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Fine needle aspiration cytology and cell-block study of various breast lumps

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Abstract: Aim of the study was to evaluate the importance of the combined use of fine needle aspiration cytology (FNAC) and cell-block in the diagnosis of different breast lesions. Patient and methods: This study was a prospective study of 310 cases (301 females and 9 males) with breast swelling, coming to cytopathology unit, faculty of medicine, Zagazig University. FNAC and cell-block were performed. Cytological findings of smears and cell-blocks were correlated to histopathological results. Results: Most of cases were diagnosed as benign lesions or normal findings. The majority of FNAC samples was diagnosed as fibrocystic disease (160 cases, 51.6%), followed by fibroadenoma in 54 cases (17.4%). Positive cases for malignancy, including non-Hodgkin lymphoma and false negative cases were encountered in 50 cases (16.1%). Studying the histopathological results showed that true positive cases by FNAC and cell-block were 47 in number (15.16%) and true negative ones were 247 cases (82.90%). False positive cases were 3 in number (0.96%) and false negative ones were also 3 cases (0.96%). Sensitivity was 94% and specificity was 98%. Positive predictive and negative predictive values were 94% and 98%, respectively. False positive and false negative rates were 1.15% and 6% respectively. Total accuracy was 98%. Conclusion: It is advised to perform cell-blocks for each case of breast FNAC to decrease the pitfalls and to improve the diagnosis and management of breast lumps.

Keywords: Breast, Histology, Pathology, FNAC, Cell-Block

1. Introduction

The breast is a specialized accessory organ of skin. It is one of the common sites of cancer in women. It is also a site for different types of benign lesions and may be subject to acute inflammation and abscess formation [1]. Therefore, the clinical personnel must be familiar for the differential diagnosis of these conditions. Misdiagnosis of breast malignancy can lead to inappropriate radical treatment with unnecessary physical and psychological hardship [2]. Triple assessment, consisting of clinical evaluation, mammography or ultrasound and fine-needle aspiration cytology (FNAC), allows a precise initial diagnosis and reduces the risk of such misdiagnosis [3]. FNAC is usually performed on palpable breast masses, especially in cases of suspected malignancy; and positive cytology is considered a sufficient evidence for diagnosis of breast cancer. Such cases can then be treated using mastectomy or breast-conserving surgery without the need for a time-consuming tumor biopsy. However, because there are false-negative cases, attributable to a variety of factors also technical failure, the diagnostic accuracy of FNAC for breast cancer is only around 91% [4]. Several studies have shown that the delay in diagnosis of breast cancer reduces survival [5,6]. Early definitive diagnosis of breast masses is important in FNAC-negative cases. On the other hand, core needle biopsy (CNB) is thought to provide a more exact diagnosis of breast tumors [7], especially in cases of non-palpable breast tumors. However, CNB is not widely used, because it takes more time, often necessitates anesthesia, and requires many staff members who are familiar with certain techniques. As a result, it is sometimes difficult to decide on the next step, that is, whether to perform a CNB, lumpectomy or more extensive surgery [4]. Combining FNAC with core biopsies has been shown to increase the diagnostic accuracy [7]. In this study, we investigate the use

of combining the breast FNAC smear with cell-blocks instead of core needle biopsy (CNB), and whether it gives the advantages of both approaches.

2. Aim of the Work

This work aimed to give a spot light on the importance of FNAC, and cell-block technique in the diagnosis of breast lesions.

3. Patients and Methods

This study enrolled 310 patients (301 females and 9 males) with breast lumps referred to cytopathology unit, faculty of medicine, Zagazig University, in the period between January 2005 and December 2012. The information about the age, sex, ultrasound findings, and mammographic findings were recorded. The technique of FNAC has been described previously [8]. Briefly, aspirates were obtained with a 22 gauge needle and a 10 mL syringe, and the needle was moved in many directions under vacuum. The needle was occasionally guided by ultrasound (US) whenever the tumor was smaller than 2 cm. At least two aspirates were collected each time. The needle was then opened and; smears were prepared from the wide end of the needle and the narrow opening of the plastic portion. Four slides smears were made for each case and immediately fixed in 95% ethyl alcohol for about 30 min. The specimens were stained with Hx&E, Giemsa, and Papanicolaou (PAP) stain. The remnants in the syringe were clotted then fixed in neutral buffered formalin for 12 hours duration, then processed as cell-blocks. Unsatisfactory smears were excluded and repeat FNAC was performed shortly thereafter. The diagnostic cytopathological classifications were: C-I, benign (including: normal budding, inflammatory, purulent, granulomatous lesions, fibroadenoma, duct adenoma, gynecomastia); C-II, probably benign (including: fibrocystic disease); C-III, equivocal (suspicious); C-IV, probably malignant (highly suspicious) and C-V, malignant (7). The results of cytological diagnosis of these patients were compared with the histological findings of the subsequent tissue specimens i.e. open biopsy or mastectomy. The sensitivity, specificity and accuracy of FNAC and cell-block for the diagnosis of malignancy were calculated [9]

