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Somatic Cell Count: A Biomarker for Early Diagnosis and Therapeutic Evaluation in Bovine Mastitis

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A B S T R A C T

The present investigation was carried out to evaluate the usefulness of somatic cell count in prompt diagnosis of bovine mastitis as well as to ascertain the effectiveness of the therapeutic regimen. Out of the 1040 selected animals, 273 no of milk samples were having a SCC of < 2 X 10 ^{4} cells / ml and considered as normal animals. 485 no. of milk samples were having SCC of > 2 X 10 ^{4} cells / ml but no visible clinical signs of mastitis. Rest 282 no. of cows were having the characteristic lesions and clinical symptoms of mastitis. Among the cases of clinical mastitis, 80 no. of milk samples were examined for SCC. Twenty normal milk samples were examined and considered as control group samples for comparative analysis. The SCC value was significantly higher ($P< 0.05$) in clinical mastitis animals as compared to subclinical and control group of animals. Out of the collected samples, 100 mastitis milk samples were subjected for culture examination and antibiotic sensitivity test followed by recommendation of treatment with suitable antibiotics and other supportive therapy. Post treatment assessment was carried out after 7 days. The recovery rate was found to be 64 per cent. There was complete remission of clinical signs with normal milk colour, negative reaction to CMT and significant reduction ($P< 0.05$) in somatic cell count. Hence it was concluded that SCC can be used as a biomarker for early detection as well as evaluation of therapeutic effectiveness in both clinical and subclinical mastitis

Introduction

Mastitis is the major lactation-associated disease condition that causes serious losses to the dairy industry in almost all parts of India. Clinical mastitis is easy to detect as visible clinical signs are evident and hence immediate treatment can be practiced. However, Subclinical mastitis (SCM), is a hidden malady, and therefore necessitates the use of routine surveillance and monitoring for its detection. Somatic cell count (SCC) is considered to be the gold standard for ascertaining clinical or subclinical mastitis (Hegde *et al.*, 2013).

However, it has been difficult to define the threshold for SCC which would be indicative of mastitis since SCC can overlap between mastitis affected and unaffected animals or even between udder quarters of the same animal (Sharma *et al.*, 2011).

Materials and Methods

The present research work was conducted in ten districts of Odisha involving the cases of mastitis in cattle and buffaloes. A total 1040 random animals were selected across 10 different districts of Odisha and milk samples were collected and subjected to California mastitis test (CMT), somatic cell count (SCC) and differential leukocyte count (DLC). Based on somatic cell count the milk samples were classified as normal (< $2X10^4$ cells / ml) and subclinical mastitis (>$2X10^4$ cells/ml) (Preethirani *et al.,* 2015). Milk samples which were positive either clinically or subclinically for mastitis considered as mastitis milk samples. Milk total somatic cell and differential leukocyte count were estimated according to general principle of Prescott and Breed method as described by Schalm *et al.,* (1971). Twenty normal milk samples were examined and considered as control group samples for comparative analysis. Out of the collected samples, 100 mastitis milk samples were subjected for culture examination and antibiotic sensitivity test followed by recommendation of suitable antibiotics and other supportive therapy. Post treatment assessment was carried out after 7 days, based on negative reaction to CMT, reduced SCC and clinical improvement observed in the animal. The data obtained from the study was analyzed by one way analysis of variance (ANOVA) test using IBM SPSS Statistics software version 10.

Results and Discussion

Out of the selected animals, 273 no of milk samples were having a SCC of < 2 X 10^4 cells/ ml, hence considered as normal animals. 485 no. of milk samples were having SCC of > 2 X 10^4 cells / ml but no visible clinical signs of mastitis. Rest 282 no. of cows were having the characteristic lesions and clinical symptoms of mastitis. Among the cases of clinical mastitis, 80 no. of milk samples were examined for SCC. The values of SCC in different group of animals are being presented in Table 1. Different cut-off values of SCC are adopted by different countries. In the European Union, Australia and New Zealand, the penalty limit for saleable milk is with a SCC of 4-9 X 10^5 cells/mL, whereas Canada, the US and Sweden use 5- 9 X 10^5, 7.5- 9 X 10^5 and 2- 9 X 10^5 cells/mL limit, respectively (Paape and Contreras, 1997). Since no standards are adopted in India taking the cutoff of 5 X 10^5 cells/mL SCC in milk, Hegde *et al.,* (2013) reported that 45 % of the 246 milk samples were positive for SCM. Sarvesha *et al.,* (2017) also reported SCC value > 5.00 Lakhs/ml of milk was taken as criteria to declare the milk or animal as subclinically mastitic or infected.

