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Original Research Paper

Pathology

A CLINICOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE NEOPLASM IN A TERTIARY CARE CENTER

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ABSTRACT **Objective:** To compare the frequency of neuroendocrine tumors in our center with that reported in the literature considering age, gender, location, degree of differentiation and proliferation index. **Method:** Search of variables from a database of neuroendocrine tumor cases diagnosed in Department of Pathology at Sri Aurobindo Medical College and PG Institute, Indore over the past 2.5 years, relating them to epidemiological data such as gender, age, distribution across organs, most-used immunohistochemical markers and presence or absence of either lymph node or distant metastases. **Results:** 51 cases were reviewed, 16 were females and 35 males, predominantly in the 24-73 years age range. Lung was the most frequent primary site. CD56, synaptophysin and chromogranin A were the immunohistochemical markers used most often along with Ki67, a marker of cell proliferation that indicates a higher or lower degree of histological malignancy. Metastases were noted either in lymph nodes or liver. **Conclusion:** The results were largely consistent with those in the literature, including age group, gender and location. Most metastases originated from high-grade tumors, with high Ki67 levels and greater impairment of the liver.

KEYWORDS : neuroendocrine tumors, neoplasms, Immunohistochemistry

INTRODUCTION

Neuroendocrine tumors (NETs) can be located in any organ and constitute a group of primitive neoplasms originating from endodermal cells that proliferate from epithelia or other tissue structures with or without endocrine action.[1-4]

Most publications agree that the sites where NETs appear most frequently are the gastrointestinal tract and pancreas (70%) followed by the bronco-pulmonary system (20-30%). Other sites such as head, neck, thymus, genital and urinary system and skin are very rare (< 10%).[1] Various studies published in the literature from centers all around the world confirmed such organ distribution, affecting males and females alike, with an age peak in the sixth and seventh decades of life.[1-3,5-7]

In Brazil, Younes et al[8] in a study using the database of the GETNE – Grupo de Estudo de Tumores Neuroendócrinos (Neuroendocrine Tumors Study Group) involving 32 centers, which comprised 1,000 patients since 1985, found that the sites most frequently affected were the thoracic cavity (71.6%) and gastro-enteric-pancreatic tract (20.2%).

The behavior and local aggressiveness of neuroendocrine tumors vary, which in turn is related to tumor size or secretion and histological grade of malignancy. In some cases there are no well-defined histopathological criteria for classifying neuroendocrine tumors in terms of prognosis or progression factors.[3,8] Irrespective of neoplasm grade, metastases may occur, more frequently to the liver, and are often present by the time of primary diagnosis in 45-95% of cases.[10,11]

Immunohistochemical markers, such as chromogranin A, synaptophysin and CD56, represented a major improvement in the diagnosis and histological classification of neuroendocrine tumors. Mitotic counts and the Ki67 protein-based cell proliferation index are critical to assess the classification and the possible disease prognosis.[12,13]

Under the 2018 World Health Organization (WHO) classification scheme, NETs are classified based on biological behavior as grade 1 (NET G1), 2 (G2 NET) and neuroendocrine carcinomas (NEC): carcinomas are subdivided into large cell neuroendocrine carcinoma (LCNEC) and small cell neuroendocrine carcinoma (SCNEC), and mixed adenoneuroendocrine carcinomas. Histologic grades are dependent on mitotic counts and the Ki-67 labeling index: when the Ki67 index is low (< 3%) or the mitotic count is less than 2/10 HPFs, it is classified as G1 NET. G2 NET has the Ki67 index values between 3 and 20% and mitotic count (2-20/10 HPFs). Neuroendocrine carcinomas (NEC) have > 20% (Ki67 index) and > 20 mitotic count in 10 HPFs. However, the classification of NETs of the lung is different, being divided into low- (typical and atypical carcinoid) and high- (large cell and small cell neuroendocrine carcinoma) grade.[7,12-14]

We intend to describe the distribution of neuroendocrine tumors and analyze the epidemiological profile of cases from a single institution using data collection that included a distribution by gender, age and primary site, as well as the existence or absence of metastases (whenever possible) and the frequency of use of immunohistochemistry for making definitive diagnoses and defining prognosis.

