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PROGNOSTIC ROLE OF KI-67 INDEX AND MITOTIC INDEX IN NEUROENDOCRINE TUMORS OF HETEROGENOUS ORIGIN

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ABSTRACT: Neuroendocrine tumors (NETs) can arise in various organs of the body and share many common pathologic features. Although there are various classification systems, World Health Organization (WHO) proposed classification of NETs based on differentiation and grading for prognostic and therapeutic implications. Ki-67 index and Mitotic index are the most important criteria for grading of NETs. Many studies have been done concerning prognostic role of Ki-67 index in NETs specific to organ systems, but fewer data are available about patients with NETs of heterogenous origin. The aim of our study was to evaluate the prognostic significance of Ki-67 index and mitotic index in NETs. A total of 50 patients undergoing multidisciplinary management were analyzed prospectively over a period of 4 years, from 2010 to 2014. Among various locations, NETs most commonly involved gastrointestinal tract. The study population was classified into three grades. Grade I (13 patients), Grade II (10 patients) and Grade III (27 patients). There was a significant association between tumor grading and metastasis where tumor grading served the most important predictor of outcome.

KEYWORDS: Grade, Ki-67 index, Metastasis, Mitotic index, Neuroendocrine tumor.

INTRODUCTION: The definition of a neuroendocrine cell has changed over the last few years as our understanding and experimental techniques have advanced. The following criteria are now generally accepted as defining neuroendocrine cells: the production of neurotransmitter, neuromodulator or neuropeptide hormone; the presence of dense-core secretory granules from which the hormones are released by exocytosis in response to an external stimulus; and the absence of axons and synapses.^[1,2] In practical terms, molecular markers are invaluable in defining neuroendocrine cells.^[3] With the discovery of neuroendocrine phenotypes developing in cells such as immunocytes and certain neoplastic cells such as small cell carcinomas, it has recently been proposed that activation of specific genetic switches leading to neuroendocrine phenotypes should also be included in the definition.^[4]

Neuroendocrine tumours (NETs) are therefore a very heterogeneous group arising from these neuroendocrine cells and may secrete hormones. NETs can arise in various organs of the body but share many common pathologic features. The World Health Organization's (WHO) definition of neuroendocrine tumours is 'morphofunctional' and is primarily based on microscopic characteristics, but incorporates immunohistological data (with such markers as the chromogranins, synaptophysin and non-specific enolase), special stains (e.g. silver), in addition to immunohistochemical stains for specific hormones which result in endocrine hyperfunction syndromes.^[5]

The classification and nomenclature of NETs is complex and confusing, in part because most studies have focused on tumors arising in a specific organ system. Site-specific proposals for nomenclature and classification differ in terminology and in the criteria for histologic grading and

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staging, and this has led to morphologically similar NETs being designated differently, depending on the site of origin. For now, there is no one single system of nomenclature, grading, or staging that is suitable for NETs at all anatomic sites. However, features such as the proliferative rate of the tumor and the extent of local spread are shared by most classification systems.^[6]

NETs can be classified by anatomic site of origin as Gastroenteropancreatic tumors, Lung and Thymus NETs, catecholamine secreting tumours (pheochromocytomas, Paragangliomas), medullary carcinoma of the thyroid, chromophobe pituitary tumours, Merkle cell tumors and small cell carcinoma cervix.

NETs can be classified by histopathology.^[7] The WHO guidelines divide NETs into 2 clinically distinct pathologic classes: Well and Poorly differentiated. Well-differentiated NETs have characteristic organoid arrangements of the tumor cells, with nesting, trabecular, or gyriform patterns.

The cells are relatively uniform and produce abundant neurosecretory granules, reflected in the strong and diffuse immuno expression of neuroendocrine markers such as chromogranin A and synaptophysin. Poorly differentiated NETs less closely resemble non-neoplastic neuroendocrine cells. They have a more sheet like or diffuse architecture, irregular nuclei, and less cytoplasmic granularity. Immunoeexpression of neuroendocrine markers is usually limited.^[8] Well-differentiated NETs can be classified as either grade I or grade II depending on proliferation and histology.

