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BIOCENOSIS OF THE MAMMARY GLANDS IN CHILDREN WITH LACTOSTASIS

Breastfeeding is one of the components of health and optimal development of a newborn child, and a woman's health. Lactation disorders can be the cause of mastitis. In conditions of violation of milk evacuation, there is a change in the microbiological state of the mammary gland in the direction of the growth of pathogenic bacteria, which later leads to the development of a mammary gland abscess. The article compares the microbiological state in different areas of the mammary glands of 67 parturients with normal lactation during the 7 days of the postpartum period with biocenosis of the mammary glands in parturients diagnosed with lactostasis. In the picture of the biocenosis of the mammary gland, attention is paid to the changes of such a microorganism as Aerococcus viridians, which is an antagonistically active saprophytic microorganism.

The conducted research makes it possible to further apply a promising method of preventing mastitis with the help of probiotics which contain Aerococcus viridians.

The aim of the study. *lactostasis during the postpartum period with women in labor who were lactating without pathology on the 3rd and 5th-7th days of the postpartum period. At the same time, separately analyze the changes in the content of Aerococcus viridians, which is a representative of saprophytic and antagonistic microflora, in different groups of parturients. Taking into account the obtained data, prove the feasibility of using probiotics, which are represented by Aerococcus viridians, in the prevention of the development of mastitis.*

Research materials and methods. *The analysis of the microbiological state of the mammary glands was carried out from the sections of the areola mammae and the papilla mammae in 33 parturients without lactostasis and in 34 parturients with lactostasis, with subsequent identification of bacterial flora. A selective indicator medium was used for sowing Aerococcus viridians.*

Results and discussion. *During the bacteriological examination of women in labor of different groups, 13 strains of bacillus and coccal microflora were detected. The spectrum of microflora included bacteria that were saprophytic for the biocenosis of the mammary gland – Staphylococcus epidermidis, Staphylococcus saprofiticus, Aerococcus viridians, conditionally pathogenic Micrococcus sp., Candida sp. and pathogenic – Staphylococcus aureus, Enterobacter sp., E. solli, Klebsiella pneumonia.*

Separately, there were singled out the so-called compensatory microorganisms, which also had high antagonistic activity in the biocenosis of the mammary glands – Bacillus sp.

Microflora analysis revealed that during the physiological course of the postpartum period, Staphilococcus epidermidis, Staphilococcus saprofiticus, and Aerococcus viridians were sown with a high percentage. In the dynamics of puerperium, the favorable growth of Staphylococcus saprofiticus, Bacillus subtilis sp., and Aerococcus viridians. Pathogenic microflora was sown from different areas of the mammary gland much less often.

In parturient women with lactostasis, there was a probable increase in the seeding of Staphylococcus aureus up to 73.5% in various areas of the mammary glands, Enterobacter sp. up to 47.0%, E. solli up to 35.3% and Klebsiella pneumonia up to 26.5%. When compared with the biocenosis of the mammary gland of parturient women during physiological lactation, in parturient women with lactostasis, the seeding of saprophytic microflora, as well as Aerococcus viridians in particular, decreased significantly to 5.9% ($p<0.05$).

The results of the conducted research make it possible to use a probiotic containing Aerococcus viridians to prevent the development of lactational mastitis.

Conclusions. *In women with the physiological course of lactation, an increase in the colonization of the mammary glands by saprophytic and antagonistic active coccal flora was observed, with a simultaneous decrease in the colonization of Staphylococcus aureus and Gram-negative enterobacteria from different areas of the mammary gland. The stability of the biocenosis of the mammary gland during physiological lactation was maintained due to the introduction of representatives of the genus Bacillus sp. In parturient women, during the development of lactostasis, the biocenosis of the mammary glands changed significantly due to an increase in the seeding of Staphylococcus aureus and Gram-negative enterobacteria, which is a significant factor in the development of lactational mastitis. Taking into account the reliable decrease in lactostasis in the biocenosis of the mammary gland Aerococcus viridians, there are prospects for the use of probiotics that contain Aerococcus viridians for the prevention of lactational mastitis.*

