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Original Research Article

Neuroendocrine neoplasms of gastro intestinal tract with special reference to immunohistochemistry markers at a tertiary care hospital

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ABSTRACT

Background: Neuroendocrine neoplasms (NEN) are rare entity comprising ~2% of all malignancies with the gastro intestinal tract (GIT) and the lung being the most common sites. They are classified as epithelial and non epithelial based on their origin. The epithelial type arise from epithelial neuroendocrine progenitor cells and non-epithelial type are derived from the neural crest. According to the latest WHO classification 2022, NEN are categorized into neuroendocrine tumors (NET), neuroendocrine carcinoma (NEC) and mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN). The above-mentioned classification is possible with the help of use of immunohistochemistry neuroendocrine markers namely synaptophysin, chromogranin-A and proliferation marker as ki67. Synaptophysin is entirely sensitive, and less specific. Chromogranin A is an excellent marker to follow tumour progression and recurrence but is less useful for the diagnosis as it can be elevated for multiple reasons and is therefore nonspecific but very sensitive.

Materials and Methods: This study includes 25 cases, with study duration of 2 years including all the cases of neuroendocrine tumours of gastrointestinal tract excluding pancreas. Routine H&E staining was done followed by Immunohistochemical markers consisting of synaptophysin, chromogranin-A and ki67 markers.

Results: In our study the affected mean age was 51.8 and there was slight male predominance (1.2:1). The most common site being duodenum followed by stomach, and least affected was oesophagus. Out of total 25 cases of Neuroendocrine neoplasms, 18 cases (72% were diagnosed as NET, which were further categorized into G1 (32%), G2(28%) and G3(12%). Neuroendocrine carcinoma was seen in 04 cases (16%) and 03 cases were of MiNEN.

Conclusion: The GI-NETs are rare but their incidence and prevalence have been increasing. Due to the improvement and advancement in the diagnostic tools and the knowledge about these tumors has helped in diagnosing more of these tumors early and accurately. For accurate grading and pathological diagnosis. It is important to carefully evaluate hot spots for the Ki-67 index, identify areas of the highest mitotic density for mitotic count, and recognize the characteristic histological features of GI-NETS.

1. Introduction

Neuroendocrine neoplasms are heterogenous group of epithelial and non-epithelial neoplastic proliferations arising in different parts of our body.[1,2] They are also called as carcinoid tumor, the name was derived from the word coined by A German pathologist "Karzinoide" to indicate the carcinoma-like appearance.[3] Carcinoids are thought to arise from the enterochromaffin cells (Kulchitsky) cells found throughout the crypts of Lieberkuhn of the gut.[4] The enterochromaffin refers to the term “ability to stain with chromium or chrome salts, a common feature of 5-Hydroxy tryptamine-containing cells.”[5] Approximately two-thirds of neuroendocrine neoplasms (NENs) arise in the GIT

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and pancreas. Endocrine cells are interspersed within the mucosa of the and comprise approximately 1% of all mucosal cells. Grossly, these tumors commonly present as polypoidal or nodular circumscribed elevations which are located within the mucosa or submucosa.[6,7] These tumor cells contain cytoplasmic granules and secrete a variety of biogenic amines and hormonal substances that regulate the GI motility and secretion. These secretory products are responsible for a variety of signs and symptoms produced by these tumors.[6] According to the degree of differentiation, NET's are classified as well differentiated tumors which are further subdivided into G1, G2 and G3 based on expression of ki-67. The cornerstone for the diagnosis of NEC is the recognition of a poorly differentiated neuroendocrine morphology which is further divided into two different subtypes, small cell NEC (SCNEC) & large cell (LCNEC). Once a poorly differentiated neuroendocrine morphology is identified, the neuroendocrine nature must be confirmed by the immunohistochemical expression of neuroendocrine markers. In the 5^{th} edition WHO classification of endocrine and neuroendocrine, a new entity named mixed neuroendocrine-non-neuroendocrine neoplasms (MiNEN's) term has been coined and accepted.[8] By definition, mixed epithelial neoplasms in which a neuroendocrine component is combined with a non-neuroendocrine one, each of which is morphologically and immunohistochemically recognized.[9] Colorectal NETs are more frequent in the Asia/Pacific region than in Europe. In Europe, small intestinal and stomach carcinoids are more prevalent. Intestinal NETs have also been found to be more prevalent among African Americans as compared to whites.[10] The main aim of the study is to determine histopathological spectrum of NENs of GIT and immunohistochemical expression of Chromogranin-A, Synaptophysin and ki67 and to grade them according to the recent WHO classification (2022).