MATERIAL AND METHODS

The study was conducted in Department of Pathology, Sri Aurobindo Medical College and PG Institute, Indore and included diagnosed cases of neuroendocrine tumors from December 2020 to November 2023 (2 ½ years retrospective and 6 months prospective).

All trucut biopsies / radical specimens received with clinical suspicion or diagnosis of neuroendocrine neoplasms along with requisition forms during the study period were subjected to histopathological examination. Clinical details and radiological findings of patients were procured from the files of the patients obtained from medical record section of the institute. The trucut biopsy was processed as per standard operating procedure. In case of radical specimens, multiple sections were taken from the tumor, surgical margins and lymph nodes.

After conventional processing and embedding in paraffin wax, sections of 5 um thickness were cut and stained using haematoxylin and eosin (H&E) for histopathological study. These H&E stained slides were studied as per the standard reporting protocol. In addition, 4um sections were cut from a paraffin block of tumor tissue and taken on a glass slide coated with adhesive poly L-Lysine for immunohistochemistry (IHC) to evaluate synaptophysin / chromogranin expression.

The technique for IHC included antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody against synaptophysin / chromogranin protein (Biogenex), linking with rabbit anti mouse secondary antibody (Biogenex), enzyme labeling with streptavidin-horseradish peroxidase, developing chromogen with diaminobenzidine (DAB) and counterstaining with haematoxylin. Positive and negative controls were run with each batch of slides. Only diffuse cytoplasmic immuno-reactivity was considered to represent positive staining.

RESULTS

Fifty one (51) cases of neuroendocrine tumors were reviewed for two and half year period. Of the 51 patients, 16 (31%) were females and 35 (69%) were males. Mean age was 53.2 years. Twenty three (45%) cases were less than 50 years and twenty eight (55%) cases were more than 50 years of age. Most common clinical symptoms were weight loss followed by pain in abdomen, dysphagia, diarrhea and back ache.

In 49 cases (98%), examination was based on biopsy alone whereas in 2 cases (2%), it was based only on surgical specimens.

Out of 51 cases, 25 (49%) had their primary site identified. In one case, the diagnosis was made in the metastatic site (liver) and the primary site (lung) was identified with the help of immunohistochemistry. The most frequent primary site was lung (10 cases). Liver (14 cases) was the most frequent site for metastasis followed by lymph Nodes (5). Appendix, spine, endometrium, epiglottis, supraglottis, gall bladder, stomach, ishchiorectal fossa, ovary, pituitary, mediastinum, vocal cords, paraortic and cavernous sinus were other primary sites making a total of 22 cases (43%) (Figure1).

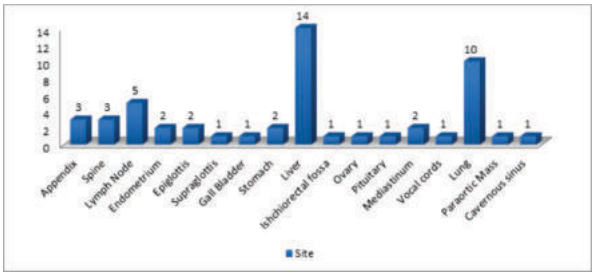

Figure 1 – Distribution Of Cases According To Site.

Immunohistochemical assays were used to identify the type and site of origin in 27 cases. The most commonly used markers were chromogranin in 26 cases, with 88 % positive results; synaptophysin in 26 cases, with 96% positive results; and CD56 in 18 cases, with 83% positive results, as shown in Table 1.

Table 1: Distribution Of The Neuroendocrine Markers.

