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ORIGINAL RESEARCH

A study of the role of electron microscopy in fine needle aspirates of neoplastic lesions

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ABSTRACT

Introduction: Evolution of cytopathology in the past few decades as a distinct adjunct in screening and diagnosis of a neoplastic process, has paralleled the development of newer ancillary techniques like immunocytochemistry, flow cytometry, karyotyping and genetic studies. But a combination of LM and IHC will not identify every tumor and some diagnostic problems go unanswered. If the differential diagnosis on routine LM includes a tumor that is known to possess distinctive ultra structure, Electron Microscopy (EM) is clearly the method to select for confirmation as a subsidiary technique.

Aim: Owing to the restricted availability of TEM in most centres, data pertaining to its utility in tumor cytopathology is limited. Hence, the current study is designed to study the role of Electron Microscopy (EM) in fine needle aspiration cytological examination of neoplastic lesions, to correlate the findings of EM with Light microscopic findings and to correlate the EM findings with immunohistochemical findings, wherever possible.

Materials and Methods: Electron microscopy was carried out in a total of 50 cases where were subjected to FNAC for suspected neoplastic primary malignant or metastatic lesions. The cellular features of material aspirated were studied and ultrastructural details of cytoplasm including the plasma membranes, organelles and nuclear features were noted. The cases were grouped histogenetically and analysed. The final cytological diagnosis were tabulated and compared with the histopathological diagnosis.

Results: Fine needle aspirations in a total of 75 cases in various categories of patients were studied. A total of 25 cases, comprising 33.3% were rejected due to gross hemodilution or grossly inadequate cellular yield on initial methylene blue screening. Ultrastructural details were studied in 50 cases which yielded adequate material on cytology. EM studies were significantly contributory in 14/50 (28%) cases, supportive of light microscopic diagnosis in 25/50 (50%) cases and not contributory in 11/50 (22%) cases. IHC was carried out in 14 out of 50 cases which was contributory in 10 out of 14

(71.4%) cases. Out of the 14 cases IHC was equivocal in 04 cases. Electron microscopic yielded significant diagnostic findings in three of these 04 cases.
Conclusions: Electron microscopy is of utmost importance as an ancillary tool in identifying the tumor histogenesis in FNACs of neoplastic lesions provided there is adequate yield on FNAC with presence of representative areas and proper processing techniques.
Keywords: Electron microscopy; fine-needle aspiration cytology; immunohistochemistry.

INTRODUCTION

Every year the approach to the laboratory diagnosis of cancer becomes more complex, advanced and places severe demands on surgical pathologists for identifying the nature or cause of certain phenomena. Evolution of cytopathology in the past few decades as a distinct adjunct in screening and diagnosis of a neoplastic process, has paralleled the development of newer ancillary techniques like immunocytochemistry, flow cytometry, karyotyping and genetic studies. But a combination of LM and IHC will not identify every tumor and some diagnostic problems go unanswered. If the differential diagnosis on routine LM includes a tumor that is known to possess distinctive ultra structure, Electron Microscopy (EM) is clearly the method to select for confirmation as a subsidiary technique. The use of enzyme histochemistry and transmission electron microscopy (TEM) expanded the primary microanatomic evaluation to include biochemical and subcellular structural features. Knoll and Ruska invented the first EM for which Ernst Ruska was awarded the Nobel Prize in Physics in 1986. It was subsequently demonstrated that the EM was capable of resolving structures to a far greater extent than that attainable by LM.

The optimal use of Transmission Electron Microscopy (TEM) in cytopathology provides a unique analytical technique which has lesser interpretative complexities as compared to IHC. Owing to the restricted availability of TEM in most centres, especially in India, data pertaining to its utility in tumor cytopathology is limited.

The use of TEM as a potent tool in accurate cellular diagnosis is well established in renal and muscle pathology and clinical virology. The value of EM in the diagnosis of tumours lies in trying to identify poorly differentiated neoplastic cell structural elements, which might be characteristic of the cell line from which the tumours arose. These include structural details beyond the reach of LM.

The advent of the fine-needle aspiration biopsy (FNAB) is responsible for cytology's new place in pathology. In the everyday practice of cytopathology, about 85-90% of the nongynecological cases can be diagnosed with the use of routine stains (i.e., Papanicolaou and Diff Quik). The other 10-15% of the cases requires the use of ancillary diagnostic techniques for a precise diagnosis. Immunohistochemistry helps solve approximately 50% of these cases, and the other half of these challenging cases are best approached and diagnosed by using electron microscopy (EM).[1]

The use of electron microscopy has been emphasised in identifying the histogenesis of cells in all poorly differentiated tumours. The tumours which have distinctive electron microscopic features that allow them to be readily identified are squamous cell carcinoma, adenocarcinoma, melanoma, neuroendocrine tumor, lymphoma and primary pulmonary tumour. The salient ultrastructural features signifying tumour histogenesis are enlisted in Table 1.

