

Spectrum of neuroendocrine neoplasms of GIT – a histomorphological study in a tertiary care centre

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ABSTRACT

Background. Neuroendocrine neoplasms are diverse in terms of sites of origin, functional status, and degrees of aggressiveness. Since neuroendocrine cells are ubiquitous in the human body, these neoplasms can arise in different organs, with gastrointestinal tract being most frequently involved. The past few years have seen a surge in the diagnosis of these neoplasms, which were earlier considered to be rare. Their nomenclature, classification, and diagnostic criteria are revamped frequently, as new knowledge emerges.

Aim. To study the histopathological spectrum of neuroendocrine neoplasms of gastrointestinal tract and assess the immunohistochemical expression of neuroendocrine markers in them.

Material and methods. Ours is a descriptive study of the distribution and pathologic characteristics of gastrointestinal neuroendocrine neoplasms in a tertiary care hospital in Kerala, over a three year period. Neoplasms were categorised based on 2019 updated WHO classification.

Results. Among the 59 cases, we observed a male predominance. (Male to female ratio – 1.8:1). Most patients were in 6th and 7th decades of life. Duodenum was most frequently involved followed by rectum and appendix. NET G2 and G1 constituted the predominant histologic grades (47% and 24% respectively). NEC and MiNEN were infrequent. All cases were positive for synaptophysin, with variable positivity for chromogranin. Ki67 helped establish the histologic grade. We also came across a rare case of neuroendocrine tumor with coexisting mucinous neoplasm in appendix.

Conclusions. With evolving knowledge and advanced imaging modalities, the incidence of these neoplasms is increasing with time. Histopathology is the mainstay of diagnosis and plays a decisive role in influencing management protocols and prognosis.

Introduction

Neuroendocrine neoplasms (NENs) are a diverse group of neoplasms composed of cells containing

dense-core neuroendocrine secretory granules in their cytoplasm. Accumulating evidence over the years has paved way for newer and updated clas-

sification of these neoplasms. The consensus meeting held in Lyon, under the auspices of the WHO Classification of Tumors Group established a unitary classification system for NENs, that was published in the 5th edition of 2019 WHO classification of tumors of the digestive system. Presently, NENs are categorised as well differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC).

Most of these neoplasms are neuroendocrine tumors and possess an indolent disease biology. 10–20% are neuroendocrine carcinomas, which are highly proliferative tumors characterized by rapid disease progression.[1]

These tumors have a diverse spectrum of clinical presentation with variable etiologies, clinical features, morphologic and genomic findings, and degree of aggressiveness.

The incidence and prevalence of NETs has increased substantially over time, with greatest rates of increase in USA, Canada and Norway.[2] Availability of powerful functional imaging modalities such as endoscopy & ultrasound guided fine needle biopsies, immunohistochemistry for a definitive diagnosis, better awareness and health care utilisation have contributed to the global rise in incidence.

Approximately two-thirds of NETs are found in the gastrointestinal tract, 25% occur in the lungs, and the remaining cases arise in other endocrine tissues, such as the thyroid.[3]

However, there are few concise reports which give the entire spectrum and prevalence of these tumors in the GI tract in our geographic area. In this study, we attempt to put forward our experience of gastrointestinal NENs.

Materials and methods

This is a two and half year retrospective and six months prospective study of all gastrointestinal neuroendocrine neoplasms reported in our hospital from July 2019–June 2022. Approval of institutional ethics committee was taken.

Clinical data, including gender, age at diagnosis, and anatomic locations, were obtained for all the cases from the hospital database. The cases were categorised based on 2019 WHO classification of tumors of digestive system (see **Table 1**).

Haematoxylin and eosin (H & E) stained sections were reviewed and histomorphological features were analysed.

Immunohistochemistry with synaptophysin (Rabbit polyclonal antibody), chromogranin (Mouse monoclonal antibody), CD56 and Ki 67 cell proliferation index (Mouse monoclonal antibody) were performed on fully automated immunostainer (Leica Bondmax) by using poly horse radish peroxidase (HRP) technique. The Ki-67 index was assessed in areas with highest nuclear labeling (hot spot areas). 500–2000 cells were counted manually for assessment.

Socio demographic data and characteristics of tumors were expressed as number, percentage and mean value.

