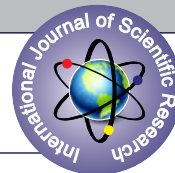


A CASE SERIES OF NEUROENDOCRINE NEOPLASMS OF GASTROINTESTINAL TRACT: A REAPPRAISAL OF NEWER CONCEPT OF THE UPDATED WHO CLASSIFICATION OF NEUROENDOCRINE LESIONS CITING A RARE & FASCINATING CASE OF COEXISTING INTESTINAL TUBERCULOSIS.



Pathology

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ABSTRACT

Neuroendocrine neoplasms are an important subcategory of tumours encountered in gastrointestinal tract and lung. Previously known as 'Carcinoid tumours', Considerable confusions prevailed regarding the nomenclature, diagnostic categories as well as prognostic classification of this group of tumours; until recently, in 2018, a uniform framework of classification was attempted by WHO. Here, we discuss nine cases of neuroendocrine neoplasms of GI tract diagnosed in our Institution over past two years, focussing on the application of this classification system, the key concepts behind it and also the points to be addressed in critical scenarios while making a diagnosis. A fascinating case of coexistent neuroendocrine carcinoma and intestinal tuberculosis is specially highlighted in this article, as probably, it is the first case reported of its genre till now, as far our knowledge goes.

KEYWORDS

Neuroendocrine tumour, carcinoid, MiNEN, synaptophysin, Ki-67 labelling index.

INTRODUCTION:

Neuroendocrine neoplasms are an important subcategory of tumours encountered in gastrointestinal tract and Lung [1], distinct from the more conventional epithelial tumours like adenocarcinoma and squamous cell carcinoma. The neuroendocrine system of cells are scattered throughout the body and are characterized by the presence of dense core neurosecretory granules in the cytoplasm on electron microscopy. Previously known as 'Carcinoid tumours', these may be associated with certain clinical syndromes due to excessive production of biologically active substances.

Considerable confusions prevailed regarding the nomenclature, diagnostic categories as well as prognostic classification of this group of tumours, until recently, in 2018, a uniform framework of classification was attempted by WHO. Here, we discuss nine cases of neuroendocrine neoplasms of GI tract diagnosed in our Institution over the last two years, elaborating the key concepts behind the latest WHO classification. A fascinating case of coexistent neuroendocrine carcinoma and intestinal tuberculosis is specially highlighted in this article, as probably it is the first case reported of its genre till now, as far our knowledge goes.

Case Reports:

Case 1: 38 years old male presented with features of acute intestinal obstruction. An exploratory laparotomy was undertaken, when the symptoms did not subside on conservative management. A growth was found in the wall of **small intestine** and segmental resection was done.

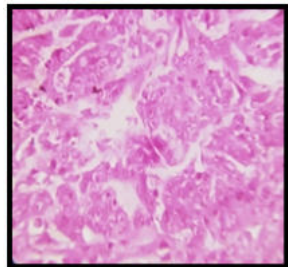


Fig 1

Fig 1: Large cell NEC, H & E, 400x (case 1)

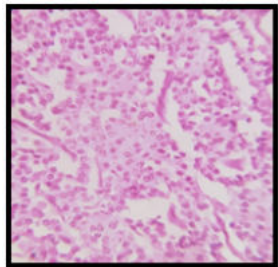


Fig 2

Fig 2: Grade 2 NET, H & E, 400x (case 2)

Gross examination revealed a 5cmx4cmx3cm mass involving the wall

of the gut, very close to one of the resection margins. No mucosal irregularity was noted. Microscopic examination revealed, a poorly differentiated tumour composed of sheets, nests and trabeculae of neoplastic cells with abundance of mitotic figures. Individual cell contained round, large, hyperchromatic nucleus with scant cytoplasm and prominent nucleoli. Focal positivity of Synaptophysin was found along with a high Ki-67 labelling index. A diagnosis of **High grade Neuro endocrine carcinoma (Large cell)**, stage T2Nx was made; the resection margin was found to be involved.

