

Diagnostic Significance of Fine Needle Aspiration Smear and Cell Block Study in Skin and Subcutaneous Nodules

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Abstract

Background: Evaluation of patients with skin or subcutaneous nodules, especially if it was multiple represents a major problem for the clinicians. But with full investigations, the problem becomes easier. However, the final diagnosis depends on pathological reports of tissue biopsy [1]. The easy access of the skin nodule provides the soil for performance fine needle aspiration cytology and taking the samples by the pathologists, or the clinicians. Smear cytology gives many defaults [2]. Performance of cell blocks was a trial to avoid these defaults and improve the diagnosis. The results of smear and cell block examinations were compared with examination of tissue biopsies.

Methodology: Two hundred twenty five skin and subcutaneous nodules from 225 patients underwent the material of this study. The patients were selected from those referred to pathology department, faculty of medicine Zagazig University, through the period between January 2018 and December 2020. Fine needle aspirations were performed utilizing the ordinary 22-gauge needle 10 cc syringes. Cell blocks were done utilizing the remnants in the syringe after performance of the smears. Histopathological examination was done using biopsies taken later on.

Results: According to tissue biopsy examination: Benign lesions constitute the commonest findings (180 cases) 80% Of cases: Lipoma 35 cases (15.5%), Adipose tissue 30 cases (13.3%), Fibrolipoma 20 cases (8.8%), Fibroma 20 cases (8.8%), Juvenile fibroma 5 cases (2.2%), Neurofibroma 5 cases (2.2%), Sebaceous cyst in 25 cases (11.1%), Implantation cyst, or dermoid cyst 20 cases (8.8%), Pilomatrixoma 5 cases (2.2%), Seborrheic keratosis 10 cases (4.4%), Madura foot (actinomycosis) 5 cases (2.2%). Intermediate lesions: Atypical lipoma 5 cases (2.2%). Malignant lesions 25 cases (11.1%): Basal cell carcinoma 10 cases (4.4%), squamous cell carcinoma 10 cases (4.4%), Basosquamous cell carcinoma 5 cases (2.2%). For the FNAC smear examination, the sensitivity test was: 83.01%, the specificity test was: 16.66%. For the cell blocks examination, the sensitivity test was: 97.77%, specificity test was: 100%.

Conclusion: Smear cytology is an easy, rapid test for diagnosis of skin nodule and performance of cell blocks improves its sensitivity and specificity outcome, but both must be attempted, because smear may be inefficient for some cases while cell block cannot be performed others.

Keywords: Biopsy • Cell Block • Skin • Subcutaneous Nodules

Introduction

Skin and subcutaneous nodules are just elevation of skin contexture more than 5 mm [3]. The depth is more important than width. The skin nodule may be mobile or fixed to the underlying tissues. The proper management needs to know about its exact nature, whether benign or malignant. These nodules are accessible for needle aspiration, to perform smears and cell blocks; this action is non-invasive, without need of operating theater, or anesthesia. Also, it is cheap and rapid procedure. The question is about its sensitivity, and specificity, and to much how we can depend on, in taking the decision of treatment.

Methodology

Two hundred twenty five patients suffering from skin or subcutaneous nodules were selected for this study. These patients were attenders in the outpatient clinics of surgery and pathology department in the period between January 2018 and December 2020. The patients underwent full history taking, general

examination for pulse, temperature, respiration rate, body weight, length. The nodules under study were examined clinically, in site, size, multiplicity, shape, consistency, color, if there's hair or not, if there's change in color, size, shape, mobility or fixation. Then, A 20cc plastic sterile syringe is opened, about 1cc of air is taken, and the cover is placed on the needle and become ready for use. The skin or subcutaneous nodule underwent gentle massage by a cotton gauze filled with 95% ethanol, then introduce the needle near the periphery of the nodule (avoid the center where there may be necrotic material that hinder proper smear taking). The needle was moved in multiple directions with suction. Then the syringe is removed gently. The site of aspirate underwent compression by clean, sterile cotton gauze for at least 10 minutes to stop bleeding. The needle is gently dislodged from the syringe, then one drop is placed from the syringe on about 4 clean glass slides. The smear is prepared by gentle moving of the blunt end of the syringe. The remaining part of fluid underwent addition of 10% neutral buffered formalin by gentle suction of the previously prepared formalin by the syringe. The smear is left to about to dry on the glass slides, then the slides were placed vertically in the staining jar, the addition of 95% ethanol, left for 20 minutes for fixation. The fixed smear slides are placed gently in water for one minute, and then placed in filtered hematoxyline for 5 minutes, then in tap water for 30 minutes, then in eosin for one minute the tap water for 20 minutes. The slide smear then, underwent placing in ascending grades of ethanol till absolute alcohol. A cover is placed and then examination of the smear by the Olympus XL 30 binuclear microscope. The pellet of cells in the used syringe, is left for one day for fixation, then underwent processing to form paraffin blocks, according to Hegazy method of tissue processing [4]. Unstained slides are prepared from the paraffin blocks then staining with Hematoxyline & eosin, Giemsa stain, Papaneacolo stain. Then slides are examined by the Olympus XL30 binuclear microscope, imaging using the digital camera on microscope. The patients in this study were underwent FNAC smears examination, cell