The SCC value was significantly higher (P< 0.05) in clinical mastitis animals as compared to subclinical and control group of animals. In subclinical mastitis, the SCC value varied significantly (P< 0.05) animals from that of control group of animals. In DLC, the neutrophil count was highest significantly higher (P< 0.05) in clinical mastitis animals as compared to subclinical and control group of animals. The macrophage and lymphocyte percentage decreased significantly (P< 0.05) in clinical mastitis as well as in subclinical cases as compared to control group of animals. These findings are in accordance with the previous reports of Sarvesha *et al.,* (2017) who reported milk leukocyte count to be the ideal biomarkers in mastitis and the colonization of mammary glands by pathogenic micro-organisms results in series of events which leads to major alterations of milk compositions secreted from cells. In the current experiment, a higher level of milk leukocytes is indicative of the activation of immune response against the microorganisms in the mastitis affected mammary gland (Djabri *et al.,* 2002; Gargouri *et al.,* 2008 and Sarvesha *et al.,* 2017).

Table.1 Values of milk SCC and DLC of control and mastitis cows

Parameters	Control (n= 20)	Subclinical mastitis (n = 485)	Clinical mastitis (n = 80)
SCC (Lakhs/mL)	1.62^{a}±0.09	7.16^{b}±0.29	13.72^{c}±0.56
Neutrophil (%)	5.7^{a}±0.48	45.15^{b}±1.30	64.5^{c}±1.85
Macrophage (%)	74.95^{a}±1.13	28.7^{b}±1.21	12.08^{c}±0.62
Lymphocyte (%)	16.2^{a}±0.55	17.83^{b}±0.69	14.3^{a}±1.47
Others (%)	3.15^{a}±0.43	8.32^{b}±0.48	9.08^{b}±0.51

* Means ±SE bearing different superscripts in a row vary significantly (P< 0.05).

Table.2 Post treatment means ± S.E values of milk Somatic cell count

Groups	SCC (Lakhs/mL)
Control (n= 20)	1.62^{a}±0.09
Pretreatment (n = 64)	9.48^{b}±0.54
Post treatment (n = 64)	1.70^{a}±0.02

* Means ±SE bearing different superscripts in a row vary significantly (P< 0.05).

Similar reports have been previously depicted by Hussain *et al.,* (2012) and Hussain *et al.,* (2013).

Out of the treated cases, 64 cases recovered. Hence the recovery rate was found to be 64 per cent. There was complete remission of clinical signs with normal milk colour, negative reaction to CMT and significant reduction (P< 0.05) in somatic cell count. The post treatment means ± S.E values of milk Somatic cell count is given in Table 2.

Hoeben *et al.,* (2000) also reported that local clinical signs, such as swelling, pain and firmness of the inflamed mammary quarters, were less severe in the treated cows. Antimicrobial therapy is one of the bases of control programs for mastitis caused by *S. aureus* organism; however, several factors such as cow, pathogen and antibiotic treatment levels affect the probability of cure in *S. aureus* IMI (Zecconi *et al.,* 2006). Therefore, the appropriate selection of an antimicrobial agent for treatment of bovine mastitis should not only include knowledge about pharmacokinetics, but also about local susceptibility patterns of the main pathogens. Furthermore, surveys on in vitro susceptibility patterns of *S. aureus*, performed by quantitative methods are required for the detection of emerging resistance worldwide (De Oliveria *et al.,* 2000). Systemic administration of antimicrobials was recommended for the treatment of severe coliform mastitis because of the risk of bacteremia (Wenz *et al.,* 2001). Effective treatment for clinical mastitis depends on different factors related to the cow, the pathogen and the drug used for treatment. Factors associated with treatment efficacy included age, stage of lactation, effectiveness of the cow's immune response, somatic cell count, number of infected quarters, chronicity and severity of the cases. Pathogen factors included inherent characteristics of the pathogen, duration of the infection, and pathogen response to antimicrobial therapy (Bradley and Green, 2009). Cure rates for subclinical mastitis caused by *S. aureus* have been shown to decrease with age (from 81 per cent for cows < 48 months of age to 55 per cent for cows >96 months), the number of infected quarters (from 73 per cent for one

infected quarter to 56 per cent for 4 infected quarters) and increasing SCC (Sol *et al.*, 1997).

From the present investigation it can be concluded that SCC can be used as a biomarker for prompt diagnosis of both clinical and subclinical mastitis as well as to ascertain the effectiveness of the therapeutic regimen. This will ensure early detection of subclinical mastitis and will enable successful implementation of mastitis control programmes to ensure quality milk production.

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