Case 2: A 42 year post menopausal old lady was undergoing evaluation for refractory anemia. Peripheral blood picture showed microcytic, hypochromic RBCs with marked anisopoikilocytosis; Serum iron profile was suggestive of iron deficiency anemia. Stool occult blood test was found to be positive. A colonoscopic procedure was undertaken, which revealed a large mucosa covered mass protruding in the lumen of ascending colon. Biopsy was taken; but before the arrival of report, the patient presented with features of intestinal obstruction and underwent radical resection.

On gross examination, an 8cmx6cmx4cm eccentric mass was found protruding in the lumen of **ascending colon**. Microscopic examination revealed, nests and ribbons of monomorphic tumour cells arranged in organoid pattern; individual cells having speckled salt pepper chromatin and granular cytoplasm. Brisk mitotic activity (8/10 HPF) was noted. Immunohistochemical examination showed strong synaptophysin positivity and a ki-67 labelling index of 10%. The tumour has not invaded the muscularis propria and no regional lymph node involvement was found. Final diagnosis was **Grade 2 Neuroendocrine tumour**, stage T2N0.

Case3 : A 48 years old male presented with features of acute intestinal obstruction. An exploratory laparotomy was undertaken, when the symptoms did not subside on conservative management. A growth was found in the wall of **small intestine** and segmental resection was done.

On gross examination, an 5cmx3cmx2cm eccentric mass was found protruding in the lumen of **small intestine**. Microscopic examination revealed, organoid nests and ribbons of tumour cells separated from the stroma by artifactual hollows; individual cells having speckled salt pepper chromatin and granular cytoplasm. Very low mitotic activity (0-1/10 HPF) was noted. Immunohistochemical examination showed strong synaptophysin positivity and a ki-67 labelling index of 2%. However, one of the two regional lymph nodes isolated showed

presence of metastatic deposit and the tumour was found to involve muscularis propria. Final diagnosis was Grade 1 **Neuroendocrine tumour**, stage T2N1.

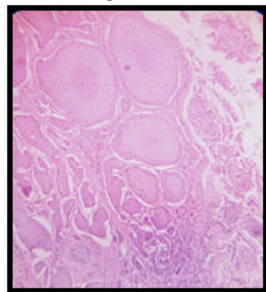


Fig 3

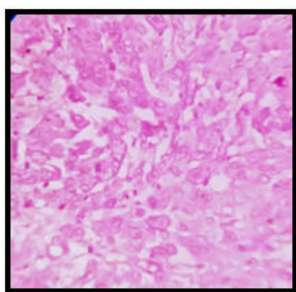


Fig 4

Fig3: Grade 1 NET, H & E, 100X (**Case 3**)

Fig 4: Large cell NEC, H&E, 400x (**Case 4**)

Case 4: An 85 year old man presented with altered bowel habit, hematochezia and spurious diarrhoea. On digital rectal examination a large, smooth, exophytic growth was noted spanning the **lower rectum and anorectal junction**. However, considering the age of the patient and associated comorbidities, radical resection was avoided; only a palliative resection was done.

Grossly an irregular polypoid mass measuring 4cmx3cmx2cm was received for histopathological examination. Microscopic examination revealed, a poorly differentiated tumour composed of sheets, nests and trabeculae of neoplastic cells with abundance of mitotic figures. Individual cell contained round, large, hyperchromatic nucleus with scant cytoplasm and prominent nucleoli. Very focal positivity of Synaptophysin was found along with a high Ki-67 labelling index. A diagnosis of High grade **Neuro endocrine carcinoma (Large cell)**, stage T2Nx was made.

Case 5: Forty years old male presented with features of acute intestinal obstruction. An exploratory laparotomy was undertaken, when the symptoms did not subside on conservative management. A growth was found in the wall of small intestine and segmental resection was done.

