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Lactation mastitis: Promising alternative indicators for early diagnosis

Huang Q *et al*. Promising indicators for sub-clinical mastitis

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Abstract

Although lactation mastitis (LM) has been extensively researched, the incidence rate of LM remains a salient clinical problem. To reduce this incidence rate and achieve a better prognosis, early and specific quantitative indicators are particularly important. It has been found that milk electrolyte concentrations (chloride, potassium, and sodium) and electrical conductivity (EC) significantly change in the early stages of LM in an animal model, which means lactic sodium, chloride, and EC increase, but potassium decreases. However, these indicators have not been reported in the diagnosis of LM in humans. This review summarizes the pathogenesis and the mechanism of LM in terms of milk electrolyte concentration and EC, and aim to provide new ideas for the detection of sub-clinical mastitis in humans.

Key Words: Lactation mastitis; Milk electrolyte concentration; Milk electrical conductivity; Pathogenesis; Mechanism; Early diagnosis

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Core Tip: It has been found that milk electrolyte concentrations and electrical conductivity (EC) significantly change in the early stages of lactation mastitis (LM) in an animal model, allowing the early and specific diagnosis of LM. These indicators have not been reported in the diagnosis of LM in humans. We summarize the pathogenesis and the mechanism of LM in terms of milk electrolyte concentrations and EC and aim to provide new ideas for the early diagnosis of LM in humans.

INTRODUCTION

Lactation mastitis (LM), which is universally described as a suite of breast conditions that present with local, and often systemic, inflammatory symptoms and signs during lactation[1]. It is an enervating and common disease that affects up to 33% of lactating women[2-4]. LM has a negative impact on both the mother and the baby. Women with LM may develop pain, localized skin redness, and can have associated systemic symptoms, including fever. However, breast pain is the most common and the most distressing symptom for mothers[5]. The symptoms of LM can lead to a compromised maternal psychological state. In addition, approximately 3% of women with LM will develop a breast abscess[6], and an incidence rate of up to 11% has been reported[7], which may cause permanent damage to the shape of the breast. In order to minimize the detrimental effects of LM, researchers have explored various strategies, including the associated risk factors and etiology[8,9]. However, none of these are known to improve clinical prognosis.

To date, the clinical diagnosis of LM has mostly relied on empirical diagnoses, such as a tender, hot, swollen, wedge-shaped area of redness on the affected breast which is associated with an elevated temperature and systemic symptoms, such as a temperature of 38.5 °C or higher, chills, flu-like aching, and systemic illness[10,11]. Due to the lack of early standardized diagnostic criteria, this causes repeated outbreaks and even progresses to a breast abscess, causing great physical and mental pain in mothers with LM. In some Western countries, for example, 73% of children born in Sweden in 1996

were breastfed for 6 mo[12], but LM frequently results in the cessation of exclusive breastfeeding in the absence of appropriate treatment. As a result, it frequently reduces the protective effect of breastfeeding in mothers. For example, related data show that breastfeeding is associated with a 24% lower risk of invasive ovarian cancer[13], and aggregate results indicate that breastfeeding is inversely associated with the risk of breast cancer[14] and reduces the incidence of osteoporosis and type 2 diabetes[15]. Furthermore, LM is linked to lower levels of fat, carbohydrate, and energy in breast milk[16], which may lead to nutritional deficiencies, lowered immunity, and mental effects in infants.

Several studies have evaluated electrical conductivity (EC) for the detection of subclinical mastitis in cows, some have even identified mastitis causing pathogens using EC[17,18]. EC, chloride (Cl^-), and sodium (Na^+) content in milk were more accurate in predicting infection status than other variables. The electrolyte concentration and EC of milk are the physicochemical properties of milk, and EC measurements were used as an experimental screening indicator for LM in animals as early as the 1990s[19]. Paudyal et al[18] showed that absolute changes in cow's milk electrolyte concentrations and EC can be used to screen breast milk samples for LM. A previous study provided evidence that when an inflammatory response occurs in the breast tissue of animals, breast permeability increases, and as a result, the potassium (K^+) concentration reduces, but the Na+, Cl- concentrations, and EC increase[20-22]. Singh et al[23] recently conducted an observational animal study and concluded that the magnitude of changes in the milk electrolyte concentration and EC may have diagnostic and prognostic values. Due to a lack of research, more data are needed to identify the variation in human milk electrolyte concentration and EC during LM.

