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P53, KI-67, CD117 expression in gastrointestinal and pancreatic neuroendocrine tumours and evaluation of their correlation with clinicopathological and prognostic parameters

STOMACH

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Original Article

ABSTRACT

Background/Aims: Gastrointestinal and pancreatic neuroendocrine tumors (GEPNETs) originate from the cells of the endocrine system. Their molecular genetic mechanism of development and progression is complex and remains largely unknown. The purpose of this study was to review the gastrointestinal and pancreatic neuroendocrine tumors and to evaluate p53, Ki-67 and CD 117 expressions with their clinicopathological correlations.

Materials and Methods: Twenty-one patients were reviewed and classified as having well-differentiated neuroendocrine neoplasm (WDET, Grade I), well-differentiated neuroendocrine carcinoma (WDEC, Grade II) and poorly differentiated neuroendocrine carcinoma (PDEC, Grade III). We performed immunohistochemical tests to characterize the expession of the immunoreactivity for synaptophysin, chromogranin, p53, Ki67 and CD 117.

Results: Median age of 21 patients was 43 years. Thirteen (61.9%) patients were male and eight (38.1%) patients were female. Tumors were located in the stomach (38.1%), appendix (38.1%), duodenum (4.8%), ileum (4.8%), colon (9.5%), and pancreas (4.8%).

Conclusion: There was a statistically significant difference between well-differentiated endocrine neoplasm (Grade I), and well-differentiated endocrine carcinoma (WDEC, Grade II) and PDEC for Ki-67 >20% ($p<0.001$) (Pearson chi-square test). There was a statistically significant difference between WDET (Grade I), WDEC (Grade II) and PDEC (Grade III) for p53 positivity ($p<0.05$) (Pearson chi-square test).

Keywords: Gastrointestinal, neuroendocrine tumors, immunohistochemistry

INTRODUCTION

Gastrointestinal and pancreatic neuroendocrine tumors (GEP-NETS) originate from the cells of the endocrine system (1-3). They comprise approximately 2% of all malignant gastrointestinal tumors (1,4). The term "neuroendocrine tumor" defines these tumors better than the previously used "carcinoid" as it reflects the origin of these neoplasms (5). GEP-NETs are rarely seen heterogenous tumors, and it is difficult to predict their behaviour and prognosis (1,6).

Of the various organs wherein neuroendocrine cells have been identified, they are most prevalent in the mucosal epithelium of the gastrointestinal tract. It is known to be populated by at least 20 or more functionally distinct neuroendocrine cell types, each of which synthesizes, stores and secretes one or more polypeptide hormones and/or biogenic amines that are regarded as their specific secretory products. Upon their release, these products act as chemical messengers to orchestrate various secretory, motor and absorptive functions of the gut. On account of the multiplicity of endocrine cell types dispersed within it, gastrointestinal mucosa has often been regarded as the most versatile, complex and perhaps even the largest endocrine organ of the body (7).

According to WHO Classification of neuroendocrine tumors of the GEP system in 2010, these tumors are divided into well-differentiated neuroendocrine neoplasms (Grade I) that show either benign behaviour or uncertain malignant potential, well differentiated neuroendocrine carcinomas (Grade II) that are characterized by

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low grade malignancy, and poorly differentiated (usually small cell) neuroendocrine carcinomas of high grade malignancy (Grade III) (3,8-12,13-15).

To date, so many studies have been performed to predict the prognosis and behaviour of these tumors. In this study, we used immunohistochemical staining for synaptophysin and chromogranin to evaluate the diagnostic value of these markers and for p53, Ki-67 and CD117 to predict their behaviour and prognosis. We investigated p53, Ki-67 and CD 117 expressions to evaluate their correlations with clinicopathological and prognostic parameters in twenty gastrointestinal and one pancreatic neuroendocrine tumors. It was important to explore the role of CD 117 in gastrointestinal tract endocrine tumors hoping that targeted therapy may be possible for these tumors.

MATERIALS AND METHODS

Twenty-one patients who had GEP-NETs were identified from the archives of The Pathology Department from 1997 till 2010. We re-examined hematoxylin and eosin slides of the patients, and classified the NETs according to the WHO Classification. We retrieved the following information such as location, and diameter of the tumors, types of procedure performed, date of the initial diagnosis, phone numbers of the patients from the surgical pathology reports. Clinicopathological data were also obtained from the reports and electronic hospital records.

