Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Clinical pathology

# A Review of The Subclinical Mastitis in Cattle with Special Reference to The New Approaches of Its Diagnosis and Control

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**ABSTRACT**

Subclinical mastitis (SCM) is an inflammation of the mammary glands that does not cause any visible changes in the milk or udder. Milk looks normal, but subclinical infected cows produce less milk and the quality of the milk deteriorates. Subclinical mastitis is considered the most economically important form of mastitis due to its higher prevalence and difficulty in identifying and destroying long-term effects compared to clinical mastitis. The disease is considered to be the most common disease in dairy cows around the world and is 15-40 times more common than clinical mastitis. Subclinical mastitis is a multi-pathogenic complex disease caused by potential risk factors rather than infectious pathogens and other non-communicable factors . The disease is usually unnoticed by farmers and cannot be identified without laboratory or field trials, as there are no visible signs. The main problem with subclinical mastitis is the potential to develop into clinical mastitis, which increases the cost from discarded milk and the cost of additional treatment and veterinarians. Most casts due to intramammary infection are subclinical and an increase in somatic cell count is the only indicator of infection. Therefore, in order to avoid persistent mammary glands infections and the spread of the disease, new diagnostic tests need to be developed to detect SCM rapidly. This contributes to the success of treatment and control.

**Key words:** *Subclinical mastitis, Dairy cattle, Milk production, Somatic cell count.*

## INTRODUCTION

Mastitis is a worldwide multifactorial disease and continues to be the most common and ambiguous disease of dairy cattle in most parts of the world. It is the most economically important illness in the dairy industry, accounting for about 38% of all direct damage (Qayyum *et al*., 2016 and Das *et al*., 2018).

Mastitis can be classified as either clinical or subclinical. Clinical mastitis is characterized by a sudden onset, changes in milk appearance, composition, decreased milk production, and the presence of inflammation as a major sign of infected udder. In contrast, subclinical mastitis (SCM) is the most common form in dairy cows, with no visible signs on the udder or milk, but reduced milk production and its composition and nutritional value. It has undesired effects, poor quality and is inappropriate for processing (Abebe *et al*., 2016).

Clinical mastitis is often diagnosed directly by visual assessment of udder inflammation and changes in the character of the milk, but SCM is a hidden form of mastitis and the body in the milk. Increased cell count (SCC) is the only indicator of inflammation (Argaw, 2016).

Various methods are used to diagnose subclinical mastitis debend on physical, chemical changes in milk and isolation of microorganisms. Diagnosis of subclinical mastitis include measuring somatic cell counts directly or indirectly by performing a California mastitis test (CMT) in a suspicious inflamed udder (Langer *et al.*, 2014).

Due to the clinical importance of SCM, alternative, rapid and reliable diagnostic methods need to be found to help avoid the financial adverse effects of SCM before developing more serious cases. Today, many studies have shown that as biomarkers for the diagnosis, treatment, and prognosis of many diseases such as mastitis with alternative agents such as acute phase protein (APP), inflammatory cytokines, and oxidative stress status in animals. Suggests treatment for inflammation (EL-Deeb, 2013; Sadek *et al.*, 2017 and Carvalho-Sombra *et al.*, 2021). The goal of this review was to provide a brief overview of the most recent knowledge about subclinical mastitis in cattle including the overall effect on blood-biochemical parameters and focusing on the clinical importance of the parameters of systemic inflammatory response and oxidant-antioxidant status as biomarker indicators which can be debend on in diagnosing the disease.

## 1. Prevalence, causative pathogens and associated risk factors of subclinical mastitis (SCM):

Dairy cattle are the most efficient of all farm livestock in converting feed protein and energy to food. Milk and tile products made from it are nutritious and highly desirable foods. Milk has been called one of nature's most nearly perfect foods, supplying humans with energy as well as essential nutrients (Hodgson, 1979).

Milk production is carried out around the world, and global demand for dairy products continues to grow due to growing populations and increasing incomes. As the demand for dairy products grows, so does the need for rational planning to improve the dairy industry. This should include planning to maintain the health status of the udder, improve milking hygiene and implementing mastitis control programs including early and quick detection of any pathological problem that would affect the quality and quantity of the milk produced (Argaw, 2016).

Mastitis is defined as an inflammation of the mammary gland that is triggered when pathogenic microorganisms in the udder produce toxins that are toxic to the mammary gland. Mastitis is mainly caused by bacterial infections and is a major cause of economic loss in dairy cattle **(**Olivares-Pérez *et al.,* 2015). Mastitis is classified as clinical or subclinical according to the visibility of the effects of inflammation of the udder.