4. Results

Lumps that were diagnosed as benign lesions (C-I) included: one case of normal findings, one lactating adenoma, one juvenile fibroadenoma, 54 cases with fibroadenoma. The case that was diagnosed as normal findings, belonged to a girl with 8 years old suspected for a unilateral enlargement of the breast; and the diagnosis was normal breast budding (Figure 1). The case of lactating adenoma was diagnosed by FNAC and cell-block (Figure 2). It was for a pregnant woman with a well-defined mass. Follow-up was performed for 6 months; and the swelling was noticed to be relieved. The case of juvenile fibroadenoma belonged to a patient with age of 12 years and cases of fibroadenoma were in the range from 14-45 years (Figures 3-6). Eight cases were diagnosed as gynecomastia (male patients 17-57 years without peak) (Figure 7). The cases, were diagnosed as inflammatory smears (14 cases, with age range from 23-45 years without peaks), underwent follow-up for 6 months, without harm and no need for biopsy. Cases showing purulent aspirates (12 cases) were diagnosed histopathologically as chronic breast abscess. Lumps diagnosed as granulomatous lesions were two cases. Their biopsy results were one of TB and the other of hydatid cyst. Cases diagnosed as duct adenoma were three in number (Figures 8-10). These cases were diagnosed by nipple discharge FNAC smear; however cell-blocks were not performed because of deficient material taken. There was one case, diagnosed as milk cyst; and biopsy was not needed. Benign fibrous or fibromyomatous tumor (one case) was underwent biopsy which gave the result of granular cell tumor (Figures 11-13). Cases, diagnosed as fibrocystic disease (FCD) (C-II), were 160 females' patients, with age ranging from 17-70 years (Figures 14-16; Tables 1,2). All cases of FCD were followed up for two years without need for biopsy, except for five cases; three of them underwent follow-up for one year and then biopsies were performed and gave the same result (FCD). Two cases underwent follow-up for only six months and became clinically free. Three false negative cases were seen. One of them was diagnosed as FCD with apocrine change and inflammatory changes (Figures 17, 18). This case underwent repeated FNAC after one month and diagnosed as highly suspicious of malignancy. Two cases were diagnosed by FNAC as FCD with epitheliosis and adenosis (Figure 19) and gave a result of comedo carcinoma in biopsy. The other case diagnosed as FCD with atypical cells in FNAC (Figure 20) showed comedo carcinoma by biopsy (Figure 21). Cases diagnosed as suspicious of malignancy (C-III) were 13 in number, with age range from 30-45 years. Three cases of the suspicious of malignancy were false positive; one of them was diagnosed in biopsy as fibroadenoma, the other case was FCD with inflammatory reaction and the last one was FCD (Table 2). The cases diagnosed as highly suspicious of malignancy (C-IV) showed atypical ductal hyperplasia in cell-blocks (Figures 22, 23), and gave carcinoma in situ in biopsy. Thirty-five cases were diagnosed infiltrating ductal carcinoma (IFDC) "C-V" by FNAC and cell-block (Figures 24-26). Their age ranged from 24-75 years. Two cases, one for male and the other for female, were diagnosed by FNAC smear and cell block as non-Hodgkin lymphoma (Figures 27-29). Cell-block mimics the biopsy in good identification and better diagnosis of the breast lesions, especially for cases with insufficient FNAC diagnosis (Table 3). True positive cases were 47 in number (15.16%) and true negative ones were 247 cases (82.90%). False

positive cases were 3 in number (0.96%) and false negative ones were 3 cases (0.96%). Sensitivity was 94% and specificity was 98%. Positive predictive and negative predictive values were 94% and 98%, respectively. False positive and false negative rates were 1.15% and 6% respectively. Total accuracy was 98% (Table 4).

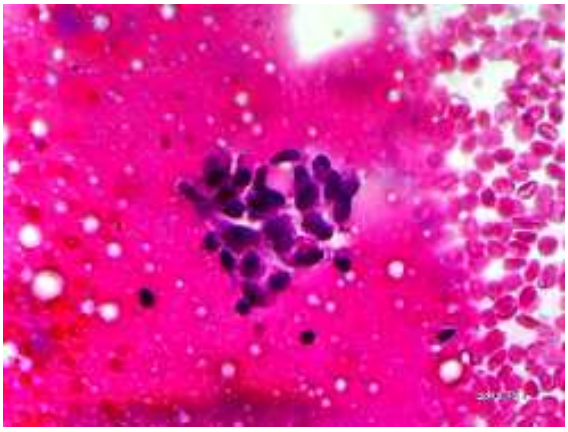

Figure 1. *Normal breast budding, FNAC smear in a young girl showing cohesive cluster of ductal cells, an orientation of an acinus. (Hx&E stain X 400)*

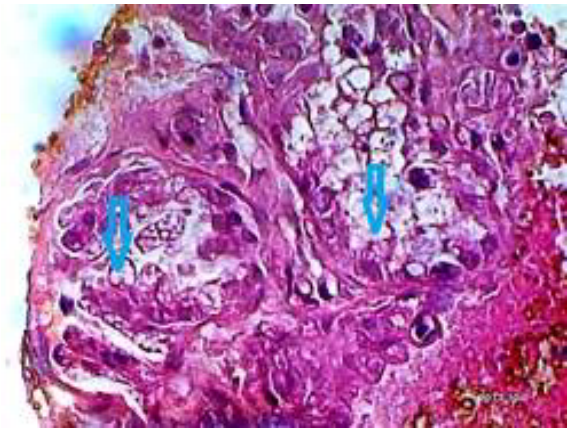

Figure 2. *Lactating adenoma, cell-block showing proliferated acini, lined by actively secreting cuboidal cells (blue arrows). (Hx&E stain X 400)*