However, it is uncertain whether human milk electrolyte concentrations and EC have a positive effect on the early diagnosis of LM. To enable more patients with LM to be effectively diagnosed and treated at an early stage of the disease, the pathogenesis of LM and the relationship between LM and electrolyte concentrations and EC (Figure 1) are discussed in this review. We investigate the changes in electrolyte concentrations

and EC from two perspectives: Altered cell membrane permeability and osmotic pressure level. This will provide new ideas for the detection of subclinical mastitis in humans.

LITERATURE SEARCH

A descriptive review was conducted on the mechanism of changes in milk electrolyte concentration and EC during LM. Pub-Med was searched for articles published between July 1966 and February 2022. The following Medical Subject Headings or free-text terms were used in the search: LM, milk electrolyte concentration, milk EC, early diagnosis, pathogenesis, mechanism, inflammatory injury, and altered cell membrane permeability. The search was limited to papers written in English, with no restrictions on the type of article.

PATHOGENESIS OF LM

The susceptibility and severity of LM are positively correlated with inflammatory factors. Some researchers consider LM to be an infectious disorder[19,24,25], but the etiology of LM has now shifted from infection to inflammation.

Infectious mechanism

Due to the characteristics of LM, it was previously considered to be an infectious disease. Based on the maturation of milk culture technology, some infectious pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus*, have all been isolated from breast milk, confirming the importance of infective pathogenic bacteria in the pathogenesis of LM[26,27]. In recent years, *Staphylococcus epidermidis*, the most common species on human skin and mucosa, has also been isolated from the milk of patients with LM and may be another causative agent of LM[28-30]. Based on the fact that a variety of infectious pathogens are present in the milk of patients with LM, researchers believed that the occurrence of LM was closely related to infectious pathogens in the milk[31]. Milk stagnation, breast trauma, excessive emptying

of the breast, nipple cracking, and dysbiosis of the breast flora can all contribute to the development of infectious LM.

Inflammatory mechanism

Recently, in-depth studies on LM have revealed that the presence of infectious agents is not positively associated with the occurrence of LM[26]. In addition, there is no positive correlation between the severity of LM and the bacterial count in milk[32]. Several studies showed that the susceptibility and severity of LM are positively correlated with inflammatory factors, including C-reactive protein, interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α)[33-35]. Elevated serum cytokines IL-1, IL-6, IL-8, and TNF-α reveal the activation of transcription factor nuclear factor-kappa B (NF-κB) in the host[36]. Furthermore, activation of the NF-κB pathway in LM hosts has been demonstrated in numerous animal studies, *in vitro* experiments, and genetic studies[37-40].

As upstream candidate receptors of NF-κB, Toll-like receptors (TLRs) are important inflammatory mediators and their isoforms include TLR1-TLR11[41,42], which are recognized as pattern recognition receptors (PRRs) by microbe-associated molecular patterns (MAMPs) or danger-associated molecular patterns (DAMPs) and activate downstream NF-κB signaling pathways[34].

MAMP-mediated TLR signaling pathway

Of all the TLRs, TLR2, TLR4, and TLR5 bind to bacterial byproducts in MAMPs, such as lipopolysaccharide, phospholipid wall acids, bacterial lipopeptides, and flagellin, activate the corresponding TLRs, which activate transcription, translation, and the release of a series of inflammatory factors, chemokines, and adhesion molecules, and recruit other molecules involved in the innate immune response (neutrophils, *etc.*) to the site of infection[33,34]. Pathogenic byproducts bind to TLRs on the surface of PRRs, and the signal is transmitted from extracellular to intracellular, activating TLRs, followed by

signal transduction, and activation of the transcription factor NF-κB. This is the pathogenesis of infectious LM via the inflammatory mechanism.

DAMP-mediated TLR signaling pathway

TLR activation *via* the DAMP pathway explains how LM can lead to disease in the absence of infectious agents. DAMPs are endogenous proteins and can activate TLRs in a sterile environment[43,44]. DAMPs can activate TLRs in two ways: first, some inflammatory mediators activated by DAMPs activate TLRs and downstream NF-κB[45]; second, DAMPs can enhance the susceptibility of the TLR immune response in a sterile environment, thus activating TLRs and downstream NF-κB leading to the development of noninfectious LM[46].

MECHANISM OF LM-INDUCED CHANGES IN MILK ELECTROLYTE CONCENTRATION AND EC

Mechanism of cell membrane permeability alteration

Changes in milk electrolyte concentration and EC are linked to the inflammatory response during LM, and pathological changes in breast tissues caused by inflammatory factors result in changes in milk composition. Our understanding of the changes in milk electrolyte concentration and EC during LM is linked to the research by Smith *et al*[47] in the 1960s.The essence of LM is the inflammatory response of the breast tissue[48], and inflammatory factors cause increased epithelial permeability and both vascular and parenchyma damage[20].