Subclinical mastitis (SCM) is defined as an inflammation of the mammary glands without visible changes in the milk or udder. Compared to clinical mastitis, considered to be the most economically important form of mastitis due to its higher prevalence, difficulty in recognition, and catastrophic long-term effects compared to clinical mastitis (Pyorala, 2003 and Pandey *et al.,* 2012). In addition, subclinical mastitic cattle considered a microbial reservoir in the herd, increase exposure of healthy cattle to clinical mastitis infections due to infectivity, and may increase the rate of new infections can be high (Kaur, 2002 and Zdunczyk *et al.*, 2003). Subclinical mastitis consider to be the most common disease in dairy cows around the world, 15-40 times more common than clinical mastitis and is thought to cause the greatest overall damage in most dairy cattle herds (Pandey *et al.*, 2012 and Kumari *et al.*, 2018). Prevalence of subclinical mastitis can also increase directly with milk production, unsanitary management practices, and increased lactation (Kumari *et al.,* 2018).

### Prevalence of SCM in Egypt

In Egypt, results obtained from studies on subclinical mastitis in dairy cows in Assiut Governorate showed a prevalence of SCM was 19.14%. The most important major causative agents isolated in this study were *Staphylococcus aureus*, *Streptococcus agalactiae and Escherichia coli from* the positive CMT samples with prevalence of 52.5, 31.25 and 16.25%, respectively (Abdel-Rady and Sayed, 2009).

In the study of Sawsan *et al.* (2017), bacteriological examination of 22 milk samples from clinical and subclinical cases of mastitis in cows and buffalo showed 50%, 36.4%, 4.5% and 9.1% gram-positive and gram-negative mixed infections, respectively. Also bacteriologically negative cases were shown. In this study, the major frequently isolated pathogens were *Staphylococcus aureus* (27.3%), followed by *Escherichia coli* (18.2%), *Klebsiella, Staphylococcus epidermidis, St. agalactia, and Shigella.* (9.1% of all).

Bacteriological milk cultures of 20 subclinical mastitic cattle with positive California Mastitis Test (CMT) showed the presence of major five types of microorganisms mainly, a mixed bacterial infections (27.7%), *Streptococcus spp* (22%), *Escherichia coli* (20%), *Corynebacterium spp* (15.3%) *and Staphylococcus spp.* (15%) (Sadek *et al.*, 2017). In dairy sheep and goat, the isolated bacterial causing SCM in ewes' milk samples were *Escherichia coli* (44.4%), *Staphylococcus aureus* (38.9%), *Streptococcus* spp. (27.8%), *coagulase negative Staphylococci* (26.0%), *Citrobacter spp.* (3.7%) and *Enterobacter spp*.

(1.9%). In contrary, the identified isolates from does' milk samples were Staphylococcus aureus (46.4%), *Escherichia coli* (26.8%), *Streptococcus spp.* (25.0%), *coagulase negative Staphylococci* (CNS) (19.6%), *Citrobacter spp.* (5.4%) and *Enterobacter spp.* (3.6%) (Abdallah et al., 2018).

Examination of California Mastitis Test (CMT) positive milk samples from 120 lactating animals (50 buffalo and 70 dairy cattle) on different farms in Ismailia revealed that the overall prevalence of *Staphylococcus aureus* was 35.9% in cattle and 31% for buffalo (Algammal et al., 2020).

In another study of dairy goats, Hany et al. (2020) found that the prevalence of apparently healthy half of SCM was 52.56%. In this study, *coagulase-negative staphylococci* (CNS) were the major bacterium (58.75%), followed by *Staphylococcus aureus* (S. aureus) (24.375%) and *streptococcus* (1.875%). In addition, culture results showed that 49.69% of the samples were single-infected, 23.9% of the samples were mixed-infected, and 26.41% of the samples were negative.

Assessment of the prevalence and causative bacterial pathogens in subclinical mastitic bovine milk herds in North Upper Egypt demonstrated that the prevalence of bacterial isolation in subclinical mastitic quarters was 90.4% (26 and 64.3% were single and mixed isolates, respectively). The most common bacterial isolates were *Escherichia coli* (49.8%), *Staphylococcus aureus* (44.9%), *Streptococcus* (44.1%), and *Staphylococcus aureus* (NAS) (37.1%) (Abed et al., 2021).

## Worldwide prevalence of SCM

Worldwide, Busato et al., (2000) found that the prevalence of subclinical mastitis in Switzerland at the quarter level were 21.2% for lactation period of 7 to 100 days and 34.5% for 101 to 305 days post-partum in organic certified dairy herds. In India, about 70-80% economic loss mainly due to subclinical mastitis alone (Dua, 2001).

Olivares-Pérez et al. (2016) found that the overall prevalence of SCM in tropical Mexico was 20.5% of all positive isolates, 97.5% were Gram-negative and 37.5% of all positive isolates were *Proteus vulgaris*, 25% *Salmonella*, 12.5% *Enterobacter aerogenes*, and 10% *Escherichia coli*. *Staphylococci, streptococci* and *Escherichia coli* were considered to be the most common bacterial pathogens involved in the pathogenesis of mastitis in cattle, sheep, and goats. In addition, in small ruminants, the prevalence of subclinical mastitis was higher than that of clinical mastitis, ranging from 5% to 30%, and in some cases over 50% (Novac *et al.*, 2020). Furthermore Sayeed et al. (2020) found that subclinical mastitis considered an important issue for the development of Bangladeshi dairy cows, with SCM prevalence of 71.9%, 67.9% and 29.5%, respectively, at farm level, individual animal level and quarterly.

