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Original Research Article

Cytologic diagnosis of neuroendocrine neoplasms including carcinoid tumours- A retrospective study

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ABSTRACT

Background: The classification of neuroendocrine neoplasms has evolved substantially over time but remains a topic of controversy and debate. Cytology has become one of the mainstays of diagnosis for these tumors, and the treatment may be entirely based on the FNA report.
Aims, Settings and Design: This is a retrospective study which aims to describe the cytological features found in different groups of Neuroendocrine neoplasms. We have also tried to enumerate the not so typical features which we have seen in our cases.
Materials and Methods: Cases of Neuroendocrine neoplasms diagnosed by cytology in the year 2018 were included in this study. The slides of these cases were retrieved, cytological features reviewed, and clinicopathological features evaluated. Histopathological correlation was done wherever possible.
Results: In this retrospective study, there were 43 cases which included FNA (n=38), Fluid cytology(n=3), Bronchial washings and Brushings(n=2). FNA sites included lung, cervical lymph nodes, scalp, liver, pancreas, and mesentery with the cytological diagnoses of Small cell carcinoma (n=22) Neuroendocrine tumor (n=7), Large cell Neuroendocrine carcinoma (n=3) and Poorly differentiated carcinoma with neuroendocrine features (n=11). Features that are of help include scanty cytoplasm, fine or coarse granular chromatin, nuclear moulding and streaking, cells adhering to vessels, inconspicuous nucleoli, nuclear debris in small cell carcinomas; larger cell size, a moderate amount of cytoplasm, coarse granular chromatin in large cell neuroendocrine carcinomas; uniformity of cell size, round to plasmacytoid cells with stippled chromatin and rosette formation in carcinoid tumors.
Conclusion: The identification of neuroendocrine morphology in cytology specimens is crucial as this would be the initial step towards using the appropriate markers for confirmation, which in turn has got therapeutic and prognostic significance.

1. Introduction

Neuroendocrine neoplasms (NEN's)are a group of heterogeneous tumors which comprise approximately 2% of all malignancies. These are divided into two main groups for functional purposes-well differentiated neuroendocrine tumors and poorly differentiated neuroendocrine carcinomas. The diagnostic criteria and classification systems are clinically relevant concerning the therapeutic and prognostic aspects.[1] The terminology of NEN's has created quite a lot of confusion among the pathologists because of the relatively frequent modifications in the classification.[2]

Cytopathology has been playing a pivotal role in the diagnostic workup of NEN's in recent times. Many of these tumors are first sampled as cytology specimens, as it is a rather straightforward method of getting a diagnostic yield that can be utilized for further ancillary techniques like

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immunocytochemistry. In Pulmonary tumors, the treatment modalities may be entirely based on the cytology report. The uncertainties present in surgical pathology are also reflected in the cytological diagnosis of these tumors.[1] Consequently, this study aimed to identify the cytological features most suggestive of neuroendocrine differentiation, the characteristic features encountered in the different categories, the utility, and limitations of ancillary techniques like cell blocks and immunocytochemistry.

2. Materials and Methods

This was a retrospective study which comprised of 43 cases cytologically diagnosed as neuroendocrine neoplasms in the year 2018, from our institute. The cases were retrieved from the register of the cytology division, slides taken, and reviewed, including cell block and IHC. The aim of this study was to find out the morphological features most suggestive of the cytologic diagnosis of the different groups of neuroendocrine neoplasms including neuroendocrine tumours [carcinoid group] and neuroendocrine carcinomas. We did histopathological correlation wherever possible. Cases were also analyzed for the age, sex, nature of specimen received, and site of the tumor. The study was conducted after the approval of the hospital ethics committee.

3. Results

During the period of study, there were 43 cases with a diagnosis of neuroendocrine neoplasms by cytology. The age group ranged from 38 to 81 years with a mean age of 61, and a predilection for males (93.02%). The specimens that we received included 38 FNA(88.37%), Fluid cytology(6.98%), Bronchial brushings, and washings(4.65%). We got FNA's from various sites including 25 from Lymph nodes(65.79%) 4 each from Liver and Lung(10.53%), 2 from Scalp(5.26%), 1 each from Pancreas and Mesentery (2.63%).

A straightforward diagnosis of Small cell carcinoma could be rendered in 15 cases (51.16%). An impression of 'suspicious of small cell carcinoma' was given in 3 cases(6.98%), which included two lymph node FNA's and one lung FNA. A diagnosis of 'possibly small cell carcinoma' was made in 4 (10.53%) lymph node FNA's. These were the cases in which all the characteristic features described for small cell carcinoma were not present in the smears. The cell block was obtained in 4 of these cases and showed positivity for neuroendocrine markers by immunocytochemistry.