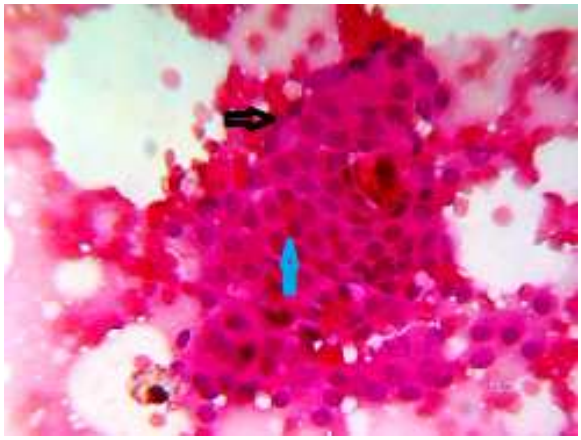

Figure 3. *A case of fibroadenoma, FNAC smear showing cohesive clusters of ductal cells with honey-comb appearance (blue arrow), rimed by myoepithelial cells (black arrow). (Hx&E X 400)*

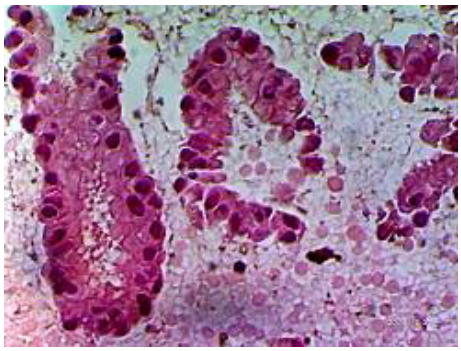

Figure 4. *A case of fibroadenoma, cell-block showing cells lining the breast acini. The cells are monotonous, without atypia. (Hx&E X 400)*

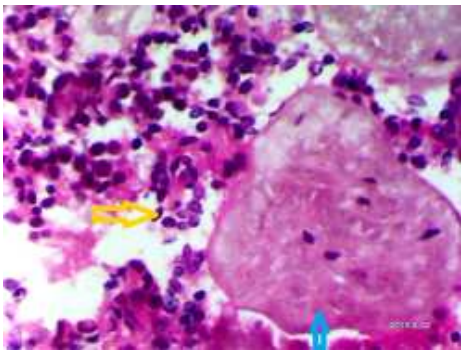

Figure 5. *A case of intra-canalicular fibroadenoma, cell block showing large spherules of fibrous tissue (blue arrow), with scattered ductal cells without atypia. Some of them are trying to form small acini (yellow arrow). (Hx&E X 400)*

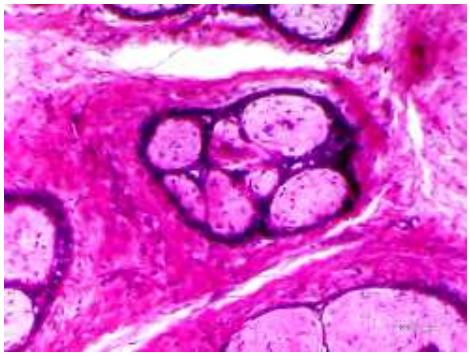

Figure 6. *The same case of intra-canalicular fibroadenoma biopsy. (Hx&E X 400)*

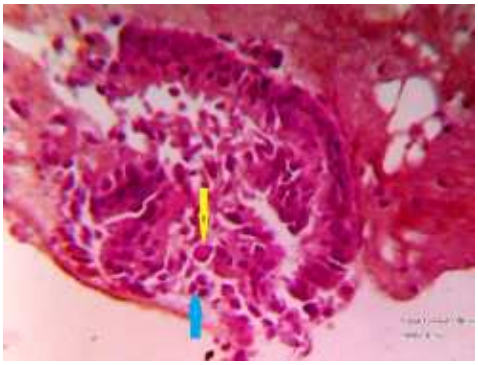

Figure 7. *A case of gynecomastia, cell-block showing a papilla formed by mixture of ductal cells (blue arrow) and myoepithelial cells (yellow arrow) without atypia. (Hx&E X 400)*

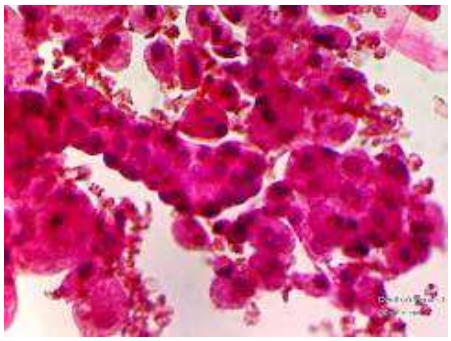

Figure 8. *A case of duct adenoma, nipple discharge smear showing ductal cells. The cells are large monomorphic with abundant esinophilic cytoplasm and central rounded nuclei, homogenously stained. They are arranged in papillae. (Hx&E X 400)*