## Etiology Of Subclinical Mastitis In Cattle

There is a known relationship between the causative agents and the form of the disease. For example, S. *uberis*, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas aeruginosa* and pyogenic bacteria are mainly considered to be causative agents of clinical mastitis (Kluytmans et al., 1997) On the other hand, *streptococcus agalactiae*, CNS and Enterococcus spp. are associated with subclinical mastitis (Kathiriya *et al.*, 2014).

Bacterial pathogens are the main threat to the mammary glands among infectious pathogens. These microorganisms are often contagious and spread near dairy cattle and increasing the prevalence of intramammary infections (Sharif and Muhammed, 2009).

The cause of infectious mastitis is infected cattle, which are transmitted from cow to cow during milking, mainly through milking equipment, milker's hands, and contaminated hand towels. The major contagious pathogens are Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium bovis and Mycoplasma species. Among these, Staphylococcus aureus, has been designated as the most frequently isolated contagious pathogen in both clinical and subclinical mastitis (Zecconi, 2006). In general, the majority of the cases of clinical and subclinical mastitis globally are caused by only a few common bacterial pathogens, namely, *Staphylococcus species, Streptococcus species, Coliforms and Actinomyces pyogenes* with the *S. aureus, S. epidermidis, St. agalactiae, St. dysgalactiae and E. coli* are the major occurring pathogens (Sharma *et al.*, 2010).

## Predisposing risk factors

Subclinical mastitis is considering to be a multi-etiological complex disease which is caused by infectious agents as well as other non-infectious agents as potential risk factors (Kumari *et al.*, 2018). Many risk factors can predispose to the development of subclinical mastitis and understanding them is considered an effective way to improve management practices and thus reduce the occurrence of subclinical mastitis in dairy herds. Predisposing risk factors can be categorized into environmental factors (temperature, humidity, season, overcrowding, poor ventilation…etc.), host factors (cow factors) including breed, age, high yielder, stage of lactation, udder defense, milking interval, milk somatic cell count, dry period, teat injuries, genetic resistance and pathogen factors (pathogenic factor, number of organisms, blind treatment) (Argaw, 2016).

In Egypt, studies of the link between subclinical mastitis and breed showed that friesian breed are the major susceptible to infection than local breeds (16.67% at cattle level) (20.43% at cattle level, 6.09% at quarterly level). Was shown. And 5%) at the quarterly level). In terms of weather and season, it was also noticed that prevalence of subclinical mastitic cows in hot climate as during summer (9.14% at the cow level and 2.64% at the quarter level) and during spring (4.86% at the cow level and 1.36% at the quarter level) was higher than in cold weather as during winter (2% at the cow level and 0.64% at the quarter level) and during autumn (3.14% at the cow level and 1.07% at the quarter level) (Abdel-Rady and Sayed, 2009).

The influence of age susceptibility as a risk factor for subclinical mastitis was also evaluated and the results showed that 5-8 years old cows (15.43% at the cow level and 4.36% at the quarter level) more susceptible than those of 2-4 years (3.71% at the cow level and 1.36% at the quarter level) (Abdel-Rady and Sayed, 2009)

Worldwide, the infection rate of mastitis in cattle with pendulous udder was higher than cattle with non-pendulous udder because the pendulous udder exposes the teat mainly to injury, and pathogens may easily adhere to the teat and get access to the mammary gland tissue (Sori *et al.,* 2005). Prevalence of bovine mastitis was higher in farms with larger herd sizes than in those with lower herd sizes (Radostits *et al.*, 2007).

Other researchers reported that 65% of coliform clinical cases which occur in the first two months of lactation are from intramammary infections that originated during the dry period. Additionally, Streptococcal infections during the dry period represented 56% of clinical cases during the first two months after calving (Green *et al.,* 2008). In addition, early lactation infection rates (87.2%) are higher than mid-lactation 65.9%, with cows with 5 or more calves being less affected by late lactation cows (73.1%) than more cows. I was affected. Medium calf (Zerihun *et al.*, 2013).

2. <u>Pathogenesis of SCM</u>

Mastitis in dairy cattle occurs when the udder becomes inflamed and bacteria invade the teat canal and mammary glands. It also produces toxins which cause damage to the milk secreting tissue, besides, physical trauma and chemical irritants. which elevate the concentrations of leukocytes, or somatic cell counts in the milk, lowering its quantity and adversely affecting milk quality and milk by products (Jones, 2006).

The teat end serves as the first line of defense against infection. From the outside, a smooth muscles sphincter of surrounds teat canal and functions to keep it closed. It also prevents milk from leaking, and bacteria from invade into the teat. From the inside, the teat canal is lined with keratin obtained from stratified squamous epithelium. The keratin is a waxy material composed of fatty acids and fibrous proteins in the teat. The fibrous proteins of keratin in the teat canal bind to mastitis pathogens, blocking the bacterial cell wall, making it more susceptible to osmotic pressure. Inability to maintain the osmotic pressure lead to lysis and death of invading pathogens (Jones, 2006).