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blocks performance if possible then histological examination of tissue biopsy.

Statistical analysis

The correlation between results of FNAC smear and that of Cell block study was examined by chi-square test and student T-test. Sensitivity test, & Specificity test for both FNAC smear and cell blocks were examined in relation to the tissue biopsy, considering the results of tissue biopsy examination as the reference positive cases. All statistical tests were done with considering the significance value of chi-square test and T-test (p value=0.05 or less) using SPSS 16.0 for Windows (SPSS Inc. Chicago, Illinois, USA).

Results

There was a wide range of age in the patients underwent this study; ranging from 2 years to 76 years old, but most of them (80%) were at the range of 40-45 years. The genders of patients were mostly female; female: male, 10:1.

Results of FNAC smear, cell block examination and Tissue biopsy examination were summarized in (Tables 1-3).

The most relevant pictures of microscopic examination were placed in figures (1-10). Sensitivity and specificity test results of both FNAC smear examination and Cell block examination are summarized in the following data,

For the FNAC smear examination:

- True positive cases= 220/225
- True negative cases= 5 /225
- False positive cases=25/225
- False negative cases= 45/225
- Sensitivity test = $220/220+45= 220/265=83.01\%$
- Specificity test= $5/5+25=5/30= 16.66\%$

For the cell blocks examination:

- True positive cases= 220/225
- True negative cases= 5 /225

Table 1. Results of FNAC smear, Cell block examination, Tissue biopsy in the benign nodules.

Smear Cytology	Cell Block Examination	Histopathology	No.	%
Inadequate smear in 5 cases	negative cell block in 5 cases	Lipoma	35	15.5%
Fat globules in 30 cases	adipose tissue in 30 cases P=0.05			
Fat globule in 10 cases	fibrofatty tissue in 10 cases	Adipose tissue	30	13.3%
Fat cells in 20 cases	Fibrofatty , fibrous bundles in 20 cases P=0.05			
Fat globules, fibroblasts in 20 cases	fibroadipose tissue in 20 cases P=0.05	Fibrolipoma	20	8.8%
Fibroblasts in 20 cases	Fibroblasts, fibrous bundles in 20 cases P=0.001	Fibroma	20	8.8%
Fibroblasts in 5 cases	Fibroblasts, fibrous bundles in 5 cases P=0.001	Juvenile fibroma	5	2.2%
Fibroblasts, nerve fibrils in 5 cases	Fibroblasts, nerve fibrils, in 5 cases P=0.001	Neurofibroma	5	2.2%
Inadequate smear 10 cases	Fibroadipose tissue in 10 cases	Sebaceous cyst in 25 cases	25	11.1%
Fat cells 15 cases	fibroadipose tissue in 15 cases P=0.05			
Inadequate smear in 5 cases	Benign cells in 5 cases	Implantation cyst, or dermoid cyst	20	8.8%
Benign cells, fluid background in 15 cases	Benign in in 15 cases P=0.05			
Degenerated cells, others mature squamous cells in 5 cases	negative in 5 cases P=0.0001	Pilomatrixioma	5	2.2%
Epithelial cells with degenerative changes and pigmentation in 10 cases	Epithelial cells with degenerative changes and pigmentation in 10 cases P=0.001	Seborrheic keratosis	10	4.4%
Inflammatory cells, sulphur granules in 5 cases	Colonies of actinomycosis, inflammatory cells around in 5 cases P=0.001	Madura foot (actinomycosis)	5	2.2%

Table 2. Results of FNAC smear, Cell blocks and Tissue biopsy in intermediate nodules.