Table 1: Salient ultrastructural features signifying tumour histogenesis

S.No.	Ultrastructural features	Histogenesis
1.	Melanosomes	Melanoma
2.	Mucin granules	Adenocarcinoma

3.	Thyroglobulin supranuclear granules	Follicular carcinoma thyroid
4.	Glycogen deposits	Ewing's sarcoma
5.	Rough endoplasmic reticulum in parallel arrays	Plasmacytoma
6.	Cell junctions, tonofilaments, desmosomes	Squamous cell carcinoma
7.	Microvilli	Adenocarcinoma
8	Mitochondria	Oncocytic neoplasm
9	Filopodia	ALCL
10	Interwoven cytoplasmic processes	Nerve sheath origin

The common diagnostic dilemmas in the everyday practice of cytology as elaborated by Elba et al [2,3] are differentiation of mesothelioma and adenocarcinoma, hepatocellular carcinoma and metastatic carcinoma in the liver and of melanoma and poorly differentiated carcinoma from lymphoma. Distinction of melanoma from adenocarcinoma, sarcoma, hepatocellular carcinoma and mesothelioma where spindling of cells may be seen. It helps in determining the primary site in metastatic tumors, subtyping of sarcomas and differential diagnosis of tumors at sites like the mediastinum, retroperitoneum and CNS.

Our aim was to study the role of Electron Microscopy (EM) in fine needle aspiration cytological examination of neoplastic lesions and to correlate the EM findings with the light microscopic findings and immunohistochemical findings, wherever possible.

MATERIALS AND METHODS

A total of 50 cases reporting to a tertiary care hospital for FNAC of suspected neoplastic primary malignant or metastatic lesions were included in this study over a period of 02 years.

Cases with inadequate yield on cytology and those reported as 'negative for malignancy' were excluded from the study. Relevant clinical details of each patient viz. age, sex, site, clinical features, and any other relevant information were recorded. All cases were followed up and histopathological diagnoses with relevant histochemical and immunohistochemical details, wherever available, were obtained.

Multiple passes were made in order to increase cellular yield. Where necessary computed tomographic and USG guided FNACs were also carried out. A rapid examination of a smear after staining with methylene blue was done to establish the adequacy and representative nature of cellular material. Smears for LM were prepared and stained with Romanovsky's stain and Papanicolaou (Pap) stain.

Additional passes were then made for EM .The sample for EM was flushed into 3% glutaraldehyde in an Eppendorf tube, which was then pelleted by centrifugation. The aspirate was post fixed in 2% OsO_4 (Osmium Tetroxide) for 45 mins –1 hr. The osmic acid was then decanted, and then the sample was washed with phosphate buffer at pH 7.4.

The cell pellet was gradually dehydrated in increasing concentrations of ethyl alcohol followed by embedding in Epon. Semithin sections of polymerised blocks of 0.5 µm thicknesses were prepared using an ultramicrotome and stained with toluidine blue to check for adequacy and representativeness of cellular yield. The blocks were trimmed as per the field of interest. Ultrathin (500-700A^{O}) or 50-70 nm sections were stained with uranyl acetate and lead citrate and then studied by EM.

The cellular features of material aspirated were studied and ultrastructural details of cytoplasm including the plasma membranes, organelles and nuclear features were noted. The cases were grouped histogenetically and analysed. The final cytological diagnosis were tabulated and compared with the histopathological diagnosis. The contribution of EM toward the light microscopic diagnosis was then determined and graded as per criteria laid down by Fisher et al.

RESULTS

Fine needle aspirations in a total of 75 cases in various categories of patients were studied. A total of 25 cases, comprising 33.3% were rejected due to gross hemodilution or grossly inadequate cellular yield on initial methylene blue screening. The 50 cases comprised 34 males and 16 females The age of patients ranged from 09 to 81 yrs with a mean of 36 yrs and a median of 65 yrs. Lymph nodes comprised the most common sites of FNAC in 29 of 50 cases (58%), followed by soft tissue and bone which comprised 7 of 50(14%) cases. Primary sites for malignancy were aspirated in 16 cases (32%) and suspected metastatic sites in 34(68%) of 50 cases. Lymph node metastases comprised the most common site of all the metastatic lesions in 26 of 34 cases (76.6%). In order to evaluate the tumor types, cases were divided into separate groups based on predominant cytomorphological features on LM [Table 2]. Data accrued were tabulated to analyze the utility of Electron microscopy.

Table 02: Correlation Of Electron Microscopy With Cytology And Histopathology

S.No.	Site of origin	Site	No. of cases	Cytology	EM	Biopsy and IHC
1	**EPITHELIAL TUMORS**					
	a. Squamous cell carcinoma	Lymph node	1	Features of SCC well differentiated	Features of SCC	Well differentiated SCC
			7	Features of SCC moderately differentiated	Features of moderately differentiated SCC	Mod differentiated SCC
			4	Poorly diff SCC		Poorly differentiated SCC
	b. Adenocarcinoma	Lymph node	4	Adenocarcinoma	Mets from GITAdenoca	Mets from GIT
			1	Mets Adenocarcinoma	Mets parotid Adenocarcinoma	Adenocarcinoma mets from parotid N/A
			2	Adenocarcinoma	Adenoca Lung	Adenocarcinoma Lung
			1	Poorly diff ca		-
		Liver	3	Adenocarcinoma	Mets from GIT	Mets from GIT
		Soft tissue	1	Adenocarcinoma	Adenocarcinoma	Mets from ovarian ca
	c. Undifferentiated Carcinoma	Lymph node	2	Malignant epithelial tumor	Malignant epithelial tumour	N/A