Fatty acids are both esterified and non-esterified and representing myristic acid, palmitoleic acid and linolenic acid which are bacteriostatic. Therefore, damage to keratin has been reported to cause increased susceptibility of teat canal to bacterial invasion and colonization. During milking, bacteria found near the opening of the teat find opportunity to invade the teat canal, lead to trauma and damage to the keratin or mucous membranes lining the teat sinus. The canal of a teat may still partially open for 1-2 hour after milking and during this period the pathogens may freely invade into the teat canal (Jones, 2006).

Neutrophils are the major cells found in the mammary glands and mammary secretions mainly in early stage of mastitis and represent >90% of the total leukocytes. The neutrophils exert their bactericidal effect through a respiratory burst and release hydroxyl and oxygen radicals that kill the bacteria. The severity of inflammatory response, however, is dependent upon species, virulence, strain and the size of inoculum of bacteria, as well as parity, age, the stage of lactation, and immune status of the animal (Jones, 2006).

### 3. Economic impact of SCM on dairy industry

Sub-clinical mastitis considered to be one of the threats to the dairy industry. Incidence of sub-clinical mastitis is often overlooked, but it is a huge economy due to reduced milk yields (about 21%), increased labor force, increased treatment costs, post-treatment milk retention, early culling) May cause loss (Sinha *et al.,* 2014 and Argaw, 2016). In addition, sub-clinical mastitis is at increased risk of developing clinical mastitis, increasing the cost of discarded milk and the cost of additional treatment and veterinarians.

As a result of inflammation, increased pH and decreased casein and lactose synthesis, as well as the amount and quality of fat (25%), alter the composition of the milk (Botaro *et al.*, 2015). This processing of milk results in poor production of fermented final products such as yogurt, cheese and other dairy products and is suboptimal (Sharif and Muhammed 2009).

Several studies revealed that clinical and sub-clinical mastitis delay the reproductive process at several levels. If clinical mastitis occurs before the first artificial insemination, delivery to the first service interval will be significantly longer than after the first artificial insemination. In addition, fertility was reduced by 44% if mastitis occurred one week before insemination, 73% if it occurred during the week of insemination, and 52% if mastitis occurred the week after insemination (Hansen *et al.,* 2004).

Isobe *et al.* (2014) investigated the effect of the number of somatic cells in milk on fertility such as prenatal pregnancy status and postpartum ovulation function of dairy cows, SCC with high prenatal stage has a high birth rate of prostaglandin F2α and progesterone. Itfound that there is a decrease and the first postpartum ovulation may have been affected by high SCC. In addition, sub-clinical mastitis is thought to affect the follicular response of cattle, reduce fertility in cattle and reduce conception (Wolfenson, *et al.*, 2015).

### 4. Public health importance of SCM

Subclinical mastitis considers one of the most zoonotic potential diseases for consumers globally (Tanmay *et al.*, 2017). Causative pathogens of mastitis with zoonotic importance may represent a health risk and a real damage to human health via the food chain (Argaw, 2016). In developing countries, milk zoonosis is very important where there is consumption of untreated milk (M and H.A, 2016).

Bacterial contamination of affected milk makes it unsuitable for human consumption and provides a mechanism for the spread of the disease. For example, ingesting staphylococcal or streptococcal endotoxin from infected milk can cause serious illness. For example, certain Staphylococcus aureus strains produce heat-resistant enterotoxins. When ingested by humans, it causes nausea, vomiting, abdominal cramps and causes food poisoning from staphylococci. Similarly, the genus Pseudomonas aeruginosa. It can be life-threatening for immunocompromised patients. Similarly, Mycobacterium bovis causes tuberculosis, E. coli causes severe gastrointestinal disorders (Galal *et al.,* 2007).

Antibiotic residues also treat the disease without waiting, as these antibiotic residues can cause unwanted effects and cause the development of antibiotic resistance in people who are allergic to these antibiotics, And because of the widespread use of antibiotics in control, it is a public health problem associated with mastitis Have a bacterial strain (Kluytmans, 1997 and Galal, *et al.,* 2007).

### 5. Diagnostic techniques of SCM

There are no visible signs of SCM udder or milk, but it reduces milk production and affects milk quality. As a general rule the disease is usually not identifiable without laboratory or field testing to detect inflammatory components and pathogens in milk without the farmer's knowledge. Therefore, it is considered a hidden form of mastitis (Kumari *et al.,* 2018).

Various methods are used to diagnose subclinical mastitis based on physical and chemical changes in milk compositions and isolation of microorganisms. These methods include measuring of somatic cell counts (SCC) directly or indirectly by performing a California mastitis test (CMT) on suspected quarters **(Langer *et al.,* 2014)**.

