Original Research Article

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

ABSTRACT

Background: Mastitis is one of the most frequent issues observed in female lactating patients, and it is mammary gland inflammation, which is characterized by milk stasis in glandular tissue. The primary risk factor for bitches suffering from mastitis is an elevated percentage of mortality in the offspring. Breast milk screening at an early stage can help identify inflammatory cells in mothers early on. All healthcare practitioners gain knowledge and awareness from the findings. ***Aim***: To cytologically screen inflammatory cells in breast milk. ***Methods***: This cross-sectional study was conducted in Shendi town using one hundred samples of women's breast milk. (50 milk smears, 50 postpartum milk) have been collected and screened cytologically. Stained by pap stain. ***Results***: Following cytology screening of breast milk, 26/50 (52%) of the women were found to be noncell secretors and 24/50 (48%) to be cell secretors. Among the cells that appeared in the secreting group were a few epithelial cells and immune cells. In postpartum milk, all 50/50 (100%) of the women were found to be cell secretors, with the high secretion of immune cells 50/50 (100%), The study group's cell secretion and breast fluid type have a significant correlation (*P. value* = 0.000), but the study group's age group and cytological diagnosis have an insignificant correlation (*P. value* = 0.064), the study group's risk factors and diagnosis have a significant correlation (*P. value* = 0.003), and the study group's fluid type and immune cell have a significant correlation (*P. value* = 0.000). ***Conclusion***: In the present study, salient findings 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, Pap stain.*

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-fatty 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].** Acute mastitis typically results from a bacterial infection and is most frequently observed during the postpartum phase. An abscess may develop when bacteria enter the breast through a nursing woman's nipple's tiny erosions. Acute mastitis may lead to chronic mastitis, or more frequently, duct ectasia is linked to chronic mastitis. The pathogenesis of chronic mastitis is unknown, however, it causes

the dilatation of large and intermediate-sized ducts along with an inflammatory infiltration of plasma cells and lymphocytes around them. Some patients develop a palpable lump that mimics cancer. Granulomatous mastitis has the typical cytologic picture of granulomas and can be infectious (i.e., tubercular or fungal) in origin. Plasma cell mastitis is a variant of chronic mastitis and is denoted by an infiltrate primarily composed of plasma cells **[5]**. "Granulomatous mastitis granulomatous reactions result from an infectious etiology, foreign material, or systemic autoimmune diseases" **[6].** "Foreign body reactions such as silicone and paraffin" **[7].** "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" **[8].** "Mammary duct ectasia, also called periductal mastitis is a distinctive clinical entity that can mimic invasive carcinoma clinically" **[9].** "Fat necrosis of the breast is a benign nonsuppurative inflammatory process of adipose tissue" **[10,11].** "Papanicolaou stain (PAP stain) is multi multi-chromatic (multicolor) cytological staining technique developed by George Papanicolaou in 194" **[12-14].** "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 is also used to stain nongynecological specimen preparations from a variety of bodily secretions and small needle biopsies of organs and tissue" **[15,16].** "Papanicolaou published three formulations of stain in 1942, 1954, and 1960" **[13].** "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" **[16].** 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 study was a cross-sectional descriptive study carried out in Shendi City, River Nile State, Sudan. From January 2023 to June 2024. To detect cytomorphological patterns, 100 lactating Sudanese women provided 50 postpartum milk samples (from the first 2–5 days after the baby is born), and 50 milk samples were obtained randomly from either

breast by manual expression, and breast milk was immediately aliquoted (after about 14 days after birth until you are done producing milk). The milk samples were then sent to Shendi University in the Department of Histopathology and Cytology, where they were processed and examined. The study population included breast-surgery patients and asymptomatic lactating women; pregnant, non-lactating, and symptomatic women were excluded. I have not used any medications to induce lactation. A questionnaire sheet was utilized to document the sample data and all participants. All of the data will then be compiled into a master sheet. And use SPSS for analysis.

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 **[17].**

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 **[17].**

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

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%**

A total of 100 study group samples were examined; 82 (82%) had a normal cytological diagnosis, 16 (16%) had acute mastitis, and 2 (2%) had HPV.