Results

A total of 59 cases including biopsies and resection specimens were studied. Mean age at diagnosis was 56.13 years, with an age range of 14 – 82 years. Individuals in 7th and 8th decades were

Table 1. WHO 2019 Classification and grading criteria for neuroendocrine neoplasms of the gastrointestinal tract [7].

Terminology	Differentiation	Grade	Mitotic rate (Mitoses/2 mm ²)	Ki 67 index (%)
NET G1	Well differentiated	Low	<2	<3
NET G2		Intermediate	2-20	3-20
NET G3		High	>20	>20
NEC (Small cell type)	Poorly differentiated	High	>20	>20
NEC (Large cell type)			>20	>20
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

NET – Neuroendocrine tumor, G 1,2 & 3 – Grade 1,2 & 3, NEC – Neuroendocrine carcinoma, MiNEN – Mixed neuroendocrine-non-neuroendocrine neoplasm.

most commonly affected. We observed a male predominance with male: female ratio of 1.8:1. The gender distribution with respect to location is depicted in **Figure 1**. The diagnosis was made on endoscopic biopsies in 42 cases and resected surgical specimens were available in 17 cases.

Location

Most common site of involvement was duodenum (28.8%) followed by rectum (22%). Esophagus and GE junction were least frequently involved.

Grading

We came across 28 cases of NET G2, 14 cases of NET G1, 4 cases of NET G3, 5 cases of NEC and 5 cases of MiNEN. Three cases showed poorly differentiated adenocarcinoma with neuroendocrine differentiation. The distribution of NEN in

different locations and their grades is shown in **Table 2**.

Of the 10 appendiceal NEN, most belonged to pathological stage pT1 (4 cases) followed by pT3 (3 cases). One case had coexisting low grade appendiceal mucinous neoplasm (LAMN, **Figure 2**). Of the 17 resected specimens, nodal metastasis were found in three. **Figures 2-5** show the different grades of NEN encountered.

Among the MiNEN, all cases had an admixture of adenocarcinomatous areas and NEC (**Figure 5**).

On IHC, all cases showed diffuse cytoplasmic positivity for synaptophysin. 81% and 14% cases showed diffuse and focal positivity for chromogranin respectively. 5% cases were negative for chromogranin, of which majority were high grade NET and NEC. CD 56 was done in 21 cases and was found to be positive.

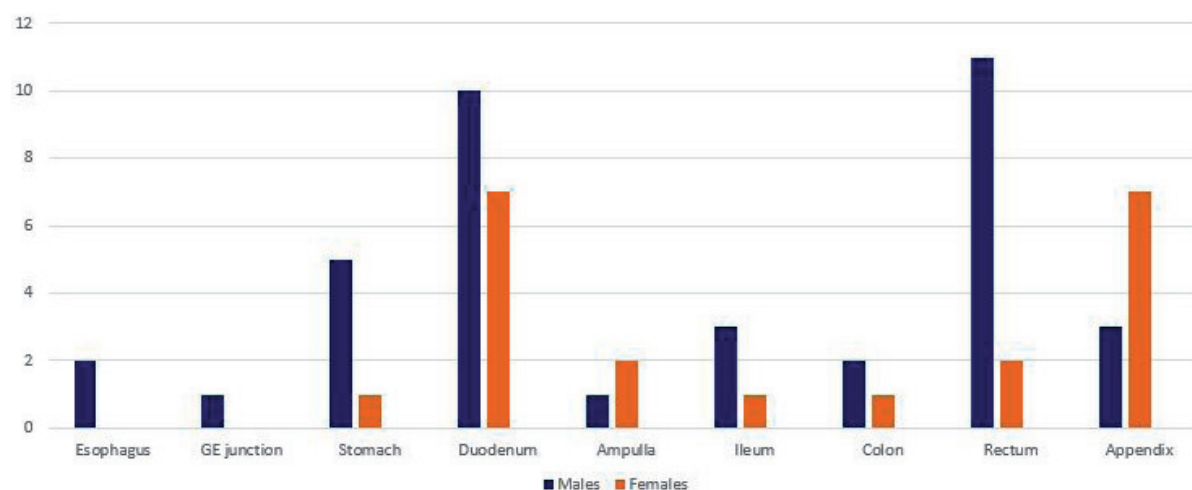


Figure 1. Gender distribution according to location.