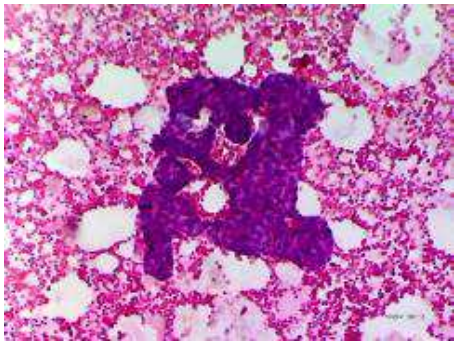

Figure 9. *A case of duct adenoma, FNAC smear showing sheet of cohesive ductal cells with card-wheel pattern. (Hx&E X 100)*

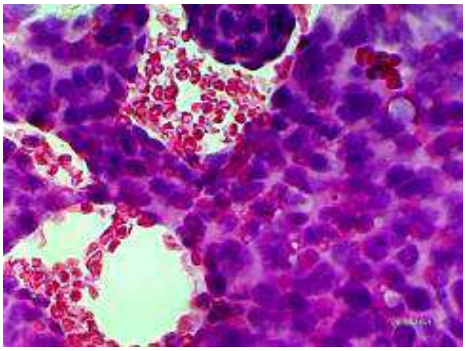

Figure 10. *The same case of duct adenoma, FNAC smear showing the cells, appeared to be hyperchromatic, but uniform, with homogenous stained nuclei. (Hx&E X 400)*

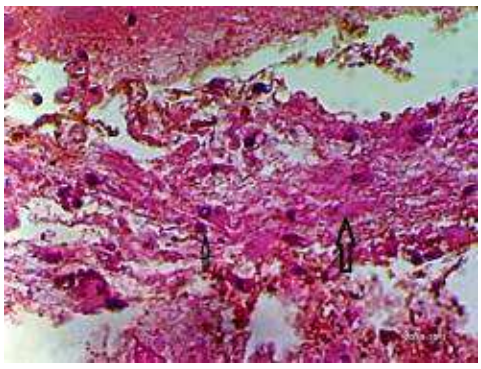

Figure 11. *A case of granular cell tumor, cell-block. The granular cells showed esinophilic granular cytoplasm (thick arrow); the nuclei are rounded without atypia (thin arrow). (Hx&E X 400)*

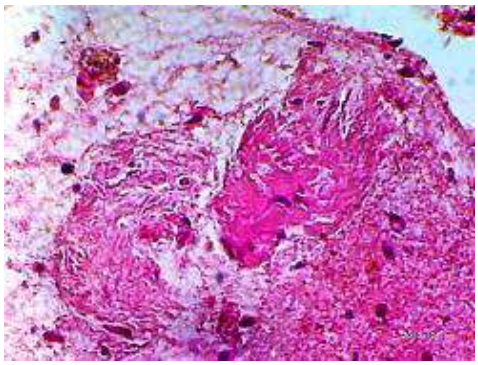

Figure 12. *The same case of granular cell tumor, another view of the cell-block showing spherules of granular cells, with deeply esinophilic cytoplasm. (Hx&E X 400).*

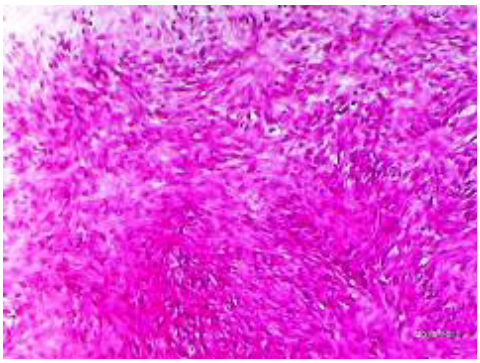

Figure 13. *The same case of granular cell tumor, biopsy. Granular esinophilic cytoplasm of the cells could be seen. Hx&E X 400)*

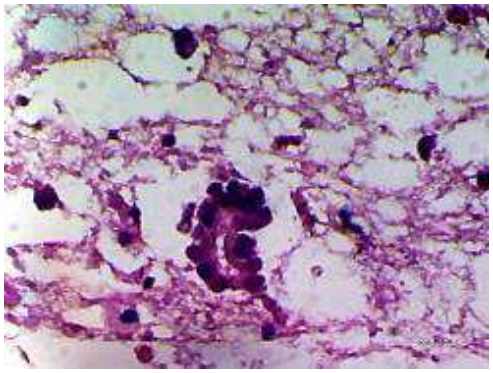

Figure 14. *A case of FCD, cell-block showing a breast acinus, lined by small acinar cells without atypia. (Hx&E X 400)*