On gross examination, an 8cmx6cmx4cm eccentric mass was found protruding in the lumen of **small intestine**. Microscopic examination revealed, nests and ribbons of tumour cells arranged in organoid pattern; individual cells having speckled salt pepper chromatin and granular cytoplasm. Low mitotic activity (2/10 HPF) was noted, however the Ki-67 labelling index was 8%. Immunohistochemical examination showed strong synaptophysin positivity. Final diagnosis was Grade 2 **Neuroendocrine tumour**, stage T2Nx, as the tumour was found to involve muscularis propria.

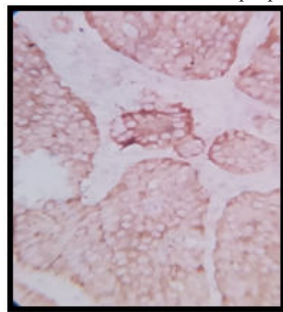


Fig 5

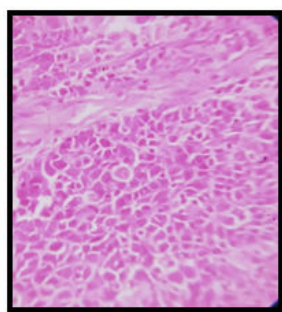


Fig 6

Fig 5: Grade 2 NET, Synaptophysin IHC, 400x (**Case 5**)

Fig 6: Small cell NEC, H&E, 400x (**Case 5**)

Case 6: A 55 year old male presented with altered bowel habit of recent onset with significant weight loss in last six months. Stool OBT was positive. Colonoscopy revealed a circumferential constricting growth in the **descending colon**. After Histopathological confirmation, a left hemicolectomy was undertaken with local nodal clearance.

On gross examination, a circumferential growth was found spanning 8 cm length of the gut. Microscopic examination revealed, a poorly differentiated tumour composed of sheets, nests and trabeculae of

neoplastic cells with abundance of mitotic figures with areas of necrosis. Individual cell contained round to oval, hyperchromatic nucleus with scant cytoplasm and prominent nuclear moulding. Focal positivity of Synaptophysin was found along with a high Ki-67 labelling index. The tumour was found to infiltrate the muscularis propria upto subserosal fatty tissue with involvement of regional lymph nodes (2/8). A diagnosis of High grade **Neuro endocrine carcinoma (Small cell)**, stage T3N1 was made.

Case 7: A 42 year post menopausal old lady was undergoing evaluation for refractory anemia. Peripheral blood picture showed microcytic, hypochromic RBCs with marked anisopoikilocytosis; Serum iron profile was suggestive of iron deficiency anemia. Stool occult blood test was found to be positive. A colonoscopic procedure was undertaken, which revealed a large mucosa covered mass protruding in the lumen of **Caecum**. Biopsy can not be taken during the procedure due to associated comorbid medical condition; repeat procedure was planned, but within this time, the patient presented with features of intestinal obstruction and underwent segmental resection.

Gross examination revealed a 7cmx4cmx3cm mass involving the wall of the gut, close to one of the resection margins. No mucosal irregularity was noted. Microscopic examination revealed, a poorly differentiated tumour composed of sheets, nests and trabeculae of neoplastic cells with abundance of mitotic figures. Individual cell contained round, large, hyperchromatic nucleus with scant cytoplasm and occasional nucleoli. Focal positivity of Synaptophysin was found along with a high Ki-67 labelling index. A diagnosis of High grade **Neuro endocrine carcinoma, Large cell**, stage T2Nx was made.