We had 7 cases (16.28%) of Neuroendocrine tumour, 3 of them from the liver, 2 from lung,1 each from pancreas and mesentery, and cell block was obtained in 4 cases. In 3 of the cases, immunocytochemical studies confirmed the neuroendocrine differentiation. One cell block preparation showed only scanty degenerated cells. In 2 cases, the smears were destained, and synaptophysin was done out of which one was positive, and the other one turned out to be inconclusive. There were 3 cases (6.98%) where a possible diagnosis of large cell neuroendocrine carcinoma was rendered. Two of them had cell blocks, and the neuroendocrine nature was confirmed with the help of markers like synaptophysin.

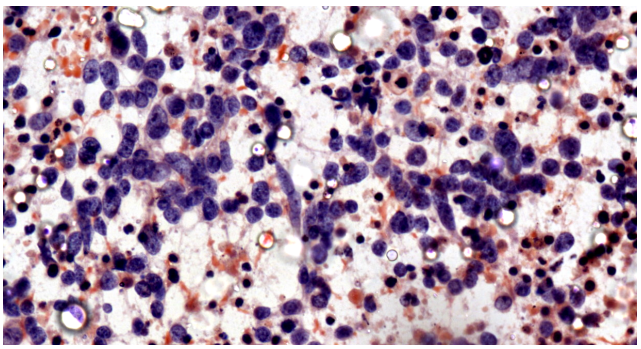

Fig. 1: Nuclear mouding and streaking in Small cell carcinoma

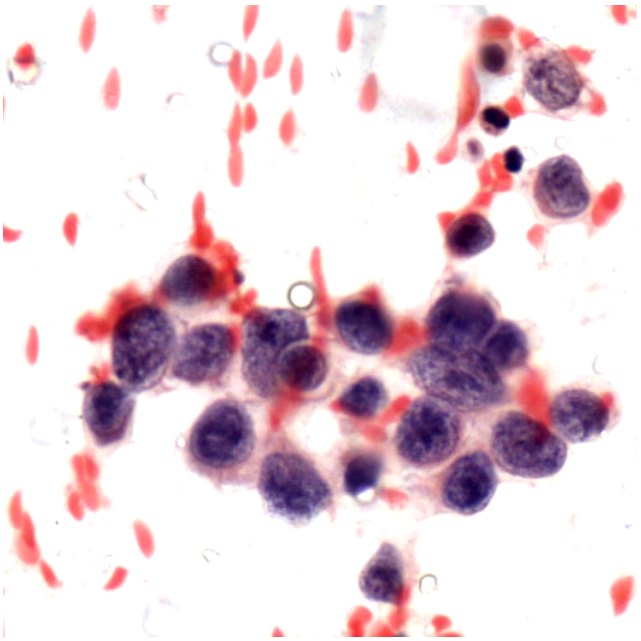

Fig. 2: Larger cells with nucleoli in Large cell neuroendocrine carcinoma

'Poorly differentiated carcinoma with neuroendocrine features' was the diagnosis given in 11 cases (25.58%) where a further categorization into Small cell, Large cell, or Mixed types could not be made. These were cases in which the neuroendocrine nature of the chromatin was appreciated, but certain features like the larger size of the cell, absence of nucleoli, presence of a few cells with moderate cytoplasm as opposed to the majority with scant cytoplasm precluded

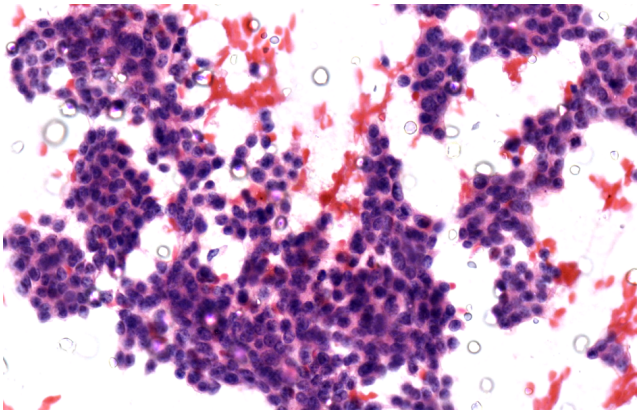

Fig. 3: Uniform cell population with stippled chromatin in carcinoid tumour

their classification into more specific groups. The cell block was present in one case in which immunocytochemistry was done to confirm the neuroendocrine nature. In two of the cases, smears were destained. One of them was positive for neuroendocrine markers, while the other turned out to be inconclusive.