Key words: *mammary gland, lactation, microbiocinosis, lactostasis.*

Василь Чуйко, Тетяна Василенко. БІОЦЕНОЗ МОЛОЧНИХ ЗАЛОЗ У ПОРОДІЛЬ З ЛАКТОСТАЗОМ

Грудне вигодовування є одною з складових здоров'я та оптимального розвитку новонародженої дитини, здоров'я жінки. Порушення лактації можуть бути причиною розвитку маститу. В умовах порушення евакуації молока виникає зміна мікробіологічного стану молочної залози в напрямку росту патогенних бактерій, які в подальшому призводять до розвитку абсцесу молочної залози. В статті проводиться порівняння мікробіологічного стану у різних ділянках мо-

лочних залоз 67 породіль з нормальною лактацією протягом 7 діб післяпологового періоду з біоценозом молочних залоз у породіль з діагностованим лактостазом. В картині біоценозу молочної залози приділяється увага змінам такого мікроорганізму, як Aerococcus viridians, котрий являється антагоністично активним сапрофітним мікроорганізмом.

Проведені дослідження дають змогу в подальшому застосовувати перспективний метод профілактики маститу за допомогою пробіотика А-бактерин, який складає Aerococcus viridians.

***Мета дослідження.** Провести порівняльний аналіз якісного стану біоценозу у різних ділянках шкіри молочних залоз породіль, у яких виник лактостаз протягом післяпологового періоду, з породіллями у яких проходила лактація без патології на 3 та 5-7 добу післяпологового періоду. При цьому окремо провести аналіз змін у різних груп породіль вмісту Aerococcus viridians, який є представником сапрофітної та антагоністичної мікрофлори. З урахуванням отриманих даних довести доцільність застосування пробіотиків, які представлені Aerococcus viridians, у профілактиці розвитку маститу.*

***Матеріали та методи дослідження.** Аналіз мікробіологічного стану молочних залоз проводився з ділянок areola mammae та papilla mammae у 33 породіль без лактостазу та у 34 породіль з лактостазом, з подальшою ідентифікацією бактеріальної флори. Для висівання Aerococcus viridians застосовувалась селективно-індикаторне середовище.*

***Результати і обговорення.** При бактеріологічному обстеженні породіль різних груп було виявлено 13 штамів паличкової та кокової мікрофлори. В спектр мікрофлори входили бактерії ,які являлись сапрофітною мікрофлорою для біоценоза молочної залози -Staphilococcus epidermidis, Staphilococcus saprofiticus, Aerococcus viridians, умовно- патогенною -Micrococcus sp., Candida sp. та патогенною – Staphilococcus aureus, Enterobacter sp., E. colli, Klebsiella pneumonia.*

Окремо виділялись так звані компенсаторні мікроорганізми, які також мали високу антаногістичну активність у біоценозі молочних залоз – Bacillus sp..

Аналіз мікрофлори виявив, що при фізіологічному перебігу післяпологового періоду з високим відсотком висівалися Staphilococcus epidermidis, Staphilococcus saprofiticus, Aerococcus viridians. В динаміці післяпологового періоду відмічалось вірогідне зростання Staphilococcus saprofiticus , Bacillus subtilis sp. та Aerococcus viridians. Патогенна мікрофлора висівалась з різних ділянок молочної залози значно рідше.

У породіль з лактостазом відмічалось ймовірне зростання висівання Staphilococcus aureus до 73.5% на різних ділянках молочних залоз, Enterobacter sp. до 47.0%, E. colli до 35,3% та Klebsiella pneumonia до 26.5%. При порівнянні з біоценозом молочної залози породіль при фізіологічній лактації, у породіль з лактостазом суттєво знизилось висівання сапрофітної мікрофлори, а також зокрема Aerococcus viridians до 5,9% (p<0,05).

Результати проведених досліджень дають можливість застосуванню пробіотика, який містить Aerococcus viridians, для профілактики розвитку лактаційного маститу.