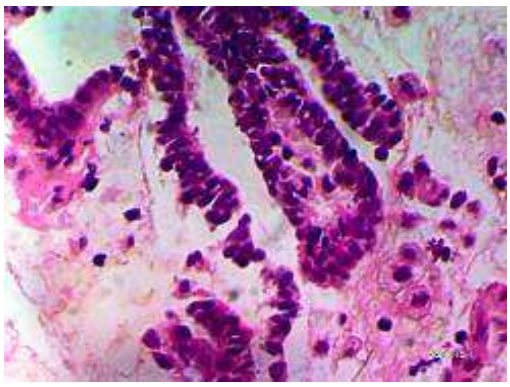

Figure 15*. A case of FCD, cell-block showing a papilla lined by mixture of ductal cells and myoepithelial cells without atypia. (Hx&E X 400)*

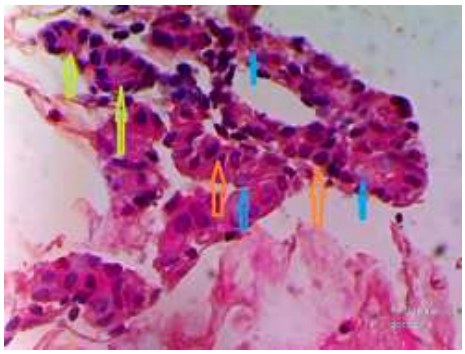

Figure 16. *A case of FCD, cell-block showing typical papillary hyperplasia, mixture of ductal cells, with dark staining (orange arrows) and myoepithelial cells with pale staining (blue arrows). Some small acini with irregular spacing could be seen (green arrows). There`s also, alignment of the cell nuclei axis parallel to the spacing axis. (Hx&E X 400)*

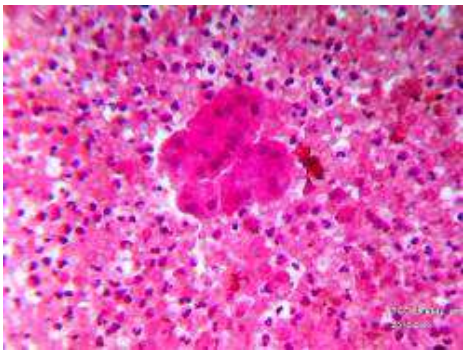

Figure 17. *A case of FCD with apocrine changes, and the inflammatory reaction could be demonstrated. (Hx&E X 400)*

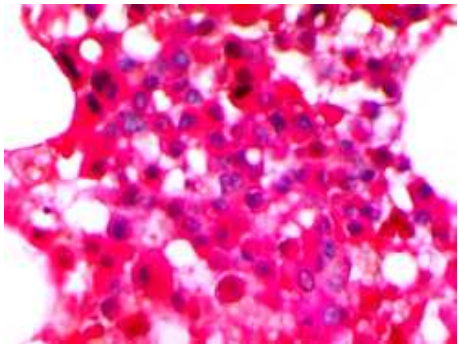

Figure 18. *The same case of FCD with apocrine changes and inflammatory reaction. Some degenerative changes could be seen. (Hx&E X 600)*

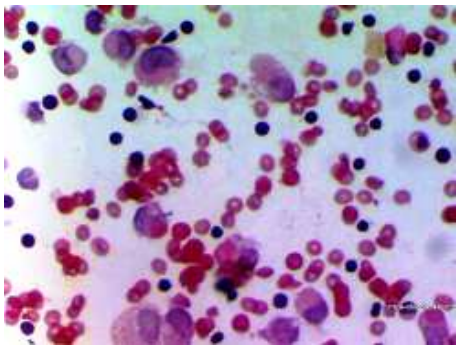

Figure 19*. A case was diagnosed in FNAC as FCD with epitheliosis and adenosis with hyperchromatic, discohesive ductal cells. (Hx&E X 400)*

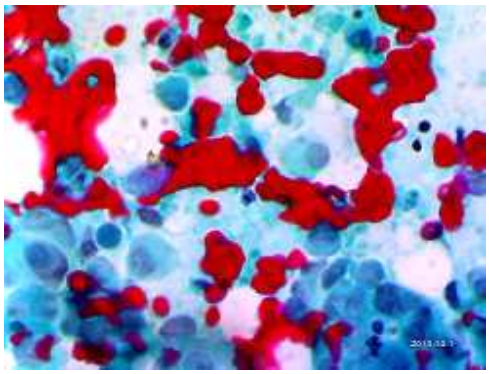

Figure 20. *A case diagnosed in FNAC as FCD with atypical cells showing discohesive ductal cells with hyperchromasia. (PAP stain X 400)*

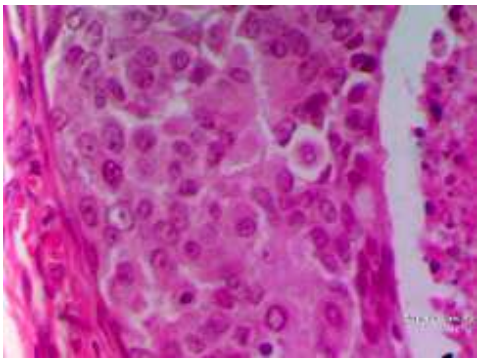

Figure 21. *Biopsy of the previous case showed comedo carcinoma. (Hx&E X 400)*

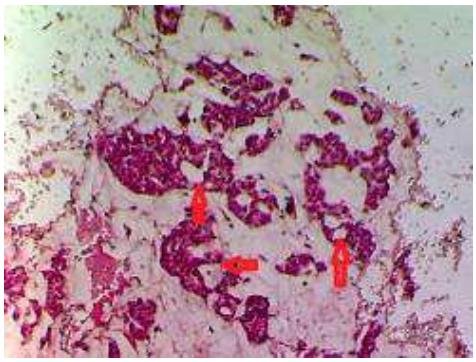

Figure 22. *A case of atypical papillary hyperplasia, cell-block. The lumina are regular, punched out (orange arrows). (Hx&E X 100)*