	d. non small cell ca lung	Lung	1	Non small cell ca	Adenocarcinoma lung	Adenocarcinoma lung
2.	**LYMPHOMA**					
	a. SLL/CLL	Lymph node	1	SLL	SLL	CLL - CD5,CD10,CD23,43,Cyclin D1-ve
	b. Hodgkin's	Lymph node	1	Hodgkin's	Hodgkin's	Hodgkin's CD15,CD30 +
	c. ALCL	Lymph node	1	High Grade NHL	ALCL	High gd NHL.IHC Non contribute as EMA—ve,CD20 + in mature cells, -ve in tumor cells.
3	**SMALL ROUND CELL TUMOR**					
	a. Ewing's sarcoma	Iliac region Chest wall	1 1	Small round cell tumor	Ewing's	Ewings, MIC 2+,EMA CK-ve
	b. Wilm's tumor - Metastasis to liver	Gluteal region	1	Small round cell tumor	Mets from Wilm's tumor	Undiff sarcoma-mets from Wilm's IHC-MIC 2+,LCA -ve
		Liver	1	Small round cell tumor	Metastases from Ewing's	Metastases from Ewing's sarcoma
4	**NEUROENDOCRINE**					
	a. Malignant melanoma	Calf	1	Malignant melanoma	Malignant melanoma	Malignant melanoma
		LN	1	Spindle cell lesion	Malignant melanoma	Malignant melanoma
	b. Amelanotic melanoma	Dorsum of tongue	1	Poorly diff sarcoma	Amelanotic melanoma	Amelanotic melanoma, IHC non contributory,S100 –ve, vimentin positive
5	**SALIVARY GLAND**					
	a. Oncocytic neoplasm	Submandibular region	2	Oncocytic neoplasm	Oncocytic & secretory differentiation	N/A

	THYROID a. Mets of MTC to LN	Lymph node	1	MTC	MTC	N/A
	b. Mets of Follicular Ca to scalp	Scalp	1	Follicular ca	Equivocal	Follicular Carcinoma
	c. Papillary carcinoma	Thyroid	1	Papillary ca ?MNG	Papillary carcinoma	Papillary carcinoma
	PLASMACYTOMA	Maxilla	1	Undifferentiated Carcinoma/ NHL	Plasmacytoma	
		Calf	1	Undifferentiated Carcinoma	Plasmacytoma	Plasmacytoma
	Breast carcinoma	Breast	1	Breast carcinoma	Equivocal	Plasmacytoma
7.		LN	2	Mets Breast Ca	Breast Ca	Intraductal carcinoma
	Aneurysmal bone cyst	Maxilla	1	Giant cell tumor	Giant cell tumor	Intraductal carcinoma
8	**Soft tissue sarcoma**	Chest wall	1	High grade STS	Osteogenic sarcoma	Aneurysmal bone cyst
		Abdominal mass	1	High grade STS	MPNST	Osteogenic sarcoma

Epithelial tumors comprised (64%) of the 50 cases studied out of which 13 cases (40.6%) were adenocarcinomas followed by squamous cell carcinomas (37.5%) and soft tissue tumours (12%). The most common histogenetic type in metastatic lesions was adenocarcinomas and squamous cell carcinomas (12/34). The primary sites of malignancy in cases of metastases were discernible in 79.4% cases.

The cases examined by ultrastructure were divided into three categories as in a previous study by Fisher et al .[4] The contribution of EM in the LM diagnosis was broadly classified into three categories where EM significantly contributed, EM was confirmatory and was non contributory as per Tables 4 to 6. The data has been divided accordingly and also summarized in Table 3.

Table 3: Summary of contribution of EM in the cytological study

Site of origin	No. of cases of FNAC	No. on which EM performed	Significantly Contributory	Non Contributory	Confirmatory
Lymph node	35	29	6	6	17
Soft tissue	16	7	2	2	3
Head and neck	6	4	2	2	Nil
Breast	3	1	Nil	1	Nil
Lung	3	1	1	Nil	Nil
Thyroid	3	1	1	Nil	Nil
Salivary gland	2	2	Nil	Nil	2
Mets to Liver	5	4	1	Nil	3
Bone	2	1	1	Nil	Nil
Total	75	50	14	11	25