#### 5.1 Somatic cell count Somatic cell counts (SCC)

Somatic cell count (SCC) is one of the most common and best ways to diagnose subclinical mastitis in dairy cattle. Timely detection of mastitis should be based on SCC values. Otherwise, subclinical mastitis can develop into a clinical disease (Sandgren *et al.,* 2008). Somatic cells consider as a normal part of the mammary gland, with lactating epithelial cells (25%) secreted from the inner layer of the gland and white blood cells (white blood cells) (75%) that enter or enter the mammary gland in response to the mammary gland damage or infection (Annonymous, 2009).

Mammary glands Inflammation accompanied by high levels of SCC in milk so, the greater the SCC in the milk, the higher the degree of inflammation in the tissue (Rodriguez et al., 2000). Accordingly, many reports have considered the Somatic cell count (SCC) as a diagnostic marker for subclinical mastitis (Dürr *et al.,* 2008). Somatic cells are expressed either as cells/ml of milk or as Somatic Cell Score (SCS). The number of cells reflects the degree of mastitis.

Milk from a healthy mammary gland usually contains less than 100,000 cells per ml of milk. If this concentration is exceeded, it is considered infected. Cell numbers above 200,000 cells / ml clearly indicate that an inflammatory response was triggered (subclinical mastitis) and are probably infected that quarter (Bytyqi *et al.,* 2010). In addition, long-term elevated SCC levels suggest that the affected quarters are in a state of chronic inflammation and may interfere with the development of lactating tissues (Nickerson, 2009).

### 5.2 California mastitis test (CMT)

California Mastitis Test (CMT) is another test that has been accepted as a quick and easy test for predicting subclinical mastitis cases from individual quarters or bulk milk. California Mastitis Test(CMT) is a simple biomarker of somatic cell count in milk. It works by destroying the cell membranes of all cells present in the milk sample. This causes the DNA of these cells to react with the test reagents to form a gel (White *et al.,* 2005).

### 5.3 Bacteriological examination

The most accurate method for detection of bacterial mastitis is the isolation of the causative microorganism(s) but it is time consuming **(Sawsan *et al.,* 2017).** Bacteriological cultures are most commonly used as a diagnostic tool for solving the problem of mastitis. However, knowledge of mammary gland infections can also be very helpful in preventing pathogen infection through early diagnosis of reservoirs **(Kumari *et al.,* 2018)**.

## 6. Clinicopathological alterations associated with SCM

### 6.1 Hematology

Accurate diagnosis is assisted primarily by evaluating animal hematological and biochemical markers. Deviations from normal blood- biochemical parameters indicate the state of the disease and are also useful in the diagnosis and prognosis of the disease (Garba *et al.,* 2019). In addition, leucograms have proven to be an essential and reliable standard for assessing animal health (Blumenreich, 1990).

Regarding the case of mastitis, Zaki et al. (2008) reported a significant decrease in RBC, Hb and PCV values and an increased total leukocytic count (TLC) with increase in absolute number of monocytes, eosinophils and neutrophils in mastitic buffalos. The same findings were recorded in cattle suffering from Subclinical mastitis (Zaki *et al.*, 2010).

Abba et al. (2013) and Siddiqe et al. (2015) found that hematology of mastitic animals revealed decrease levels of ESR, RBC, WBC, PCV and Hb than healthy animals while, differential leucocytes count revealed higher neutrophil and lymphocyte, monocyte and basophil count in mastitic cows than control cows.

Sadek *et al.* (2017) and Sarvesha *et al.* (2017) also observed high level of leukocyte and granulocyte count along with lymphopenia and anemia in mastitic and subclinical mastitic cows suggesting that the changes in blood-biochemical parameters were observed in the physiological or pathological state of the animal (It suggests that it can be used as useful indicator of mastitis).

Referring to the type of pathogen causing mastits, Smith et al. (2001) found that routine hematological analysis has been documented to be useful in predicting pathogen types in cows with clinical mastitis which facilitating treatment decisions. In this regard, the authors found that cows with gram-negative mastitis had significantly lower blood leukocytes, segmented neutrophils, monocytes, and lymphocytes in compare to cows with gram-positive mastitis, and blood. He reported high levels of hemoglobin and hematocrit inside.

As a result, Sawsan et al. (2017) reported mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in cows suffering from gram-negative bacteria-induced udder inflammation have significantly increased red blood cell counts than in cows suffering from Gram-positive bacteria. Turned out to be low. However, contrary to the results of Smith et al. (2001), they observed higher neutrophil count and higher neutrophil / lymphocyte ratios in Gram-negative cattle than in Gram-positive mastitis cattle.

On the other hand, some authors have found that hematological parameters in animals suffering from mastitis do not show a particular tendency (Khan et al., 1997 and Sischo et al., 1997). Similarly, Shin et al . (2014). In contrast to healthy cattle, we reported no significant changes in Hb and RBC levels in CM-affected animals. In a study by Carvalho Sombra et al. (2021), circulating leukocytes were elevated in cows with subclinical mastitis and exceeded the reference values, but this increase was not significant.