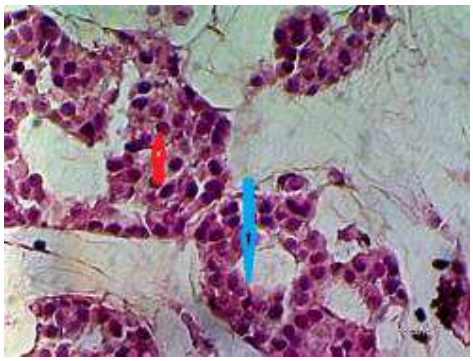

Figure 23. *The same case of atypical papillary hyperplasia, cell-block. The cells are uniform (no myoepithelial cells) (orange arrow). There`s also alignment of the cell nuclei perpendicular to the axis of spacing (blue arrow). (Hx&E X 400)*

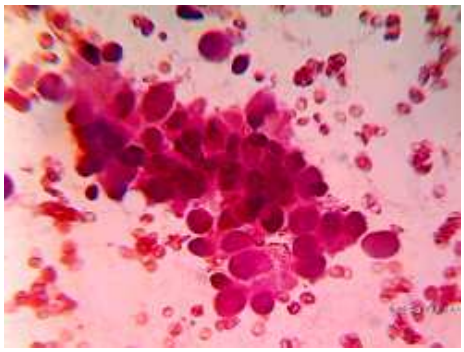

Figure 24. *A case of IFDC, FNAC smear. The cells are pleomorphic hyperchromatic, overlapping (discohesive). (Hx&E X 400)*

Figure 25*. The same case of IFDC, cell block. The hyperchromatism and pleomorphism become more obvious. There`s also abnormal mitotic figures. (Hx&E X 400)*

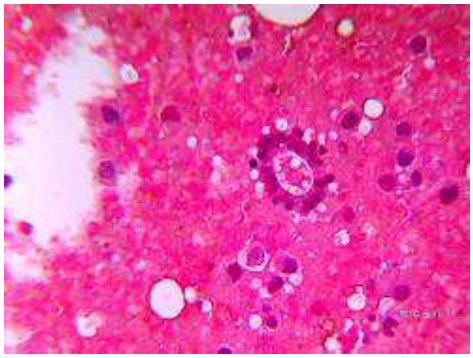

Figure 26*. A case of IFDC, cell block. An acinar pattern could be seen but the lining cells are highly pleomorphic. (Hx&E X 400)*

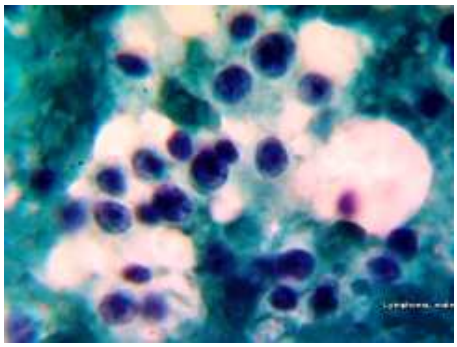

Figure 27. *A case of non-Hodgkin lymphoma, male breast FNAC smear. (PAP stain X 400)*

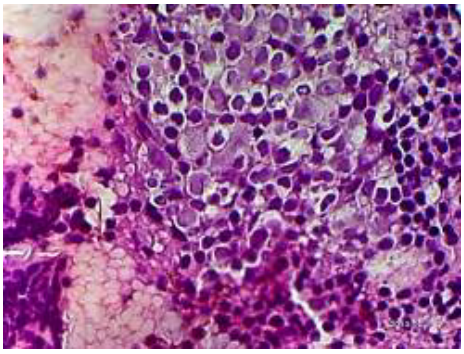

Figure 28. *A case of non-Hodgkin lymphoma, female breast cell-block. The lymphocytes are large, pleomorphic, with clear zone around. (Hx& E X 400)*

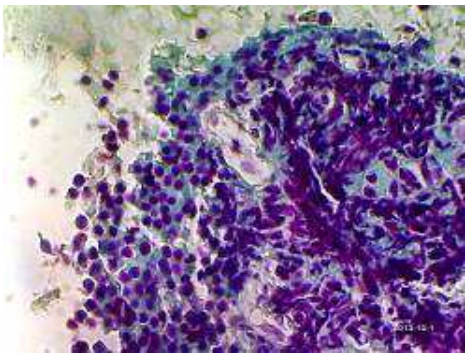

Figure 29. *The same case of non-Hodgkin lymphoma, female breast cell-block. The nuclei are dark blue. There is no evidence of Hodgkin cells (binuclear with prominent red nucleoli. (PAP stain X 400)*

