COMPARISON OF CELL BLOCKS AND SMEAR EXAMINATION WITH FINE NEEDLE ASPIRATES IN THE DIAGNOSIS OF SUPERFICIAL PALPABLE HEAD AND NECK LESIONS

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ABSTRACT

OBJECTIVE: To compare the findings of cell blocks and smear examination with fine needle aspirates (FNA) in the diagnosis of superficial palpable head and neck lesions taking histopathology as the gold standard.

METHODS: This cross-sectional comparative study was conducted at Pakistan Institute of Medical Sciences, Islamabad and Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. Eighty patients of all age groups, having superficial clinically palpable lesions of head and neck region were recruited in the study from August 2014 to August 2015. FNA cytology, cell block and open biopsy were performed in all cases and compared with the histopathological examination. The data was recorded in a proforma and analyzed through SPSS Version-23.

RESULTS: Out of 80 patients, 44 (55%) were males and 36 (45%) females. The age of patients ranged from 01-81 years with a mean age of 45.68 ± 20.43 years. Lesions involving lymph nodes (n=31; 38.8%) and salivary gland (n=24; 30%) were common in head and neck region. Tuberculosis (n=15: 48.38%) and pleomorphic adenoma (n=13; 54.16%) were the common lesions involving lymph nodes and salivary glands respectively. Overall, 49 (61.2%) cases were benign/reactive and 31 (38.8%) cases were malignant on histopathology. Sensitivity, specificity, positive predictive value and negative predictive value was 74.2%, 95.9%, 92% & 85.5% for FNA smears and 83.9%, 98%, 96.3% and 90.6% for cell block respectively in diagnosing head and neck lesions.

CONCLUSION: Cell block with better diagnostic accuracy can be used as adjunct to FNA smears in diagnosis of superficial palpable head and neck lesions.

KEY WORDS: Cell-block cytology (Non-MeSH); Biopsy, Fine-Needle (MeSH); Fine needle aspiration cytology (FNAC) (Non-MeSH); Head and neck (Non-MeSH); Cell Biology (MeSH); Histology (MeSH).

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one is at the risk of making indeterminate or false positive/negative diagnoses.[11,12]

Cell blocks, per se, are not a new phenomenon. Being introduced more than a century ago in 1896, they gained better acceptance in 1947. At that time, they were used to enhance the cellularity of aspirates from pleural effusions.[13] Over the years, their use extended to all kinds of cytology specimens including cavity fluids, washings, FNAs, cyst contents and liquid-based cytology (LBC) specimens. The cell block preparation technique is simple and all it requires is fixing of the residual fluid or blood clot left in the needle hub in 10 % formal saline. After fixation, it is sent to the histopathology laboratory and processed routinely. For the hemorrhagic smears, the results of cell block technique are promising.[14,15] Cell blocks are a useful adjunct to cytology smears for reaching a more definitive diagnosis. With cell blocks technique, multiple sections of the same material can be examined for routine, special and even immunohistochemical staining. In addition to that, molecular and cytogenetic studies can also be conducted. Therefore, with the simple procedure of making cell block from FNA smears, chances of cancer detection are increased. Moreover, the cell block preparation may give a clue to the histologic pattern of the neoplasms. The usefulness of cell blocks is further enhanced when the preserved tissue is used for microbiological, histochemical and immunohistochemical stains if required. The former is used for identi-

INTRODUCTION

The head and neck is an important site for many reactive processes, inflammatory lesions, benign, and malignant tumors.[1] Numerous metastatic tumors metastasize to cervical lymph nodes can also be detected in this area,[2,3] most of which are aggressive metastatic tumors that originate from the upper aerodigestive tract.[4,5] Early detection of these tumors is promising and ensures better management of the patient. Head and neck cancers stand 10th most common cancer throughout the world.[4] The prevalence of head and neck cancers in Pakistan is 18.74%.[6]

The prevalence of head and neck cancers in the Indian subcontinent is 25 to 30% of all cancers which is contrary to 3-4% in the Western World.[7]