***Висновки.** У жінок з фізіологічним перебігом лактації спостерігалось збільшення колонізації молочних залоз сапрофітною та антагоністичною активною коковою флорою при одночасному зменшенні колонізації з різних ділянок молочної залози Staphilococcus aureus та Грам- негативних ентеробактерій. Стабільність біоценозу молочної залози при фізіологічній лактації підтримувалась за рахунок інтродукції представників роду Bacillus sp.. У породіль при розвитку лактостазу біоценоз молочних залоз змінювався суттєво за рахунок збільшення висівання Staphilococcus aureus та Грам- негативних ентеробактерій, що є в подальшому значним фактором в розвитку лактаційного маститу.*

З урахуванням достовірного зниження при лактостазі в біоценозі молочної залози Aerococcus viridians, виникають перспективи застосування пробіотика А-бактерина в профілактиці лактаційного мастита.

***Ключові слова:** молочна залоза, лактація, мікробіоциноз, лактостаз.*

Introduction. Breastfeeding is a very important social and medical factor affecting the health of the mother and child. Strengthening the function of the mammary gland in the postpartum period and reducing the immunological reactivity of the body cause changes in the microbiological composition of the mammary gland during this period. The lactation process begins in the parturient woman, and can be accompanied by pathological conditions – lactostasis, which can lead to the development of lactational mastitis.

According to the literature, the incidence of acute lactational mastitis to the number of deliveries ranges from 0.5 to 6% [3, 12].

In 85.8% of mastitis observations lactostasis preceded it, that's why this is the main "starting" mechanism for the development of the inflammatory process in the mammary gland. If the symptoms persist for 12-24 hours, the growth of microorganisms occurs in the conditions of impaired evacuation of milk, which leads to infectious lactational mastitis, which can be complicated by an abscess [14, 16, 20].

The main causative agent of purulent mastitis is Staphylococcus aureus, including methicillin-resistant S. aureus (MRSA), which was isolated from pus in monoculture in 90.8% of patients and in association with other microflora in 2.5%. Inflammation of the mammary gland can be caused by such opportunistic microorganisms as Streptococcus pyogenes (group A or B), Escherichia coli, Bacteroides species, Corynebacterium species? and coagulase-negative staphylococci (for example, Staphylococcus lugdunensis) [8, 16, 18, 21].

Since 2007, based on the Department of Obstetrics and Gynecology of the Dnipro State Medical University, studies of microbiocinosis of the mammary gland in pregnant women before childbirth and in women in labor in the dynamics of the postpartum period have been conducted. It showed microbiological changes in various areas of the mammary glands before childbirth and in the postpartum period, taking into account the determination of the condition one of the antagonists of pathogenic bacteria – Aerococcus viridians.

It was established that in the microbiocenosis of the mammary glands of pregnant women before childbirth, with a frequency of 26.5% – 64.7%, representatives of saprophytic and antagonistic microflora – Aerococcus viridans are present in the amount of 10^2–104 CFU/ml.

The obtained strains of Aerococcus viridans had a high ability to produce hydrogen peroxide [4, 5].

In the postpartum period during the formation of lactation, mostly opportunistic microorganisms that are most often the cause of the development of lactational mastitis (Staphylococcus aureus, Enterobacter sp., E. colli, Klebsiella pneumonia) were sown on the skin of the mammary glands. Later, it was established that there are changes in the microbiological state of women in labor, which are accompanied by the growth of representatives of normal microflora [5].

The aim of the study. To study the qualitative state of the microbial flora in different areas of the skin of the mammary glands of women in labor who developed lactostasis during the postpartum period, as well as the content of Aerococcus viridans, as one of the expressed bacteria- antagonists of the pathogenic flora. And also to conduct a comparative analysis of biocenoses of the mammary glands in women in labor with and without lactostasis on the 3rd and 5th–7th day of the postpartum period.

Materials and research methods. The microbiological examination was carried out on the 3rd and 5th to 7th days of the postpartum period in 33 mothers who had a normal delivery with physiological development of lactation, who fed the child exclusively by breast, with the absence of extragenital pathology, acute and chronic infectious diseases. Also, the determination of the state of micro biocenosis of the mammary glands was carried out in 34 parturient women who were diagnosed with lactostasis.