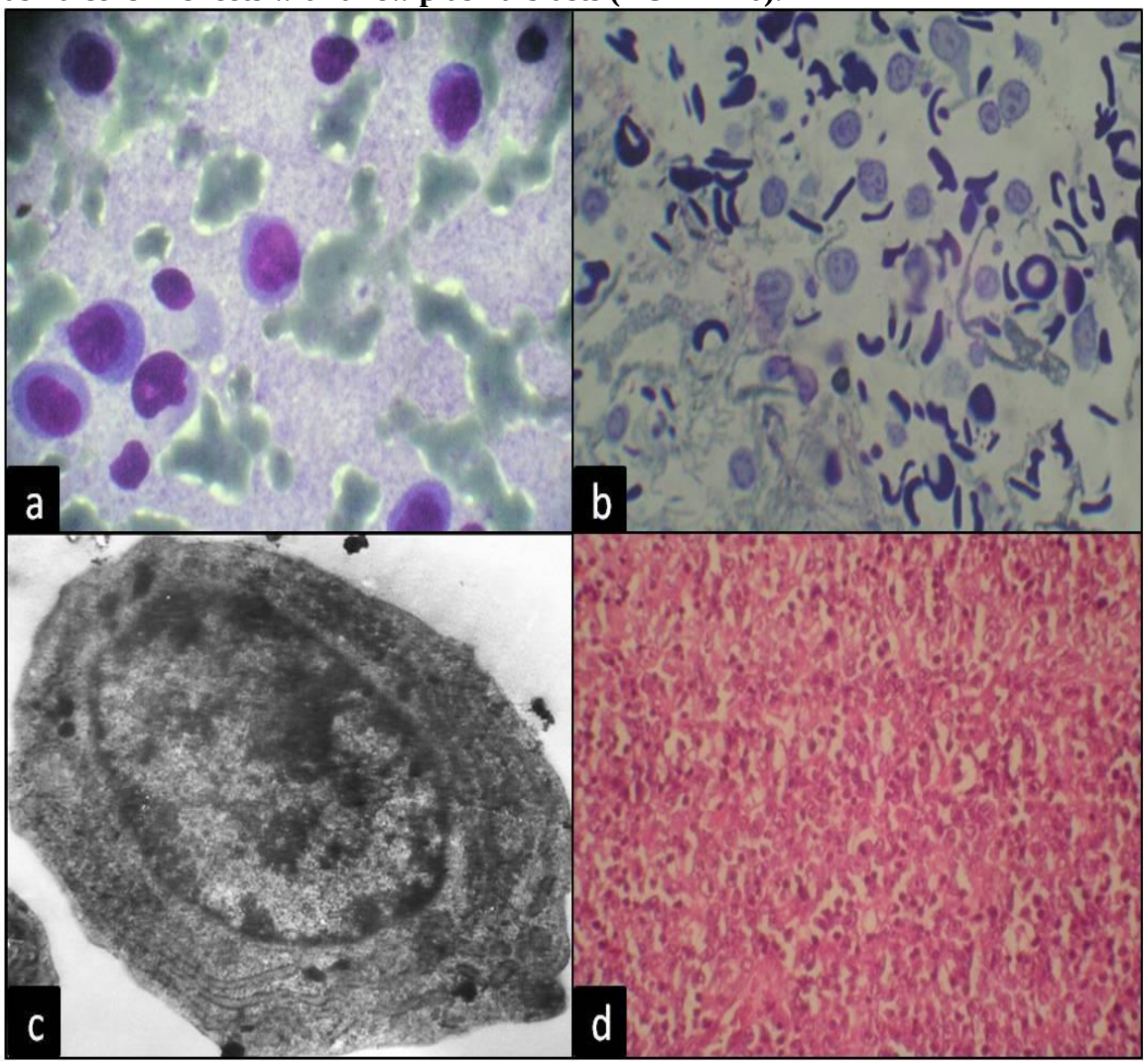
There were 28% (14/50) cases in which ultrastructural findings significantly advanced or narrowed a problematic or incomplete light microscopic diagnosis. The details of the cases are given in Table 4.

Table 4: Summary of cases where EM contributed significantly to the diagnosis

Site of origin	Cytological Diagnosis	No of cases	Final diagnosis
Lymph node	1. Poorly differentiated carcinoma	1	Adenocarcinoma Lung
	2. Adenocarcinoma	2	Adenocarcinoma from GIT
	3. High Gd NHL	1	ALCL
	4 Spindle cell tumor	1	Malignant melanoma
	5.Mets from ? Squamous cell carcinoma(Breast ca old)	1	Mets of poorly differentiated SCC
Soft tissue	Spindle cell lesion	1	MPNST
	Small round cell tumor	1	Ewing's
Head and neck	Plasmacytoma/high gd salivary gland tumour	1	Amelanotic melanoma
	Poorly differentiated carcinoma/Non Hodgkin's Lymphoma	1	Plasmacytoma
Lung	Non small cell carcinoma	1	Adenocarcinoma
Thyroid	MNG ? Papillary carcinoma	1	Papillary carcinoma
Liver	Adenocarcinoma	1	Adenocarcinoma Colon
Bone	Poorly differentiated carcinoma	1	Plasmacytoma