We re-examined the slides and surgical pathology reports and for each patient's type, location, surgical margins, local-distant metastases, diameter, pattern of the tumor, (Table 1), presence of atypia, necrosis, solid pattern, angio-perineural invasion, mitosis, dimensions of the tumor cell, depth of invasion and the microscopical findings around tumor.

Immunohistochemistry: We re-examined H&E slides and a 4-µm section was cut from a chosen block, kept at room temperature for 10-15minutes, and then incubated in an incubator at 60 ^{0}C for 45-50 minutes. Afterwards, we placed the slides in Ventana Benchmark XT autostainer (AEC v3 protocol). Then the slides were exposed to deparaffinization, cell condition, titration for 1 hour, Ab blockage, and staining with standard hematoxylin, standard Bluing Reagent. After these procedures, the slides were washed by hot soapy water, rinsed and dried. Then the slides were covered with liquid-based mounting medium.

p53 and Ki-67 showed nuclear staining, and CD 117 cytoplasmic staining. We accepted any nuclear staining of p53 for positivity. We scored Ki-67 nuclear staining based on the percentage of the area stained as 0 (no staining), 1(>2%), 2 (>20%). We also accepted any cytoplasmic staining of CD 117 for positivity.

Statistical analysis

While evaluating the data that we acquired from the study, SPSS (Statistical Package for Social Science) for Windows 17.0 programme was used for statistical analysis. Sociodemographical features and characteristics of the tumors were expressed as number, percentage (%) and median value. Pearson chi-square test was used for determination of difference between groups. We used Spearman correlation test for determination of the relationship between age, tumor diameter, Ki-67 index and the grade of the tumor The results were evaluated within 95% confidence interval and significance level of $p<0.05$.

RESULTS

The median age of 21 patients included in the study was 43 years (15-79), while 61.9% (n=13) of them were males, 38.1% (n=8) were females. From the present data, tumors were located in in the stomach (38.1%), appendix (38.1%), duodenum (4.8%), ileum (4.8%), colon (9.5%) and pancreas (4.8%). Besides, the cases were classified as Grade I (66.7%), II (19%), and III (14.3%) (Table 1). Distribution of other histopathological features of the cases are given in Table 1.

In Table 2, comparisons of the study cases with respect to their demographical and histopathological features are given. When we examined the characteristic features in the table, there was no statistical relationship between age and gender of the patients and types of the tumors ($p>0.05$).

We categorized the pathological atypia as negative-mild and medium-severe. Besides 85.7% (n=12) of the cases in Grade I, and 14.3% (n=1) in Grades II-III demonstrated negative-mild atypia with a statistically significant difference between them ($p<0.01$). Thirteen of 21 patients were categorized as WDET, Grade I (n=13) (Figure 3), WDEC, Grade II (n=4) (Figure 2), and PDEC, Grade III (n=4) (Figure 4).

Tumours with a diameter of ≥10 mm were also detected in 14.3% (n=2) of Grade I cases, 50% (n=2) of the cases. The diameter of the tumor was determined as ≥10 mm in 14.3% (n=2) of Grade I, 50% (n=2) of Grade II and 100% (n=3) of Grade III cases. Therefore statistically smaller number of Grade I tumors had diameters of ≥10 mm, when compared with Grade II, and III tumors with a statistically significant difference between them ($p<0.01$). Besides Spearman Correlation Analysis showed a statistically significant and positive (r=0.619) corre-

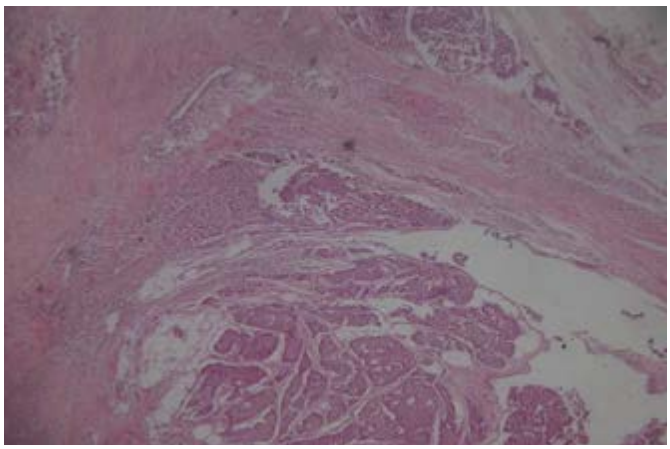

Figure 1. High p53 expression in poorly differentiated endocrine carcinoma (p53 x40).