Smear Cytology	Cell Block Examination	Histopathology	No.	%
Fat cells in 10 cases	pleomorphic atypical lipoblasts in 10 cases	Atypical Lipoma	20	8.8%
Atypical cells (pleomorphic, hyperchromatic) in 10 cases	atypical lipoblasts in 10 cases P=0.05			

Table 3. Results of FNAC smear, Cell blocks, and Tissue biopsy in malignant nodules.

Smear Cytology	Cell Block Examination	Histopathology	No.	%
Malignant cells in 10 cases	Malignant cells, basal cell carcinoma with peripheral palisading in 10 cases P=0.001	Basal cell carcinoma	10	4.4%
Tad pole cells, hyperchromatic cells in 10 cases	Tad pole cells and carcinoma cells in 10 cases P=0.001	Squamous cell carcinoma	10	4.4%
Malignant cells in 5 cases	Malignant cells, basaloid cells, squamous cells with cell nest and keratin pearls in 5 cases P=0.001	Basosquamous cell carcinoma	5	2.2%
Total of all examined cases			225	100%

- False positive cases= 0/225
- False negative cases=5/225
- Sensitivity test= $\frac{220}{220+5} = \frac{220}{225} = 97.77\%$

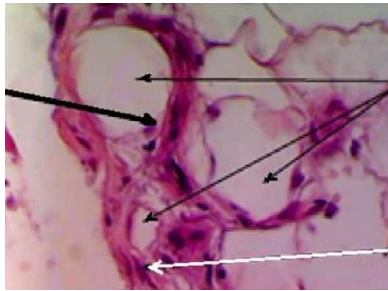


Figure 1. High power view of the same case of fibrolipoma showing mature fat cells (thick arrow), fat globules (thin arrows), fibroblasts and collagen bundles (white arrow), H&E stain.

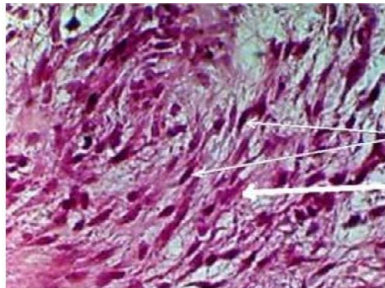


Figure 2. Cell block from a case of juvenile fibroma, showing: fibroblasts (thin arrows), thick homogenous collagen bundles (white arrow), H&E stain.

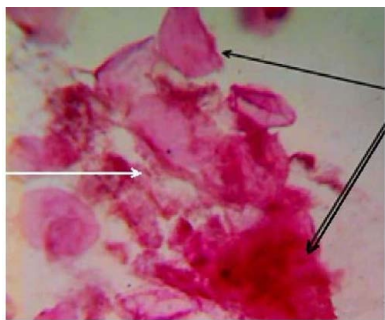


Figure 3. FNAC smear from a case of dermoid cyst, showing benign squamous degenerated cells (thin black arrow), keratinous material (double arrow) in a background of cellular debris (white arrow), H&E stain

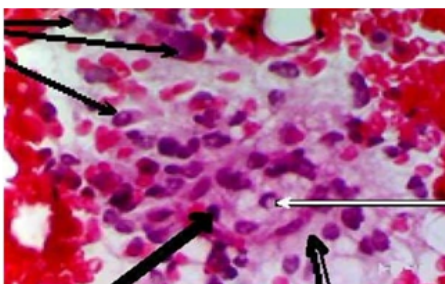


Figure 4. FNAC smear from case of Atypical lipoma, the mass was 2x2x3cm, recurrent after one year, the smear showed pleomorphic hyperchromatic cells (thin black arrows), some with pseudoinclusion in nuclei (thin white arrow), some apoptotic bodies (thick black arrow) could be seen, but absent mitosis. The background was mucinous with slight vacuoles (double arrow), H&E staining.

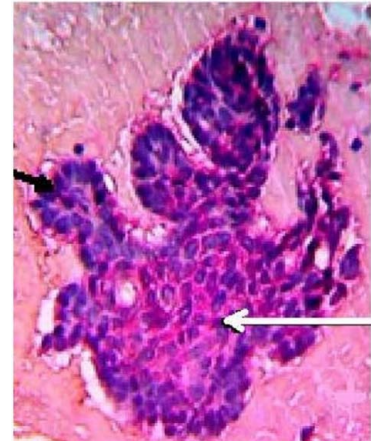


Figure 5. Cell block from a case suspected of basal cell carcinoma, showing malignant basaloid cells (white arrow), peripheral palisading (black arrow), H&E stain.