Fine needle aspiration cytology (FNAC) is a simple, quick, reliable and cost-effective method of determining pathology of superficial lesions of head and neck e.g., salivary glands, lymph nodes and thyroid nodules. It is well tolerated by the patients and is a cosmetically acceptable method.[10] However, at times, FNAC yields non-diagnostic smears and

TABLE I: TYPES OF LESION BY FINE NEEDLE ASPIRATE SMEARS, CELL BLOCK AND HISTOPATHOLOGY OF LYMPH NODE LESIONS (N=31)

Type of lesion	FNA smears		Cell Block		Histopathology	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Tuberculosis	7	22.58%	9	29.03%	15	48.38%
Reactive Hyperplasia	14	45.16%	10	32.25%	2	6.45%
Suspicious for malignancy	2	6.45%	-	-	-	-
Metastatic Carcinoma	3	9.67%	8	25.80%	8	25.80%
Hodgkins Lymphoma	2	6.45%	2	6.45%	4	12.90%
Non-Hodgkins Lymphoma	2	6.45%	2	6.45%	2	6.45%
Hemorrhagic aspirate	1	3.22%	-	-	-	-
Total	31	100%	31	100%	31	100%

fication of infectious etiology and the latter for classification of various neoplasms.[1,16-19]

The usefulness, cost effectiveness and sensitivity of FNAC is well established, however, its limitation is that it doesn't yield an accurate diagnosis. Cell block prepared from residues of fine needle aspirations can be a useful supplement to smears for determining a more precise cytology diagnosis. They are of particular help in tumor categorization, a field, where smears generally lag behind. This study was conducted to compare the clinical utility of cell block with routine FNA smears in the final cytopathologic diagnosis of superficial palpable head and neck lesions.

METHODS

This cross-sectional comparative study was conducted from August 2014 to August 2015 at the histopathology section of Pathology Department, Pakistan Institute of Medical Sciences (PIMS), Islamabad and Department of Histopathology, Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar. A total of 80 clinically palpable superficial lesions of head and neck region were included in the study for which FNA smears, cell block and open biopsy were performed.

Patients of all age groups, both sexes, clinically palpable lesions in the head and neck region and cases in which ample material was available to make cell blocks were included in the study. Patients were recruited in the study through non-probability, convenient sampling technique.

Patients not willing/uncooperative for FNA procedure, the history of bleeding diathesis and cases in which preparing the cell block was not possible due to insufficient material were excluded from the study.

Before performing FNA, the procedure was explained to the patient and informed consent was taken. After recording their demographic profile (i.e. age, sex, occupation, and socioeconomic status), relevant history regarding nature, intensity, and duration of the symptoms was acquired. A directed examination for any positive signs was performed. Findings of specific investigations e.g. Chest X-Rays, CT scan and MRI were recorded when available. The record was maintained for all samples received from patients. Fine Needle Aspiration was performed; four smears were made on clean glass slides. All of them were immediately fixed in 95 % ethanol for 30 minutes to 1 hour and stained with Hematoxylin & Eosin stains. The residual material mixed with procedural blood in the needle hub was allowed to clot spontaneously for 1 hour. Afterwards the clot was tapped and removed from the needle hub, wrapped in a filter paper and then fixed in 10% formal saline solution for 2 hours. This is called "one step spontaneous clot method" which does not involve centrifugation. After fixation, the clot was processed like routine tissue processing for preparation of cell blocks.

Subsequent confirmation of diagnosis was done by histopathological examination of representative section of the lesion which was taken as the gold standard. The data was recorded in a Proforma/Data collection form and then entered in SPSS computer software version 23.