Table 2. Distribution of neuroendocrine neoplasms by location and grade.

Site	No. of cases n (%)	G1	G2	G3	NEC	MiNEN	Neuroendocrine differentiation
Esophagus	2 (3.4)	0	0	0	2	0	
GE junction	1 (1.7)	0	0	0	0	0	1
Stomach	6 (10.2)	2	2	1	1	0	
Duodenum	17 (28.8)	4	12	1	0	0	
Ampulla	3 (5.1)	1	0	0	0	2	
Ileum	4 (6.8)	1	3	0	0	0	
Colon	3 (5.1)	0	0	0	0	3	
Rectum	13 (22)	1	7	2	2	0	1
Appendix	10 (16.9)	5	4	0	0	0	1

GE junction – Gastroesophageal junction, G1,2,3 – Neuroendocrine tumor grade 1, 2, 3, NEC – Neuroendocrine carcinoma, MiNEN – Mixed neuroendocrine-non-neuroendocrine neoplasm.

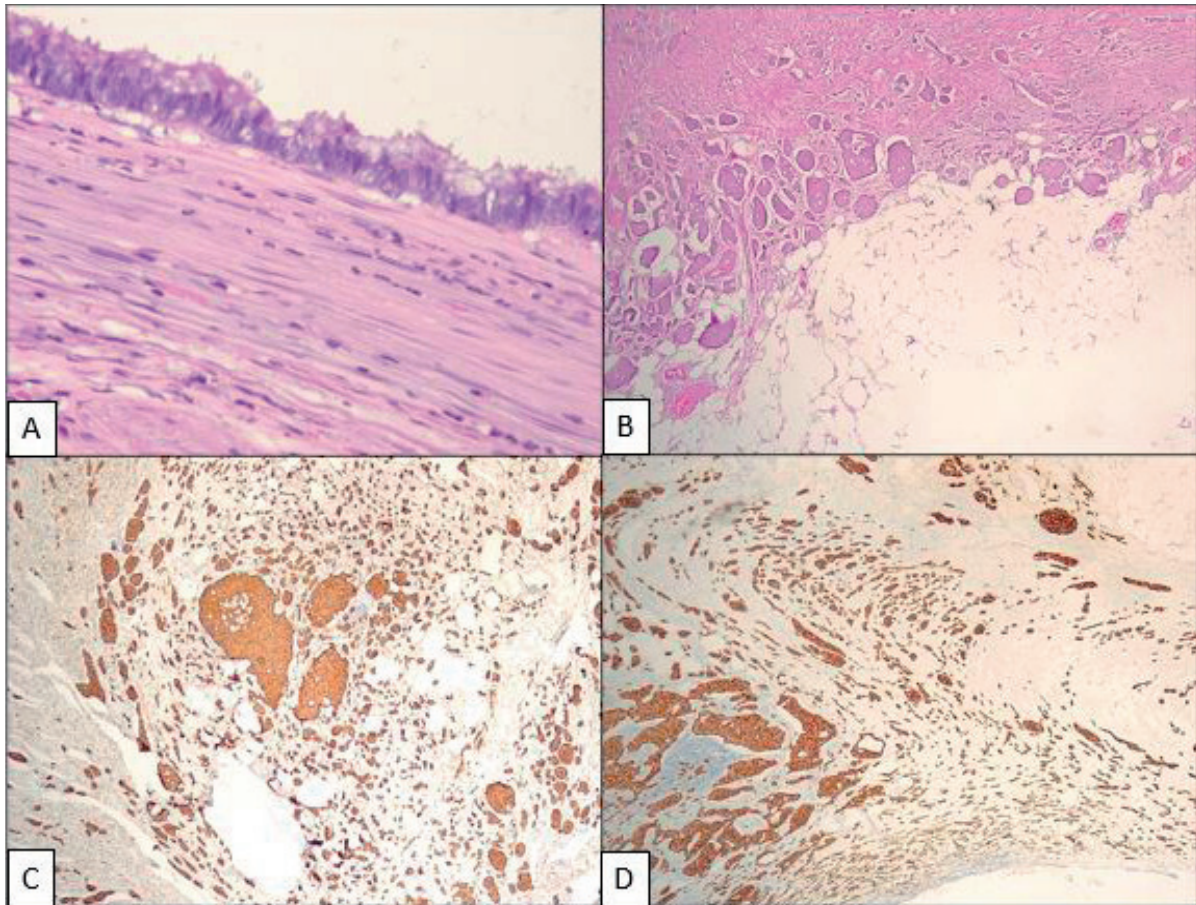


