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Bacterial factors of mastitis in lactating women and its effect on the physical properties and chemical composition of breast milk

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Abstract: Mastitis is a complication seen in some breastfeeding mothers and is the most common inflammatory lesion of the breast in breastfeeding mothers. In this complication, breast milk undergoes chemical and physical changes. It can lead to a drop in breastfeeding, weight loss, and, consequently, stunted growth of infants. Bacteria are the main cause of breast inflammation. Therefore, in this study, bacterial factors of mastitis were evaluated in lactating women. Also, their effects were considered on the physical properties and chemical composition of mothers' breast milk. For this purpose, 210 breastfeeding mothers referred to health centers were randomly selected, and their milk samples were collected. In addition to collecting mothers' demographic information by a questionnaire, the chemical composition (sugar, protein, and fat) and the physical properties (pH, density, and freezing temperature) of milk were measured. Bacterial evaluations were performed on the milk of these mothers by catalase test, coagulase test, and mannitol salt agar. Data were analyzed by SPSS software, Chi-square, Mann-Whitney U test, and T-test. The results showed that 56 mothers had mastitis, and Staphylococcus aureus and coagulase-negative staphylococci were the main bacteria in the milk of these mastitis mothers. These bacteria caused physical and chemical changes in breast milk so that mothers with Staphylococcus aureus mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, Staphylococcus aureus may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

Key words: Bacterial factors; Breast milk; Lactating women; Mastitis.

Introduction

Mastitis is an inflammation of the breast that usually occurs during breastfeeding and is therefore commonly referred to as lactational mastitis (1). Mastitis usually occurs in the second or third week after delivery. Most reports indicate that 74 to 95% of mastitis cases occur in the first 12 weeks after delivery and are rarely seen after the twelfth week. Mastitis is relatively common and affects 33-35% of women during breastfeeding (2). The main causes of mastitis are milk stasis and infection. Milk stasis occurs when the milk does not come out completely during breastfeeding (3). Inadequate position of the baby during breastfeeding, the insufficient ability of the baby to suckle, limited frequency and duration of breastfeeding and obstruction of the urethra and ducts can lead to milk stasis. As a result of milk accumulation, suitable conditions are provided for the growth of bacteria (4).

The most common organisms found in mastitis are *Staphylococcus aureus* and coagulase-negative staphylococci. Also, sometimes, gram-negative bacilli (including Escherichia coli) are found in mastitis (5). But rare species of Salmonella, Mycobacterium, Candida and Cryptococcus have also been found. Ways of entering the organism include the entry of the organism through the milk-carrying ducts to the lobe, through the fissures and cracks of the nipple, and then spread through the blood (6).

The diagnosis of mastitis is usually based on clinical signs. Usually, one of the breasts is red, painful, swollen and hard, and there are signs of local inflammation and a decrease in milk. General symptoms may include 38.5°C or higher fever, lethargy, and chills (6). However, it is not possible to distinguish infectious mastitis from non-infectious ones through clinical signs. If possible, the culture of milk samples is recommended to determine infectious agents. A sample with more than 10^6 leukocytes and more than 10^3 bacteria per milliliter of milk indicates infectious mastitis (7).

Mastitis is important for several reasons. First, it reduces milk production, and about a quarter of mothers avoid breastfeeding their babies because of mastitis, and weaning will affect the baby's health (8). Second, mastitis increases the risk of mother-to-child transmission. In the case of retroviruses, the risk of transmission increases by 2 to 4 times (9). Also, because the disease affects the alveoli of the breast, breast milk undergoes chemical and physical changes. For example, the ratio of sodium to potassium in breast milk increases, and in some cases the baby does not suckle from the inflamed breast. This may be due to a change in the taste of breast milk (10). If the infant is exclusively breastfed, this can lead to reduced breastfeeding and weight loss, resulting in impaired growth (11).