TABLE II: TYPE OF LESION BY FINE NEEDLE ASPIRATE SMEARS, CELL BLOCK AND HISTOPATHOLOGY OF SALIVARY GLAND LESIONS (N=24)

Type of lesions	FNA smears		Cell Block		Histopathology	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Pleomorphic Adenoma	14	58.33%	14	58.33%	13	54.16%
Necrotizing Sialometaplasia	1	4.16%	-	-	-	-
Warthins tumor	-		2	8.33%	2	8.33%
Reactive hyperplasia	1	4.16%	-		-	
Mucoepidermoid carcinoma	4	16.68%	6	25%	5	20.83%
Squamous cell carcinoma	1	4.16%	2	8.33%	2	8.33%
Non-Hodgkins Lymphoma	2	8.33%	-	-	-	-
Carcinoma, Ex-Pleomorphic Adenoma	-	-	-	-	1	4.16%
Adenoid cystic carcinoma	-		-		1	4.16%
Suspicion of Malignancy	1	4.16%	-		-	
Total	24	100%	24	100%	24	100%

TABLE III: TYPE OF LESION BY FINE NEEDLE ASPIRATE SMEARS, CELL BLOCK AND HISTOPATHOLOGY OF MISCELLANEOUS GROUP (N=25)

Type of lesion	FNA smears		CELL BLOCK		HISTOPATHOLOGY	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Lipoma	6	24%	6	24%	6	24%
Epidermal inclusion cyst	4	16%	5	20%	4	16%
Benign spindle cell lesion	2	8%	-	-	-	-
Colloid Goiter	3	12%	4	16%	4	16%
Suspicious for malignancy	2	8%	-	-	-	-
Papillary Thyroid Carcinoma	1	4%	1	4%	1	4%
Hemangioma	-	-	1	4%	1	4%
Schwannoma	-	-	1	4%	1	4%
Neurofibromatosis	-	-	1	4%	1	4%
Hemorrhagic aspirate	2	8%	-	-	-	-
Metastatic Squamous cell carcinoma	3	12%	4	16%	5	20%
Metastatic Ewings sarcoma	1	4%	1	4%	1	4%
Small blue cell tumor	1	4%	1	4%	1	4%
Total	25	100%	25	100%	25	100%

RESULTS

The age of patients ranged from 01-81 years with a mean age of 45.68±20.43 years. There were 44 (55%) male and 36 (45%) female patients.

Out of 80 lesions from head and neck region, lymphadenopathy was predominant, accounting for 31 (38.8 %) cases, followed by 24 (30 %) cases of salivary gland lesions and 25 (31.3 %) cases of miscellaneous lesions.

The different types of lesions diagnosed by FNA smears, Cell block, and Histopathology in the lymph node are presented in table I. The most common type of lesions diagnosed in the lymph nodes were Tuberculosis (n=15: 48.38%) and metastatic carcinoma (n= 8; 25.80%).

Out of total salivary gland cases in the study, 15 (18.7%) were benign and 9 (11.2%) malignant. The most common salivary gland lesions were pleomorphic adenoma (n=13; 54.16%) and mucoepidermoid carcinoma (n=5; 20.83%) respectively (table II).

The cases included in the miscellaneous group were 25 (31.3 %) in number. Types of lesion by FNA smears, cell block and histopathology of benign and malignant lesions in the miscellaneous group are presented in table III. The most common lesions on histopathology in miscellaneous category were lipoma (n=6; 24%) and metastatic squamous cell carcinoma (n=5; 20%).

Frequency of malignant lesions and benign or reactive lesions as detected by the technique used to diagnose is shown in table IV. On histopathology, malignant cases were diagnosed in 31 (38.8%) cases respectively.

Findings of fine needle aspirate smears and cell block were compared with histopathological findings (gold standard) for presence of malignant and benign/ reactive lesions (Table V). Sensitivity, specificity, positive predictive value and negative predictive value was 74.2%, 95.9%, 92% & 85.5% for FNA smears and 83.9 %, 98 %, 96.3 % and 90.6 % for cell block respectively in head and neck lesions.

