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RELATIONSHIP BETWEEN SERUM AMYLOID A3 IN SERUM AND MILK OF MASTITIC COWS

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ABSTRACT

Bovine mastitis, an important disease of dairy cattle, causes a significant change in normal milk composition as well as changes the overall blood biochemistry. We grouped the animals of Jersey and Holstein Frisian (HF) crossbred into normal, subclinical and clinical mastitis groups (n = 10 each) based on clinical history and diagnostic tests. The serum albumin showed a non-significant variation between the groups of both breeds following mastitis at subclinical and clinical stages. Mammary assosiated serum amyloid A3 (M-SAA3) protein in serum of Jersey crossbred cows showed non-significant difference between the groups, while in milk significant rise in M-SAA3 levels between mastitic groups was observed. In HF crossbred, there was significant increase in M-SAA3 levels in both milk and serum. The study revealed that M-SAA3 is a positive acute phase protein (APP) in both milk and serum and could be used as an adjunct biomarker to diagnose subclinical mastitis.

Key words: Acute phase protein, biomarker, albumin, bovine, mastitis, M-SAA3

INTRODUCTION

Mastitis, an inflammation of mammary gland, is a widespread disease of the dairy cattle associated with variations in the physical, chemical, pathological and bacteriological quality of milk and glandular tissues (Radostits *et al*., 2000). In response to the infection or inflammation, a rapid and non-specific reaction known as the acute phase response (APR) is generally elicited by the host, characterized by production of cytokines (Suffredini *et al*., 1999). Locally, cytokines mediate leukocyte recruitment, particularly neutrophils and mononuclear cells, to the sites of inflammation. During a mastitis episode, defense cells migrate from blood to mammary gland to combat infectious agents, which invariably increase milk SCC (Rainard *et al*., 2000). Consequently, SCC is currently used to screen for intramammary infection (IMI) status at herd level, because SCC is widely available to the dairy farmers (Schukken *et al.,* 2003). Somatic cell count is used worldwide as an indicator of subclinical mastitis and is considered an important test to assess the efficiency of a mastitis control program in dairy herds (Schukken *et al.,* 2003). Moreover, the other screening tests related to bovine mastitis like CMT and electric conductivity (EC) are also frequently performed to separate the mastitic animals from non-mastitic ones. However, these screening tests do not confirm the stage of mastitis (*i.e.* clinical and subclinical) confidently as there are chances of getting false positive results based on these screening tests. Therefore, the current study was aimed to find out other possible biomarkers like acute phase proteins (APPs) to diagnose mastitis at right stage with

lowest possible error. Systemically, cytokines act on extra hepatic and hepatic tissue where they alter the gene expressions of acute phase proteins (APPs). In cattle some APP are synthesized extra-hepatically like production of haptoglobin (Hp) and serum amyloid A (SAA) in mammary gland during mastitis (Molenaar *et al.,* 2004). Among various isoforms of serum amyloid, mammary assosiated serum amyloid A3 (M-SAA3) protein has been found helpful in determining the status of inflammatory diseases in farm animals. Further, the concentration of M-SAA3 protein in milk of cattle suffering from mastitis has been reported to increase during mastitis specifically. Bovine serum albumin synthesis in liver is down- and up-regulated in mammary tissues during mastitis (Shamay *et al*., 2005). The possibility of using APPs as markers of inflammation has triggered broad research in cattle diseases. In present study, the role of serum albumin and M-SAA3 protein has been determined to assess their possible role as biomarkers in detection of mastitis in local Jersey and HF crossbred cattle in Kashmir valley.

MATERIALS AND METHODS

The present study was conducted from August, 2013 to June 2014 at the Division of Veterinary Biochemistry, SKUAST-K, Shuhama. Apparently healthy lactating animals (4 to 6 years) of Jersey and Holstein Frisian (HF) crossbred cattle presented at Clinical Complex were employed in this study. Crossbred animals of either breed were divided into three groups (n = 10 each) *viz.*, normal, subclinical and clinical mastitis based on physical examination of animals and screening of milk by California Mastitis Test (CMT), electric conductivity (EC) and pH. Animals were examined for mastitis on the basis of physical appearance of milk and udder. CMT of milk samples was carried out as per Schalm *et al. (*1971) and the result scored as “negative”, “trace”, “1”, “2”, or “3”. EC of milk samples was measured by digital electric conductivity meter (Eutech, Singapore) at an average temperature of 26^{o}C immediately after the collection of milk sample (Norberg *et al.,* 2004). The pH of milk samples was determined by using digital electric pH meter.