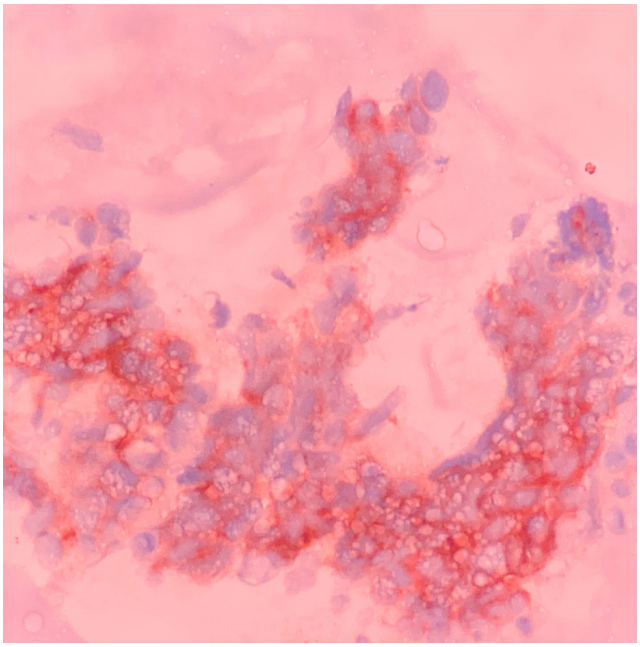

Fig. 4: IHC synaptophysin on cell block

We received biopsy specimens in 11 cases (25.58%), and the histopathological diagnosis was correlating with the cytology, in all of them. One case showed additional foci of squamoid differentiation, which was not present in the cytology smears.

Serum chromogranin assay was done in one case of metastatic neuroendocrine tumor of the liver and showed elevated levels.

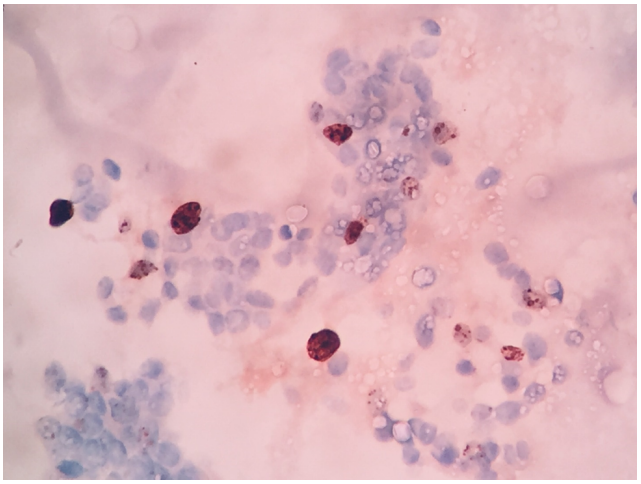

Fig. 5: IHC MIB1 on cellblock

4. Discussion

Identification of neuroendocrine morphology becomes relevant in the present scenario where many of the neoplasms are first sampled as cytology specimens. In some cases of lung tumors, these may be the only specimen available for diagnosis and thus become significant for the further treatment and prognosis.[2] The neuroendocrine neoplasms we commonly encounter in practice are Small cell carcinoma, large cell neuroendocrine carcinoma, and Neuroendocrine tumors.

The characteristic cytologic features of Small cell carcinoma include identification of a more or less uniform population of predominantly dispersed cells, at low power. At higher power, we can appreciate the nuclear pleomorphism and the fine or coarse granularity of the chromatin.[3] Scant cytoplasm, nuclear moulding, streaking, background necrotic material, brisk mitotic figures, and cells clinging to capillaries are the other helpful features. Even though the cell size accepted in cytology is 1.5 times the small lymphocyte diameter, that does not always help. Sparseness of cytoplasm rather than the size, gives an initial clue to the diagnosis.[4] The intermediate type of Small cell carcinoma can be misdiagnosed as adenocarcinoma or poorly differentiated component of a non-small cell carcinoma. But neuroendocrine nature of the chromatin helps. Also, combined Small and non-small cell carcinomas may be missed if we do not sustain a high index of suspicion.[3] Another finding which can arouse confusion is the presence of a few very large cells occasionally seen in smears of otherwise typical small cell tumors. These are usually present singly scattered. Combined tumors need to be considered only if these larger cells are numerous or are seen in clusters.[5] A diagnosis of mixed small and large cell neuroendocrine carcinomas can be very difficult as a subtle gradation in size is not that easily appreciated in cytology specimens.[3] The differentials for small cell carcinoma other

than neuroendocrine tumors include nonspecific chronic inflammation, other small round blue cell tumors including lymphoma, merkel cell carcinoma, PNET and poorly preserved, often necrotic non-small cell carcinoma.[5]