DISCUSSION

Head and neck region is an important area for various benign and malignant lesions. The different types of primary and metastatic neoplasms are diagnosed by FNA smears in this area. FNA smears is the first line of investigation for head and neck masses.[20] FNA smears is helpful in making a provisional diagnosis of many pathological conditions but some of which are difficult to classify specially those of soft tissues as they need tissue architecture and histopathological examination for proper diagnosis. The cell block uses the sediments, blood clot, and tissue fragments obtained through FNA smears. This material is processed like histopathological tissue and may provide architectural details. So cell block bridges the gap between FNA smears and histopathology.[21]

In the present study, the age ranged from 01-81 years with a mean of 45.68±20.43 years. This is comparable to studies by Padia B and Dhokia M and Khetrapal S, et al., they included the patients from the age range of 1 month to 85 years and 2 months to 80 years respectively.[20,22]

In our study, the cell blocks were found to be effective in reducing the cases generally regarded 'inadequate' or 'hemorrhagic' on FNA alone. Thereby, enhancing the diagnostic yield of the procedure. Three (3.75%) of our cases were given as hemorrhagic on smear examination but when their cell blocks were examined one of them was a case of hemangioma, one was of the colloid goiter and the other was metastatic adenocarcinoma in the lymph node. All of the three hemorrhagic cases (1 in the lymph nodes and 2 in the miscellaneous group) were subsequently confirmed on histopathology. The cell block also helped

TABLE IV: OVERALL FREQUENCY OF BENIGN AND MALIGNANT LESIONS WITH RESPECT TO THE TECHNIQUE

	FNA smears	Cell block	Histopathology
Malignant	25 (31.2%)	27 (33.8%	31 (38.8%)
Benign/Reactive	55 (68.8%)	53 (66.3%)	49 (61.2%)
Total	80	80	80 (100%)

TABLE V: COMPARISON OF FINE NEEDLE ASPIRATE SMEARS AND CELL BLOCK WITH HISTOPATHOLOGY (GOLD STANDARD) FINDINGS FOR PRESENCE OF MALIGNANT LESIONS

		HISTOPATHOLOGY		
		MALIGNANCY (N=31)	BENIGN/REACTIVE (N=49)	TOTAL (N=80)
FNA SMEARS	MALIGNANCY	23	2	25
	BENIGN/REACTIVE	8	47	55
CELL BLOCK	MALIGNANCY	26	1	27
	BENIGN/REACTIVE	5	48	53

in the identification of neoplasm type i.e. cases in which benign spindle cell lesion was given on cytological examination because of the limitation of FNA smears. Subsequent evaluation of their cell blocks revealed them to be neurofibroma and schwannoma (figure 1).

Similarly, the cases in which FNA smears raised the suspicion for malignant cells, the cell block reduced the grey zone, and we were confident to give the diagnosis of malignancy on the basis of the architectural details provided by the cell block and we were able to classify it either squamous cell carcinoma or adenocarcinoma.

Three (3.75%) cases of tuberculosis were diagnosed with the help of cell block examination as these cases showed reactive hyperplasia on FNA smears. Sometimes, a cytopathologist misses the classic aggregates of epithelioid cells and Langhan type of giant cells. In other instances, the representative areas were not sampled or the material was too small. Therefore, an additional needle pass dedicated for cell block may not only secure more material but also increases the chances of yielding the classic histopathological findings of caseous necrosis and granulomas. Nonetheless, this technique enhances the confidence of pathologist to impart

a definitive diagnosis and may prove helpful in screening the underreported extra-pulmonary tuberculosis.[23,24]

The cell block proved to be a valuable tool for discriminating malignant lesions from benign lesions in four (5%) cases. A case of mucoepidermoid carcinoma misdiagnosed as necrotizing sialometaplasia, a case of Hodgkin's lymphoma misdiagnosed as tuberculosis and two cases of metastatic squamous cell carcinoma misdiagnosed as reactive hyperplasia on smears.

In two (2.5%) benign cases, the cell block was beneficial, as cytological smears wrongly diagnosed them as malignant. These two cases were of the Warthin's tumor and pleomorphic adenoma which were erroneously diagnosed as Non-Hodgkin's lymphoma and 'suspicious for malignancy' respectively (figure 2).