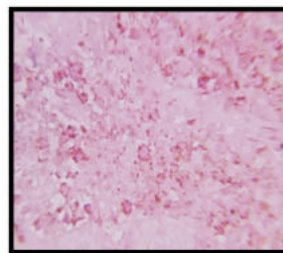


Fig 7

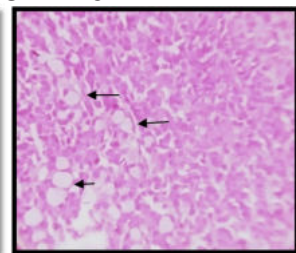


Fig 8

Fig 7: NEC, focal Synaptophysin Positivity, 400x (**case 7**)

Fig 8: MiNEN, H&E, 400x (signet cells shown with arrow) (**case 8**)

Case 8: A 62 years old lady presented with features of intestinal obstruction. An abdominal exploration was planned on failure of conservative management and a large mass in the **cecum**, in close relation to the ileo-cecal junction was revealed. The patient underwent radical resection.

On gross examination, a circumferential constricting growth was noted in the cecal wall spanning a length of 8 cm, obstructing the ileo-cecal orifice. No mucosal abnormality was noted grossly.

Microscopic examination revealed, a poorly differentiated tumour mass arranged in sheets and trabeculae with nuclear moulding and high mitotic activity. Overlying mucosa was free and devoid of any dysplastic change. The neoplastic cells were intensely intermingled with signet ring cells in different area of same sections, which showed strong cytoplasmic Periodic Acid Schiff (PAS) positivity.

Immunohistochemical examination showed focal Synaptophysin positivity, especially in the areas showing neuro-endocrine differentiation with a high Ki-67 labelling index. It was evident that two distinct population of neoplastic cells were there and each component exceeded the cut off criteria of 30%. So, a diagnosis of **MiNEN**, Stage T2Nx; comprising components of small cell neuroendocrine carcinoma and signet ring cell adenocarcinoma was rendered.

Case 9:

A fifty five years old male patient presented with abdominal distension, colicky abdominal pain, vomiting and absolute constipation in the emergency of a tertiary care hospital of East India; within 18 hours of onset of symptoms. On abdominal auscultation, the bowel sounds were increased and abdominal x-ray in the erect posture revealed multiple air-fluid levels. A provisional diagnosis of acute intestinal obstruction was made and conservative management with intravenous

fluid , Ryle's tube drainage and catheterization was initiated under appropriate medicinal coverage. However, exploratory laparotomy was decided to be done soon, when the patient showed no signs of symptomatic improvement.

Abdominal exploration inside OT revealed a closed loop obstruction, centered around an eccentric growth involving the wall of the small bowel. A segmental intestinal resection was carried out with end jejunostomy and mucous fistula creation;- the resected specimen was sent for histopathological examination.

On gross examination, a 5cm x 4cm x 4cm solid growth noted arising from the intestinal wall without any evidence of mucosal involvement. The growth was yellowish white on cut section and no serosal breach was noted. Multiple sections were submitted from the growth as well as from the normal looking mucosa.

Microscopic examination revealed, a poorly differentiated tumour composed of sheets, nests and trabeculae of neoplastic cells with abundance of mitotic figures. Individual cell contained round, large, hyperchromatic nucleus with scant cytoplasm and occasional nucleoli. The intestinal mucosa was absolutely normal without the slightest evidence of dysplasia. A provisional diagnosis of High grade Neuroendocrine Tumour/ Neuroendocrine carcinoma was made, which has involved the subserosal fatty tissue penetrating muscularis propria.

Now a startling finding made us bewildered. In several sections, we found multiple epithelioid granulomas intensely intermingled with the neoplastic cell population. The granulomas were morphologically identical to classical Tubercular granuloma, consisting Langhans giant cells and foci of micro-caseation.

Meanwhile the immunohistochemical examination was carried out. The tumour showed only focal synaptophysin positivity with a high (70%) Ki-67 labelling index, favouring the diagnosis of **Neuroendocrine Carcinoma, Large cell** (Stage pT3Nx).

Finally, It was diagnosed as a case of Neuroendocrine carcinoma (Large cell) with co existing Intestinal Tuberculosis. Ancillary investigations were suggested to confirm Koch's aetiology.