Out of the 14 cases, EM was significantly contributory in 06 cases from lymph nodes of which the diagnosis was advanced from a LM diagnosis of metastasis from poorly differentiated carcinoma to adenocarcinoma lung in one case in view of the presence of neoplastic glandular cells with secretory granules and short microvilli, with absence of well formed desmosomes and tonofilaments and intranuclear inclusions. Similarly in 02 cases of adenocarcinoma the site of origin could be detected to be the GIT based on specific ultrastructural features of presence of glandular cells with mucus granules and prominent terminal bars with tight junctions. A high grade NHL was diagnosed to be an ALCL on EM because of the presence of poorly differentiated large cells with pleomorphic large and indented nuclei, finely dispersed chromatin, prominent and multiple nucleoli, organelle rich cytoplasm and many filopodia on the cell surface. The diagnosis of a metastasis from spindle cell tumour to the inguinal lymph node was changed to malignant melanoma in view of presence of diffusely dispersed melanosomes and neurosecretory granules. A high grade spindle cell lesion was diagnosed as MPNST as infoldings of cell membrane with lamellar configuration and presence of abundant basal lamina material was demonstrated on EM. A round cell tumour was diagnosed as Ewing's sarcoma based on presence of focal deposits of glycogen in tumour cells, occasional mitochondria and abundant polyribosomes and absence of cell processes, microtubules and dense core granules. A case initially diagnosed as a high grade salivary gland tumour of the dorsum of the tongue or possibly a plasmacytoma on LM was confirmed to be an amelanotic melanoma based on the presence of discrete sparse solitary melanosomes, light cytoplasmic matrix, well developed Golgi complex and absence of tonofilaments and desmosomes. One case which was diagnosed as NHL/ poorly differentiated carcinoma of the maxilla was finally diagnosed as a plasmacytoma [Fig. 1] based on typical features of clock-face pattern of heterochromatin aggregates, occasional prominent nucleoli and organelle rich cytoplasm with presence of abundant vesiculated rough endoplasmic reticulum in parallel arrays.

Figure 1: (a) FNAC mass lesion left maxilla shows large cells with high N/C ratio, eccentrically placed nucleus and basophilic cytoplasm (Leishman-Giemsa x 400); (b) Semithin Section (Toluidine Blue stain x 400) shows atypical cells in dyscohesive clusters; (c) Plasmacytoma :Electron micrograph (uranyl acetate & lead citrate) showing an immature plasma cell with clock phase chromatin, prominent nucleoli and organelle rich cytoplasm which comprises abundant RER; (d) Plasmacytoma shows plasma cells in sheets with a few plasma blasts (H& E x 40).



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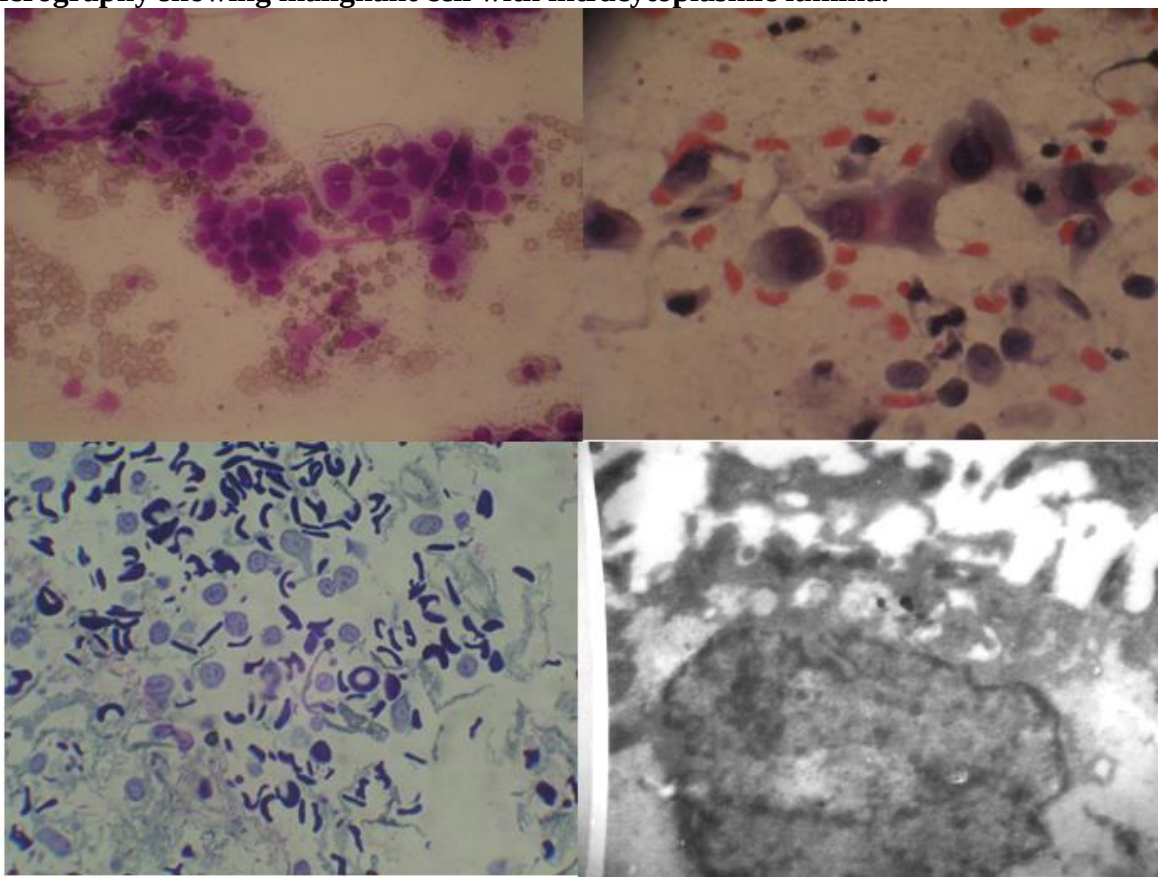
In this study one case of an adenocarcinoma in a lymph node in a background of a history of pleomorphic adenoma was identified. This was based on the presence of intracytoplasmic lumina with microvillous processes, abundant secretory granules and abundant euchromatin suggestive of secretory differentiation. There were no features suggestive of mucoepidermoid carcinoma or adenoid cystic carcinoma. The follow up biopsy revealed an adenocarcinoma NOS in the metastatic lymph node [Fig.2].