The usefulness of this technique can be spectacularly exemplified in the identification of the double-layered arrangement of neoplastic cells in the case of Warthin's tumor and the presence of Verocay Bodies along with Antoni A & Antoni B areas in case of schwannoma (figure 1 & figure 2).

In the present study, the overall sensitivity of the cell block was 83.9%. A number of studies have compared the value of cell blocks with smears. The sensitivity of cell blocks varies from 60% to 99%, depending on sampling type and size, type of specimens, and aspiration techniques used.

A study was done by Dey et al. on 50 patients with effusions showed a sensitivity of 88.88% justifying our sensitivity statistics.[25] According to a study conducted by Desai et al. on cell block technique in head and neck pathology the sensitivity was 95.56%.[1] A study conducted by Vinayakamurthy, et al. on 66 cases of imageguided FNA of abdominal masses showed that the sensitivity of cell block
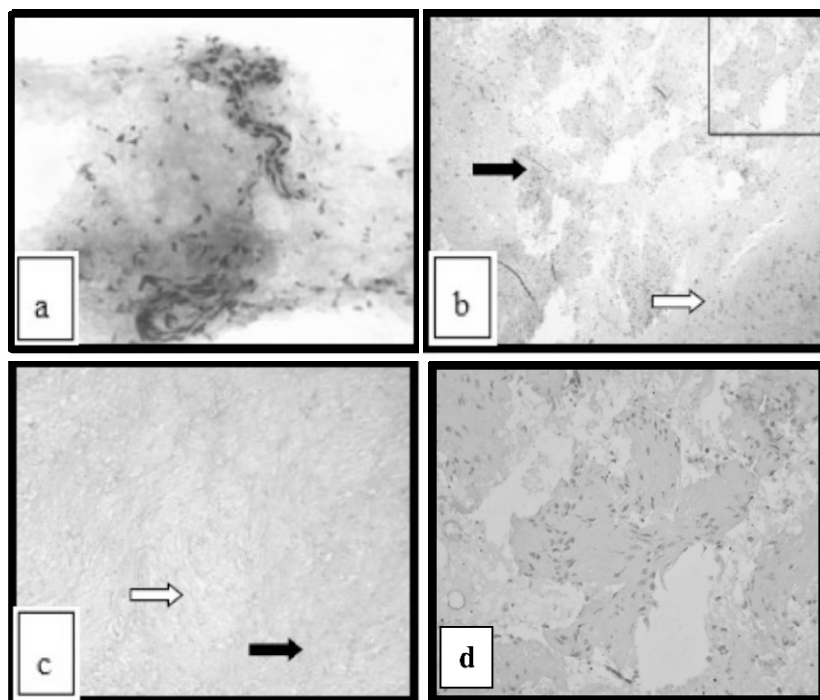