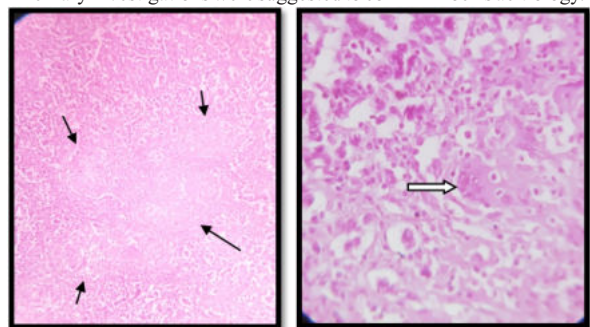


Fig-9

Fig-10

Fig 9: Neuroendocrine carcinoma with intermixed granuloma with microcaseation, 100x, H&E

Fig 10: Neuroendocrine carcinoma(in the left) with granuloma(right), 400x, H&E [Langhans giant cell is shown by arrow]

Summary:

Serial No.	Age (year)	Site	Diagnosis	Grade	Synapto physin staining	TNM stage	Associated features
case 1	38	small intestine	NEC, large cell	3	Focal	T2Nx	-
case 2	42	ascending colon	NET	2	complete	T2N0	-
case 3	48	small intestine	NET	1	complete	T2N1	-
case 4	85	rectum	NEC, large cell	3	Focal	T2Nx	-
case 5	40	small intestine	NET	2	complete	T2Nx	-
case 6	55	descending colon	NEC, small cell	3	focal	T3N1	-

case 7	42	caecum	NEC, large cell	3	Focal	T2Nx	-
case 8	62	caecum	MiNEN	3	Focal	T2Nx	-
case 9	55	small intestine	NEC, large cell	3	Focal	T3Nx	TB Granuloma

DISCUSSION:

Neuroendocrine tumour or NET (formerly known as carcinoid) and the neuroendocrine carcinoma(NEC), previously regarded as closely related neoplasms; are recently emerging as two completely divergent entities with respect to pathogenesis, clinical behaviour and long term prognosis.[2] As per the recent WHO classification, a uniform population of cells with stippled chromatin arranged in nests, cords, acini, ribbons and having positive expression of Synaptophysin or chromogranin A are essential criteria for designation of the term 'neuroendocrine tumour'. Neuroendocrine carcinoma most often shows only focal positivity of these two markers. Sheets and trabeculae of poorly differentiated cells, resembling the pattern of pulmonary Small cell or Large cell carcinoma with a high mitotic activity or Ki-67 proliferation index; is the only necessary criterion for Neuroendocrine Carcinoma. Necrosis is a common accompanying feature in NECs.

Another entity, previously known as MANEC (Mixed adeno neuroendocrine carcinoma) is now been renamed as MiNEN (Mixed Neuroendocrine-Non neuroendocrine Neoplasm); which necessitates presence of at least 30% of each component determined by both morphology and immunohistochemistry.[2] We encountered a similar tumour comprising components of both small cell neuro endocrine carcinoma and signet ring cell adenocarcinoma in **case 8**.

Metastasis and invasion are no longer important discriminatory features between NET and NEC. Even a grade 1 (well differentiated) Neuroendocrine tumour can metastasize, as evident from the **case no 3** in our study. So the differentiation between carcinoid and Atypical carcinoid in this regard is no longer in vogue, actually the ambiguous entity of 'Atypical Carcinoid' is not endorsed by WHO in the GI tract, in the latest edition.

A different staging system has been proposed for grade 1 and grade 2 NETs, in contrast to the grade 3 NET and NECs, which are to be staged according to the staging protocol for carcinoma in respective organs. Accordingly in **case 2**, the stage of NET was ascertained as stage T2 even if no muscularis propria involvement was found as the greatest dimension of tumour was 8 cm. (NET greater than maximum dimension of 2 cm are categorized as stage T2 irrespective of muscularis propria invasion).