The scrub-wash method of Williamson and Kligman in the modification of S.I. Klimnyuk and S.I. Sytnyk [1] was used to collect the material from two areas of the mammary gland: areola mammae and papilla mammae, and colostrum and milk were sieved. Identification of the bacterial flora was carried out by a colorimetric system for research by the company «Liofilchem» (Italy). Aerococcus viridans cultures were identified by additional criteria: growth on a selective indicator medium and biochemical activity on mediums with selenium and tellurium salts, lactate oxidase, and superoxide dismutase activity [2].

Research results and discussion. The age of the women who were under surveillance ranged from 18 to 40 years, which corresponds to the most active period of reproduction.

Microbiological analysis showed that the microbiocenosis of the skin of the mammary gland in parturient women was represented by various coccal and bacilli flora (Table No. 1).

When studying the nature of the microflora of the mammary glands in parturients of the examined groups, it was found that the bacteriological composition is represented by 13 types of pathogenic (Staphylococcus aureus, Enterobacter sp., E. solli, Klebsiella pneumonia), conditionally pathogenic (Micrococcus sp., Candida sp.), and saprophytic microflora (Staphilococcus epidermidis, Staphilococcus saprofiticus, Bacillus sp., Aerococcus viridians).

During the physiological course of the postpartum period, in the dynamics of the puerperium, a high percentage of Staphilococcus epidermidis, Staphilococcus saprofiticus, and Aerococcus viridians were sown from the areola mammae from 27.8% to 46.7%, and from rapilla mammae – from 44.4 to 93.3%. When comparing the microflora on day 3 and day 5-7 of the puerperium, favorable growth of Staphylococcus saprofiticus (44.4% compared to 80.0%, $p<0.05$), Bacillus subtilis (11.1% compared to 40.0%, p<0, 05), Bacillus licheniformis (5.5% compared to 20.0%, $p<0.05$). A high percentage (from 27.8% to 88.9%) was seeded with Aerococcus viridians.

Taking into account the beneficial growth of Bacillus sp. in the dynamics of the postpartum period, it must be interpreted as a compensated state of micro biocenosis of the mammary gland, thanks to the species spectrum of aerobic spore-forming.

Pathogenic microflora was seeded much less often and was determined in Staphilococcus aureus up to 11.1% of seeding, Enterobacter sp. – 5.5%, E. solli – 16.7%, Klebsiella pneumonia – 11.1%.

A different microbiological picture was observed in parturient women with lactostasis, where a probable increase in the seeding of Staphylococcus aureus from 52.9% to 73.5% in different areas of the mammary glands was noted. It confirms the importance of this pathogenic flora in the etiological development of lactational mastitis [6, 10, 13, 19]. The specific gravity of the growth of the sowing of Enterobacter sp. is up to 47.0%, E. solli up to 35.3%, and Klebsiella pneumonia up to 26.5%.

When comparing the biocenoses of women in labor with and without lactostasis, a significant increase in the area of areola mammae and papilla mammae of Staphylococcus aureus was noted from 52.9% to 11.1% on the 3rd day of the physiological puerperium ($p<0.05$), Enterobacter aerogenes from 41.2% to 5.5% ($p<0.05$), E. solli 29.4 to 16.7% ($p<0.05$), as well as Klebsiella pneumonia 26.5% to 11.1% ($p<0.05$) At the same time, it is necessary to note a significant decrease of Aerococcus viridians from different areas of the mammary gland to 5.9% in relation to its sowing from the mammary glands of healthy mothers in labor ($p<0.05$).

