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Original Research Article

NEUROENDOCRINE NEOPLASMS OF GASTROINTESTINAL TRACT – A CORRELATIONAL STUDY OF HISTOPATHOLOGICAL FINDINGS AND IHC MARKERS WITH CLINICAL PROFILE

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Abstract

Background: Neuroendocrine neoplasms (NENs) of the digestive system are epithelial tumors with primary neuroendocrine differentiation. They originate from the diffuse endocrine system found in the - Gastrointestinal (GI) tract, Pancreas and Hepatobiliary system. Notably, their incidence has been steadily increasing. However, diagnosing neuroendocrine neoplasms poses significant challenges. The objectives were to classify NENs according to histomorphological subtypes and staging as per WHO criteria and TNM staging and to correlate histomorphology of NENs of digestive tract with the Immunohistochemical (IHC) markers like Chromogranin, Synaptophysin, Neuron specific enolase (NSE) and Ki67. **Materials and Methods:** This was a cross-sectional study of 31 cases of primary NENs of digestive tract, which were diagnosed and operated between 2008 to 2017. All slides were diagnosed by two senior histopathologists. Staging was done using WHO 2010 criteria and TNM staging. The proposed grading was based on proliferation has three tiers (G1, G2, and G3) with following definitions of mitotic count and Ki67 index. **Result:** Majority (64.5%) of patients had Grade III tumour. Most common tumor type was NEC (39%), followed by NET (35%) and MANEC (26%). The variables mitotic count and Ki67 showed very strong correlation using spearman's correlation analysis with rs value of 0.91. Mitotic count showed strong correlation with ki67 and therefore both the techniques are equivalent in terms of their predictability. Spearman's correlation analysis with tumor grade and ki67 showed strong correlation with rs value of 0.86. Out of the 31 cases studied, 28 (90%) cases showed Chromogranin A positivity. Synaptophysin and NSE positivity was seen in all 31 cases. **Conclusion:** Ki67 was helpful in grading these neoplasms, which in turn helps to predict prognosis and outcome of the disease. IHC markers – Chromogranin A, Synaptophysin and NSE help in diagnosis of NENs of digestive system.

INTRODUCTION

Neuroendocrine neoplasms (NENs) of the digestive system are epithelial tumors with primary neuroendocrine differentiation. They originate from the diffuse endocrine system found in the - Gastrointestinal (GI) tract, Pancreas and Hepatobiliary system. NENs account for approximately 2% of all GI tumors. Notably, their incidence has been steadily increasing. However, diagnosing neuroendocrine neoplasms poses significant challenges. Diagnosing certain NENs can be significantly delayed, taking up to 5-7 years from symptom onset. This lag results in advanced disease stages at diagnosis, with up to 50% of NEN patients having regional or distant metastases.[1] Patients with well-differentiated Grade 1/Grade 2 NETs with distant metastases have a median survival of 33 months. Median 5-year survival probability for these patients is only 35%.[2] The objectives of the study were to correlate histomorphology of NENs of digestive tract with the Immunohistochemical (IHC) markers like Chromogranin, Synaptophysin, Neuron specific enolase (NSE) and Ki67.

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MATERIALS AND METHODS

This was a cross-sectional study of 31 cases of primary NETs of digestive tract, which were diagnosed and operated between 2008 to 2017 in a tertiary medical center in Maharashtra. All the data used in the present study was obtained from the records of histopathology section of the department of pathology. The tissues of the test population received were evaluated by histopathological processing and examination (HPE). All the slides were evaluated by two senior histopathologists. The diagnosis of neuroendocrine tumor was made on both biopsies as well as resected specimens.