Figure 1: Photomicrographs of smear, cell block and histological section from the case of Schwannoma. (a) Smears from a case of schwannoma reveal spindle cells with wavy nuclei against a myxoid background. (b) Section of cell block showing schwannoma with many Verocay Bodies. The Antoni A (black arrow) and Antoni B areas (white arrow) are visible. The inset is showing a Verocay body. (c) Histopathological section of schwannoma showing Antoni A (black arrow) and Antoni B areas (white arrow). (d) The Verocay body in the inset shown in figure 1(b) is further zoomed for clarity (H & E stain, a,b & c x200).
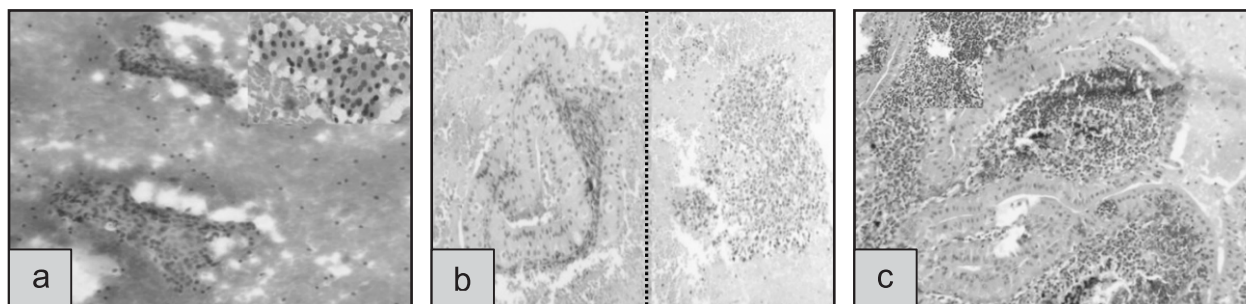
Figure 2: Photomicrographs of smear, cell block and histological section of Warthin's Tumor of salivary gland: (a) Smears of Warthin's tumor of salivary gland are showing clusters of epithelial cells along with scattered lymphocytes in the background. The inset is showing oncocytic epithelium typical of Warthin's tumor. (b) Merged fields from the same case separated by dotted line showing section of cell block of Warthin's tumor of salivary gland. Glands are lined by double layered epithelium (Left) and numerous lymphocytes are also seen (Right). (c) Sections of Warthin's tumor showing glands lined by double layered epithelium and the subepithelial tissue is showing lymphocytes. (H & E staining, a,b & c x400).

was 91.6%.[26] The overall specificity of cell block according to our study was found to be 98.4% which is almost in accordance with studies done by Mathew et al.[27], Bhowmik et al.[24] and Guldaval et al.[28]

Different studies done on FNA smears in Pakistan have used cell block as an adjuvant to cytological diagnosis. According to Mamoon et al. in their study of evaluating the diagnostic utility of endoscopic ultrasound-guided fine needle aspiration cytology in the diagnosis of mediastinal and abdominal lesions.[29] They were of the opinion that cell block should be prepared whenever possible as it helps in establishing the final diagnosis. They also commented on the application of immunohistochemistry on cell blocks in difficult cases. Jahangir S, in 2015 have also commented on the usefulness of the cell block in their study on FNAC of pseudopapillary tumor of pancreas.[30]

Naz et al. in 2015 have also commented on the usefulness of the cell block in their study on Fine Needle Aspiration Cytology of adenoid cystic carcinoma in which they have used stains for basal lamina and CD117.[31]

According to Sadaf in 2017 the cell blocks were very helpful in a case of classic Hodgkin's lymphoma on EUS-FNA where cell showed positivity of CD30 and CD15 in RS cells. In addition, they recommended the use of cell block material for FISH and molecular studies.[32]

There are certain limitations of the cell block technique worth mentioning. This technique requires increased processing time and costs incurred are obviously increased. At times multiple passes are required to get enough material for cell block which is annoying and could be unacceptable to the patient. In low yielding lesions like benign fibrous lesions, the cellular material gained is low and hence the quality of cell block and its advantage of providing additional architectural details are compromised. The cell blocks cannot be made in the centers where histological processing facilities are not available. Since the material is too small and tiny, there remains the threat of loss of material during tissue processing. Cases of necrosis, hemorrhage and inadequate material limit the usefulness of cell block. Like routine FNA smears, cell blocks only provides information about a limited area of the lesion, hence we get a partial version of the whole picture.

Nevertheless, the use of cell block technique has broadened the canvas of cytopathologists and opened new avenues to look for non-conventional adjuvants in addition to routine cytology examination.

CONCLUSION

Cell block has better diagnostic accuracy than FNA smears in diagnosis of superficial palpable head and neck lesions. Adjuvants use of cell block with FNA smears will improve the diagnostic value of these techniques in superficial palpable head and neck lesions.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

MK: Conception and study design, acquisition of data, critical review, approval of the final version to be published.

FAB: Analysis and interpretation of data, critical review, approval of the final version to be published.

WS: Conception and study design, Analysis and interpretation of data, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA SHARING STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.