Poorly differentiated grade III NETs are characterized by rapid dissemination, resistance to therapeutic interventions, and a highly aggressive course. Grade I NETs are relatively slow growing. Grade II NETs have a less predictable, moderately aggressive course. Grade III NETs can be highly aggressive. Mitotic rate and proliferative index of the tumor are the most important features used for grading. Mitotic index (MI) is assessed by counting mitotic figures, usually expressed as the number of mitoses per 10 high-power microscopic fields (HPF). Proliferative index is expressed as the percentage of tumor cells labelled by immunohistochemistry for the proliferation marker Ki-67 (Ki-67 index).^[9]

The WHO guidelines also address tumor-node-metastasis (TNM) staging of NETs. NETs can be classified by whether or not they can produce hormonal substances. Functional NETs are associated with symptoms that can be attributed to the secretion of specific hormones or peptides.^[10] Symptoms usually include flushing, fatigue, diarrhoea, hypoglycemia, skin changes, abdominal pain/discomfort, and wheezing. Nonfunctional NETs, on the other hand, are only associated with symptoms related to increasing mass.

Although there are various classification systems, WHO proposed classification of NETs based on differentiation and grading for prognostic and therapeutic implications.^[7,11,12] Ki-67 index and number of Mitotic figures per 10 HPF are the features used for grading. The aim of our study was to classify NETs of heterogenous origin according to WHO [Table 1] and to assess the correlation of biological behavior with tumor grade.

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Grade	Gastroenteropancreatic NETs (WHO 2010)	Lung and Thymus (WHO 2004)	Others (WHO)
Well differentiated Grade I	MI <2/10 hpf and <2% ki-67 index	MI <2/10 hpf and <2% ki-67 index	MI <2/10 hpf and <2% ki-67 index
Well differentiated Grade II	MI 2-20/10 hpf and 3-20% ki-67 index	MI 2-10/10 hpf and 2-10% ki-67 index	MI 2-20/10 hpf and 3-20% ki-67 index
Poorly differentiated Grade III	MI >20/10 hpf and >20% ki-67 index	MI >10/10 hpf and >10% ki-67 index	MI >20/10 hpf and >20% ki-67 index

Table 1: WHO classification of NETs

MI – Mitotic index.

MATERIAL AND METHODS: A total of 50 cases undergoing multidisciplinary management in MNJ Institute of Oncology, Hyderabad were studied prospectively over a period of 4 years, from June 2010 to May 2014. Samples included were 14 biopsies and 36 surgical resection specimens. Available clinical data including patient age, sex, Imaging and surgical findings, details of therapy were recorded. Hematoxylin-eosin (H&E) staining was done for all cases. Neuroendocrine differentiation was confirmed immunohistochemically using antibodies directed against chromogranin and CD56.

Proliferation marker ki-67 immunohistochemistry was done for all the cases using MIB-1 antibody. Mitotic index was assessed by counting mitotic figures under high power (400x) and average was expressed as number of mitotic figures per 10 HPF. Ki-67 index was assessed by counting at least 1000 nuclei at high magnification (400x) without recounting the same area and the average was expressed as percentage.

Foci of necrosis were excluded. H & E and Immunohistochemistry slides were viewed and cases were classified according to WHO classification. Statistical analysis was done using SPSS 17 version software. Relationship between Tumor grade and metastasis was assessed by chi square test and p value. The results were considered statistically significant if the P value was <0.05.

RESULTS: Age of the patients ranged from 19 to 80 years, with age predilection in fourth decade (32%) followed by sixth decade (26%). 27 patients were males and 23 were females (M: F ratio = 1.2: 1). Males were commonly affected in fourth decade (20%), whereas females were in sixth decade (14%). NETs most commonly involved Gastrointestinal tract (16 cases, 32%) and majority were in ileum (4 cases).

Classification was done according to WHO [Table 2], of the 50 cases, 13 cases (26%) were well differentiated grade I [Fig 1], 10 cases (20%) were well differentiated grade II and 27 cases (54%) were poorly differentiated grade III [Fig 2].

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Figure 1: A: Well differentiated grade I NET showing organoid pattern arrangement of tumor cells with very low mitotic activity, B: Immunohistochemistry - Ki-67 index <2%.

Figure 1A, 1B

Figure 2: A: Poorly differentiated grade III NET showing sheet like arrangement of tumor cells with very high mitotic activity, B: Immunohistochemistry – Ki-67 index 65%.