Figure 2: (a) Malignant cells in nests and acinar pattern (Leishman-GiemsaG stain x 40); (b) Papanicolaou stain showing malignant cells lying individually (Leishman-Giemsa stain x 40);z
(c) Semithin section showing malignant cells admixed with RBCs; (d) Electron micrography showing malignant cell with intracytoplasmic lumina.



One case of non small cell carcinoma on LM from the lung was further classified as being an adenocarcinoma due to the presence of neoplastic glandular cells with presence of secretory granules, intracytoplasmic lumina with microvilli and prominent cell junctions. One case of MNG showed atypical cytological features in light microscopy, EM revealed classical intranuclear cytoplasmic inclusions with marked nuclear convolutions suggestive of a papillary carcinoma. The primary site of metastasis to the liver was identified in 1 of 3 cases as being the colon based on presence of filamentous microvilli. These cells did not show homogenous electron dense bodies, vesicles or membranous whorls.
25 out of 50 cases (50%) evaluated showed that EM was merely supportive of a light microscopic diagnosis [Table 5].

Table 5: Summary of all cases where EM was confirmatory of the LM Findings

Site of origin	Cytological Diagnosis/EM diagnosis		No of cases
Lymph node	Well differentiated SCC		1
	Mod differentiated SCC		7
	Poorly differentiated SCC		2
	Adenocarcinoma		3
	SLL		1
	Mets of MTC		1
	Breast Ca		2

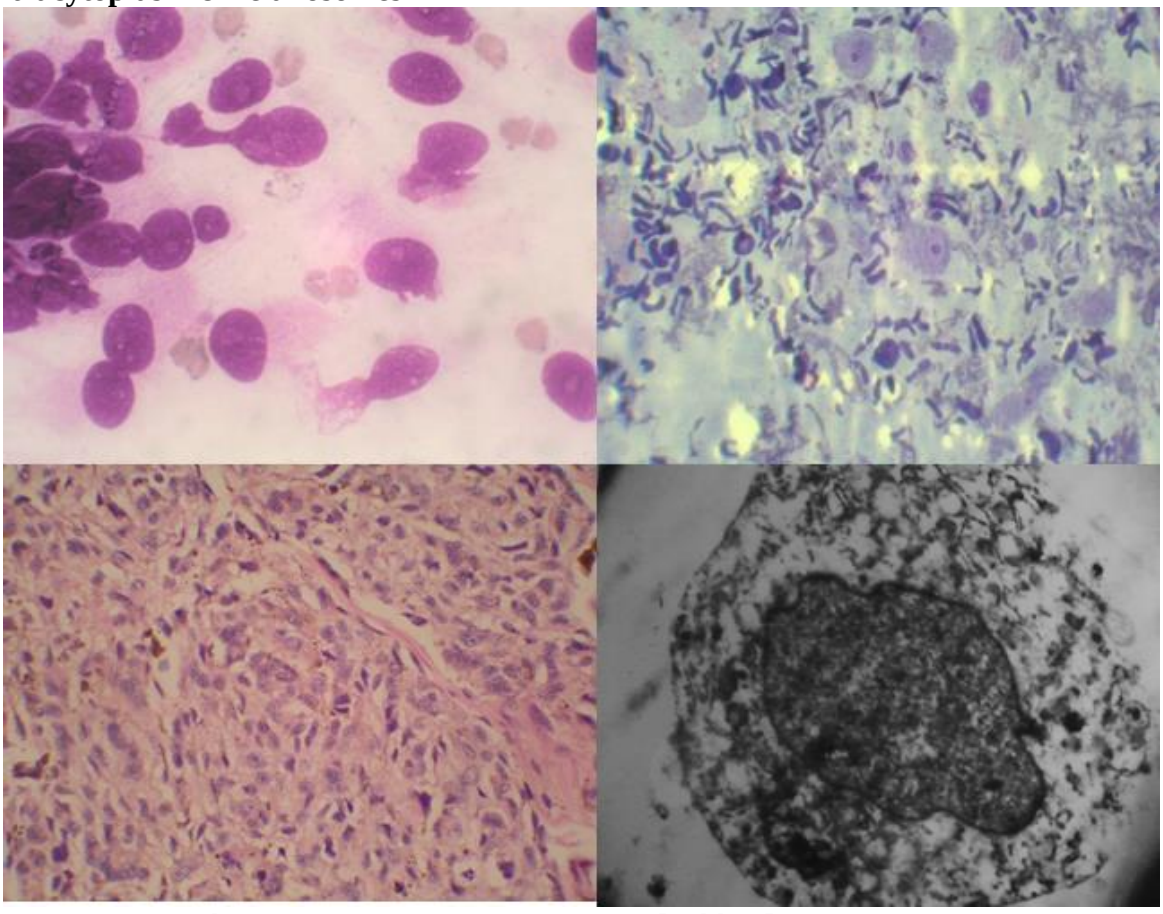
Soft tissue	Ewing's		1
	Adenocarcinoma		1
	Malignant melanoma		1
Salivary gland	Oncocytic neoplasm		2
Mets to liver	Small round cell tumor		1
	Adenocarcinoma		2
Total			25

