk can be categorized into ones with higher concentrations, such as phosphoproteins (91 kDa), and iron-binding glycoprotein (80 kDa; Baggiolini *et al*., 1970), and constituents with lower concentrations, such as calcium-binding protein (75 kDa; Hogarth *et al*., 2004) and α-antitrypsin (54 kDa; Mao *et al*., 2001).

There are some contraversies about mastitis effect on the milk constituents.In this respect Ashowr *et al*. (1967) discussed that Negasawa and Tanahashi (1963) did not observe any changes in this regard. It has been argued that the composition of milk, especially proteins and protease activity, changes in the presence of mastitis (Moussaoui *et al*., 2002; 2003; Moussaoui *et al*., 2004) Lecce and Legates (1959) reported that the concentrations of α-lactalbumin and ß-lactoglobulin proteins (14 and 18.4 kDa, respectively) decreased in the milk of cows with acute mastitis. The analysis of proteins in milk has a potentially major diagnostic significance (Hogarth *et al*., 2004).

Haptoglobin (HP) and serum amyloid A (SAA; molecular weights (MW) between 11.7 and 12.5 kDa) are well-known bovine acute phase proteins (APP), and their concentrations increase in the milk of mastitic cows (Ilzeka and Stelmasiak, 2000). HP is a major APP in ruminants and it has been suggested as a diagnostic marker for mastitis (Eckersall *et al*., 2001; McDonald *et al*., 2001). The measurement of SAA in milk samples could also be a useful marker for the diagnosis of subclinical mastitis. The most research on milk proteins has focused on the proteins of milk with molecular weights between 2,300 and 2,400 Da. However, some minor proteins of milk, such as HP, SAA and C-reactive protein (CRP) have also received much interest due to their potential practical value for diagnosis of inflammation. (Patton, 1999; Hiss *et al*., 2004).

Polymorphisms in the gens that encode milk proteins and the quantitative differences in the expression of variant alleles have been studied by gene analysis as well as by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) methods (Medrano and Aguilar-Cordova, 1990; Lum *et*

al., 1997).

A comparison of the constituents of milk proteins between healthy and mastitic cows could identify potential markers for the early diagnosis of subclinical mastitis. Therefore, the purpose of this present study was to evaluate and compare the milk proteins of healthy animals with those of cows with sub-clinical mastitis using the SDS-PAGE method.

Materials and Methods

Milk samples from 30 cows with subclinical mastitis and 10 healthy cows were collected in the summer months of 2006 from the Research Farm of the University of Tehran in Aminabad, Tehran, Iran. Cows were aged between two and eight years old. Cows were assessed and diagnosed with subclinical mastitis on the basis of clinical signs and the California Mastitis Test (CMT) in accordance with the study by Randy *et al*. (2003). Samples were transferred immediately to the Department of Biochemistry, Faculty of Veterinary Medicine, University of Tehran on an ice bag. The concentration of proteins was determined by the method described by Lowry *et al*. (1951).

Each sample was divided into two aliquots; one was used for the microbiological studies and the other was frozen at -80°C until electrophoretic studies were performed in accordance with the methods described by Bowen and Lawrence (2005). Briefly, 1.5 ml of raw milk was incubated at 75°C for15 minutes and centrifuged at 15,000 ×g. The supernatant was collected and diluted (1:1) with sample buffer (3% SDS, 5% 2ME, 10% glycerol, 0.005% BPB, 6% Tris base, pH 7.3). They were then incubated at between 90 and 100°C for 5 mins and then again centrifuged at 15,000 ×g for 5 mins. The resultant supernatant was applied to polyacrylamide gels that contained 12.5% SDS (Laemmli and Faver, 1973). Gels were stained with comassie blue and the pattern of protein bands were compared with the standard protein marker (MW-SDS-200; Sigma). The number of bands in each rate of flow (RF) were shown as the maximum and minimum and compared with the Mann-Whitney test using Sigma Stat 2.(Systat software Inc, point Richmond, CA, USA)The α in all cases was < 0.05.

Results

Clinical findings of milk production rate and CMT values were shown in Table 1.

Figure 1 shows the electrophoretic band patterns of different milk proteins. The bands had different patterns when the healthy and mastitic samples of milk were compared. Moreover, the numbers of bands in different RFs are shown in Table 2. The RFs of bands were determined as the distance from the well (the starting point of the sample) to the bottom of the protein band. This distance was divided by the distance from the well to the tracking dye band (Goodrich *et al*., 1993).