The test slides were examined along with the control sections in all batches of IHC. Positive control: Chromogranin A – Lung, Synaptophysin - Small Bowel, Neuron specific enolase – Stomach. Ki-67 - Breast cancer tissue which previously showed unequivocal strong immunoreactivity for HER2/Neu. Negative control – slides prepared and examined without adding primary antibody. Evaluation of IHC: A strong expression on cytoplasmic membrane and in the cellular cytoplasm to anti- chromogranin A antibody represented by brownish color is considered as positive for Chromogranin A. Synaptophysin was considered positive if there is cytoplasmic positivity. NSE was considered positive if there is cytoplasmic positivity. Classification of Neuroendocrine neoplasms of digestive system: Neuroendocrine neoplasms of digestive system were classified according to WHO 2010:3 1. NET G1 (Carcinoid), 2. NET G2, 3. NEC (Large cell/Small cell type), 4. Mixed Adenoneuroendocrine Ca (MANEC), 5. Hyperplastic and Preneoplastic lesion. Grading was performed based on morphological criteria and assessment of proliferation fraction. The proposed grading was based on proliferation has three tiers (G1, G2, and G3) with following definitions of mitotic count and Ki67 index.

RESULTS

There were 31 cases of NET diagnosed in the study period which included 17 male and 14 female patients. The diagnosis was made on biopsies in 13 cases and resected surgical specimens were available in 18 patients. [Table 1] shows the distribution of cases based on characteristics age, location, size of tumour, and gross features. The age of patients ranged from 18 to 82 years, with a mean of 53.12 years. The highest numbers of cases were seen in the age group of 41-60 years (42%). The grading was done according to WHO 2010 criteria. It included 6 cases (19.4%) of grade I, 5 cases (16.1%) of grade II and 20 cases (64.5%) of grade III. [Table 2] shows the grading according to of Neuroendocrine tumours in the study. Table 3 shows distributions of cases based on TNM staging. [Figure 1] shows the Correlation between mitotic count and Ki67. [Figure 2] shows the Correlation between Ki67 and tumor grade. The variables mitotic count and Ki67 showed very strong correlation using spearman's correlation analysis with rs value of 0.91. Mitotic count showed strong correlation with ki67 and therefore both the techniques are equivalent in terms of their predictability. Spearman's correlation analysis with tumor grade and ki67 showed strong correlation with rs value of 0.86. Similarly, correlation coefficient between mitotic count and tumor grade was 0.85 indicating strong correlation between two variables. [Table 4] shows the Distribution of cases according to IHC marker expression. Out of the 31 cases studied, 28 (90%) cases showed Chromogranin A positivity. Synaptophysin and NSE positivity was seen in all 31 cases.

Table 1: Descriptive characteristics of study participants (N=31).

Variable	Number (n)	%	Variable	Number (n)	%
Age in years	n	%	Location	n	%
0-20	1	3	Oesophagus	1	3.2
21-40	6	20	Stomach	4	13
41-60	13	42	Ampullary region	4	13
61-80	10	32	Small Intestine	3	9.6
>80	1	3	Appendix	4	13
Size of tumor (in cms)	n	%	Colon & Rectum	6	19.3
0-4	10	55.5	Anal Canal	0	0
4.1-8	7	39	Liver and intrahepatic biliary duct	5	16.1
>8	1	5.5	Gall bladder & Extrahepatic biliary duct	1	3.2
Gross features	n	%	Pancreas	2	6.4
Polypoidal growth	7	38.9	Multicentric (Involving ileocaecal junction, caecum, appendix)	1	3.2
Nodular growth	4	22.3	Grade (WHO 2010 criteria) (n=31)	n	%
Ulceroproliferative growth	5	27.8	I	6	19.4
Thickened wall	1	5.5	II	5	16.1
Cystic lesion	1	5.5	III	20	64.5

Table 2: Grading according to location of Neuroendocrine tumours in the study.