2. Materials and Methods

The present study was conducted at the Kamineni academy of medical sciences and research centre, LB Nagar, Hyderabad in the Department of Pathology. It is a prospective and retrospective study for a period of 2 years starting from June 2021 to May 2023. The present study included 25 cases of NENs of the GIT (excluding pancreas) reported in our department. The clinical data of the patient were recorded and samples were collected in 10% Neutral buffered formalin for routine histopathological examination. After overnight fixation, the specimen was grossed with 3 to 4 sections taken from the tumor. The tissue was processed and 4–5 micron thick sections were cut on microtome and stained by routine hematoxylin and eosin stain. Immunohistochemistry (IHC) was done in all NENs. Chromogranin-A, Synaptophysin and ki67 markers were performed on all the tissue sections. Expression of Ki-67 was assessed by staining with MIB-1 antibody which binds to the Ki-67 antigen. It shows nuclear positivity. The Ki-67 index has to be assessed in areas where the highest nuclear labeling is observed (hot spot areas) and at least 500-2000 cells are to be counted.[11] If there is any discrepancy between mitotic index and Ki67 index, the higher value should be considered for the grading.[12] Chromogranin-A and synaptophysin are usually combined along with Ki67 as a neuroendocrine marker panel. Grading for cytoplasmic staining of synaptophysin and chromogranin - A is evaluated as given in the Table 1.[13–15]

Table 1:

Grading	
0	No expression
+1	<2% of tumour cells
+2	3-30% of tumour cells
+3	31-50% of tumour cells
4+	>50% of tumour cells

3. Results

The present study included 25 cases of Neuroendocrine neoplasms of GIT (excluding pancreas) reported. The age range of the patients ranged from 23 to 81 years with most common affected age group being 51-60 years (24%) as shown in Figure 1. The mean age being 51.8 with male : female ratio being 1.2:1.

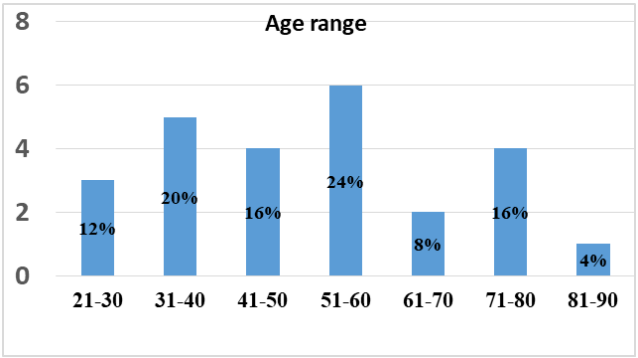

Figure 1: Age range of patients

In our study, 21 cases were symptomatic and 4 were asymptomatic. Most common symptom being pain abdomen followed by heartburn. The most common site was duodenum (08 cases-32%), followed by stomach (07 cases-24%), colon (04 cases-16%), rectum (02 cases-8%), appendix (02 cases-8%) and gastroesophageal junction (02 cases 8%) and oesophagus (01 case-4%) as shown in Figure 2.

Out of total 25 cases of NENs, 18 cases (72%) were diagnosed as well-differentiated NETs out of which, G1 comprised 08 cases (32%), G2 comprised 07 cases (28%)

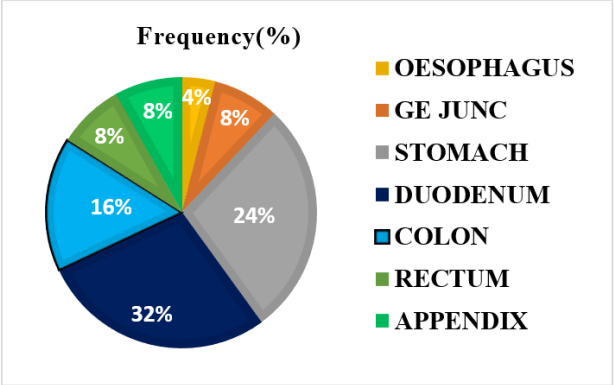

Figure 2: Distribution according to site

and G3 were 03 cases (12%) and 04 cases (16%) were of NEC. Out of which, 3 cases (12%) were of small cell neuroendocrine carcinoma and 1 case (4%) of large cell neuroendocrine carcinoma. There were 03 cases (12%) of MiNEN as described in table number 2 and in Figures 3 and 4.

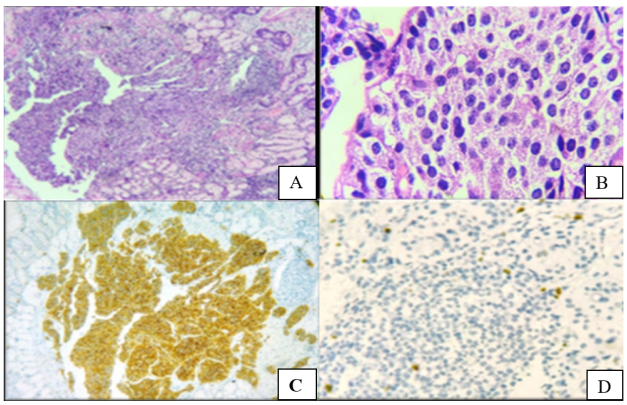

Figure 3: A): Well differentiated duodenal NET (H&E -40X); **B)**: Histomorphology of duodenal NET showing salt and pepper chromatin (H&E-400X); **C)**: Duodenal NET showing 100% synaptophysin cytoplasmic staining (IHC -100X); **D)**: Duodenal NET showing KI67 proliferative index as 6-8%, grade 2 (ki67 IHC-100X)