Milk sample (17 mL each) from all quarters were pooled aseptically and about 15 mL used for milk diagnostic tests whereas 1.5 mL milk was collected in Eppendorf tube and stored at -80^{o}C for the analysis of M-SAA3 protein. Blood sample (5 mL) was also aseptically drawn from each animal through jugular vein puncture into clot activator vacutainer (RAPID^{TM}) for serum extraction. The requisite serum was harvested and transferred into 1.5 mL microfuge tube and stored at -80^{o}C till albumin and M-SAA3 analysis.

Serum albumin was determined by using commercial reagent kit (Accurex, USA) based on BCG (bromocresol green) method (Dumas *et al.,* 1997). M-SAA3 protein was estimated in milk and serum by using bovine M-SAA3 protein ELISA kit (Sincere Biotech Co., China). The data from screening and biochemical tests was statistically assessed by descriptive analysis and one-way ANOVA using SPSS (2012).

RESULTS AND DISCUSSION

Cattle of Jersey and HF crossbreds were selected in normal, subclinical and clinical groups. CMT score of normal group ranged between zero and trace, subclinical mastitis between 1 and 2, clinical mastitis was $\geq$ 3 in both crossbred cattle. EC values in Jersey cattle in normal, subclinical and clinical groups were 4.05 ±0.29, 5.43 ±0.15 and 6.98 ±0.17 mS cm^{-1}, respectively. The pH values in Jersey breed for respective groups were 6.38 ±0.05, 6.62 ±0.03 and 7.44 ±0.22. In HF crossbred, the

EC values in normal, subclinical and clinical groups were 4.61 ±0.27, 5.56 ±0.16 and 7.07 ±0.24 mS cm^{-1}, respectively, and the milk pH values for respective groups were 6.59 ±0.143, 7.17 ±0.17 and 7.54 ±0.12. The EC and pH values of milk differed significantly between the same groups of two crossbred cattle indicating different ionic composition of milk between these breeds.

The albumin protein from serum samples of Jersey and HF crossbred cattle, normal or suffering from mastitis, by semi auto-analyzer (Photometer 5010 V5+) at 630 nm revealed minimum and maximum serum albumin concentration in Jersey groups as 2.16 and 2.71 g dL^{-1} in normal, 2.26 and 2.50 g dL^{-1} in subclinical and 2.20 and 2.60 g dL^{-1} in clinical group. The mean albumin concentrations in three groups of Jersey breed decreased non-significantly between normal, subclinical and clinical cases (2.42 ±0.05, 2.30 ±0.02 and 2.39 ±0.05 g dL^{-1}, respectively) indicating that it could be weak diagnostic serum APP in mastitis. In HF crossbred, the mean serum albumin values showed non-significant difference in decreasing trend among the normal, subclinical and clinical groups with respective mean values as 2.359 ±0.087, 2.378 ±0.046 and 2.267 ±0.064 g dL^{-1}. Further, there was no significant variation in mean concentrations of serum albumin between two breeds revealing its weak role in differentiating the different forms of mastitis.

The mean concentrations of M-SAA3 in bovine serum of normal, subclinical and clinical Jersey groups was 9.37 ±0.90, 13.87 ±2.89 and 16.82 ±2.61 ng dL^{-1}, respectively (Table 1). There was non-significant variation in M-SAA3 protein levels in serum among different groups of Jersey breed. Contrarily, significant increase in M-SAA3 protein concentration in milk samples was observed within the same animal group ($p \leq 0.01$). The mean values of M-SAA3 protein in milk samples of respective groups were 4.71 ±0.34, 21.30 ±2.72 and 68.01 ±4.90 µg dL^{-1}, which suggested that M-SAA3 protein is highly sensitive biomarker of mastitis in milk than in serum of local Jersey crossbred cattle. Between the groups the difference with respect to increase in M-SAA3 protein in serum was significant in normal and clinical groups of Jersey breed (Table 2).

Table 1: M-SAA3 protein levels in serum (ng dL^{-1}) and milk (µg mL^{-1}) of Jersey and HF breed

Sample	Status	Jersey		HF	
		Mean ± SE	p value	Mean ± SE	p value
Serum	Normal	9.37 ±0.90	0.10	6.82 ±6.06	0.00
	Subclinical	13.87 ±2.89		16.44 ±3.81	
	Clinical	16.82 ±2.61		20.29 ±6.51	
Milk	Normal	4.71 ±0.34	0.00	10.62 ±21.38	0.00
	Subclinical	21.21 ±2.72		35.30 ±18.86	
	Clinical	68.09 ±4.90		72.20 ±25.27	

p value < 0.05 is statistically significant

Table 2: Comparative difference in mean between the subgroups of two respective breeds

