Original Research Article

Cytological Screening of Inflammatory Cells in Breast Fluid among Lactating Women in Shendi Town, Sudan

ABSTRACT

Background: Mastitis variety of inflammatory and reactive changes that can be seen in the breast. While some of these changes are a result of infectious agents. Acute mastitis usually occurs during the first three months postpartum as a result of breastfeeding. The cytological evaluation of milk could be quite helpful in diagnosing mammary gland inflammations. **Aim**: To cytological screening of inflammatory cells in breast milk. ***Methods***: This is a cross-sectional study conducted in Shendi town, with 100 samples of breast milk from women. (50 milk smears, 50 postpartum milk) have been collected and screened cytologically. Stained by pap stain. ***Results***: After cytology screening of breast milk in, milk 26/50(52%) of women were noncell secretors and 24/50(48%) of women were cell secretors. Cells that appeared among the secreting group were a few epithelial cells and immune cells. In postpartum milk all women 50/50 (100%) were cell secretors, with the high secretion of immune cells 50/50 (100%) which found a statistically significant value (0.000). In milk smear 6/50(12%) of women were suffering from acute mastitis. In postpartum milk, 10/50(20) of the women were suffering from acute mastitis. In this study, HPV infection cytomorphological change (koilocyte) was detected in breast fluid 2/100 (2%). ***Conclusion***: Cytology of the breast is a simple, safe, rapid test that is acceptable to patients and shows the ability to detect inflammatory cells and immune cells from asymptomatic volunteers. Practiced anatomic pathologists and pathology trainees will find the information provided here useful in gaining a better understanding of particular morphologic features and overcoming differential diagnosis difficulties associated with pathology reporting of inflammatory lesions of the breast.

Keywords: *Screening, Cytological, Breast fluid, Inflammatory cells, Antimicrobial, Lactating, Women, Sudan.*

1. INTRODUCTION

From menarche to menopause, the breast is an organ that is influenced by steroid hormones. Numerous hormone levels fluctuate during pregnancy and lactation. Increased ductal and vascular proliferation is caused by elevated estrogen levels. Progesterone affects the proliferation of acinaria. The breast's fibro-lipoma substrate diminishes as ductal-lobular units grow **[1].** Human milk is a dynamic physiological fluid that contains not only the necessary nutrients for the optimal growth of the infant but also a lot of different kinds of live cells. The role of human milk in the overall development of the neonate is established beyond doubt. The short-term as well as long-term benefits of human milk are already proven. However, there are very few studies on the cytological evaluation of human milk our original study has documented the presence of the multi-potent mesenchymal stem cells in the human milk **[2].** Apart from these cells; breast milk is thought to harbor epithelial cells and immune cells. Human colostrum contains a significant number of immune cells consistent with the higher immunological needs of the neonate in the early postpartum period. However, within the first two weeks after birth, their number is decreased to 0-2% of the total cells, which is maintained throughout the lactation. The present study is an attempt to study the cytology pattern of human milk in the first week of lactation. The importance of the present study is for the clinical correlation of the significance of the colostrums and their physiological role in the development of the neonate with the background of its cytological composition **[3].** Mastitis variety of inflammatory and reactive changes that can be seen in the breast. While some of these changes are a result of infectious agents. Acute mastitis usually occurs during the first three months postpartum as a result of breastfeeding **[4].** Granulomatous mastitis granulomatous reactions result from an infectious etiology, foreign material, or systemic autoimmune diseases **[5].** Foreign body reactions such as silicone and paraffin **[6].** Recurring subareolar abscess (Zuska’s disease) is a rare bacterial infection of the breast that is characterized by a triad of draining cutaneous fistula from the subareolar tissue **[7].** Mammary duct ectasia, also called periductal mastitis is a