Figure 2A, 1B

Organ	Number	WD Grade-I	WD Grade-II	PD Grade-III
Gastrointestinal tract	16	7	3	6
Thyroid	10	--	5	5
Lungs	7	1	--	6
Cervix	7	--	--	7
Adrenal Gland (Pheochromocytoma)	4	2	1	1
Carotid body tumor (Paraganglioma)	3	3	--	--
Breast	2	--	1	1
Larynx	1	--	--	1
Total	50	13	10	27

Table 2: Organ specific Grading of NETs

WD – Well differentiated, PD – Poorly differentiated.

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Of the 50 cases, 17 cases (34%) showed metastasis, of which 4 cases belonged to grade II and 13 cases to grade III [Table 3]. Association between grade and metastasis was calculated using 2x2 table. 4 cases showed metastasis among 10 grade II cases. P value was 0.65(>0.05) and chi square test value 0.2.

Among 27 grade III cases, 13 cases showed metastasis. P value was 0.02(<0.05) and chi square test value 5.2. Among 4 cases of grade II, 3 cases showed regional metastasis and 1 case showed distant metastasis. Among 17 cases of grade III, 4 cases showed regional metastasis and 13 cases showed distant metastasis.

Organ	Number	Metastasis	Grade II	Grade III
Gastrointestinal tract	16	5	3	2
Thyroid	10	5	1	4
Lungs	7	5	--	5
Cervix	7	1	--	1
Adrenal Gland (Pheochromocytoma)	4	1	--	1
Carotid body tumor (Paraganglioma)	3	--	--	--
Breast	2	--	--	--
Larynx	1	--	--	--
Total	50	17	4	13

Table 3: Organ specific Metastasis and Grade

DISCUSSION: NETs predominantly involve Gastrointestinal tract.^[12] We found similar result in our study (G.I.T- 32%) as described in the literature. Uncommon sites like Breast (2 cases), Larynx (1 case) NETs have been found in our study.^[13,14] Current consensus statements set forth by several organizations (WHO, College of American Pathologists, and ENETS) recommend evaluating the MI and Ki-67 proliferation index to predict behavior of these rare lesions.^[15] In our study, majority of the cases 27/50 (54%) belonged to grade III.

P value and chi square test were calculated using SPSS software to analyze association between tumor grade and metastasis. Metastasis in relation to grade II cases showed p value 0.65 which was >0.05 indicating insignificant association between tumor grade and metastasis. Chi square test value was also low (0.2). There was a significant association between tumor grade and metastasis in relation to grade III cases with a p value 0.02 which was <0.05 and chi square test value of 5.2. Regional and distant metastasis were analyzed in relation to tumor grade, 25% (1 case) of grade II tumors showed distant metastasis whereas 62% (13 cases) of grade III tumors showed distant metastasis indicating grade of the tumor was intimately associated with disease progression and extent of involvement.

In a study by Ashlie Nadler et al,^[16] 184 cases were classified according to WHO. Grade I-50%, grade II-36% and grade III-14% of cases. 36% of grade II cases showed distant metastasis where as 62% of grade III cases showed distant metastasis. In a study by Antonio Bianchi et al,^[17] 54 cases were analyzed and classified according to WHO. Grade I-75%, grade II-25% and grade III-5% of cases. All cases of grade III showed distant metastasis. Similar results were observed in our study in terms

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of most common location being Gastrointestinal tract and intimate association of biological behaviour of the tumor with tumor grade.

WHO recommends the use of tumor grade as it has been repeatedly shown to provide significant prognostic information. Management primarily depends on grading.^[9] Surgery is the main stay of treatment for localised resectable cases. Grade I and II tumors are treated by somatostatin analogues, radionuclide therapy and are kept on surveillance. Grade III tumors require aggressive therapy and chemotherapeutic agents like Everolimus, sunitinib, doxorubicin etc are administered.

Many studies have shown that survival rate was significantly affected by tumor grade and metastasis. Among Grade I tumors, 5 year survival rate was 82% where as in Grade III tumors, 5 year survival rate without distant metastasis was 38% and with distant metastasis survival rate dropped to 4%.

CONCLUSION: Though there are multiple classification systems for NETs, grading forms the basis of many systems. Proliferative rate, extent of disease – local spread, regional/distant metastasis are critical factors and are intimately associated. So Grading and staging of NETs should be included in the pathology reports as prognosis and management entirely differ for each grade.

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