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Table 1. Demographical and histopathological features (n=21).

Features	n	%	Features	n	%
Median age	43(15-79)		Necrosis		
≤40 years	10	47.6	Positive	5	23.8
>40 years	11	52.4	Negative	16	76.2
Genus			Loss of chromogranin A expression		
Female	8	38.1	Positive	5	23.8
Male	13	61.9	Negative	16	76.2
Tumor type/grade			Solid pattern		
Grade I	14	66.7	Positive	7	33.3
Grade II	4	19.0	Negative	14	66.7
Grade III	3	14.3	Perineural invasion		
Lokalization			Positive	8	38.1
Stomach	8	38.1	Negative	13	61.9
Appendiks	8	38.1	Anjioinvasion		
Colon	2	9.5	Positive	7	33.3
Duodenum	1	4.8	Negative	14	66.7
Ileum	1	4.8	Mitosis		
Pancreas	1	4.8	≤2	12	57.1
Surgical border			>2	9	42.9
Negative	16	76.2	Kİ-67 (%) index		
Positive	5	23.8	≤15	17	81.0
Atypia			>15	4	19.0
Negative	5	23.8	p53		
Milde	8	38.1	Positive	6	28.6
Medium	6	28.6	Negative	15	71.4
Severe	2	9.5	CD117		
Median tumor diameter(mm)	5(1-130)		Positive	8	38,1
≤10 mm	14	66.7	Negative	13	61.9
>10 mm	7	33.3	Chromogranin A		
Multifocality			Positive	16	76.2
Focal	19	90.5	Negative	5	23.8
Multifocal	2	9.5	Synaptophysin		
Tumor cell dimension			Positive	21	100
Small	8	38.1	Negative	0	0.0
Medium	10	47.6	Depth of invasion		
Large	3	14.3	TI	8	38.1
Local-distant metastasis			TII	7	33.3
Positive	6	28.6	TIII	4	19.0
Negative	15	71.4	TIV	2	9.5

lation between grade and tumor diameter (mm) ($p<0.01$). In consideration of these data, we thought that the tumor grade increased with the diameter of the tumor (Table 3).

When the dimensions of the cells were classified as small and medium-large, in 42.9% (n=6) of Grade I, and 100% of Grade II-III cases (n=7; 100%) medium-large tumor cells were found with a statistically significant difference between the groups ($p<0.05$).

Any evidence of necrosis was not found in Grade I cases, while in 50 % of Grade II (n=2), and 100% (n=3) of Grade III tumoral necrosis was detected with a statistically significant difference

Table 2. The demographical and histopathological features of the cases according to tumor type (n=21)

	Grade I		Grade II		Grade III			
	n	%	n	%	n	%	X^2	p
Age								
≤40 years	8	57.1	2	50.0	0	0.0	3.245	0.197
>40 years	6	42.9	2	50.0	3	100.0		
Genus								
Female	6	42.9	1	25.0	1	33.3	0.454	0.797
Male	8	57.1	3	75.0	2	66.7		
Atypia								
Negative-mild	12	85.7	0	0.0	1	33.3	10.904	0.004**
Medium-severe	2	14.3	4	100.0	2	66.7		
Tumor diameter (mm)								
<10	12	85.7	2	50.0	0	0.0	8.786	0.012*
≥10	2	14.3	2	50.0	3	100.0		
Cell dimension								
Small	8	57.1	0	0.0	0	0.0	6.462	0.040*
Medium-large	6	42.9	4	100.0	3	100.0		
Nekrosis								
Present	0	0.0	2	50.0	3	100.0	15.488	<0.001**
Absent	14	100.0	2	50.0	0	0.0		
Loss of chromogranin A								
Present	3	21.4	0	0.0	2	66.7	4.331	0.115
Absent	11	78.6	4	100.0	1	33.3		
Solid pattern								
Present	1	7.1	3	75.0	3	100.0	13.446	0.001**
Absent	13	92.9	1	25.0	0	0.0		
Perineural invasion								
Present	2	14.3	3	75.0	3	100.0	10.550	0.005**
Absent	12	85.7	1	25.0	0	0.0		
Anjioinvasion								
Present	1	7.1	3	75.0	3	100.0	13.446	0.001**
Absent	13	92.9	1	25.0	0	0.0		
Mitosis								
<2	10	71.4	2	50.0	0	0.0	6.385	0.041*
≥2	4	28.6	2	50.0	3	100.0		
Ki-67 index								
≤20	14	100.0	3	75.0	0	0.0	16.136	<0.001**
>20	0	0.0	1	25.0	3	100.0		
p53								
Positive	2	14.3	1	25.0	3	100.0	8.925	0.012*
Negative	12	85.7	3	75.0	0	0.0		
CD117								
Positive	6	42.9	2	50.0	0	0.0	2.221	0.329
Negative	8	57.1	2	50.0	3	100.0		
ChrgA								
Positive	11	78.6	3	75.0	2	66.7	0.197	0.906
Negative	3	21.4	1	25.0	1	33.3		
Depth of invasion								
≤TII	14	100.0	1	25.0	0	0.0	17.325	<0.001**
>TII	0	0.0	3	75.0	3	100.0		
Local-distant metastasis								
Present	0	0.0	3	75.0	3	100.0	17.325	<0.001**
Absent	14	100.0	1	25.0	0	0.0		