distinctive clinical entity that can mimic invasive carcinoma clinically **[8].** Fat necrosis of the breast is a benign nonsuppurative inflammatory process of adipose tissue **[9,10].** Papanicolaou stain (PAP stain) is multi multi-chromatic (multicolor) cytological staining technique developed by George Papanicolaou in 194 **[11-13].** The Papanicolaou stain is one of the most widely used stains in cytology (10) pap stain is not only used to detect cervical cancer, but it is also used to stain nongynecological specimen preparations from a variety of bodily secretions and small needle biopsies of organs and tissue **[14,15].** Papanicolaou published three formulations of stain in 1942, 1954, and 1960 **[12].** Papanicolaou stain, which has become the most popular stain for gynecological cytology, Papanicolaou stain provides a good differential stain and as a result, is used widely for other routine cytological smear **[15].** One of the most common disturbances noticed during the lactation period in females is mastitis, which is described as an inflammation of the mammary glands accompanied by milk stasis in glandular tissue. Lactating mastitis can cause fatigue which makes it difficult to care for the infant sometimes mastitis causes a mother to wean her baby before she intends to. Milk cytology comes to the aid of diagnostic protocol.

2. METHODOLOGY

This hospital-based analytical cross-sectional study was conducted in Shendi City, River Nile State, Sudan. From January 2023 to June 2024. One hundred Sudanese women samples were collected from lactated women, and then the collected samples were transferred to the histopathology and cytology lab at Shendi University, where they were processed and examined.

2.1 Sample collection and processing

Milk fluid was obtained with a Sartorius aspirator, as previously described. After cleansing the nipple with isopropanol to unclog ducts, the aspirator was placed over the nipple, and negative pressure (90 mmHg) was applied for 45 seconds. Before and during the procedure, the subject gently massaged her breast from the periphery toward the center of the breast. Aspiration was attempted on both breasts. If fluid appeared, a direct smear was made on a microscope slide, wet-fixed in 95% ethanol, and stained by the standard Papanicolaou **[16].**

2.2 Papanicolau staining technique

Each fixed smear was rehydrated in 90%, 70%, and distilled water for 2 minutes in each. After rehydration, the slide will be stained with Harris's hematoxylin for 5 minutes. Then

the smear will be differentiated in 1% acid alcohol, will be blued in a running tap for rinse, then the smear will be rinsed in 70% and 95%, then the smear will be stained in orange G6 for 2 min, then the smear will be washed in 95% ethanol, eosin azure 50 stain will be applied for 3 minutes, then the slide will be dehydrated in absolute ethanol, cleared in xylene, and mounted in Dixterene A plasticizer and xylene. The smear will then be screened under a light microscope by the researchers and confirmed by well-trained cytologists independently **[16].**

2.3 Data Analysis

After examination of the sections, the results of the laboratory investigation as well as the demographic data from the patient's records were processed using the Statistical Packages for Social Sciences (SPSS) computer program. Frequency, mean, and chi-square test values were calculated at <0.05 and considered statistically significant.

3. RESULTS AND DISCUSSION

One hundred cytological smear samples obtained from lactated women were prepared and stained by Papanicolaou stain the results are as follows:

Table 1. The frequency of cytological diagnosis among the study group.

Diagnosis	Frequency	Percentage %
Normal	82	82%
Acute mastitis	16	16%
HPV	2	2%
Total	**100**	**100%**

Table 2. The correlation between cytological diagnosis and type of fluid among study groups.

Type of fluid	Cytology diagnosis		Total
	Normal	Abnormal	
Milk	44	6	50
Postpartum	38	12	50
Total	**82**	**18**	**100**

P. value =0.20

Table 3. The correlation between cell secretion and type of breast fluid among the study group.

Type of fluid	Cells secretion		Total
	Yes	No	
Milk	24	26	50
Postpartum	50	0	50
Total	**74**	**26**	**100**

P. value = 0.000

Table 4. The Frequency of cytological diagnosis of HPV among the study group.

HPV	Frequency	Percentage %
Not exist	98	98%
Exist	2	2%
Total	**100**	**100%**

Table 5. The distribution of age group among the study group.

Age group	Frequency	Percentage %
Less than 35	81	81%
More to 35	19	19%
Total	**100**	**100%**

Table 6. The correlation between cytological diagnosis and age group among the study group.

Age group	Diagnosis		Total
	Normal	Abnormal	
Less than 35	68(83.9)%	13(16)%	81
More than35	14(73.5)%	5(26.3)%	19
Total	**82**	**18**	**100**

P. value = 0.064

Table 7. The Frequency of women's intake of contraceptive drugs in the study group.