**6.2 <u>Blood biochemistry</u>**

**<u>Basic biochemical profile in SCM</u>**

During mastitis, the level of most blood components changes due to the disruption of the blood-milk barrier, in addition to impaired production and secretion of breast epithelial cells. udder epithelial cells lead to changes in milk composition in animals with mastitis. Therefore, the effects of subclinical mastitis on dairy cow blood biochemistry have been extensively studied. (Pandey et al., 2012 and Krishnappa et al., 2016).

In this respect, biochemical analysis of blood samples from subclinical mastitic dairy cows revealed a significant elevation in serum levels of total protein (TP) which was related to the significant increase in serum globulin levels (Dwivedi et al., 2004; Pandey et al., 2012; Singh et al., 2014; Krishnappa et al., 2016; Ali et al., 2017and Sarvesha et al., 2017). Shifting to albumin, significant decline was observed in the serum levels of albumin in cows having subclinical mastitis as compared to healthy cows (Singh et al., 2014)

On the other hand, no significant changes were found in the serum levels of TP, globulin and albumin in dairy cows with subclinical mastitis (Carvalho-Sombra et al., 2021) as well as in subclinical and clinical mastitis buffaloes (Krishnappa et al., 2016).

Blood urea nitrogen consider the only reliable biochemical indicator that is positively correlated with the type of bacteria present in mastitis. Cattle suffering from mastitis infected with Gram-negative bacteria have been found to have higher blood urea nitrogen levels than cattle infected with Gram-positive bacteria Smith et al. (2001).

Investigated serum samples from sub-clinically and clinically mastitic cows demonstrated significant elevation in serum AST (Zaki et al., 2010; Pandey et al., 2012 and Sarvesha et al., 2017). Consistently, Krishnappa et al. (2016) observed elevation in serum AST activity in subclinical and clinical mastitic buffaloes.

Interestingly, in relation to type of pathogen, Sawsan et al. (2017) stated that serum concentrations of alanine aminotransferase (ALT) was lower in cattle with gram- negative mastitis than in those with gram- positive mastitis. On the other hand, no significant change was observed in ALT values in mastitic cattle (Sarvesha et al., 2017) and in subclinical and clinical mastitis affected buffaloes (Krishnappa et al., 2016). Both serum alkaline phosphatase (ALP) (Katsoulos et al., 2010) and lactate dehydrogenase (LDH) (Zaki et al., 2010 and Sharma et al., 2016) activities were also increased in SCM.

**<u>Minerals, PH and electrolytes profile in SCM</u>**

Mineral estimation in subclinical and clinical mastitis affected- dairy cows revealed significant increase in calcium (Ca) (Singh *et al.*, 2014; Siddiqe *et al.*, 2015; Sarvesha *et al.*, 2017 and Das *et al.*, 2018) and inorganic phosphorus level (Siddiqe *et al.*, 2015) in affected cows as compared to healthy control. In subclinical and clinical mastitis buffaloes, Krishnappa *et al.* (2016) reported significantly higher levels of serum Ca while, serum inorganic phosphorus was significantly reduced. In some studies, Friesian suffering from subclinical mastitis showed a notable decrease in serum calcium however; serum phosphorous level did not exhibit obvious changes (Zaki *et al.*, 2010). On contrary, Sarvesha *et al.*, (2017) observed significant increase in serum phosphorus concentration in mastitis-infected cattle compared with healthy animals.

Higher serum values of magnesium (Mg) were recorded in the subclinical and clinical forms of mastitis in buffaloes compared with healthy animals (Singh, 1999 and Krishnappa et al., 2016). Singh *et al.* (2014); Siddiqe *et al.* (2015) and Sarvesha *et al.*, (2017) did not reported significant differences in Mg concentrations between the mastitic and healthy cows.

Ibtisam et al. (2006) recorded significant increase of copper (Cu) and significant decrease of zinc (Zn) level in blood serum samples of subclinically-and clinically mastitis-infected cows. Similarly, serum copper concentrations were also reported to be significantly increased in cows affected with clinical mastitis (Kleczkowski *et al.*, 2008). Moreover, elevation in the mean concentrations of iron (Fe) and Zn in milk samples of SCM affected cattle was well documented (Gera *et al.*, 2001 and Wenz *et al.*, 2006).

In relation to ph and electrolytes, elevated pH in association with higher levels of citrate and bicarbonate in mastitis milk was reported by Ogola *et al.* (2007). Significant decline was observed in the level of Na, K and Cl in cows having subclinical mastitis as compared to healthy cows (Pandey *et al.*, 2012). Krishnappa et al. (2016) recorded the same results in subclinical and clinical mastitis buffaloes but serum sodium levels were significantly higher in affected animals compared to control. Additionally, Sarvesha *et al.* (2017) reported significantly higher average values of Na, Cl, and K levels in mastitis-infected cattle.