Due to the importance of mastitis in the health of infants, it is necessary for women with symptoms of mastitis during breastfeeding to be identified and treated immediately (12). In cases of infectious mastitis, the best way for treatment is to culture the milk sample for recognizing the exact bacteria to determine the appropriate antibiotic (13). This study aimed to evaluate mastitis in lactating women and to diagnose the associated bacterial infection and the effect of these bacterial agents on the physical properties and chemical composition of breast milk.

Materials and Methods

Demographic characteristics

The present study was a cross-sectional descriptive study. The study population consisted of all breastfeeding mothers who were less than 6 months after delivery, and they were referred to health centers. 210 mothers were randomly selected and their milk samples were collected. The data collection tool was a questionnaire. The questionnaire included 41 questions related to demographic factors, physical and chemical characteristics, bacterial factors that cause mastitis and physical characteristics of breast milk. After recording the demographic information of the mothers, they were asked to pour a sample of their milk into disposable sterile containers after washing their hands with soap and water without contacting the nipple with the sampling container. Then, the milk samples were stored in the refrigerator. At the end of the same day, the samples collected in the vicinity of the ice were transferred to the laboratory for microbiological tests and determination of chemical and physical properties.

Physical properties and chemical composition

The physical characteristics of breast milk in this study include milk pH, milk density, milk freezing point, the presence of blood and coagulation clots, and milk color. The chemical properties of milk in this study refer to the amount of fat, protein, and sugar.

Microbial assessments

For microbial culture, the samples were inoculated into a blood agar medium and incubated at 37°C for 24 hours. After the growth of bacteria on the culture medium, a direct slide was prepared from the colonies and the following differential tests were performed to detect the bacteria.

A. Catalase test: The catalase test is an important diagnostic key for identifying and differentiating bacteria. For example, streptococci (negative catalase) can be differentiated from staphylococci and micrococci (positive catalase). It is used to differentiate Clostridium (catalase-negative) from Bacillus (catalase-positive) and Listeria monocytogenes (catalase-positive) from beta-hemolytic streptococci (catalase-negative) (14). The protocol for performing this test is using 3% hydrogen peroxide as a substrate. With a wooden applicator, some microbial samples were transferred from the center of a colony to the surface of a glass slide. A few drops of 3% hydrogen peroxide were added to it and mixed. The positive reaction of catalase was characterized by the production of oxygen gas bubbles at the slide surface.

B. Coagulase test: The coagulase test is used to differentiate Staphylococcus aureus (coagulase-positive) from coagulase-negative staphylococci. Coagulase is an enzyme produced by Staphylococcus aureus that converts fibrinogen (soluble) in plasma to fibrin (insoluble) (15). For this purpose, one-quarter (1ml of plasma, 4ml of sterile saline) was prepared from rabbit serum plasma coagulase. Then a thick suspension of bacteria was prepared and placed in an incubator at 37°C for 2 to 4 hours. In the case of coagulation, a positive result was considered.

C. Mannitol salt agar: High salt concentration (7.5%) inhibits the growth of most gram-negative and gram-positive strains except *Staphylococcus aureus*. Staphylococcus can ferment mannitol (mannitol is the only carbohydrate in the culture) and produce acid. This leads to a decrease in pH and discoloration of phenol to yellow (16). In this experiment, staphylococcal colonies are distinctly yellow and surrounded by a yellowish halo, but other staphylococci and micrococci do not have the ability to ferment mannitol. They create red colonies with a purple-red halo by breaking down the peptone in the environment.

To assess bacterial susceptibility to antibiotics, 0.5 McFarland bacterial serum suspension was prepared. The bacteria were then cultured in a Mueller-Hinton agar medium. Finally, antibiotic discs were placed at appropriate intervals on the culture. Bacterial susceptibility was measured to the antibiotics amoxicillin, tetracycline, erythromycin, azithromycin, flucloxacillin, dicloxacillin, cloxacillin, cephalothin, and co-trimoxazole.

Statistical Analysis

All obtained information was calculated by SPSS 16 statistical software. Chi-square, Mann-Whitney U test, and t-tests were also used for correlation and the results were reported at a significant level of 5%.