*=p<0.05, **=p<0,01; Chi-Square Tests (Pearson Chi-Square)

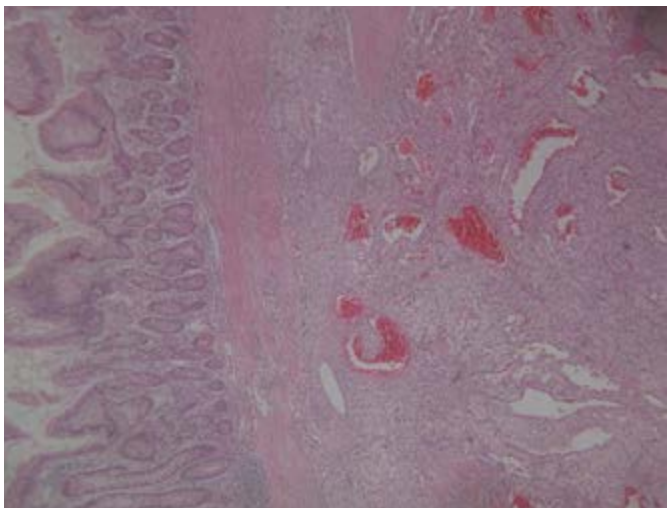

Figure 2. Well differentiated endocrine carcinoma (intestine) (H&E x100).

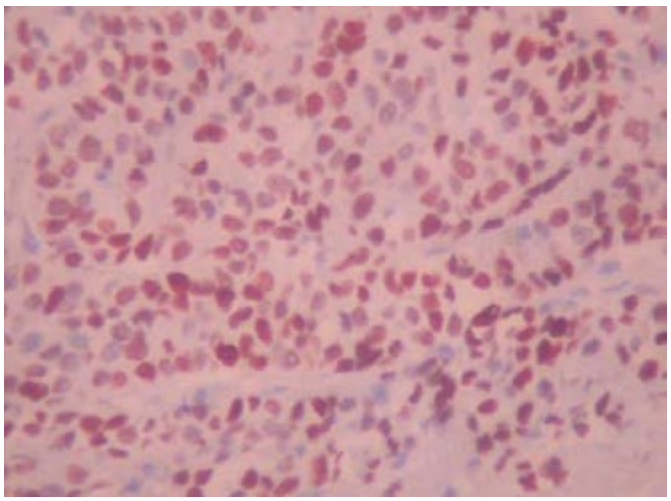

Figure 4. Poorly differentiated endocrine carcinoma (H&E x100).

between Grade I, and Grade II-III cases ($p<0.01$). There was no statistically significant correlation between the grade of the tumor and loss of chromogranin A expression ($p>0.05$).

We determined solid pattern in 7.1% (n=1) of grade I, but in 75% (n=3) of grade II and in 100% (n=3) of grade III cases. Angioinvasion was more frequently encountered in Grade I cases, with a statistically significant difference between Grade I and Grades II–III cases ($p<0.05$).

There was perineural invasion in 14.3% (n=2) of Grade I, in 75% (n=3) of Grade II, and 100% (n=3) of Grade III cases. Higher percentage of patients in Grade I had perineural invasion relative to Grade II, and III cases. The difference between them was statistically significant ($p<0.01$).

We determined angioinvasion in 7.1% (n=1) of Grade I, in 75% (n=3) of Grade II and 100% (n=3) of Grade III cases. Angioinvasion was more frequently encountered among Grade I cases with a statistically significant difference between Grade I, and Grades II-III cases ($p<0.05$).