The NETs are graded according to the mitotic index (number of mitotic figures per 10 HPF or 0.2 mm² area) and the Ki-67 labelling index; when there is discordance between the two, the one associated with higher grade is taken into account. As an example, in **case 5**, the grade should be 1 if the mitotic count (2/10HPF) is taken into account, but considering the Ki-67 labelling index of 8% final grade was determined as grade 2.

All NECs are considered as high grade neoplasm by definition. The difficulty arises in the discrimination between grade 3 NET and Neuroendocrine carcinoma. Certain NET can present with very high ki-67 labelling index, but this alone does not justify the use of term NEC. NECs, on other hand, may present with low ki-67 index especially after chemotherapy. So, the differentiation based on ki-67 index is not straight forward. The chance of inter-observer variability remains in this particular scenario, even after endorsing this newer classification.

Again no optimum cut off was set regarding assessment of synaptophysin/chromogranin positivity. So, the dilemma persists in case of a poorly differentiated carcinoma, wheather to or not to fix a neuroendocrine category; as subtle neuroendocrine differentiation is common even cases of conventional poorly differentiated adenocarcinoma. Presence of dysplasia in the lining epithelium favours the diagnosis of Adenocarcinoma, but again it becomes a vexing issue in case of MiNEN.

Special discussion on case 9: Tuberculosis is a major community

health problem in the developing countries like India. Especially after the emergence of Multi Drug Resistant and Extensively Drug Resistant strains, the drug therapy has become challenging. Primary intestinal tuberculosis is rare now-a-days, most often intestinal TB emerges as a sequelae of pulmonary Tuberculosis due to swallowing of the expectorated sputum. Caseating epithelioid granuloma with Langhans giant cells is the histological hallmark of Tuberculosis.

Many Tumours are known to present with florid granulomatous reaction, which often creates confusion regarding diagnosis especially based on cytology alone. A beautiful article focussing on this issue is published by Chhabra S et al.[3] This type of presentation is hitherto not reported in cases of neuroendocrine neoplasms. Coexisting other diseases like intestinal Crohn's or sarcoidosis may present with granuloma, but the classical picture with caseation and Langhans giant cells are very unlikely. Few such cases with coexistent sarcoidosis and neuroendocrine tumour are reported by Mungo N et al.[4]

Tuberculosis may mimic intestinal carcinoid both clinically and in imaging studies,[5] but the co-occurrence of these two is not commonly found. Only two cases are found in existing literature, again by Chhabra S et al from PGIMER, Chandigarh.[3] Similar case occurring in appendix is reported by L. Ionescu et al [6] and two case reports are found focussing on coexistence of colorectal carcinoma with intestinal tuberculosis-one from India [7] and another from Bangladesh.[8]

Probably, this is the first case report of co-occurrence of neuroendocrine carcinoma of small intestine and Intestinal tuberculosis. Further follow up regarding the final outcome could not be carried out as the patient reported to a different Institute for better treatment

CONCLUSION:

Genetic background, so far deciphered, has corroborated with the concept of this classification. NETs are commonly associated with ATRX, MEN1, DAXX mutations, which, on the other hand, are rare in NECs. They, more often, are associated with common mutations like RB and TP53. This is also supported by the good clinical response achieved with platinum based chemotherapy in NECs, which is not found in cases of NETs.[2]

Several case series focussing on gastro intestinal neuroendocrine tumours have been published till date.[9][10][11][12] Though clinically and morphologically related, (i.e just include the word related, otherwise the sentence does not make any sense) neuroendocrine neoplasms in different organs are heterogeneous from various perspectives. Specific morphological entities are associated with specific organs in some cases. New vistas are unfurling on the research front everyday--probably this would enable us to decrypt the evolving pathology of this group of tumours in a more comprehensive manner.

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