Contraceptive intake	Frequency	Percentage %
Yes	45	45%
No	55	55%
Total	**100**	**100%**

Table 8. The correlation between risk factors and diagnosis among the study group.

Risk factors	Diagnosis		Total
	Not exist	Exist	
Normal	2	80	82
Abnormal	3	15	18
Total	**5**	**95**	**100**

P value = 0.003

Table 9. The Show correlation between type of fluid and immune cell.

Type of Fluid	Immuno cells		Total
	Yes	No	
Milk	8	42	50
Postpartum	50	0	50
Total	**58**	**42**	**100**

P value = 0.000

In Sudan, breast cancer is a common disease, and because early screening programs are lacking and awareness of the condition is low, the majority of patients are discovered when the disease is advanced. A large proportion of breast cancers start in the milk duct epithelium. The majority of breast cancers are thought to start as slow-growing precancerous cells, which exhibit observable nuclear and cellular abnormalities under a microscope **[17].** In our study, one hundred samples from lactated women and breast fluid collected were fifty milk smears and fifty postpartum milk. All smears were fixed in 95% ethyl alcohol and then stained using the Papanicolaou staining protocol. After cytology screening of milk, fifty-two percent of women were noncell secretors and forty-eight percent were cell secretors. Normal cells appeared among the secreting group, with few epithelial cells and immune cells. In the postpartum milk smear, all of the women were cell secretors; thirty percent of them had high secretion of immune cells, which was observed in 100 percent, which was found to be statistically significant (0.000). All cytological findings in milk among lactated women in this study were in agreement with Satish and his colleague in 2014, who studied the cytology pattern of human milk in the first week of lactation. They described normal cells found in human milk, and they thought that milk harbors epithelial cells and immune cells. The immune cells in human milk consist of macrophages (large lipid-laden macrophages), neutrophils, and lymphocytes, of which the majority are T cells **[2].** Also, this study's results go with what was stated by Hassiotou and

his colleagues who have also observed that 70% of the total human milk cells in the first two postpartum weeks consist of immune cells **[2].** In this study of human milk, twelve percent of women suffered from acute mastitis, and in postpartum, twenty percent suffered from acute mastitis, which found a statistically significant *value* of 0.02. This study agreed with VasiuI in the 2018 study of the prevalence of mastitis in female lactating dogs by interpreting the milk cytology in the diagnostic protocol. Milk samples from 89 nursing bitches, ranging in age from 10 to 168 months, were physically collected and placed in sterile vials. Using the squash and May-Grünwald-Giemsa (MGG) procedures, milk smears were produced. All forms of inflammation included foamy cells associated with mastitis. Neutrophils that had swallowed bacteria were less common in subclinical cases of mastitis. The type of mastitis that develops is greatly influenced by the lactation phase. Our findings indicate that the breastfeeding phase affects several forms of inflammations of the mammary glands **[18].** Human papillomavirus (HPV) infections are common and associated with a wide spectrum of benign mucosal and cutaneous lesions, cancer precursors, and cancer. In 2/50 (4%), breast milk samples in this investigation showed cytomorphological changes (koilocytes) indicative of HPV infection. The HPV prevalence in breast milk was reported to be relatively low. HPV prevalence in breast milk was reported to be relatively low, 1/70 (1.4%). According to a study from Italy, Karolina and her colleagues in 2017 stated that HPV in breast milk is prevalent among lactating mothers, and HPV can also persist in breast milk **[19].**

4. CONCLUSION

Our study demonstrated cytology of the breast is a simple, safe, rapid test that is acceptable to patients and shows the ability to detect inflammatory cells and immune cells from asymptomatic volunteers. Nonetheless, in the appropriate clinical and radiologic context, subspecialized breast pathologists and anatomic pathologists practicing breast pathology have a unique opportunity to play a critical role in identifying key histologic features suggestive of specific entities and in directing the most effective and timely clinical management.

CONSENT

The patient’s written consent has been collected.

ETHICAL APPROVAL

The study was approved by the Department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area of study. The aims and the benefits of this study were explained with the assurance of confidentiality.

Disclaimer (Artificial intelligence)

As a result, the Author (s) declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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