Results

Demographic characteristics

Out of 210 samples, 56 mothers (26.66%) had mastitis. The mean age of healthy mothers was 28.71±4.6 years and the mean age of mothers with mastitis was 32.02±3.2 years. There was a significant difference between the mean age of mothers in the two groups ($P < 0.05$). Mothers with and without mastitis were evaluated and compared in terms of the number of children, the number of deliveries, and the duration of previous breastfeeding (Table 1). The results showed that there was a significant relationship between the previous history of breastfeeding and mastitis ($P < 0.05$).

About 38.7% of mothers with mastitis fed their children with other foods and fluids in addition to breast milk. In relation to maternal breast problems, all mothers with mastitis had clinical signs of mastitis in the form of pain, swelling, and redness. The method of delivery in 75.8% of mothers with mastitis was a cesarean section. Also, in relation to contraception, 31.2% of mothers with mastitis used the IUD method, 45.2% of mothers used natural methods of contraception and the rest of the mothers used other methods of contraception.

There was no significant relationship between contraceptive use and maternal mastitis. In addition, none of the mothers with mastitis had an underlying disease (diabetes, hypertension, and heart disease). In addition, 51 (91.07%) of mothers with mastitis did not receive breastfeeding training during pregnancy or after delivery. The gender of the child in 51.78% of mothers with mastitis was male, which was 53.7% for non-infected mothers. There was no significant relationship between child gender and mastitis.

According to the central indices related to height, head circumference, birth weight, and current weight of children of mothers with mastitis and healthy mothers, it was observed that there was a significant difference between the average height and head circumference of children in the two groups ($p < 0.05$) and in other cases no significant difference was observed (Table 2).

Physical properties and chemical composition

The chemical properties of milk in this study include milk fat, protein, and sugar (Table 3). There was a significant difference between the mean amount of sugar and protein in the two groups ($P = 0.003$).

Table 4 compared the physical properties of milk, including pH, density, and freezing point of milk for affected and non-affected mothers. As can be seen, there was a significant difference between the mean pH and density between the two groups. Regarding the appearance of breast milk, 83.9% of mothers with mastitis had clots in their milk, while no clots were found in any of the samples of healthy breast milk. There is a significant relationship between mastitis and clots in milk ($p < 0.001$).

Microbial assessments

Out of 210 patients, 56 (26.66%) were positive cultures and 154 samples (73.34%) were negative cultures. Of 56 culture-positive samples, 51 samples (91.1%) were coagulase-negative for staphylococci and 5 samples (8.9%) were positive for *Staphylococcus aureus*. The number of *Staphylococcus aureus* bacteria isolated was more than 10^3 per ml of milk. In the case of coagulase-negative staphylococci, out of 51 positive culture samples, 21 samples had more than 103 colonies per ml of milk, and in 30 samples, the number of colonies was less than 10^3 per ml.

The susceptibility and resistance of Staphylococcus aureus isolated from 5 samples to different antibiotics are shown in Table 5. Staphylococcus aureus isolated from all five samples was sensitive to the antibiotics flucloxacillin, dicloxacillin, and cloxacillin. Of the 5 samples, 2 samples were sensitive to amoxicillin, 3 samples to tetracycline, 3 samples to erythromycin, 3 samples to azithromycin, 2 samples to cephalothin, and 1 sample to co-trimoxazole.

Coagulase-negative staphylococci isolated from all 21 samples were sensitive to the antibiotics flucloxacillin, dicloxacillin, and cloxacillin. Of the 21 samples, 15 samples were sensitive to amoxicillin, 16 samples to tetracycline, 17 samples to azithromycin, 18 samples to cephalothin, and 13 samples to co-trimoxazole. Table 6 shows the frequency distribution of isolated coagulase-negative staphylococci in terms of resistance and sensitivity to various antibiotics.

Milk samples of mothers with *Staphylococcus aureus* mastitis and mothers with coagulase staphylococci were compared in terms of physical characteristics and chemical composition (Table 7). The results showed

Table 1. Comparison of mothers with mastitis and healthy mothers.