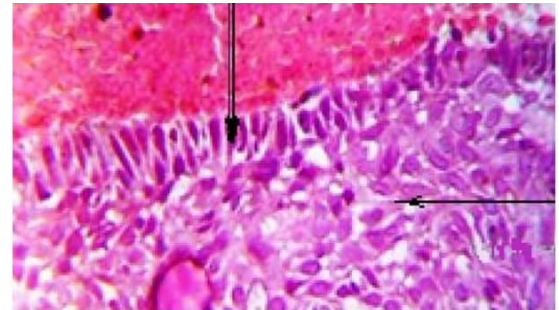


Figure 6. Cell block from a case of basal cell carcinoma, showing malignant basaloid cells (double arrow), peripheral palisading (arrow), H&E stain.

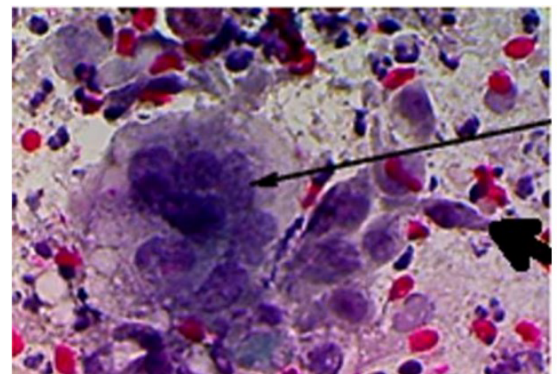


Figure 7. Cell block from a case of squamous cell carcinoma, showing: tap pole malignant squamous cell (thick arrow), overlapped malignant squamous cells (thin arrow), PAP stain.

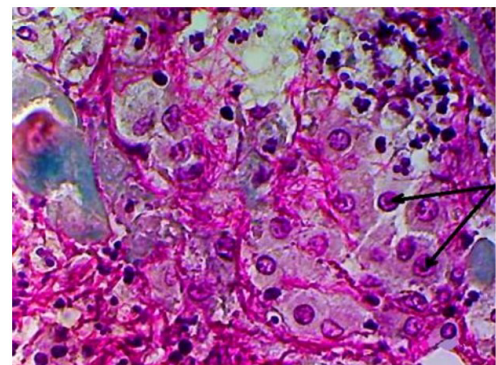


Figure 8. Cell block from a case of Squamous cell carcinoma, showing malignant squamous cells with prominent nucleoli (arrows), PAP stain.

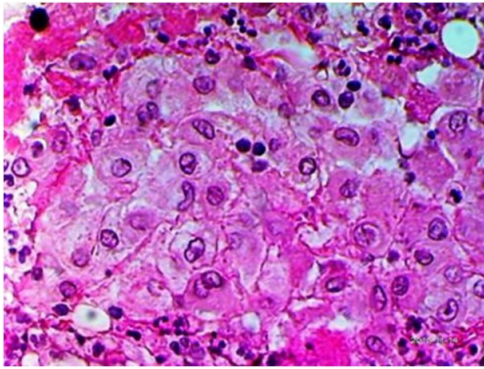


Figure 9. Cell block from a case of Squamous cell carcinoma, showing: pleomorphic malignant squamous cells, H&E stain.

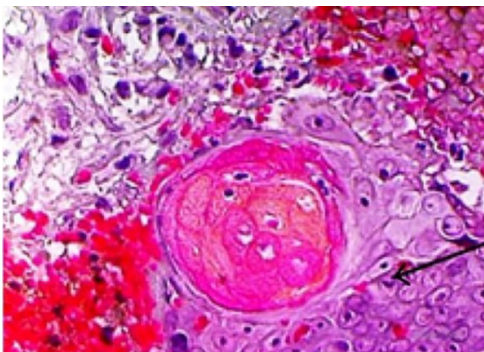


Figure 10. Cell block from a case of squamous cell carcinoma, showing malignant squamous cells with prominent nucleoli (thin arrow), keratin pearls, H&E stain.