Multiple comparison between subgroups of two breeds			Jersey	HF
Sample	Subgroup	Status	Mean difference	Mean difference
Serum	Normal	Subclinical	-4.41	-9.61*
		Clinical	-7.47*	-13.47*
	Subclinical	Normal	4.41	9.61*
		Clinical	-2.97	-3.84
	Clinical	Normal	7.47*	13.47*
		Subclinical	2.97	3.84
Milk	Normal	Subclinical	-16.58*	-24.69*
		Clinical	-63.38*	-61.59*
	Subclinical	Normal	16.58*	24.69*
		Clinical	-46.80*	-36.90*
	Clinical	Normal	63.38*	61.59*
		Subclinical	46.80*	36.90*

**represents statistically significant difference, p<0.005.*

In HF breed, unlike Jersey, there was significant increase in M-SAA3 serum protein in normal, subclinical and clinical mastitis groups with mean respective values 6.82 ±6.06, 16.44 ±3.81 and 20.29 ±6.59 ng dL^{-1} (Table 1). There was a significant increase in M-SAA3 concentration in both milk and serum of HF crossbred cattle ($p \leq 0.05$). The mean values of M-SAA3 protein in milk were 10.61 ±21.38, 35.30 ±18.85 and 72.20 ±25.26 µg dL^{-1} in normal, subclinical and clinical groups respectively (Table 1). Between the groups of HF crossbred, mean serum protein values showed significant variation ($p \leq 0.05$), except between clinical and subclinical groups (Table 2). There was also significant difference in

increase in mean value of M-SAA3 protein in milk between subclinical groups of two breeds ($p \leq 0.05$) when the same groups of two breeds were compared.

Current research is focused on the identification of reliable tool or biomarker(s) for mastitis diagnosis. Bovine serum albumin (BSA) is one of the APP whose concentration in serum during mastitis decreases but increases in milk (Shamay *et al*., 2005). The down regulation of hepatic production of albumin protein during inflammation provides potent source of amino acids for positive APPs. Recent studies have revealed that APPs act as modulators of immune system during APR by interacting with pathogens and defensive cells. BSA-immunoglobulin complex in milk has been linked to immune defense reactions occurring during early stages of udder infections (Lieske *et al*., 2005). The present study revealed no significant variation in serum albumin concentration of normal to clinical mastitis and severity of mastitis in both crossbred animals. Our findings are supported by Matei *et al.* (2010) who found increase in total protein concentration with increase in serum globulin content but decrease in serum albumin content which act as negative APP in serum during subclinical mastitis. Matei *et al.* (2010) got the mean concentration of albumin from normal and subclinical cases as 3.50 ±0.20 and 3.40 ±1.06 g dL^{-1}, respectively. Some researchers have suggested possible role of serum albumin as biomarker in bovine mastitis and inflammatory conditions of small animals (Ottenjann *et al*., 2006; Pradeep, 2013). In present study, we may conclude that bovine M-SAA3 is differentially produced by mammary epithelial cells in response to bacterial infection leading to the over-production of bovine M-SAA3 in milk and serum. There was marked increase in the concentration of M-SAA3 protein in milk during clinical and subclinical mastitis which is in agreement with McDonald *et al.* (2005), who found increased expression of M-SAA3 protein in mammary tissue by infecting the cows with Gram negative and Gram positive bacteria. However, in present study there was non-significant increase in M-SAA3 protein concentration in serum of Jersey breed; and the concentration of M-SAA3 protein in serum was too low to detect which is in agreement with McDonald *et al.* (2005) who found undetectable amount of M-SAA3 protein in serum of animals suffering from mastitis. Remarkably, albumin protein is down regulated (negative APP) in serum during APR, but on contrary up-regulated (positive APP) in extrahepatic tissues like mammary tissue during inflammation. In this study, there was increase in M-SAA3 protein concentration in milk and serum of HF crossbred cattle suffering from mastitis, but only in milk of Jersey crossbred cattle. Milk and serum M-SAA3 levels were elevated in all mastitic serum and milk samples of HF cattle but only in milk samples of Jersey mastitic animals. The observations are in agreement with Eckersall *et al*. (2001). The recent discovery of a mammary-associated SAA3 expressed and secreted in colostrum (McDonald *et al.,* 2001) supports the theory that APP may be locally produced within the udder rather than entering the mammary gland by traversing the blood-mammary barrier. M-SAA3 can be considered as sensitive and reliable markers of clinical and subclinical mastitis and could be used as an early bio-marker of the disease prior to the onset of clinical signs.

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