Site	Total	Synapto-physin	Chromo-granin	Cd56
Appendix	3	3/3	2/3	1/3
Endometrium	2	2/2	2/2	2/2
Larynx	4	3/4	3/4	4/4
Cavernous sinus	1	1/1	1/1	1/1
Stomach	2	2/2	2/2	1/1
Gall bladder	1	1/1	1/1	1/1
Ischilorectal fossa	1	1/1	1/1	-
Pituitary	1	1/1	1/1	1/1
Mediastinal	2	2/2	2/2	1/1
Ovary	1	1/1	1/1	-
Para-aortic mass	1	1/1	1/1	-
Lung	8	7/7	6/7	3/5
Total	27	25/26 (96%)	23/26 (88%)	15/18 (83%)

Other markers, depending on the organ where the tumor originated, were important for diagnosing the primary site. TTF1 was used in 6 cases with 100% positives, AE1/AE3 in 30 cases (41.2%) with 26/30 (86%) positive results, CD99 in 8 cases with 7/8 (87%) positive results, NSE in 13 cases with 12/13 (92%) positive results; as well as other markers, such as CD138, SATB2, CDX2, CA-19.9, FLi1, P53, CK7 and Ck20.

The proliferation index was evaluated based on the Ki67 marker in 51 cases, with low index (NET G1) results in 5 cases, moderate index (NET G2) in 6 cases, and high-grade (NEC) in 40 cases.

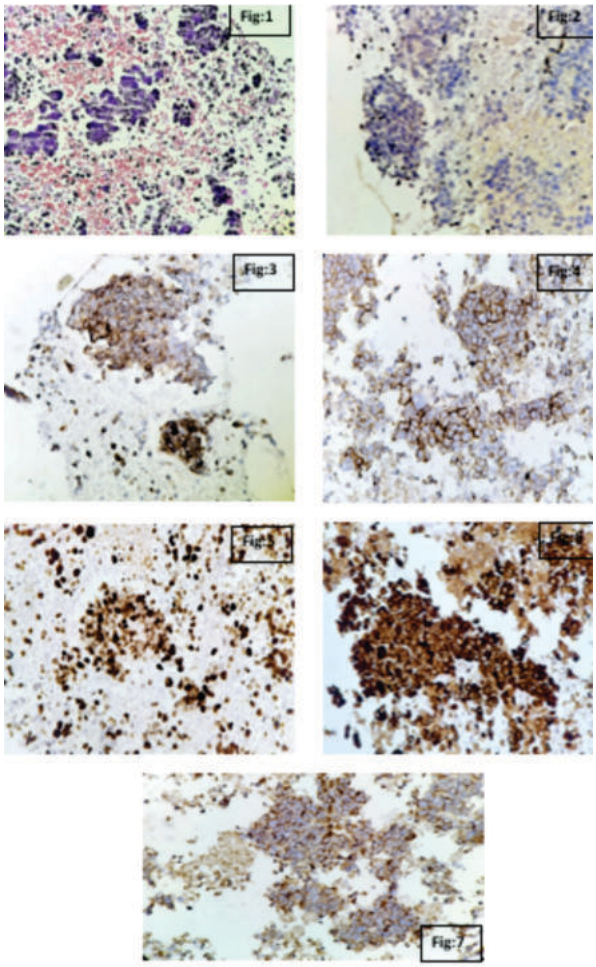

Fig1: Tumor cells forming nest and sheets having round oval nucleus with moderate cytoplasm., **Fig2:** CEA –ve, **Fig3:** Chromogranin +ve, **Fig4:** CD56 +ve, **Fig5:** Ki 67(>80%), **Fig6:**NSE +ve, **Fig7:**Synaptophysin +ve

DISCUSSION

In our study, we present data similar to the current literature regarding the age distribution of neuroendocrine tumors, gender predominance, and increased incidence in recent years, mainly due to greater access to complementary diagnostic tests, such as imaging and endoscopic procedures, and the availability of immunohistochemical markers that are

specific for neuroendocrine neoplasms.[1-3,6,9,12-15]