**Hormonal profile in SCM**

Zaki et al. (2010) suggested a higher values of cortisol levels in serum samples from Friesian cattle with subclinical mastitis compared to healthy cattle. The pituitary gland is a major source of prolactin (PRL), although the hormone is produced by many other tissues, including the brain, placenta, cells of the immune system, and mammary gland epithelial cells (Freeman et al., 2000). Higher levels of prolactine have been reported in mastitis milk, mainly in the quarters with higher SCC levels (Freeman et al., 2000).

**Oxidant-antioxidant status, cytokine profile and acute phase proteins**

The standard diagnosis of mastitis is based on somatic cell count and culture. In the case of acute mastitis, the diagnosis is not difficult because the clinical changes are clear and the chemical composition of milk changes. Because the problem occurs in cases of subclinical mastitis that shows no clinical signs and there are no observable changes in milk composition, making an accurate diagnosis is often a challenge for veterinarians (Argaw, 2016). A number of current studies suggest that mastitis can be diagnosed with alternative agents such as acute-phase proteins (APP), proinflammatory cytokines and animal oxidative stress conditions as biomarkers for the diagnosis, treatment and prognosis of many diseases such as mastitis (EL-Deeb, 2013; Sadek *et al*., 2017 and Carvalho-Sombra *et al.*, 2021).

Oxidant-antioxidant status in SCM

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant mechanisms. Oxidative stress begins when ROS production exceeds its protective antioxidant capacity, resulting in oxidative damage to macromolecules, including DNA, lipids, and proteins (Sordilo and Aitken, 2009). Moreover, ROS can reduce the immune system's response to infection through its deleterious effects on immune cells (Spears and Weiss, 2008). Therefore, the antioxidants synthesized by body, have the purpose of maintaining the integrity of organs and tissues including health of mammary glands and their resistance to mastitis (Sadek *et al.,* 2017 and Carvalho-Sombra *et al.,* 2021).

Alterations in the blood oxidative stress markers with decreased level of antioxidant protection in the blood of cows with clinical and subclinical mammary gland inflammation have been reported (Ranjan *et al.,* 2005; Kizil *et al.,* 2007 and Jhambh *et al.* 2013).

Increased free radical production and increased total oxidative capacity have been reported along with decreased total antioxidant capacity during clinical and subclinical mastitis in cows where there may be oxidative stress. (Lykkesfeldt and Svendsen, 2007 and Atakisi *et al.*, 2010). Subclinical mastitis in cows was also accompanied with a compromised antioxidant defense evidenced through the significant increase in malondaldhyde (MDA) levels along with the significant decrease in total antioxidant capacity (TAC) and catalase (CAT) levels (Celi, 2011). Sadek et al. (2017) found that two forms of bovine mastitis were associated with oxidative stress. This has been demonstrated by significant increases in serum and milk malondialdehyde and nitric oxide levels, as well as reduced glutathione and a significant decrease in overall antioxidant capacity. Early diagnosis of subclinical mastitis has also been reported by evaluating the relationship between malondialdehyde and enzymatic antioxidants such as superoxide dismutase and glutathione peroxidase (Abdel-Hamied and Mahmoud, 2020).

Studies of putative biomarkers for mastitis in cows, including pro-inflammatory cytokines and biomarkers of oxidative stress, have shown significant increases in serum malondialdehyde and nitric oxide levels and decreased levels of the antioxidant enzyme glutathione peroxidase. showed Superoxide dismutase and catalase in asymptomatic cows and clinical mastitis compared to healthy animals (Huma et al., 2020).

Novac et al. (2020) has been demonstrated that mastitis correlates with increased concentrations of nitric oxide, nitrites, nitrates and other oxidative compounds, resulting in nitrifying stress. Carvalho Sombra et al. (2021) reported a positive correlation between malondialdehyde levels and somatic cell counts in cows with subclinical mastitis.

**Cytokine profile in SCM**

Cytokines are a group of regulatory molecules secreted by cells that regulate the behavior, proliferation, differentiation and function of other cells to modulate and control the inflammatory response. They are glycoprotein in nature and produced predominantly by macrophages and lymphocytes but can be synthesized by other cell types as well. They are listed in five groups which are interleukins (ILs), interferons (INFs), tumor necrosis factor (TNF), colony-stimulating factors (CSF) and chemokines (Hamilton, 2008). Their role in inflammation is complex. They are responsible for mobilization and activation of leukocytes as well as proliferation of B and T cells. Cytokines also were known to mediate the acute phase response through stimulating the liver to synthesize acute-phase proteins. Further, they contributed to production of free radicals in large amounts which have an important role in inflammatory process (EL-Deeb, 2013).

The study conducted by Hagiwara *et al.* (2001) confirmed high levels of IL-6 in cows with acute mastitis than that in the samples from apparently healthy cows particularly in the first stage of infection. In addition, the concentration of IL-6 at the onset of clinical mastitis was higher in the milk samples than in the serum samples. Osman et al. (2010) examined 25 cows with subclinical mastitis and 15 cows with clinical mastitis infected with coagulase-positive Staphylococcus spp. and reported higher values of IL-6 in the milk of cows with subclinical mastitis than in the milk of cows with clinical mastitis and healthy cows. Similar results were also reported by Sakemi et al. (2011).