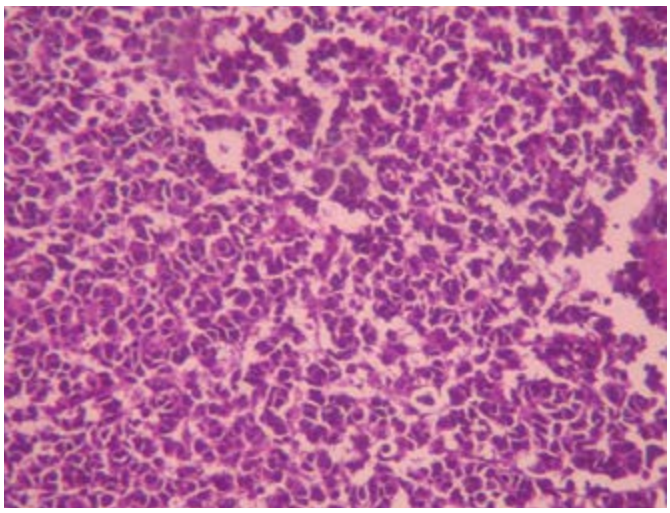

Figure 3. Well differentiated endocrine tumor (uncertain malignant potential) (H&E x40).

Table 3. The Relation Level between age, tumor diameter and Ki-67 expression index with tumor grade (n=21)

	Tumor type		
Features	**n**	**r**	**p**
Age	21	0.324	0.152
Tumor diameter	21	0.619	0.003*
Kİ-67	21	0.684	0.001*

*=p<0.01; Sperman correlation.

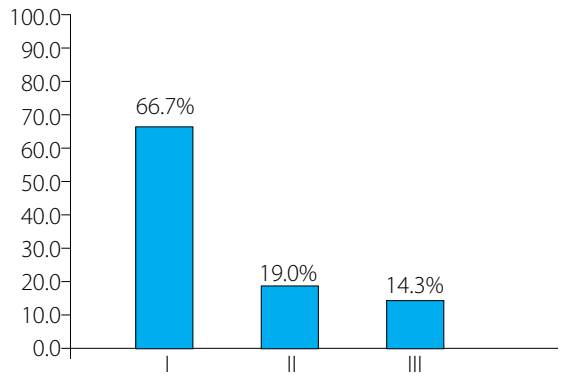

Figure 5. Tumor grades.

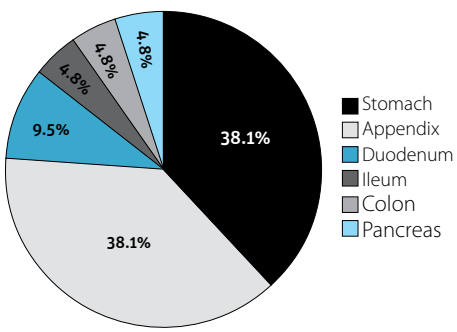

Figure 6. Tumor Localization.

When we scored Ki-67 index as <20, and ≥20 and the cut-off value was accepted as 20 and more; Ki-67 index was 0% (n=0) in Grade I, 25% (n=1) in Grade II and 100% (n=3) in Grade III cases. The Ki-67 index in grade III cases was higher than Grade I- II cases. Difference among them was statistically significant (p<0.001). Besides by Spearman Correlation Analysis; there was a positive (r=0.684) and statistically significant correlation between the degree of grade and Ki-67 index (p<0.001). Based on these data, we examined that as the tumor grade increased Ki-67 index also increased (Table 3).

We determined p53 expression positivity in 14.3% (n=2) of Grade I, in 25% (n=1) of Grade II, and 100% (n=3) of Grade III cases. The ratio of p53 expression positivity in grade III cases was higher than Grade I–Grade II cases which was statistically significant (p<0.05).

There was no statistically significant correlation between CD 117 expression and loss of chromogranin expression with the degree of the tumor grade (p>0.05).

When we grouped the depth of invasion as ≤T II and ≥T II, ≥T II was more frequently observed in Grade III cases [0 (n=0), 25 (n=1), and 100% (n=3), in Grades I, II, and II, respectively with a statistically significant difference between them (p<0.05).

No local-distant metastases was detected in Grade I cases. Local-distant metastases were found in 75% (n=3) of Grade II, and 100% (n=3) of Grade III cases. The number of local-distant metastases in Grade II -III cases was higher than Grade I cases with a statistically significant difference between them (p<0.001).