	Healthy mothers	Mothers with mastitis	*P*-value
Age (year)	28.71±4.6	32.02±3.2	0.04
Number of deliveries	2.02±1.2	3.3±1.5	0.001
Number of children	1.9±1.5	3.2±1.5	0.002
Duration of the previous breastfeeding	12.4±11.8	18.7±8.5	0.04

Table 2. Comparison of children of mothers with mastitis and healthy mothers.

	Healthy mothers	Mothers with mastitis	*P*-value
Child height (cm)	63.7±7.2	53.2±6.5	0.001
Child head circumference size (cm)	41.8±3.7	38.6±4.5	0.03
Child age (weak)	39.7±2.1	39.8±1.4	0.21
Child weight (g)	7345.2±1987	6754.3±2615	0.52
Birth weight of Child (g)	3287.3±321	3209±412	0.43

Table 3. Comparison of chemical compounds in the milk of mothers with mastitis and healthy mothers.

	Healthy mothers	Mothers with mastitis	*P*-value
Fat (%)	4.2±0.8	3.6±0.6	0.19
Protein (%)	1.7±0.31	1.3±0.22	0.007
Sugar (%)	6.7±0.31	6.3±0.18	0.002

Table 4. Comparison of physical characteristics in the milk of mothers with mastitis and healthy mothers.

	Healthy mothers	Mothers with mastitis	*P*-value
pH	6.9±0.23	6.7±0.31	0.001
Density (µg/ml)	1037±1.8	1036±1.4	0.02
Freezing point (°C)	-0.054±0.05	-0.055±0.07	0.21

Table 5. Sensitivity and resistance of 5 positive samples of *Staphylococcus aureus* isolated from patients to different antibiotics.

Sample	Amoxicillin	Tetracycline	Erythromycin	Azithromycin	Flucloxacillin	Dicloxacillin	Cloxacillin	Cephalothin	Co-Trimoxazole
1	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
2	Resistant	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Resistant	Resistant
3	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
4	Resistant	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
5	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant

Table 6. Absolute and relative frequency distribution of coagulase-negative staphylococci isolated in terms of resistance and sensitivity to different antibiotics.

	Resistant		Sensitive		Total	
	Number	Percent	Number	Percent	Number	Percent
Amoxicillin	6	28.6	15	71.4	21	100
Tetracycline	5	23.8	16	76.2	21	100
Erythromycin	4	19	17	81	21	100
Azithromycin	4	19	17	81	21	100
Fluoxacillin	0	0	21	100	21	100
Dicloxacillin	0	0	21	100	21	100
Cloxacillin	0	0	21	100	21	100
Cephalothin	3	14.3	18	85.7	21	100
Co-Trimoxazole	8	38.1	13	61.9	21	100

Table 7. Comparison of milk samples of mothers with Staphylococcus aureus mastitis and mothers with coagulase staphylococci in terms of physical characteristics and chemical composition.

	Staphylococcus aureus	coagulase-negative staphylococci	*P*-value
Fat (%)	3.5±0.7	3.7±0.8	0.22
Protein (%)	1.5±0.12	1.1±0.32	0.004
Sugar (%)	5.9±0.07	6.7±0.29	0.002
pH	6.71±0.23	6.79±0.39	0.6
Density (µg/ml)	1036±0.9	1036±1.9	0.91
Freezing point (°C)	-0.054±0.05	-0.056±0.08	0.21

that there was significantly less sugar in the milk of mothers with *Staphylococcus aureus* mastitis, and mothers with coagulase-negative staphylococci had less protein in their milk.