Large cell neuroendocrine carcinomas can cause diagnostic dilemma because they can be mistaken for Adenocarcinomas. The cells show moderate amount of cytoplasm, pleomorphic vesicular nuclei with conspicuous nucleoli.[2] The rosettoid patterns and the peripheral palisading seen in them may be mistaken for glandular differentiation.[4] Close observation of the nuclear features ie. granularity of the chromatin; along with poor cell cohesion, nuclear stripping and moulding are of help in distinguishing these from adenocarcinoma in most cases.[2] A cellblock with immunocytochemical markers can be of real value as the diagnosis of Large cell neuroendocrine carcinoma is really challenging, especially in small cytology samples.[6] Rarely, they can be confused with Small cell carcinomas.[7] In such cases, larger cell size along with presence of nucleoli and presence of rosettes are features that can help. Atypical carcinoids can also pose diagnostic problem because of the pleomorphism and necrosis, and we may not be able to sort this out cytologically.[2]

The cytologic characteristics of Neuroendocrine tumors include uniformity of cell population with round to plasmacytoid cells, stippled chromatin and rosette formation. Necrosis is not present and there are scanty mitotic figures.[2] Vascularity can be a prominent feature in pulmonary carcinoid tumors.[8] One may mistake them for well-differentiated adenocarcinoma, but the stippled chromatin and confirmation by neuroendocrine markers help us in arriving at a correct diagnosis. Spindle cell carcinoids have to be distinguished from mesenchymal tumors. Carcinoids with prominent plasmacytoid features may resemble a plasma cell neoplasm.[9] In bronchial brush cytology, the bland nuclei and resemblance to bronchial cells can be pitfalls. The identification of terminal bars helps distinguish bronchial cells from neuroendocrine cells. Crushed samples may result in an overdiagnosis of small cell carcinomas.[10]

Pancreatic neuroendocrine tumors and solid pseudopapillary neoplasms share many cytological features. Aspirates from both tumors may yield moderate to very high number of cells. Both demonstrate single cells with low NC ratios and may have a plasmacytoid appearance.[11]

Neuroendocrine tumors in head and neck can be divided into those with epithelial differentiation like carcinoids, small cell carcinomas etc. and neurally derived tumors including paragangliomas and olfactory neuroblastomas. Merkel cell carcinoma is an uncommon primary cutaneous small cell carcinoma with predilection for head and neck. Another tumor with neuroendocrine features is medullary carcinoma. Also, neuroectodermal tumors and mucosal melanomas can come in the differentials. In this context, Immunocytochemistry can help.[12]

The role of cell block preparation in diagnostic cytopathology is of immense significance. Optimal preservation of the tumor tissue is ensured in most cases.[13] Although cell block gives additional material to resolve diagnostic difficulties, the main effectiveness of this is to provide formalin fixed paraffin embedded tissue for ancillary studies such as Immunocytochemical studies and molecular testing.[14]

Cell block preparation plays a significant role in neuroendocrine tumors as one can attempt a MIB 1 marker, which helps in their grading. Tumor morphology and proliferative rates are key aspects of tumor prognostication in Pancreatic NETs. FNA with cell blocks (CB) may offer advantages for Ki-67 assessment as the technique obtains highly cellular, well preserved specimens with potential for broader tumor sampling.[15] Although FNA is associated with a higher diagnostic yield, the presence of single cells or small dispersed groups puts some limitations on the assessment of tumour grade. Accurate quantification is curtailed by the disruption of architecture.[16] Grade 2 Pancreatic NETs can be under graded when Ki-67 index is evaluated on CB material.[17]

The relevance of identifying cytologic features mentioned above is that it helps to raise the suspicion of neuroendocrine differentiation, and once the pathologist reaches that point, the cell block and immunomarkers will be of great help.

When adequate cells are present in the cell block, relevant immunocytochemical markers can be done. The epithelial nature of the tumor cells can be confirmed by the Cytokeratin, which produces a focal dot like positivity in the para nuclear position. Synaptophysin and Chromogranin A are the first-choice markers, rather than CD56 and NSE, which has got limited specificity. Both Synaptophysin and Chromogranin have a cytoplasmic distribution, and the epitopes may be sensitive to the fixation procedures applied, especially synaptophysin. This may explain the inconclusive results that were seen in some of our cases. In small samples, none of the neuroendocrine markers is reliable.[6]

Serum Chromogranin A is a sensitive and effective noninvasive lab test for clinical detection and management of NETs.[18] Chromogranin assays may be useful in some cases, but the levels may vary according to the degree of tumor differentiation.[19]

5. Conclusion

The cytologic diagnosis of neuroendocrine neoplasms, both high and low grade, can be difficult. Proper recognition of the neuroendocrine morphology along with adjuncts like cell block and immunocytochemical markers helps in avoiding errors and arriving at a correct diagnosis.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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