4. Discussion

According to Sippel et al. the estimated prevalence of NETs is 1–2 cases per 100,000 people, of which Gastrointestinal tract is the most common site.[16] The NETs are rare neoplasms, but their incidence has been increasing probably because of advancement in various diagnostic modalities. The main aim of the study was focused on these rare tumours. Most affected patients were of 51-60 years of age, with a mean age being 51.8 years. It was in concordance with studies done by Amrapurkar et al.[17] Zeng et al.[18] Lee

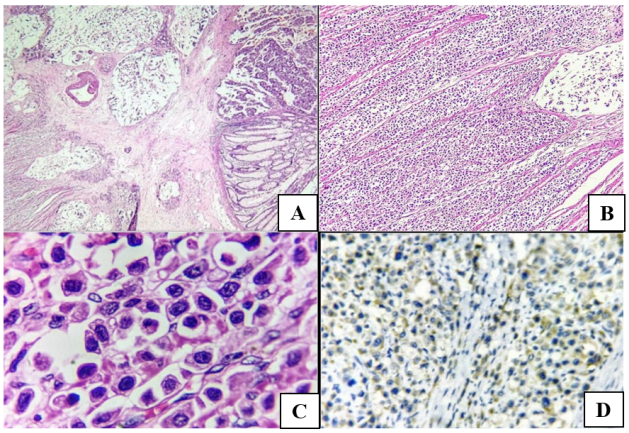

Figure 4: A): Histomorphology of rectal MiNen (Left side of the picture shows Neuroendocrine component and right side shows adenocarcinoma component- [H&E -40X]); **B)**: Histomorphology of neuroendocrine carcinoma, arranged in trabeculae and cords (H&E 100X), **C)**: High power view showing salt and pepper chromatin and prominent nucleoli (H&E -400X), **D):** Neuroendocrine component showing cytoplasmic synaptophysin positivity –(IHC-SYN-400X)

Table 2: Histopathological grading of neuroendocrine neoplasm

S. No	Differentiation of NEN	Grading	% of cases in my study
1	Well differentiated	G1	32%
		G2	28%
		G3	12%
2	Small cell neuroendocrine carcinoma		12%
3	Large cell neuroendocrine carcinoma		4%
4	MiNEN		12%

et al.[19] The chief symptom in our study was pain abdomen which was also seen in the study conducted by Zeng et al.,[9] abdominal pain was the most common symptom which was present in 99 patients out of a total of 122 patients (77.9%). In a study by Niederle et al.,[12] the most common presenting symptom was also abdominal pain (29.5%) followed by diarrhoea (8.7%). In the present study, most common site involved was duodenum, which was seen in about 32% of cases, followed by stomach seen in 24% of cases and least was seen in oesophagus in about 4% of cases. This was in concordance with the study of Albores et al.[20] Based on the recent WHO classification 2022, a more emphasis is been given on MiNen. A diagnosis of MiNEN has a clinical impact on patient management and treatment, in turn depending on the two tumor components.[18] To diagnose as Minen, there should be at least 30% component of each of the two i.e neuroendocrine and a non- neuroendocrine component.[21] Although the prognosis

seems to be related to the biologically more aggressive component, both MiNEN components, independently of their morphology, may progress and metastasize, so they need to be characterized, quantified, graded separately and to be considered in terms of treatment.[9] In general, the MiNen neuroendocrine component, which is a NEC are expected to behave more aggressively than MiNEN's with a NET component. In Our study 3 cases of MiNen were diagnosed each in stomach, colon, rectum. NETs in the present study were analysed immunohistochemically for expression of Chromogranin A, synaptophysin and Ki-67/MIB-1. All 25 cases (100%) of the cases on IHC showed cytoplasmic positivity for Synaptophysin and chromogranin –A. Based on IHC staining features ,the NEN are sub divided into various subcategories as shown in table 2. Our study was in concordance with study conducted by Zeng et al. The most common was well differentiated neuroendocrine tumor Grade 1. Zeng et al[18] they found that G1 tumours comprised 55.7% of cases (68/122), G2 comprised 26.2% of cases (32/122), and 18.1% of cases (22/122) were G3 tumours. In a study conducted by Lim et al.,[22] the most common histological grade was G1 (74.5%) followed by G2 (13.7%) and G3 (11.8%) which was in concordance with our study. The IHC markers used also showed comparable results with most other studies. However, our study suggest that atleast two of the IHC markers one of them preferably being synaptophysin should be used in the analysis of NEN, which helps in resolving dilemma about the diagnosis. Furthermore, Ki-67 proved to be helpful in grading these neoplasms and it has prognostic implications, which is a subject for research.

5. Conclusion

The study was focused on NENs of the GIT. Due to the improvement and advancement in the diagnostic tools and the knowledge about these tumors has helped in diagnosing more of these tumors early and accurately. For accurate grading and pathological diagnosis, it is important to carefully evaluate hot spots for the Ki-67 index, identify areas of the highest mitotic density for mitotic count, and recognize the characteristic histological features of GI-NENs. Also, new entities like MiNEN should be diagnosed carefully.

6. Source of Funding

None.

7. Conflict of Interest

None.

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