EM confirmed the light microscopic diagnosis of squamous cell carcinoma in 10 of 25 cases. Adenocarcinomas comprised 6 of 25 cases. 17 of 25 cases occurred in lymph nodes and the remaining in soft tissue, salivary gland and liver. De novo melanomas are also described. 02 of the cases in this study occurred as metastasis to a lymph node and the soft tissue of calf which showed the presence of melanosomes on ultrastructure [Fig. 3].

Figure 3: (a) FNAC, Metastasis of malignant melanoma to calf showing cells with a high N/C ratio, granular chromatin, prominent nucleoli and light staining osmophilic cytoplasm (Leishman-Giemsa x 400); (b) Semithin Section (Toluidene Blue X 400x) shows atypical cells with prominent nucleoli in a background of RBCs

(c) **H &E X 40x) shows atypical melanin containing cells in sheets**

(d) **Electron micrograph shows malignant cell with prominent nuclear membrane and Intracytoplasmic melanosomes**



EM was not contributory in 11/50 cases (22%) cases [Table 6].

Table 6: Cases in which EM was Non Contributory

Site of FNAC	Light Microscopic Diagnosis	No of Cases
Lymph Node	Poorly differentiated SCC	1
	Malignant Epithelial tumor	2
	Hodgkin's lymphoma	1

	Adenocarcinoma	2
Head and Neck	Aneurysmal bone cyst Follicular Neoplasm	1 1
Breast	Infiltrating Ductal Carcinoma	1
Soft Tissue	Wilm's tumor Osteogenic Sarcoma	1 1
TOTAL		11

IHC was carried out in 14 out of 50 cases [Table 7].

Table 7: Results of IHC done in representative cases

	Electron microscopy	**IHC**	**No of cases**
Lymph Node	1).SLL 2).ALCL 3).Hodgkin's Lymphoma 4).Malignant melanoma 5).SCC 6).Adenocarcinoma	CD5+, CD10+,CD23+,43+,Cyclin D1- EMA-, CD 20+ in mature cells, CD 20- in immature cells CD 15+,CD30 + S100+,HMB45+ CK +, EMA- Calretinin -, CK+, EMA+	1 1 1 2 1 2
Soft tissue	1). Ewing's Tumour 2). Wilm's Tumour 3). Osteogenic Sarcoma 4).MPNST	MIC2+,EMA-,CK- MIC 2+, LCA- Vimentin+, CK- Patchy S100+	2 1 1 1
Head and Neck	1).Amelanotic Melanoma	Vimentin +,CK,EMA,S100,SMA,Desmin,CD34-	1
TOTAL			14

IHC was contributory in 10 out of 14 (71.4%) cases. Out of the 14 cases IHC was equivocal in 04 cases. These cases included- Amelanotic Melanoma, ALCL, MPNST and Wilm's tumor. Electron microscopic yielded significant diagnostic findings in three of these cases except Wilm's tumor in which the findings were not contributory.

DISCUSSION

Percutaneous fine needle aspiration biopsy for routine cytologic examination is a widely accepted tool. The role of electron microscopy in the field of surgical pathology is well established. However, its role in examination of tissue obtained by fine needle biopsy has not been adequately emphasised. Hence this study attempted to determine the utility of ultrastructural evaluation in fine needle aspirates of ambiguous neoplastic lesions

ROLE OF EM IN MALIGNANCY

The major role of EM in solving diagnostic problems in surgical pathology and fine needle aspiration cytology has been identified in 18-57% of cases. [4] In our study EM was significantly contributory in 14 of 50 (28%) cases. Our figure matches with those of the study by Wills et al (33%), as against 18% cases in a study by Fisher et al. Dardick et al [5] concluded that EM played a major role in 7.7% cases, Mackey [6] in 10% of cases and Kuzela [7] concluded that EM was more diagnostic in 27% of cases while one study showed 44% cases in which EM was of a significant clinical value. [8]