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

For 50 milk samples (44 with normal cytology diagnoses and 6 with abnormal cytology diagnoses) and 50 postpartum milk samples (38 with normal cytology diagnoses and 12 with abnormal cytology diagnoses), there is no significant correlation between the type of fluid and the cytological diagnosis (*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

Between the study groups, there is a significant correlation between cell secretion and the type of breast fluid (*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%**

There are 98 (98%) of the samples are HPV-Negative and 2 (2%) positive HPV.

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%**

In the age group, 19 (19%) were aged more than 35 years, and 81 (81%) were aged less than 35 years.

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

Among the research group, there is no correlation between age group and cytological diagnosis. *P value* = 0.064. Within the age category of under 35 years, 68 (83.9%) have a normal cytological diagnosis, whereas 13 (16%) have an abnormal one. Additionally, of the age group over 35, 5 (26.3%) have an aberrant cytological diagnosis and 14 (73.5%) have a normal one.

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%**

There are 45 women (45%) take contraceptive pills, and 55 women (55%) do not take contraceptive pills.

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

Among the study group, there is a significant association between risk variables and

diagnosis. *The P value* is 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

There are significant correlation between the type of fluid and immune cell *P. value* = 0.000. Only eight milk samples included immune cells; however, postpartum milk samples contained immune cells in every sample that was obtained.