Figure 2. Low grade appendiceal mucinous neoplasm with neuroendocrine tumor grade 2. A – Appendix lined by mucinous cells with low grade nuclear atypia, atrophy of underlying lymphoid tissue and effaced muscularis mucosae, H&E, 100×. B – Neuroendocrine tumor extending into periappendiceal fat, H&E, 100×. C – Tumor cells showing diffuse positivity for synaptophysin, 100×. D – Tumor cells showing diffuse positivity for chromogranin, 100×.

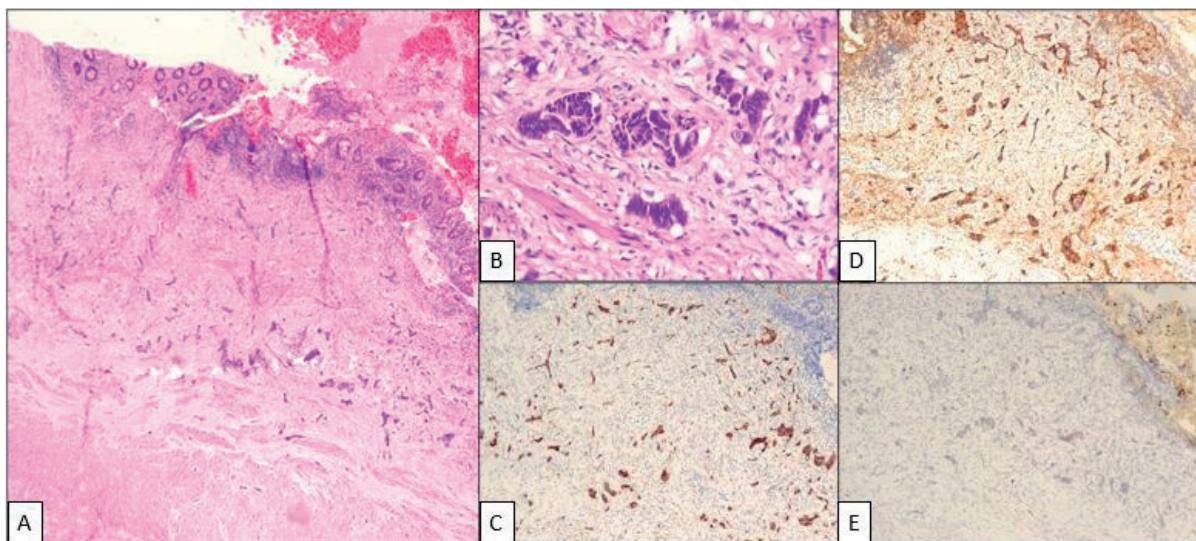


Figure 3. Neuroendocrine tumor grade 1, appendix. A – Appendix showing NET, H&E, 100×. B – Nests of neuroendocrine cells, H&E, 400×. C – Synaptophysin – diffuse positivity, 100×. D – CD56 – diffuse positivity, 100×. E – Ki 67 – 1%, 100×.

