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Original Research Article

Cytological Screening of Breast Cancer Using Breast Fluids among Women in Shendi Town at River Nile State, Sudan

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Abstract: ***Background*:** Breast cancer is common in Sudan and most patients are detected at later stages of the disease due to the lack of awareness and absence of early screening programs. The majority of breast cancers originate in the epithelium lining the milk ducts. It is believed that most breast canceras are slow growing and progress from precancerous cells, which have cellular and nuclear changes that can be identified microscopically. ***Aim*:** To cytological screening of breast cancer in breast fluid. ***Methods*:** This is a cross-sectional feasible study conducted in Shendi town, 200 samples of different breast fluids from asymptomatic women. (100 nipple aspiration fluid, 50 milk smear, 50 postpartum milk) have been collected and screened cytologically. Stained by pap stain. ***Results*:** After cytology screening of breast fluid in, milk 30/50(60%) of women were non-cell secretors and 20/50 (40%) 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, abnormal cytomorphological changes were in 15/50 (30%) of them, and high secretion of immune cells 50/50 (100%) which found to statistically of significant value (0.000). NAF was not produced in 78/100. Among women who produced NAF22/100, women were produced NAF 6/22 (27.3%) were not cell secretors (category 0), benign nonhyperplasic ductal epithelial cells (category 1) 6/22(27.3%), benign hyperplasic ductal epithelial cells (Category II) 5/22(22.7%), atypical ductal epithelial cell (category III) 3/22 (13.7%) atypia (Category IV) 2/22(9%). cytological atypia 21/25 (84%) appears among women more than 30 years old and there was a strong statistical of significant value *P. value* =(0.000). Also, risk factors (family history, contraceptive intake, and HPV) statistically have a significant correlation with cytological atypia among study group *P. value* = (0.036). In this study HPV infection, cytomorphological change (koilocyte) was detected in breast fluid 4/200 (2%). ***Conclusion*:** cytology of the breast is a simple, safe, rapid test that is acceptable to patients and showed the ability to detect benign and pre-neoplastic ductal epithelial cells from asymptomatic volunteers.

Keywords: Breast Cancer, Breast Fluid, Screening, cytological, Shendi, Sudan.

INTRODUCTION

Breast cancer is caused by the development of malignant cells in the breast. The malignant cells originate in the lining of the milk glands or ducts of the breast (ductal epithelium). Cancer cells are characterized by uncontrolled division leading to abnormal growth and the ability of these cells to invade normal tissue locally or to spread throughout the body, in a Process called metastasis [1]. Breast cancer is among the most common causes of cancer deaths today, coming fifth after lung, stomach, liver, and colon cancers. It is the most common cause of cancer death in women [2]. Breast cancer mortality is high in Sudan and most patients are detected at later stages of the disease due to the lack of awareness and absence of screening programs [3]. More than 90%of breast cancer-related mortalities are caused not by the primary tumor, but by its metastases at distant sites [4]. The diagnosis of breast cancer is accomplished by the biopsy of any suspicious lump or mammographic

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abnormality that has been identified [1]. Surgery, radiation, and chemotherapy are all used in the treatment of breast cancer [5]. A common technique used for the diagnosis of breast cancer is a physical examination of the breast; mammography, fine needle aspiration, and paraffin-embedded block contain tissue obtained by surgical procedure from tumor site using histopathology and immune histochemical stain to detect estrogen, progesterone receptors, and HER2/ Neu [6,7]. Breast fluids are usually produced in connection with childbearing, but occasional spontaneous fluid production in non-pregnant, non-lactating women has been recognized for centuries. It was thought to be abnormal and was labeled in textbooks as 'galactorrhea' [8-10]. Papanicolaou described the cells contained within these breast fluids and how they could be used diagnostically to identify breast neoplasms [11, 12]. Nipple fluid or secretions, usually aspirated from the breast ducts, is a protein-rich material termed nipple aspirate fluid (NAF) which can be microscopically examined for the presence of atypical ductal epithelial cells. Nipple fluid can be obtained from many women, with reports of NAF production ranging from 25% to more than 95% of women [13, 14]. There are a variety of factors associated with the ability to produce nipple fluid, particularly intrinsic breast characteristics [15]. Nipple fluid acquisition methods are various, including manual breast compression, either followed by a manual breast pump or a syringe-type device with suction, sometimes repeated up to 10 minutes on each breast. Nipple aspirate fluid may better detect breast cancer earlier than current methods used for screening such as mammograms and breast examinations [16]. Papanicolaou stain (PAP stain) is a multi-chromatic (multicolor) cytological staining technique developed by George Papanicolaou in 1942 [17-19]. The Papanicolaou stain is one of the most widely used stains in cytology pap stain is not used to detect cervical cancer, it is also used to stain nongynecological specimen preparations from a variety of bodily secretions and small needle biopsies of organs and tissue [20, 21]. Papanicolaou published three formulations of stain in 1942, 1954, and 1960 [18]. 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 smears [21].