This microscopic picture of a significant change in the biocenosis of the mammary gland probably indicates a decrease in the immunological protection of the woman in labor in the postpartum period against the background of milk stagnation, infection of the mammary glands from the center of a chronic infection, as well as when microorganisms enter from the outside

Table 1

Comparative analysis of microflora of mammary glands in parturient women with lactostasis

Microorganisms have been studied From mammary glands	Physiological course 3 days (n=18)				Physiological course 5–7 days(n=15)				Women in laborwith lactostasis (n=34)			
	Areola mammae		Rapillamammae		Areola mammae		Rapilla mammae		Areola mammae		Rapil la mammae	
	district	%±m	district	%±m	district	%±m	district	%±m	district	%±m	district	%±m
Staphylococcus epidermidis	7	38.9±11.5	14	72.2±10.5	6	40.0±12.6	14	93.3±6.5	9	26.5±7.5	6	17.6±6.5+
Staphylococcus saprophyticus	6	33.3±11.1	8	44.4±11.7	7	46.7±12.9	12	80.0±10.3*	6	17.6±6.5	8	23.5±7.3
Staphylococcus aureus	2	11.1±7.4	–	–	–	–	1	6.7	18	52.9±8.6+	25	73.5±7.6
Micrococcus sp.	5	27.8±10.6	6	33.3±11.1	4	26.7±11.4	6	40.0±12.6	12	35.3±8.2	16	47.0±8.5
Enterobacter aerogenes	1	5.5±5.4	–	–	1	6.7	–	–	14	41.2±8.4+	16	47.0±8.5
Enterobacter hafnia	–	–	–	–	–	–	–	–	3	8.8±4.8	2	5.9±4.0
Enterobacter cloacae	–	–	–	–	1	6.7	1	6.7	2	5.9±4.0	4	11.8±5.5
E. coli	3	16.7±8.8	1	5.5±5.4	1		2	13.3±8.8	10	29.4±7.8+	12	35.3±8.2+
Candida sp.	1	5.5±5.4	–	–	1	6.7	–	–	1	2.9	2	5.9±4.0
Klebsiellapneumoniae	2	11.1±7.4	1	5.5±5.4	–	–	–	–	9	26.5±7.5+	8	23.5±7.3+
Bacillus subtilis	3	16.7±8.8	2	11.1±7.4	5	33.3±12.2*	6	40.0±12.6*	2	5.9±4.0+	1	2.9
Bacillus licheniformis	1	5.5±5.4	1	5.5±5.4	3	20.0±10.3*	2	13.3±8.8	1	2.9	–	–
Aerococc us viridans	**5**	**27.8±10.6**	**16**	**88.9±7.4**	**6**	**40.0±12.6**	**13**	**86.7±8.8**	**2**	**5.9±4.0+**	**2**	**5.9±4.0+**

Note: * – p<0.05 in comparison with the sowing of microorganisms from different areas of the mammary glands on each day of the postpartum period.
+ – p<0.05 in comparison with the sowing of microorganisms from different areas of mammary glands with and without lactostasis.

from patients with various manifestations of purulent-inflammatory infection or from carriers of pathogenic microflora [10,16].

A significant decrease in lactostasis in the biocenosis of the mammary gland of the antagonistically active bacterium Aerococcus viridians makes it possible to substantiate the use of the probiotic that contains Aerococcus viridians in the prevention of lactational mastitis in women with lactostasis. Based on numerous literature data [7, 11, 12, 15, 17], the use of probiotics is a very promising method of preventing the development of lactational mastitis

Conclusion.

1. In the dynamics of puerperium in women with a physiological course of childbirth and lactation, an increase in the colonization of the mammary glands with saprophytic and antagonistic active coccal flora was observed, a significant part – in the areas of the papilla mammae. At the same time, a decrease in the colonization of Staphylococcus aureus and Gram-negative enterobacteria from different areas of the mammary gland was observed.

2. The dynamic stability of microbiocinosis of the mammary gland in women with the physiological course of puerperium was also supported by the introduction of representatives of the genus Bacillus sp., the probable growth of which was observed on the 5th–7th day of the postpartum period.

3. During the development of lactostasis in parturients, the biocenosis of the mammary glands changed significantly.

A significant growth of Staphylococcus aureus and Gram-negative enterobacteria was observed, which indicates a change in the microbial state of the mother in labor or infection of the mammary glands from patients with various manifestations of purulent- inflammatory infection and from carriers of pathogenic microflora, which can lead to the development of lactational mastitis.

4. A significant reduction of Aerococcus viridians from areas of the mammary gland in parturient women with lactostasis opens up the prospect of using the probiotic which contains Aerococcus viridians in the prevention of lactational mastitis.

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