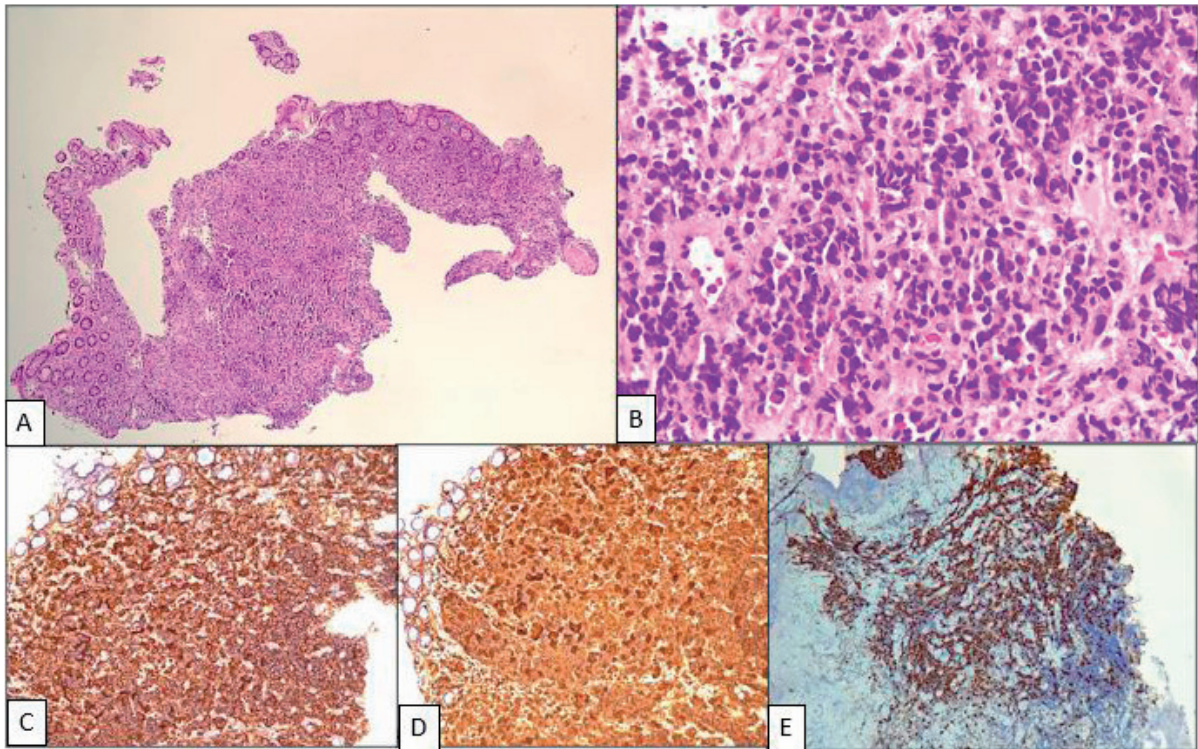


Figure 4. Neuroendocrine carcinoma, Rectum. A – Rectal mucosa showing infiltration by atypical cells, H&E, 40×. B – Small mildly pleomorphic atypical cells, H&E, 400×. C – Tumor cells show diffuse positivity for synaptophysin, 100×. D – Tumor cells show diffuse positivity for chromogranin, 100×. E – Ki 67 (86%), 100×.