Location	Grade 1	Grade 2	Grade 3	MANEC
Oesophagus	-	-	1(100%)	-
Stomach	1(25%)	-	3(75%)	-
Small intestine	2(40%)	-	1(20%)	2(40%)
Ampulla	-	-	1(50%)	1(50%)

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Appendix	1(25%)	3(75%)	-	-
Colon and rectum	-	2(33%)	1(17%)	3(50%)
Anal Canal	-	-	-	-
Liver and IHBD	-	-	4(80%)	1(20%)
Gall bladder and EHBD	-	-	-	1(100%)
Pancreas	1(50%)	-	1(50%)	-

Table 3: Distribution of cases based on TNM staging.

Variable	Number (n)	%	Variable	Number (n)	%
Location	**Number of cases**	**TNM Grading (Stage)**	**Location**	**Number of cases**	**TNM Grading (Stage)**
Oesophagus	1	T2N1Mx (Stage IIB)	4. Ampulla	1	T1N0Mx (Stage IA)
Stomach	1	T3NxMx (stage IIB)		1	T3N0Mx (Stage IIA)
	1	T2NxMx (Stage IIA)	5. Appendix	2	T1NxMx (Stage I)
	1	T3N1Mx (Stage IIIB)		2	T1NoMx (Stage I)
Small Intestine	1	T4N1Mx (Stage IIIB)	6. Colon and rectum	1	T3N0Mx (Stage IIB)
	1	T3N1Mx (Stage IIIB)		2	T3N1Mx (Stage IIIB)
	1	T1N0Mx (Stage I)	7. Pancreas	1	T2N0Mx (Stage IB)

Table 4: Distribution of cases according to IHC marker expression.

IHC markers	Positive n	(%)	Negative n	(%)
Chromogranin A	28	90	3	10
Synaptophysin	31	100	0	0
NSE	31	100	0	0

Table 5: Summary of expression of IHC markers (Chromogranin A, Synaptophysin and NSE) in neuroendocrine neoplasm in comparison to our study.

IHC markers	Jin-Hu Fan et al,[10]	Zhang et al,[11]	Uppin et al,[8]	Present study
Chromogranin A (Positivity in %)	67.6	97.7	95	90
Synaptophysin (Positivity %)	90	48.7	83.3	100
NSE (Positivity in %)	78.8	-	100	100

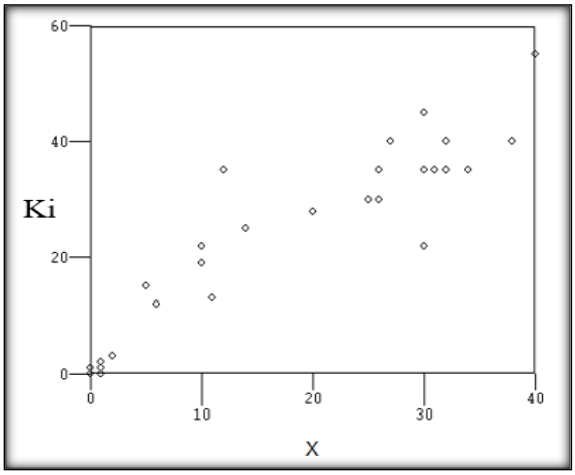

Figure 1: Correlation between mitotic count and Ki67.

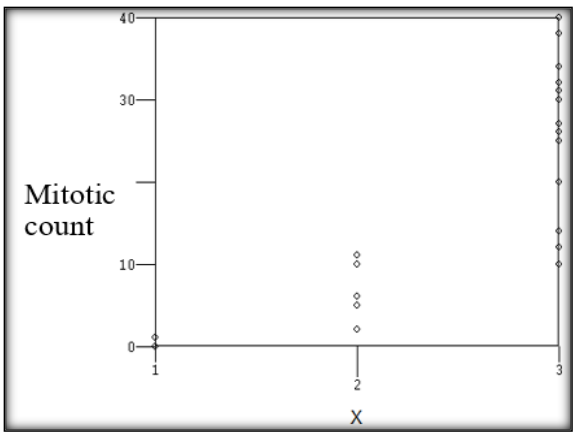

Figure 2: Correlation between Ki67 and tumor grade.