The results showed that there was a protein (MW=97 kDa) in the mastitic samples, which was absent in the healthy samples of milk (Figure 1).There was also a specific protein band in mastitic samples (MW=18.5 KDa). It was shown that a protein band with a MW of 205 kDa is present in healthy samples of milk, whereas it was not present in mastitic samples. In the RF of 0.2-1.2, the normal electrophoretic pattern showed protein bands in 220 KDa whereas the range in the mastitic cows was between 18.5-220 K Da.

Discussion

Despite considerable research, mastitis remains one of the most important diseases of dairy cattle in the world (De Graves and Fetrow, 1993). Milk protein can be considered as a useful marker for monitoring of bovine mastitis. (Hogarth *et al*., 2004). In normal milk, the most abundant proteins in the whey are β-

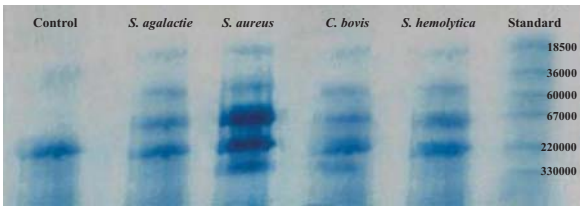
Table 1: Status of bacterial contamination in the milk samples (23) and isolated bacterial strains.

Isolated bacteria	Number of samples	Positive CMT
Coryne bacterium bovis	9	+
Streptococcus agalactiae	3	+
Streptococcus hemolytica	3	+
Staphylococcus aureus	4	+
No growth of bacteria	4	-

Table 2: Number of bands in different RF for milk samples of infected and healthy cows that were electrophoresed using SDS-PAGE.

Isolated bacteria in subclinical mastitis	Number of bands in RF 0.2-0.4		Number of bands in RF 0.2 -0.4		Number of bands in RF 0.4 -.6		Number of bands in RF 0.6 -0.8		Number of bands in RF 08 – 1		Number of bands in RF 1-1.2	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Coryne bacterium bovis	4	5	3	5	1	2	2	3	2	4	1	3
Streptococcus agalactiae	3	4	2	4	1	3	0	2	2	3	0	1
Streptococcus hemolytica	4	5	4	4	2	1	2	3	2	4	0	1
Staphylococcus aureus	3	4	3	5	0	2	0	2	2	3	0	1
No growth of bacteria	———		0	1	1	2	0	2	1	2	1	2

Figure 1: Electrophoretic pattern of different bacteria in infected milk compare to the control and standard using SDS-PAGE.



Lactoglobulin (β-La), α-Lactalbumin (α-LG) and Blood Serumalbumin (BSA) , as identified in by two dimensional gel electrophoresis (2-DE) (Hogarth *et al*., 2004).

In the present study, protein bands of 30 mastitic cows and 10 healthy ones were determined, as shown in Table 1. We showed a decrease in the milk production in both clinical and subclinical mastitic conditions, as has also been reported by Hogarth *et al*. (2004) and Moussaoui *et al*. (2004). Moussaoui *et al*. (2003, 2004) also showed changes in milk composition, including the level and type of protein constituents, enzymatic activities, and the number of polymorphonuclear (PMN) cells.

E. coli is one of the most important pathogens that causes mastitis in dairy cows (Bradley, 2002). However, the quality and quantity of the proteins is not the same in different severities of mastitic conditions. In this respect, significant differences have been found in the milk concentration of lactoferrin (LFC) among milk samples from normal cows and those with subclinical and clinically obvious mastitis by Kawai *et al*. (1999). The ranges of milk LFC in cases of mastitis were higher than those reported in normal cows, and the LFC in the milk of cows with subclinical mastitis was significantly lower than those from cows with clinical mastitis (Kawai *et al*., 1999).

In conclusion, we have shown that there are differences in the electrophoretic patterns of milk from cows that were normal or had subclinical mastitis in denaturing conditions. We propose that SDS-PAGE can be applied as a suitable method for the diagnosis of mastitis in cows.

Acknowledgments

I would like to thank Dr. Mahmood Bolorechi for assisting me in the collection of milk samples.

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