Of the 51 cases in our study, the location of the primary tumor was not known in 24 cases. In the study done by Silveira et al[1] on 250 cases, location of the primary tumor was not known in eight cases (3%). In the work conducted by Alsina et al[2], 4.7% of the cases involved an unknown primary site, while the work by Taal and Visser[6] presented no case with an unknown site. Liver was the most affected site, with an unknown primary site, consistent with the data in the literature.[9,14]

Neuroendocrine tumors can affect any organ, with case reports involving the pancreas, bladder, esophagus, larynx, retroperitoneum, cervix, ear and liver.[1-4,9,14] Such findings are in concordance with our study. The finding in our study coincides with that reported by Silveira et al.[1], Alsina et al.[2] and Calderella et al.[3] , with lung being the most frequently affected organ. In the study carried out by Taal and Visser[6], the appendix was the most frequent primary site (27%).

Immunohistochemistry panel of CD56, synaptophysin and chromogranin is useful in diagnosis of neuroendocrine tumors; nevertheless, it was used in 100% of cases.

The distribution of lung neuroendocrine tumors was similar to that in the literature, with small-cell carcinomas being the most frequent type, and liver being the site most frequently affected by distant metastasis.[13,16.]

With regard to gastric NETs, the diagnostic diversity encountered in this group is due to a change in nomenclature, and the subjectivity of each pathologist as well as particularities in writing histopathological reports, which could lead to bias, especially regarding data collection. Classification has changed over the years. In 2000, the WHO classified them as well-differentiated endocrine tumors (secretory or not); well- and poorly differentiated endocrine carcinoma; and tumor-like lesions (hyperplasia and dysplasia). In the current classification (2018), the nomenclatures well-differentiated neuroendocrine tumor (NET G1 and NET G2) (secretory or not) grade 1 (low grade, synonymous with carcinoid) and grade 2 (well -differentiated neuroendocrine carcinoma, intermediate), and poorly differentiated neuroendocrine carcinoma (NEC) were subdivided into small- and large-cell carcinomas. An example of how classification has changed is the fact that low-grade neuroendocrine neoplasm used to be interpreted as a low proliferative index neuroendocrine tumor and, according to the old classification system, the term used was a carcinoid.[8,12,14] No cases of mixed adenoneuroendocrine tumors (MANETs) and carcinomas (MANECs) were observed in this database. In the case of tumors of the GI tract, NET GII was the most frequent type, consistent with the literature.[12,14]

As for tumors of the caecal appendix, the most frequent diagnosis was that of carcinoid tumors (nomenclature observed in the reports pathological), none of which presented with metastases,[6,12,14] therefore also consistent with findings reported in the literature. According to them, neuroendocrine tumors of the cecal appendix usually have a good prognosis, and are classified as carcinoid tumors (NET G1 or NET G2) or neuroendocrine carcinoma. The most frequently affected group is that below 50 years of age, Ki67 > 3% and mitotic index > 2 mitoses/mm2.[9]

Of all NETs of the pancreas, neuroendocrine carcinoma was the most frequently encountered, and some cases presented as functional tumors. According to the literature, these pancreatic tumors develop in the islets of Langerhans and are generally well- or moderately differentiated and classified according to the type of hormone or peptide secretion. They may secrete insulin (insulinoma), gastrin (gastrinoma), glucagon (glucagonoma), vasoactive intestinal polypeptide (VIP – VIPoma) or somatostatin (somatostatinoma). The diagnosis of those that have no detectable hormonal secretion must be made exclusively on an anatomic-pathological basis.[9,12,14]

CONCLUSION

Our results were largely consistent with those in the literature, especially regarding age group, gender and location. Most metastases originated from high-grade tumors, with high Ki67 levels. The IHC panel of Synaptophysin, Chromogranin and CD56 is highly recommended for diagnosis of neuroendocrine tumors. The lung is the most common site of primary tumor and liver and lymph nodes are the most common sites of metastasis.

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