DISCUSSION

The nomenclature and classification of neuroendocrine neoplasms has undergone significant change in last few years. The neuroendocrine neoplasms of digestive system are rare accounting for 2.5 to 5 cases per 100000.[4] The incidence of tumor is on rise especially the gastric and rectal tumors. However, there are very few concise reports which give entire spectrum and prevalence of these neoplasms in digestive system. In this study, we attempt to put forward our experience of neuroendocrine tumors of digestive system. All the cases diagnosed in the present study were sporadic and we did not find any case in association with MEN's syndrome.

In a study done by Joo Young Kim et al., they observed distribution patterns of neuroendocrine neoplasms in digestive system seem to be different between eastern and western population. The rectum (48%) was most frequent site of neuroendocrine neoplasms in GI tract of patients in Korea followed by stomach (15%) which was same as our study.[5] Like in our study, Kenichi Hirabayashi et al observed in their study that tumor cell nests in neuroendocrine neoplasms of GI tract were arranged in trabecular or sheet like pattern. They also observed that tumor cells possessed round or oval nuclei with salt and pepper chromatin and granular eosinophilic cytoplasm.[6]

In present study there was strong correlation between mitotic count and Ki67 labelling index. In a study done by Kimiloglu Sahan et al., on 21 cases of

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gastroenteropancreatic neuroendocrine neoplasms showed significant correlation with spearman's correlation analysis with r=0.684, similarly in our study also there was correlation between the two with r=0.91.[7] In another study done by Megha S. Uppin et al. on 28 cases of gastroenteropancreatic neuroendocrine neoplasms, showed significant correlation between Ki67 & mitotic count, Ki67 & tumor grade and mitotic count & tumor grade by spearman's correlation analysis. They had r value of 0.88, 0.52and 0.51 respectively. Similarly in our study there was significant correlation between them with r value of 0.91, 0.86 and 0.85 respectively.[8] Bruna Estrozi studied 773 gastroenteropancreatic neuroendocrine tumors out of which 566(73.2%) were of grade 1, 81(10.5%) were of grade 2 and 126(16.3%) were of grade 3. They also observed that all appendiceal neuroendocrine neoplasms were of grade 1 and 92.1% of oesophageal neuroendocrine neoplasms were of grade 3, while in our study 75% of appendiceal neuroendocrine neoplasms were of grade 2 and 25% were of grade 1. There was only one case of oesophageal neuroendocrine neoplasm in our study and it was of grade 3 i.e. neuroendocrine carcinoma.[9]

In the present study, Chromogranin A, NSE and Synaptophysin done on all 31 cases, showed 28(90%) of cases showed Chromogranin A positivity, 31(100%) cases showed Synaptophysin and NSE positivity. A study done by Jin-Hu Fan et al. observed that Chromogranin A was positive in 1243(67.6%) cases, Synaptophysin in 1296(90.0%) cases and NSE was positive in 612(78.8%) cases.[10] In another study done by M Zhang et al. they noted rate of positive immunohistochemical staining for Synaptophysin was 97.7% and for Chromogranin it was 48.7%, which indicated that Synaptophysin has high sensitivity and Chromogranin A has high specificity.[11] In a study done by M Uppin et al. they observed Chromogranin A was positive in 38 out of 40 cases (95%), Synaptophysin in 20 out of 24 cases (83.3%) and NSE was done on 4 cases all of which were positive.[8]

CONCLUSION

In conclusion, colon and rectum followed by stomach was the most common site for neuroendocrine neoplasms of digestive system. Majority of the neoplasms were of grade 1 and classified according to WHO 2010 classification. This study showed a significant correlation amongst Ki67, mitotic count and tumor grade. Thus, Ki67 was helpful in grading these neoplasms, which in turn helps to predict prognosis and outcome of the disease. IHC markers – Chromogranin A, Synaptophysin and NSE help in diagnosis of neuroendocrine neoplasms of digestive system.

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