Table 1*. FNAC diagnosis in relation of age and sex*

Classification	No.	Percentage (%)	Sex	Age (years)	FNAC diagnosis groups
Class-I	1	0.32	F	8	Normal breast budding
	1	0.32	F	32, Pregnant	Lactating adenoma
	1	0.32	F	12	Juvenile fibroadenoma
	3	0.97	F	14-16	Fibroadenoma
	24	7.74	F	18-21	
	13	4.19	F	22-25	
	14	4.51	F	26-45	
	54	*17.42*	*Total*		
	8	2.58	M	17-57	Gynecomastia
	14	4.51	F	20-45	Inflammatory smear

Classification	No.	Percentage (%)	Sex	Age (years)	FNAC diagnosis groups
	12	3.87	F	34-85	Purulent aspirate
	2	0.65	F	30,35	Granulomatous inflammation
	3	0.97	F	28-30	Duct adenoma
	1	0.32	F	22	Milk cyst
	1	0.32	F	24	Benign fibrous or fibromyomatous tumor
	4	1.29	F	17-19	Fibrocystic disease (FCD)
	70	22.58	F	20-32	
	36	11.61	F	33-39	
Class-II	34	10.97	F	40-48	
	16	5.16	F	49-70	
	160	*51.61*	*Total*		
	1	0.32	F	30	FCD with epitheliosis
	1	0.32	F	35	FCD with atypical cells
Class-III	13	4.19	F	30-43	Suspicious of malignancy
Class-IV	1	0.32	F	30	Atypical ductal hyperplasia
	5	1.61	F	24-32	Malignant (IFDC)
	23	7.42	F	40-50	
Class-V	6	1.94	F	51-75	
	34	*10.97*	*Total*		
	2	0.65	M,F	33y	Non-Hodgkin lymphoma
	310	100		Total	

Table 2. *FNAC diagnosis in relation to measures taken (biopsy and follow-up)*

FNAC diagnosis	No.	Biopsy results	Remarks	Follow up
Normal breast budding	1	No biopsy	Negative	No
Lactating adenoma	1	No biopsy, Relief	Negative	6 months
Fibroadenoma	1	Juvenile adenoma	Negative	No
Fibroadenoma	54	Fibroadenoma	Negative	No
Gynecomastia	8	No biopsy	Negative	No
Inflammatory smear	14	No biopsy, Relief	Negative	6 months
Purulent aspirate	12	Chronic breast abscess	Negative	No
Granulomatous inflammation	2	One T.B, one Hydatid cyst	Negative	No
Duct adenoma	3	Duct adenoma	Negative	No
Milk cyst	1	No biopsy, Relief	Negative	6months
Benign fibrous or fibromyomatous tumor	1	Granular cell tumor	Negative	No
	154	No biopsy, Without harm	Negative	2 years
Fibrocystic disease	3	Biopsy after follow-up, Same result	Negative	One year
	2	No biopsy	Negative	6 months
FCD with apocrine changes and inflammatory reaction	1	Highly suspicious of malignancy (C-IV)	False negative	One month
FCD with epitheliosis	1	IFDC	False negative	No
FCD with atypical cells	1	Comedo carcinoma	False negative	No
	3	Comedo carcinoma	Positive	No
	7	IFDC	Positive	No
Suspicious of malignancy	1	Fibroadenoma	False positive	No
	1	FCD with inflammatory reaction	False positive	No
	1	FCD	False positive	No
Atypical ductal hyperplasia	1	Carcinoma in situ	Positive	No
C-V: IFDC	34	IFDC	Positive	No
Non-Hodgkin lymphoma	2	Non-Hodgkin lymphoma	Positive	No
Total	*310*			

Table 3. *Cases with insufficient FNAC diagnosis and their cell-block and histopathological diagnosis*

Cytological diagnosis	No. of cases	FNAC	Cell-block	Histopathological diagnosis
Lactating adenoma	1	Not enough diagnosis	Enough and definite diagnosis	Not done
Granulomatous inflammation	2	Not enough diagnosis	Done, but no identification of the specific lesion	One is TB and the other is Hydatid cyst
Benign fibrous or fibromyomatous tumor	1	Not enough diagnosis	Granular cell tumor	Granular cell tumor
Atypical ductal hyperplasia	1	Not enough diagnosis	Enough diagnosis	Comedo carcinoma
Non-Hodgkin lymphoma	2	Not enough diagnosis	Enough diagnosis	Non-Hodgkin lymphoma

Table 4. *Statistical results*

Negative cases (257)	82.90%
Positive cases (47)	15.16%
False negative cases (3)	0.96%
False positive cases (3)	0.96%
Sensitivity	94%
Specificity	98%
Positive predictive value	94%
Negative predictive value	98%
False positive rate	1.15%
False negative rate	6%
Accuracy	98%