The sodium pump on the basolateral membrane, which keeps intracellular K^+ high and Na^+ low, and the distribution of these ions according to the gradient of electric potential across the luminal membranes are the most important characteristics in terms of Na^+ and K^+[49]. The Na^+/K^+ ratio of the intracellular fluid is maintained at approximately 1:3 and the ratio of the extracellular fluid is maintained at approximately 3:1 by active transport of the sodium–potassium ion pump. As milk is electrically positive compared to the interior of the cell, the concentrations of these ions are lower in

milk. However, because milk is nearly isosmotic to plasma[50], the ratio between them is similar, with a Na^+/K^+ ratio of about 1:3[51,52].

When an inflammatory response occurs in the breast tissue, all of the inflammatory factors produced damage the ducts and secretary epithelial cells, disrupt the tight junctions between secretary cells, and increase capillary permeability. As a result, higher levels of Na^+ and Cl^- in the extracellular fluid enter the mammary gland alveolar lumen through the tight junctions that are opened, while the K^+ concentration decreases in order to maintain the osmotic pressure of milk in the alveolar lumen[53]. The increase in ionic concentrations in breast milk in the presence of mastitis, such as Na^+ and Cl^-, is consistent with other animal studies[54-56].

Mechanism of the milk osmolality level
Milk is rich in lactose. Lactose excretion may provide a reliable basis for fateful changes in breast permeability as lactose in food contributes minimally to the circulation[54]. Fetherston *et al*[57] conducted relevant studies, and the 24-h urinary excretion of lactose during LM has been extensively discussed. The correspondingly low steady-state urinary excretion of lactose demonstrates that these variations are not the result of increased paracellular pathway permeability. It indicates that the higher than normal concentrations of milk Na^+ and Cl^- observed in "normal breasts" could be a normal physiologic response to a lower concentration of lactose, ensuring that the osmolality of milk remains isotonic with plasma[20]. This has significant implications for the supposition that a raised Na^+ concentration is an outcome of subclinical LM.

ADDITIONAL DIAGNOSTIC PROSPECTS: MILK ELECTROLYTE CONCENTRATION AND EC

The electrolyte concentration and EC are physical properties of body fluids that are used to assess human health status and disease severity[58,59]. Previous animal studies have indicated that they can be used as an alternative method for the early diagnosis of LM[60]. In addition, Kitchen *et al*[61] demonstrated that the measurements of electrolyte

concentration and EC in milk samples for the diagnosis of LM in animals were comparable to other diagnostic methods.

Similar to the pathological response in animals with LM[62,63], when a woman is diagnosed with LM, inflammatory factors, such as procalcitonin, IL-1, IL-6, IL-8, and TNF-α[36], cause disruption of the tight junctions between cells. Furthermore, as a result of both the decrease in available glucose, particularly during severe symptoms, and the damage or death of lactocytes due to inflammation, lactose synthesis decreases[20]. Both of these factors can lead to changes in the electrolyte concentration and EC of milk. As mentioned earlier, numerous animal studies have indicated that milk electrolyte concentration and EC can be used in the early diagnosis of LM; therefore, based on the similar pathological responses, it is feasible to use milk electrolyte concentration and EC to diagnose early LM in humans.

In a recent study, we collected milk specimens from approximately 30 healthy women and 15 patients diagnosed with LM, and measured the electrolyte concentration (including Na^+, K^+, and Cl^-) and EC of bilateral breast milk in all patients. The final test results revealed that the concentrations of Na^+, Cl^- and EC in breast milk were significantly higher in women with LM than in healthy women, and in some cases several times higher. In addition, in women with LM, the concentrations of Na^+, Cl^- and EC in breast milk were markedly higher on the affected side than on the healthy side. Furthermore, we also found that the concentrations of Na^+, Cl^- and EC in milk were increased to varying degrees in patients who had symptoms but were not diagnosed with LM.

CONCLUSION

The early diagnosis and prevention of LM still face many challenges. We summarized the pathogenesis of LM, the mechanism of LM-induced changes in milk electrolyte concentration and EC, and found that changes in milk electrolyte concentration and EC in humans were primarily correlated with LM. It is clear from animal studies that there is a correlation between LM and milk electrolyte concentration and EC, and from the

significant changes in these indicators, LM can be diagnosed at an early stage and thus achieve a better prognosis. However, there have been few studies carried out on this topic. As a result, more data are required to verify these findings. If the changes in milk electrolyte concentration and EC are beneficial, these will have an enormous impact on clinical practice.

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