- Specificity test= $5/5+0=5/5= 100\%$ (Figures 1-10)

Discussion

In this study, we concentrate on cell block performance, and its importance in diagnosis and differentiation of the lesion because of its simplicity, reproducibility. We perform FNAC smear first, as it is a rapid test, to find out the presence of cells, their types generally. Also to compare the features found in FNAC smear and that of cell blocks. We utilized the clot method for preparation of cell blocks, other methods were described by, Shidham2019 Satturwar and Pantanowitz 2021 [5,6]. These include the *gel-based method* (including gelatin-based, agar based,

Histogel methods), *coagulation-based methods* (including Chemical mechanism: Egg albumin-Alcohol, Alginate-calcium, Shandon Cytobloc method, Picrate-based, Enzymatic coagulation (Enzyme-based methods, Plasma/fibrinogen-thrombin The preformed supporting media (including: Celloidin method, Millipore filters, Various foams such as Gelatin foam and foam core device. Other methods E.g: cellient alcohol, in addition to the clotting method. We prefer the clotting method because the structural forms of cells and tissues are more preserved. We utilize the same clotting method in preparation of cell blocks in two previous studies and gave excellent results [7,8]. The patients in this work ranged in age, between 2 to 76 years with more shift to the old age (>40 was about 70%), and female to male ratio was 3:2, without significant outcome. This meant that the appearance or the complaint of skin or subcutaneous nodules occur more in females and in older ages >40 years). For FNAC smear results; the test processed a sensitivity about 83%, that is a significant percentage but lower than that of cell block (97.77%), this meant that cell block is more sensitive test than FNAC smear. Also, Cell block technique is more and more specific than FNAC smear, (100%) for cell block, and 16.66% for FNAC smear). These results alluded to the role of cell block in appearance of cell orientations, which give a picture resembling the tissue biopsy.

In case of benign cyst; the cyst is filled of fluid, so the smear contain scanty

cells, which may not appear, and the smear gives insufficient result, Cell block gives more accurate result, but the performance of cell block may fail because of low cells, and need gentle handling. In case of seborrheic keratosis; the smear was insignificant, because the cells were degenerated, with more brown pigment out cells, the features of cells were vague. On contrast; in cell block; the features of cells (benign features are more obvious). This picture gives a confidence in diagnosis as a benign lesion. In cases of lipoma, and fibrolipoma; the smear showed fat globules and/or mature fat cells, sometimes, fibrous elements a picture found by other authors [9], these features give a confidence of benign lesion, but the specific diagnosis cannot be achieved. The specific diagnosis of lipoma, fibrolipoma can be given easily in cell block. In cases of fibroma; the smear give a suggestion of benign fibrotic lesion (spindle-shaped fibroblasts and some collagen fibers), but the cell block showed well-formed fibroma. In cases of juvenile fibromas; the smear may be negative because of the dense contents, however the cell block is specific for diagnosis, moreover, it takes another importance to exclude juvenile fibrosarcoma and fibromatosis (the absence of mitotic activity). On contrary of the previous lesions; FNAC smear gives a satisfactory results that differentiate a benign lesion, and also specific for neurofibroma; because it gives us a picture of twisted nuclei, angulated and a hair-like background of neurofilaments a feature also found by Abdellatif and Kamel [10]. In cases of pilomatricoma; the smear gives a picture of benign lesion (ghost cells, mature epithelial cells, giant cells) the same picture found by Bansal et al. [11], but the cell block failed in most cases because of the presence of admixed amounts of degenerated, or shadow cells. In cases of dermoid cysts or implantation cysts; the smear showed a mixture degenerated epithelial cells and keratinous material a picture found also by Vaughan and Wisell [12], but the cell block also usually fails, due to the keratinous material. In cases of atypical lipomas; FNAC smear gives us a very useful picture; pleomorphic, hyperchromatic lipoblasts, mucoid background, the cell block dose not add a more information. In cases of basal cell carcinoma; FNAC smear showed malignant cells with basophilic cytoplasm the same results were found by Pasquali et al., but the orientation of cells appear in the cell block (peripheral palisading) [13]. In cases of squamous cell carcinoma; the malignant epithelial cells and the individual cell keratinization appear in the smear. The FNAC smear gives a good picture but the specific feature of cell nests and keratin pearls appear in the cell block. In cases of FNAC smear of basosquamous cell carcinoma; the smear gives a picture of malignant epithelial cells, but the specific orientation of basosquamous cell carcinoma appear in the cell block. In cases of Madura foot; the smear showed mixed inflammatory cell infiltrate which is non-specific, but cell block is very useful to clarify the mycetoma colonies with peripheral eosinophilic clubs appear, however Afroz et al., could found this feature in the FNAC smear [14].

Conclusion

Cell block study gives a more specific and more orientation of the cells like that of tissue biopsy. FNAC smear may give a useful features. Some lesions give negative results in smears, others give negative results in cell block, Therefore we recommend to perform FNAC smear and also cell blocks whenever possible.

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