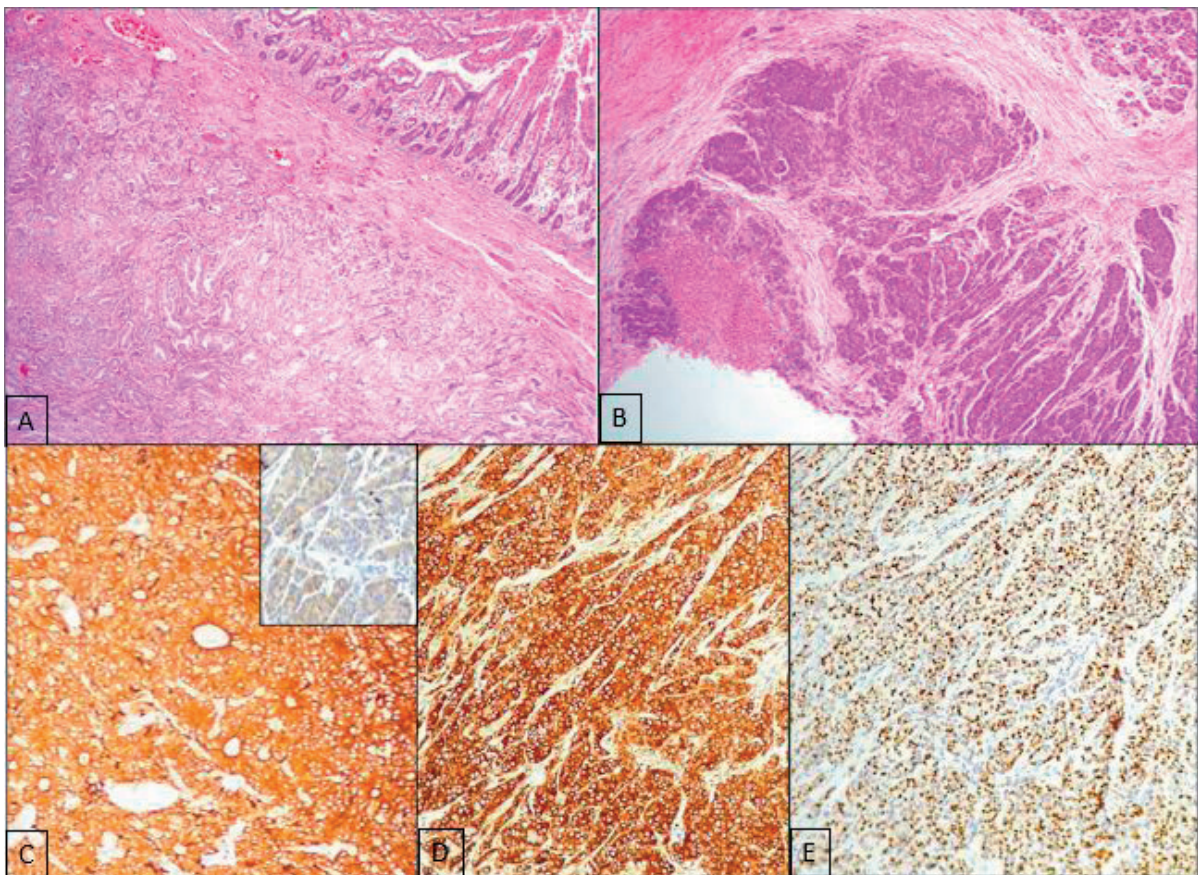


Figure 5. Mixed neuroendocrine non neuroendocrine neoplasm. A – Adenocarcinomatous component, H&E, 40×. B – Solid nests of neuroendocrine cells, H&E, 40×. C – IHC showing positivity for CK7 in glandular component, 400×. Inset shows CK7 negativity in neuroendocrine component, 100×. D – IHC showing positivity for synaptophysin, 100×. E – Ki 67 (60%), 100×.

Discussion

The neuroendocrine cells of GIT are derived from the neural crest, neuroectoderm, and endoderm. Endocrine cells are interspersed within the mucosa of the GIT and comprise approximately 1% of all mucosal cells.[4]

Pathologically, neuroendocrine differentiation is defined as architectural and cytological patterns reminiscent of non-neoplastic neuroendocrine cells (nesting or trabecular growth pattern and coarsely stippled chromatin) and production of characteristic neurosecretory proteins that can be detected by immunohistochemistry. These include synaptophysin and chromogranin A. Some authorities consider CD56 and neuron specific enolase (NSE) as adequate evidence of neuroendocrine differentiation.[5]

The World Health Organisation, in 2018, proposed a uniform classification framework for all NEN, according to which NEN are categorised into NET and NEC. The two groups differ in clinical, epidemiological, genetic and prognostic factors. Tumors with a non neuroendocrine component in addition to NEN (each component should be >30%) are categorised as MiNEN. NET and NEC are subcategorised as shown in **Table 1**.

The new category "NET G3" was first introduced for pancreatic tumors in 2017, and later extend to all gastrointestinal NEN in 2019. In the earlier classifications, NET G3 was considered to be synonymous with poor differentiation, i.e NEC. However, recent evidence suggests that NET G3 show differences in morphology, genotype, clinical features, and treatment response, compared to NEC.[6]

NECs have a less nested architectural pattern and abundant necrosis. They are said to arise from precursors that give rise to non neuroendocrine carcinomas of respective organs. Emerging genomic data on pancreatic NENs suggests differing mutation profiles in NET and NEC of pancreas. NETs more frequently harbour mutations in MEN1, ATRX, and DAXX; while TP53, Rb and SMAD4 are more commonly inactivated in NEC. Genomic comparisons in NEN of GIT are still emerging.[7]

Clinically, prognosis of NET G3 seems closer to that of NET G2 rather than that of NEC, but with a worse overall survival. Localised tumors are treated by surgery. For advanced tumors with

Ki67 index <55% alkylant based regimens are used, while platinum based chemotherapy is preferred for those with Ki67 index >55%.[6]

Studies show that NET G-3 are more often found in the pancreas with a frequency ranging from 10% to 65%. Other main tumor sites are the colon/rectum and stomach, with frequencies ranging from 8% to 24% and 8% to 29%, respectively.[6]