In this study, features favouring malignancy essentially magnified the suspicions raised at light microscopic observation. Extreme convolution of nuclei (cases of papillary carcinoma thyroid, melanoma, ALCL, Hodgkin's Lymphoma, Squamous and Adenocarcinomas), excess

of euchromatin with large irregular and eccentric nucleoli (Ewing's Tumour, Plasmacytoma, Follicular carcinomas) and simplification of cytoplasm and its contents all contributed at various stages in the assessment of these tumors. EM was confirmatory of LM findings in 50% (25/50) cases which correspond with the figures brought out by previous studies ranging from 8-45%. [5,7,9,10]

According to Fisher et al, EM was of least value in further characterisation of carcinomas. According to Fisher et al EM failed to provide a clue to 14/16 cases of adenocarcinomas as far as their site of origin was concerned. In this study differentiation between squamous and glandular neoplasms could be detected in almost all metastatic carcinomas. All cases of squamous cell carcinomas were correctly diagnosed on ultrastructure. [12]

The role of electron microscopy in metastatic tumours was seen to mainly contribute in establishment of histogenesis. In the metastases of adenocarcinoma to lymph node, some cases had specific features for indicating the organ of origin, as in origin from GIT and salivary glands. This was useful in diagnosing metastatic neoplasms of unknown primary sources. Since this study included poorly differentiated adenocarcinomas metastatic to lymph nodes, the site of origin could not be deciphered on the basis of ultrastructural evaluation alone in the majority of cases and an appropriate clinical correlation was sought. This has also been stated in previous studies. [4,8]

EM in FNAC is likely to benefit when identification of cell type or differentiation is unequivocal as in small round cell tumors, mesotheliomas etc. In neuroendocrine malignancies and adenocarcinomas, TEM helps to document neuroendocrine or melanocytic differentiation in poorly differentiated tumors. It helps in answering general questions of cell type as in small cell malignancies and spindle cell tumors.[8] Results were non contributory in 22% cases as against 30% in a previous study [8] because of either the presence of necrotic material or poorly preserved morphological appearances on EM.

Electron microscopy is of utmost importance in identifying the tumor histogenesis in undifferentiated carcinomas, spindle cell tumors, identification of the primary site of poorly differentiated metastatic deposits and cases wherein the presence of more than one cell type was seen on LM. Similar results have been reported in literature. [8,16,17,18,19,20,21,22] Hence its use is routinely recommended in such cases. Ultrastructural features like large polygonal cells with a high N/C ratio, presence of multiple prominent irregular nucleoli, marginated heterochromatin, irregular and indented nuclear membranes are the most important features in diagnosis of a malignant lesion and reflect light microscopic suspicions.

CORRELATION WITH IMMUNOHISTOCHEMISTRY

In this study ultrastructural features contributed to narrowing the cytologic diagnosis and correlating with the histology even when IHC was non contributory in (28%) (4/14) cases. This corresponds with the figures stated in previous studies ranging from 3-52.6%. [5,6,7,10,11]

IHC was non contributory due to poor differentiation, excessive necrosis and haemorrhage or non availability of a more specific marker. These cases included amelanotic melanoma, ALCL, MPNST and Wilm's Tumour. IHC was non contributory due to poor differentiation, excessive necrosis and haemorrhage or non availability of a more specific marker. However, EM significantly contributed in 3 of these cases of ALCL, melanoma and MPNST. EM findings in the case of Wilm's Tumour were non contributory.

The main difficulty of EM as applied to FNAC is ensuring that the material observed on thin sections corresponds to the neoplastic cells examined with LM. [10,25] Large heterogenous lesions may not yield representative material and would not be apparent on a small FNA specimen. The site of the aspiration was seen to play a vital role in bigger and heterogeneous lesions and hence in such cases, an aspiration under ultrasound guidance is recommended.

Another major disadvantage was the lack of specimen adequacy for ultrastructural evaluation as has been stated by previous studies. [5,13,14,15]

The cellular sample obtained by fine-needle aspiration biopsy is usually small and therefore requires extreme care during processing for electron microscopy. The most significant technical problem is due to contamination of the sample by red blood cells, which tend to dilute the samples.[15] Ensuring that the sample is representative, it is possible to find pointers towards an unsuspected diagnosis on ultrastructure.[15] Besides, the interpretation of EM often requires more judgement and expertise. [16] It is useful to preserve tissue in appropriate fixative and review the necessity of EM at a later stage after LM assessment.

To conclude, ultrastructural evaluation was of utmost importance in identifying the tumor cell lineage in case of adenocarcinoma, squamous cell carcinoma, malignant melanomas, plasmacytoma, lymphoma, oncocytic neoplasms and small round cell tumors. Electron microscopy can confirm a light microscopic finding in majority of the cases except in those where there is an overlap in the ultrastructural features like intracytoplasmic lumina, microvilli, and dense granules. Hence such features must be interpreted with caution keeping in view the clinical and the light microscopic features.

For EM to be productive in cases of aspirates of neoplastic lesions we must carefully select the cases, ensure that sampling is representative, process the sample properly in order to avoid morphological alteration at ultrastructural level and have an expertise in evaluation.

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