MATERIALS AND METHODS

Study Design

This was a descriptive cross-sectional study.

Study Sample

Breast fluid smear sample was taken from each participant to detect a cytomorphological pattern.

Study Area

This study was conducted in Shendi. Samples will be collected from breast fluid then the collected samples were transferred to Histopathology and Cytology lab at Shendi University where they were processed and examined.

Study Duration

This study was performed during the period from July to September 2021.

Study Populations

The study population included only asymptomatic, non-pregnant, non-lactating women, and lactating women with no history of breast cancer, and breast surgery.

Sample Size

Two hundred samples were taken from participants.

Data Collection Tool

A questionnaire sheet was used to record all participants and sample data. After that, all data will be gathered in a master sheet and analyzed by using SPSS.

Samples Collection and Processing

Nipple aspirate 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 s. 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].

Papanicolau Staining Technique

Each fixed smear was rehydrated in 90% and 70% and distilled water for 2 minutes in each. After rehydration slide will be stained in Harris's hematoxylin for 5 min Then the smear will be differentiated in 1% acid alcohol, then 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 3Minutes, then the slide will be dehydrated in absolute ethanol, cleared in xylene and mounted in Dixterene A plasticizer and Xylene. The smear will be then screened under a light microscope by the researchers and confirmed by well-trained cytologists independently [16].

Interpretation of Results

Benign Duct epithelial cells within (normal limits. Foam cells. Apocrine Metaplastic cells). Hyperplasia changes (including slight cell and nuclear enlargement. Chromatin remains finely granular and evenly distributed. Small and regular nucleoli are sometimes present. Cell distribution predominately in groups and cohesive) Moderate to typical hyperplasia

severe abnormalities with (distinct nuclear enlargement, increasing nuclear to cytoplasmic ratio, irregular nuclear borders, and nuclear variation. Coarsely granule chromatin. Prominent chromocenters and hyperchromasia. Cell distribution in groups with some papillary formations. Increased numbers of single atypical cells) [16].

Ethical Consideration

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

RESULTS

Two hundred cytological smear sample obtained from lactated and non-lactated women were prepared and stained by Papanicolaou stain the result shown as follow:

Table 1: Show Frequency of cytological diagnosis among study group

Diagnosis	Frequency	Percentage (%)
Normal cell	175	88%
Abnormal cell	25	12%
Total	**200**	**100%**

Table 2: Show correlation between cytological diagnosis and type of fluid group among study group

Type of fluid	Cytology diagnosis		Total
	Normal	Abnormal	
NAF	92	8	100
Milk	48	2	50
Postpartum	35	15	50
Total	**175**	**25**	**200**

P. value= (0.000)

Table 3: Show of frequency cytology finding of women secreted NAF among study group

Cytology finding	Frequency	Percentage (%)
Non-cellular cell Categories (0)	6	27.3%
Benign ductal non-hyperplasic cell Categories (1)	6	27.3%
Benign ductal hyperplasic cell Categories(11)	5	22.7%
atypical ductal epithelial cells Categories(111)	3	13.7%
atypia Categories(1V)	2	9%
Total	**22**	**100%**

Table 4: Show correlation between cell secretion and type of breast fluid among study group

Type of fluid	cells secretion		Total
	Yes	No	
NAF	16	84	100
Milk	20	30	50
Postpartum	50	0	50
Total	**86**	**114**	**200**

P. value= (0.000)

Table 5: Show Frequency of cytological change of HPV among study group

HPV	Frequency	Percentage (%)
Not exist	196	98%
Exist	4	2%
Total	**200**	**100%**

Table 6: Show distribution of age group among study group

Age group	Frequency	Percentage (%)
Less than 30	115	58%
More to 30	85	42%
Total	**200**	**100%**

Table 7: Show correlation between cytological diagnosis and age group among study group

Age group	Diagnosis		Total
	Normal	Abnormal	
less than 30	111(96.5)%	4(3.5)%	115
More than30	64(75.2)%	21(24.7)%	85
Total	**175**	**25**	**200 (100%)**

P = (0.000)

Table 8: Show frequency of women she has family history of breast cancer among study group

Family history	Frequency	Percentage (%)
Yes	6	3%
No	194	97%
Total	**200**	**100%**

Table 9: Show frequency of women intake contraceptive drug among study group.