Discussion

Mastitis is an inflammation of the breast and usually occurs during breastfeeding. The prevalence of mastitis in breastfeeding mothers is 9.5-10% in the first year after delivery (6). In the current study, out of 210 samples, 56 mothers (26.66%) had mastitis. The mean age of healthy mothers was 28.71±4.6 years and the mean age of mothers with mastitis was 32.02±3.2 years. There was a significant difference between the mean age of mothers in the two groups ($P < 0.05$). In this regard, Hartmann (17) cited the causes of mastitis are primiparous mothers over 30 years of age. Another study showed the same results. According to this study, out of 56 mothers with mastitis, 38 (67.85%) were primiparous (18). The results of many studies in the field of mastitis indicate the effectiveness of counseling and teaching proper breastfeeding techniques in the prevention of this complication (19). Because optimizing the method of breastfeeding as well as frequent and complete emptying of the breast is effective in preventing the occurrence of this disease (20). Also, in the present study, 51 (91.07%) of mothers with mastitis did not receive breastfeeding training during pregnancy or after delivery.

The duration of the previous breastfeeding is also effective in causing mastitis. In the present study, a statistically significant relationship was found between the duration of previous breastfeeding and the previous history of breastfeeding with mastitis. In addition, the long interval between breastfeeding or the use of other foods and fluids with breast milk is another risk factor, which causes the milk to not drain completely from the breast and milk stasis in the breast. Also, 38.7% of mothers used other foods and fluids with their milk to feed the baby, which can lead to long-distance breastfeeding or irregular breastfeeding. This can cause mastitis in mothers (1).

According to the results of this study, the average height, weight, and head circumference of children of mothers with mastitis were lower than those of healthy mothers. There was a significant difference between the mean height and head circumference of children in the two groups. The results of Kasonka *et al.* research also reflected the same findings (18).

Another factor that affects the growth of children of mothers with mastitis is the change in the amount of sodium to potassium and other elements of milk in infected mothers, which can lead to changes in the taste of milk and as a result, inadequate nutrition of the child (3). In some studies, the elements of breast milk from mastitis-infected and healthy mothers were compared. The results showed that the amount of sodium to potassium in the milk of mothers with mastitis increased and the amount of glucose in the milk of these mothers decreased. The amount of these elements in the milk of the two groups of mothers showed a statistically significant difference (21-25). In the present study, there was a significant difference between the mean amount of sugar and protein in the two groups. In addition, there was a significant difference between the two groups regarding the physical properties of breast milk, i.e. pH and milk density.

In several reports, researchers have named *Staphylococcus aureus* and coagulase-negative staphylococci as the most common causes of mastitis (26). In the present study, out of 56 culture samples, 5 samples were *Staphylococcus aureus* and 51 samples were coagulase-negative staphylococci. Bacteria can be isolated from breast milk samples without the clinical signs of mastitis, but the range of these isolated bacteria is usually very similar to the bacteria present on the surface of the skin. Therefore, contamination of samples with skin surface bacteria should be avoided as much as possible. In this study, we tried to provide suitable conditions during sampling.

Also, in the present study, it was found that *Staphylococcus aureus* and coagulase-negative staphylococci played an important role in the development of infectious mastitis. These bacteria caused physical and chemical changes in breast milk so that mothers with *Staphylococcus aureus* mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, *Staphylococcus aureus* may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

In general, mastitis is common in different populations and breastfeeding is important for the baby and therefore the health of the baby. Proper breastfeeding methods (such as close contact between mother and baby, the proper way of hugging the baby while breastfeeding, and proper frequency and duration of breastfeeding) are good ways to prevent the flow of milk and cause infection.

In this study, mastitis in lactating women and the associated bacterial infection were investigated. The effect of these bacterial agents was also investigated on the chemical composition and physical properties of breast milk. The result showed that *Staphylococcus aureus* and coagulase-negative staphylococci played an important role in the development of infectious mastitis. These bacteria caused physical and chemical changes in breast milk so that mothers with *Staphylococcus aureus* mastitis had less sugar in their milk, and mothers with coagulase-negative staphylococci had less protein in their milk. Therefore, *Staphylococcus aureus* may reduce milk sugar by consuming milk sugar, and coagulase-negative staphylococci may also target milk protein. But to confirm these results, a larger population of mothers with mastitis is needed. Further studies are also needed to prove this result.

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