El-Deeb (2013) reported significant increases in the IL-6, IL-1β, and (TNF-α) in does with gangrenous mastitis compared to healthy ones. Sadek et al. (2017) also found higher values for serum (TNF-α, IL-1, IL-2, and IL-6) in the clinical and subclinical mastitic cows compared with the values in the control cows.

Recently, Huma et al., (2020) studied the putative biomarkers of mastitis including pro-inflammatory cytokines and oxidative stress biomarkers of the mammary gland in healthy and diseased animals and they revealed that interleukin-1∝, and interleukin-8 were significantly increased in both blood serum and milk whey in subclinical and clinical mastitis cows.

**Acute phase proteins profile in milk and serum in SCM**

A new trend now in the diagnosis of subclinical mastitis aims to identify reliable biomarkers of the states of inflammation in the mammary tissue, and to use these biomarkers to design new diagnostic tests for the rapid detection of mammary gland infections. Among, these biomarkers acute phase response or reaction (Benic *et al.*, 2018). The acute phase reaction is a non-specific and complex response of the organism to infection, trauma, stress or neoplasia (Eckersall, 2004 and Arthington *et al.*, 2013).

Acute phase proteins (APPs) are considered non-specific innate immune response that are produced mainly by the hepatocytes and released into the blood stream upon stimulation of pro-inflammatory cytokines in response to external or internal stimuli (Quinton *et al.,* 2009 and Slavov *et al.,* 2011). APPs play an important role in protecting the body from pathological damage, in re-establishing homeostasis, and in the regulation of inflammation and hindering microbial development in the period before an acquired immune response creates (Cray et al. 2009).

Acute phase proteins (APPs) in dairy cattle have been tested under different inflammatory and non-inflammatory conditions and also in experimental contaminations and common illnesses (Sadek et al., 2017). However, the most important application of acute phase protein analyses in cattle was suggested to be in the diagnosis and monitoring of bovine mastitis (Ceciliani *et al.,* 2012).

The major APPs in ruminants were found to include haptoglobin (Hp), serum amyloid A (SAA), fibrinogen and ceruloplasmin (Eckersall *et al.,* 2006 and Thomas *et al.,* 2015). In bovines, the Milk Amyloid A (MAA) and Hp are the major APPs synthesized and secreted into milk during inflammation and are specific diagnostic markers of mastitis, since concentration of these APPs in the milk from healthy quarters is low or undetectable (Whelehan *et al.*, 2011; Ceciliani *et al.,* 2012; Kumar et al., 2014 and Thomas, 2015).

Moreover, it is now established that the major bovine MAA and Hp are synthesized locally in the mammary gland thereby improving the potentials of these APPs as biomarkers for subclinical mastitis (Eckersall et al., 2006;

Larson et al., 2006; Thielen et al., 2007; Lai et al., 2009; Whelehan et al., 2011 and Thomas, 2015).

Lauzon *et al.* (2006) supported these findings that Hp is synthesized locally by mammary gland and that its presence in milk is not due to leakage of the blood milk barrier alone. This hypothesis was further recently confirmed by Sadek et al. (2017) who found that Hp and MAA genes were up-regulated in both clinical and subclinical forms of mastitis, while fibrinogen gene expression was absent, indicating that MAA and Hp were locally expressed in mammary gland tissue and that their expression was increased during mastitis.

The usefulness of MAA in the diagnosis of clinical and subclinical mastitis was investigated (Kovac *et al.,* 2011). Positive relationship was also detected between the major bovine APPs with other inflammatory indices (particularly SCC) during mastitis and even with milk composition and protein quality and with severity of the intramammary infection (IMI) (Nielsen *et al.,* 2004; Akerstedt *et al.*, 2008 and Pyorala *et al.,* 2011). In accordance, correlation has been found between APPs, particularly SAA and Hp, with somatic cell count and bacteriological test results, regardless of whether the mammary gland infection occurs naturally or experimentally (Kleczkowski *et al.*, 2017). In the study of Sadek *et al.* (2017), clinical and subclinical mastitic cows showed higher values for serum and milk acute phase proteins including haptoglobin, serum amyloid A, ceruloplasmin and fibrinogen compared with the values in the control cows. Recently, Huma *et al.* (2020) documented that haptoglobin was significantly increased in both blood serum and milk whey in subclinical and clinical mastitis cows.

Conclusion

SCM is accompanied by many changes in the hemato-biochemical parameters that can be used as important indicators of the disease in dairy herds. The higher prevalence and the clinical importance of subclinical mastitis among dairy animals alarming that attention should be directed toward the necessity for designing new diagnostic tests for the rapid detection of SCM to avoid persistent udder infection and the spread of the disease which contribute to facilitate successful treatment and control.

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