Contraceptive intake	Frequency	Percentage (%)
Yes	54	27%
No	146	73%
Total	**200**	**100%**

Table 10: Show correlation between risk factors (family history, contraceptive and HPV) and diagnosis among study group

Risk factors		Diagnosis		Total
		Not exist	Exist	
	Normal	127	48	175
	Abnormal	13	12	25
Total		**140**	**60**	**200**

P. value = (0.036)

Table 11: Show correlation between type of fluid and immune cells

		Immune cells		Total
		Yes	No	
Type of fluid	NAF	6	94	100
	Milk	3	47	50
	Postpartum	50	0	50
Total		**59**	**141**	**200**

P. value =0.00

DISCUSSION

Breast cancer is common in Sudan and most patients are detected at later stages of the disease due to the lack of awareness and absence of screening programs. The majority of breast cancers originate in the epithelium lining the milk ducts. It is believed that most breast cancers are slow growing and progress from precancerous cells, which have cellular and nuclear changes that can be identified microscopically [6]. Two hundred Healthy women were successfully enrolled, (100) were lactated women, and (100) non-located women. Breast fluid collected were milk smears (50), postpartum milk (50) smears those samples from lactated women, and NAF from non-lactated women (100) smears. All smears were fixed in 95% ethyl alcohol and then stained using the Papanicolaou staining protocol. After cytology screening of milk, sixty percent (30/50) of women were non-cell secretors and forty percent (20/50) of women were cell secretors. Normal cells appears among the secreting group were few epithelial cells and immune cells, abnormal cells appeared in this group were epithelial cells with HPV cytomorphological changes of four percent (2/50), and the final cytological description of this group was normal cytomorphology. In postpartum milk smear, all women hundred percent (50/5) were cell secretors with abnormal cytomorphological changes thirty percent (15/50) of them and high secretion of immune cells was observed hundred percent (50/50) which found statistically significant value (0.000). All cytological findings in milk among lactate women in this study were in agreement with Satish and his colleges who study the cytology pattern of human milk in the first week of lactation. They describe normal cells found in human milk and they thought that milk harbors epithelial cells and immune cells. The immune cells in the human milk consist of macrophages (large lipid-laden macrophages), neutrophils, and lymphocytes of which the majority are T cells, also this study's results go with what stated with Hassiotou *et al.*, have also

observed that 70% of the total human milk cells in the first two postpartum weeks consist of immune cells [22]. In this study NAF was produced twenty- two percent (22/100) this result is different from what was reported by the proctor at that. Thirty-eight percent (190/500) of healthy women produce NAF, Among them (27.3%) were not cell secretors (category 0), benign non-hyperplasic ductal epithelial cells (category1) (27.3%), benign hyperplasic ductal epithelial cells (Category II) (22.7%), atypical ductal epithelial cell (category III)(13.7%) atypia (Category IV) (9%)**.** All results are varied from the study done by Proctor *et al.*, (2005), in which 500 healthy women were successfully enrolled. Thirty-eight percent (190/500) produced fluid and 187 were available for cytologic analysis. Cytologic classification of fluid producers showed 50% (93/187) Category (insufficient cellular material), 38% (71/187) Category I (benign non-hyperplasic ductal epithelial cells), 10% (18/187) Category II (benign hyperplasic ductal epithelial cells), 3% (5/187) Category III (atypical ductal epithelial cells) and none were Category IV (unequivocal malignancy). Overall, 19% of the subjects produced NAF with adequate cellularity **[6]**. This difference can be explained by the higher number of women, skills, and tools for sample collection in their study. In the current study, women without fluid and who produce normal cells (interpret as No malignant cells found) were eighty-eight percent (175/200)**.** All cytological findings described above in this study agree with what was mentioned by many authors about the possible role of breast fluid cytology in risk stratification and clinical decision-making for women who are at high risk for breast cancer development. The mean age in this study was 28.62 and ranged (from 18-61) years old, women with less than 30 were fifty–eight percent (115/200), and more than 30 years were forty-two percent (85/200). Noticeably, cytological atypia eighty-four percent (21/25) appears among women above 30 years old and there was a strong statistical of significant value P= (0.000)**.** Also, risk factors (family history, contraceptive intake, and HPV) statistically have a significant correlation with cytological atypia among women P= (0.036). Human papillomavirus (HPV) infections are common and associated with a wide spectrum of benign mucosal and cutaneous lesions, cancer precursors, and cancer. In this study HPV infection, cytomorphological change (koilocyte) was detected in breast fluid eight percent (4/50). 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%) Accordingly a study from Italy and Karolina *et al.*, (2017) state that HPV in breast milk is prevalent among lactating mothers and HPV can also persist in breast milk. HPV in this study has no correlation with cellular atypia or type of breast fluid [23].

CONCLUSION

Cellular atypia can be detected in breast fluids among asymptomatic women. Immune cells were exces sively secreted in postpartum milk. HPV can persist in b reast fluid. Cellular atypia in breast fluids increases wit h age development. Family history, contraceptive intake , and HPV infection are risk factors for Cellular atypia o f breast cells among women. Breast cytology is technica lly a feasible method for early detection of breast cancer .

CONFLICT OF INTERESTS

The authors have not declared any conflict of i nterest.

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