The most frequent primary sites of gastrointestinal NETs vary in different regions of the world. An epidemiological study by Satya Das et al[2] found small intestinal and colorectal NETs to be most common in North America. In Europe, small intestine and pancreas were found to be most frequently involved. Rectal, gastric, and pancreatic NETs occurred in highest frequencies in Asia. The reason for these differences could be a combination of environmental factors and biological differences due to differing national demographics.[2]

A multicentre longitudinal NET registry from India reported the most common primary sites of NET to be pancreas (42.9%), small intestine (22.1%), colorectum (9%), and appendix (2.7%) in diminishing order.[8]

In our study, small intestine and rectum were most frequently affected, similar to other Indian studies.[9,10,11] Studies in Brazil and Turkey found gastric NENs to be most frequent.

NETs of the esophagus are rare, representing only 0.04 -1% of all the gastrointestinal NETs reported.[7] Studies conducted in different regions show this anatomical location to be rarely involved, with a higher frequency in males, [3,9] as our study concurs.

The most common age group at presentation was 61-80 years, with a mean age of 56 years, comparable to Uppin et al and Zeng et al.[10,12] Studies conducted in Brazil[3], Kashmir[9], Japan [12], and Turkey[13] found a female predominance. Our study and Uppin et al[10] observed a male predominance, attributing no specific gender predilection.

Most NET were low grade tumors (G1 and G2), in concordance with studies across different regions. [3,9,10] NET G3 and NEC involved rectum predominantly. NECs are rare in the large bowel, representing approximately 0.6% of all carcinomas in this location. Nevertheless, they are more

common in rectum than in any other part of the intestine[3] as seen in our study as well.

MiNEN represent a rare diagnosis of the GI tract. Compared with the previous definition of mixed adeno neuroendocrine carcinomas (MAN-EC), MiNEN better represents the spectrum of variability of differentiation and morphology of these neoplasms.[7]

For a neoplasm to qualify as MiNEN, both neuroendocrine and non neuroendocrine component should be morphologically and immunohistochemically recognisable. Each component should constitute >30% of the neoplasm. In GI tract, both components are usually carcinomas. Rarely, the neuroendocrine component may be well differentiated.[7] These neoplasms may arise as a combination of two neoplastic clones or as the proliferation of one precursor cell with divergent differentiation. Molecular and genetic studies point towards a monoclonal origin of both components. The behaviour is thought to be driven by the neuroendocrine component.[14,15]

Li et al analyzed the pathologic characteristics of mixed colorectal glandular-neuroendocrine tumors in 87 cases. Majority of the lesions were located in the right colon (56%) and the left colon (41%), while rectum was uninvolved. Another case series by Guerrero et al[17] found colon to be most frequently affected. In our study as well, colon was most frequently involved, followed by ampulla. Three cases showed neuroendocrine differentiation, but did not fulfil the criteria for MiNEN, hence were categorised as adenocarcinomas with neuroendocrine differentiation.

Appendiceal collision tumors are extremely rare with very few cases reported in literature.[18] They result from proliferation of two different cellular lines. We came across one such case in a 40 year old male with a low grade appendiceal mucinous neoplasm (LAMN) and well differentiated NET G2. Management options for such cases depend on nodal status, pathological stage and margin status.

Markers of neuroendocrine differentiation include synaptophysin (imparts a diffuse cytoplasmic staining), chromogranin A and CD56. Chromogranin A is an acidic glycoprotein of the granin family, being expressed in well to moderately differentiated NENs and tends to be only focally positive in PD-NECs/SC-NEC.[19] CD56 is sensitive, but not highly specific marker for

NENs because it is often expressed in several non-NENs.

Conclusion

Our study focusses on NEN of the GIT, based on files of a single institution in Kerala. Mean age at presentation was 56.13 years with a male predominance. Duodenum was most commonly involved. Majority of the tumors were well differentiated NET grade 2. Tumors from colorectal region were mostly NEC. We also encountered a rare case of collision tumor in appendix. The present study represents a concise overview of spectrum of GI NEN, based on WHO 2019 criteria.

Histomorphology plays a pivotal role in tailoring treatment options in this diverse spectrum of gastrointestinal neoplasms.

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Conflict of interest statement

The authors declare no conflict of interest.

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