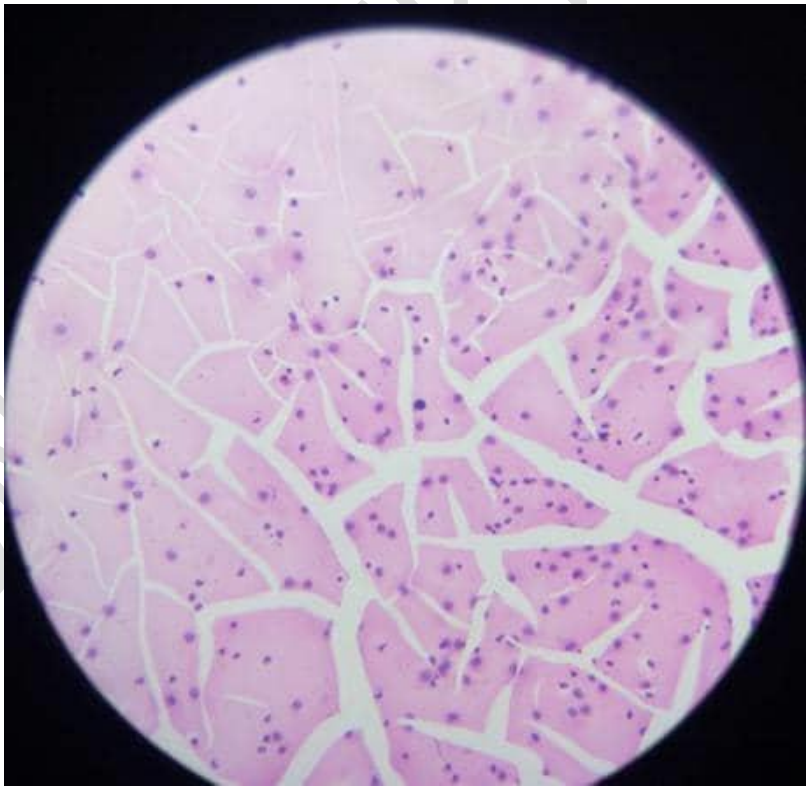

Figure 1. Inflammatory cells in postpartum milk samples

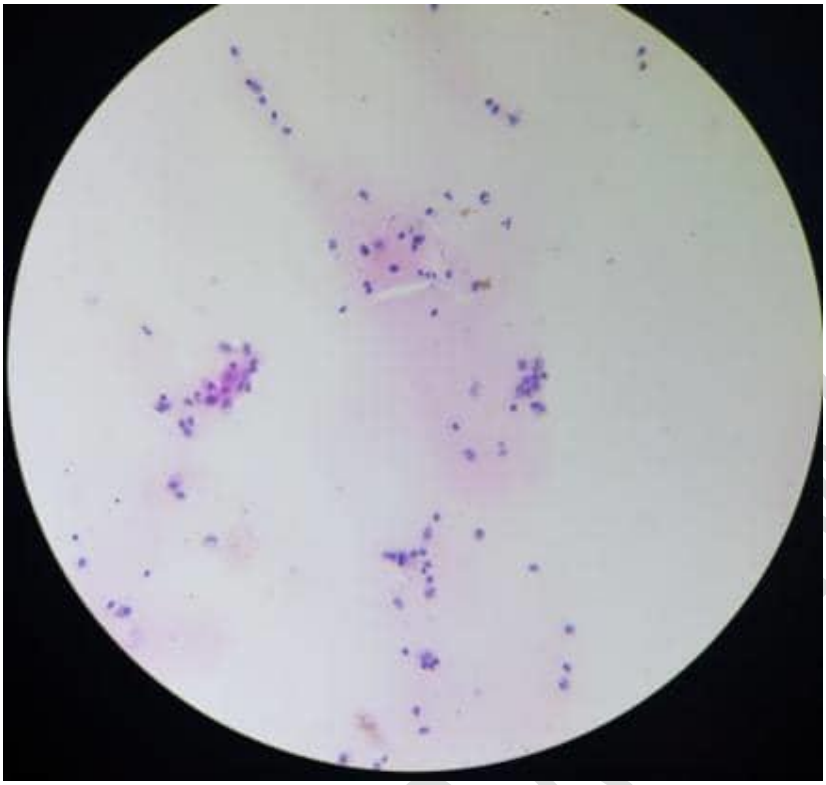

Figure 2. HPV in breast milk samples.

4. DISCUSSION

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 **[18].** 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. Large epidemiological studies suggest that clinically apparent mastitis occurs in about 20 to 33% of lactating women at some time of 20% during the first 6 months after delivery **[21].** A study of American women suggested that about one-third during lactation, mostly within the first 2 months after delivery. A prospective cohort study of 1,075 women in Australia found a crude incidence rate of mastitis in women who breastfeed develop mastitis **[23].** In Finland, 24% of 664 breastfeeding women developed mastitis **[20].** Another prospective cohort study of 306 breastfeeding mothers showed that 27% developed mastitis within 3 months of delivery

[19]. Earlier studies may have underestimated the incidence of mastitis, as these investigations relied upon the presentation of women with mastitis at hospitals and many women seek attention from midwives and general practitioners outside the hospital setting **[21,23].** The incidence of mastitis is highest within the first 2 months after delivery **[19-22].** 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].** The presence of HPV has been found in breast milk in 2.5–28.8% of the samples **[24–27].** However, Mammas *et al.* (2011) were not able to detect any high-risk HPV types (HR-HPV, including HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) in breast milk samples **[24].** In this study, HPV was detected in 2% of the studied breast milk samples, implicating the possible role of breastfeeding in the mother-to-infant transmission of HPV. The presence of HPV in breast milk can potentially increase the anxiety of mothers with HPV-related cervical neoplasia, discouraging them from breastfeeding their babies. The presence of HPV in breast milk and its possible clinical relevance regarding HPV transmission in children need to be clarified. Our finding was relevant to the results of the study by Sarkola *et al.* **[28].** Who showed no connection between HPV infection in breast milk and mother cervical cancer. To date, vertical mother-to-infant transmission of HPV has been proposed by various studies **[29].** It is yet unknown, though, how breastfeeding affects HPV transmission. A search of the literature does not appear to provide any primary evidence to support the involvement of breastfeeding in HPV transmission. At the moment, no evidence exists to support the avoidance of breastfeeding due to possible mother-to-infant transmission of HPV via breast milk.

5. CONCLUSION

In the present study, salient findings show that 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 in 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.

6. Recommendations:

1. Compiling cohort studies and multi-center prospective observational clinical trials with asymptomatic women to assess breast milk output and identify mastitis and HPV in epithelial cells.
2. Additional research testing: the source of the cells, genetics, chemical makeup, biomarkers, and milk infections.
3. A standard protocol for following patients with sub-clinical.

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.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests, non-financial interests, or personal relationships that could have appeared to influence the work reported in this paper.

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