5. Discussion

Breast FNAC is a very useful test, relatively rapid and inexpensive, less invasive owing to the fine needle size, and is easier and safer in certain lesions, such as very small lesions, lesions just under the skin or very close to the chest wall compared with core biopsy. In addition, FNAC maintains tactile sensitivity; and allows multidirectional passes enabling a broader sampling of the lesion and immediate reporting whenever necessary [10]. Also, there is decreased morbidity, including hematoma, infection, pain, and risk of seeding of the biopsy track. Nevertheless, the past decade has seen a decline in breast FNAC in favor of more aggressive core biopsy techniques. Some pathologists prefer the histologic evaluation of core biopsies because they can be analyzed relatively quickly and easily, and they allow immunohistochemistry (IHC) to be applied. Cell blocks of breast FNAC offer these same advantages. Combining FNAC with core biopsies has been shown to increase diagnostic accuracy [11]. This study suggests that combining a smear preparation of breast FNAC with the cell-block can also combine the advantages of both approaches. The sensitivity was 94%, specificity was 98%, positive predictive value was 94%, and negative predictive value was 98%. False positive rate was 1.15%, false negative rate was: 6%, Total accuracy was: 98% (Table 3). These results are in general agreement with other study of 62 cases of breast carcinoma [12]. Regarding the sensitivity rates of core biopsy, there were some variations in previous results. A previous report showed a sensitivity rate of 90% in the diagnosis of breast malignancy and a specificity of 100%. [13]. In another study, the sensitivity was 98.7% [14].

Benign breast lesions account for the majority (90%) of referrals to the breast clinics [15]. Similar results were found in the current study where negative cases were detected in 260 cases (84%), including three false positive cases. In cases of fibrocystic disease, age ranging from 17-70 years, with a peak at 20-32 years agreed with others [16, 17].

Fibroadenomas are an abnormality of breast development, but are not considered to be neoplasms [18]. These cases were detected in 54 patients (17.42%) with age peaks at 18-21 years. Similar results regarding the age peaks in cases of fibroadenomas were recorded by other studies [7,15]. Smith and Burrows added that patients aged below 25 years with the classical ultrasound features of fibroadenoma do not need core biopsy [19]. A case of false positive result was recorded in this study. It was diagnosed as suspicious of malignancy, and by biopsy as fibroadenoma. Mottahedeh *et al.*, stated that many diagnostic pitfalls can be avoided by the realization that certain benign processes present some features of malignant neoplasm [20]. These may include high cellularity, less cohesion, and nuclear anisocytosis. Cell-block of the current study could solve this problem. In case of cellular fibroadenoma, there were numerous small acini, lined by monotonous cells; whilst in case of intracanalicular fibroadenoma, spherules of fibrous tissue in-between numerous monotonous ductal cells were noticed (Figure 5).

Male cancer breast accounts for about 1% of all carcinoma of the breast. This fact must not be forgotten when examining the male breast [1]. Only one case from the nine male specimens studied in this study was for non-Hodgkin lymphoma. The other eight cases were diagnosed as gynecomastia. In these cases, FNAC smear was sufficient for giving a diagnosis with confidence; however, cell-block whenever possible also gave a result of breast acini, or ductal hyperplasia without atypia. In cases of duct adenoma, cell-block could not be obtained because of the small lesion and its location near the nipple, where the introduction of the needle was so painful. FNAC smear of the duct adenoma resembles that of the fibroadenoma; except for the cells sometimes are plumper but still monomorphic (Figures 8,9). Nipple discharge smear for cases of duct adenoma were taken whenever possible; which showed groups of ductal cells; plump but monomorphic and may give a pattern of papillae. The case

that was diagnosed as milk cyst was diagnosed on basis of FNAC smear, without need of cell block or biopsy that was relieved later on after one month. Cases diagnosed as FCD underwent follow-up for two years. Apantaku suggests that FNAC should be used as the initial diagnostic test, and that if needle cytology and imaging studies are negative, the mass should be closely monitored by a repeat physical examination in 2 months and repeat imaging in 6 months [21]. Cell-block was very useful in such cases; we found some acini lined by ductal cells without atypia, in a background of fibro-fatty tissue (Figure 15). Sometimes, we found papillary lesions; cell-block was of great importance here; as we could differentiate typical and atypical hyperplasia. In cases of suspicious of malignancy, age ranged from 30-45 years, without peak, while cases diagnosed as malignancy were with age range from 24 -75 years with peak at 40-50 years. The same age peak for malignancy was mentioned in other study using different methods for screening [22]. Patients younger than 35 to 45 years old at the time of diagnosis of invasive breast cancer have been found to have a worse prognosis than older patients [23]. False negative cases could be seen, one that diagnosed as fibrocystic disease, with apocrine change and inflammatory changes, underwent repeated FNAC after 2 months which was diagnosed as highly suspicious of malignancy. In this case, the inflammatory reaction and accompanying degenerative changes may obscure or mask the malignant changes. Cases that diagnosed as FCD by FNAC smear with atypical cells and that diagnosed as FCD with epitheliosis and adenosis showed carcinoma in situ (comedo carcinoma) in biopsy. In cases of IFCD, cell-block showed disorientation of the cells, hyperchromatic, pleomorphic and sometimes microacini lined with hyperchromatic, pleomorphic cells (Figure 26).

6. Conclusions

Combined use of FNAC smear and cell-block can be useful for establishing a more definitive cytopathologic diagnosis. It is suggested to perform cell-block for each case of breast FNAC, to decrease the pitfalls and to improve the diagnosis and management of breast lumps.

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