Survival analysis

According to Kaplan-Meier test results, one of 21 patients (4.8%) included in this study died during follow-up, 12 months after diagnosis of the disease. The survival rate corresponding to this time interval was 95.2% and its standard deviation was 0.046. The median survival time was 60 months for 21 patients.

During median 60 months follow-up, general survival rate was 100% for grade I, and II, and 75% for grade III patients. Based on these results, there was a statistically significant difference between the survival rates of these two groups (Log-Rank test=4.250; p=0.039).

DISCUSSION

Neuroendocrine tumors of gut have been known by their more popular name of "carcinoids" ever since Oberndorfer coined this term to designate tumors that resembled carcinomas but behaved as if they were benign (3). However, it is now well accepted that these tumors are not truly benign, but potentially malignant neoplasms (7). The term "carcinoid" has been so deeply rooted in the literature that even though other alternatives eg, 'APUDomas, endocrine tumors, neuroendocrine tumors (WD-NETs)', have been proposed, the WHO has recently recommended that the term 'Neuroendocrine Tumors' be used generically for these tumors and it is this terminology that shall be used in this article (7).

Gastrointestinal neuroendocrine tumors (GI-NETs) are currently subclassified on morphologic grounds into well-differentiated neuroendocrine neoplasms (WD-NETs or NETs, Grade I-Grade II) that have an indolent clinical course and the poorly differentiated ones that, on account of their outspoken malignant characteristics and aggressive clinical behaviour, are designated as poorly differentiated neuroendocrine carcinomas (PD-NECAs or NECAs, Grade III) (23). In this classification, tumors that were hitherto referred to as 'carcinoids' would correspond to the WD-NETs (7). According to this classification, 13 WDETs (Grade I), 4 WD-NECAs (Grade II) and 4 PD-NECAs (Grade III) were included in this study. Twelve WDETs were referred as 'carcinoids' before in our pathology reports and 1 of them (WDET) was reported as neuroendocrine tumor of uncertain malignant potential. We categorized this case in WDET group according to WHO Classification.

NETs are relatively uncommon, accounting for 2% of all gastrointestinal tract and pulmonary neoplasms. Previous studies have used a variety of immunohistochemical (IHC) markers in an attempt to define organ specific profiles for neuroendocrine tumors with poor results, and emphasized the need for new IHC markers to help distinguish the neuroendocrine tumors of different organs (13).

Regardless of the histological type, most neuroendocrine tumors showed immunoreactivity to either synaptophysin or chromogranin A (8). All the tumor specimens used in this study were also stained for either synaptophysin or chromogranin A or both of them.

Recent advances in molecular biology have provided new tools to determine the metastatic potential of human malignancies. Both mutations in the p53 gene and the abnormal expression of p53 protein are among the most common molecular abnormalities that have been detected in human malignancies. However, over-expression of p53 has been uncommonly identified in gastrointestinal neuroendocrine tumors (8).

Dogusoy et al. (14), in their study of 32 neuroendocrine tumor cases of stomach, suggested that gastric neuroendocrine tumors expressed neuroendocrine markers. They also reported that the expression of p53 and PCNA were associated with the stage of differentiation of neuroendocrine tumors. Like Dogusoy et al. (14), we found similar results for p53 in our study. P53 positivity and the type of tumor correlated well (p<0.05) (chi-square test), and this correlation was statistically significant in our study of 21 patients (Table 2) (Figure 1).

The cellular proliferation marker Ki-67 has been suggested as one of the potential indicators of malignant behaviour in neu-

roendocrine tumors. Several studies have supported the validity of the Ki-67 proliferation index as a prognostic indicator for gastrointestinal neuroendocrine neoplasms. In addition, high labeling of Ki-67 has also been reported in high-grade gastrointestinal neuroendocrine carcinomas (8). Based on chi-square test results, in our study group of 21 patients, there was a positive (r=0.684) and statistically significant difference between the degree of grade and Ki-67 index (p<0.001). As the Ki-67 proliferation index increased, the malignant potential of the tumor also increased (Table 2).

The c-kit protein (also known as CD117), a type III receptor tyrosine kinase, has been implicated in many human cancers, including pulmonary small cell carcinoma, with the presence of CD 117 expression in 40% to 89% of poorly differentiated neuroendocrine carcinomas of lung (9). Mitosis and necrosis are the basic criteria to differentiate well -differentiated from poorly differentiated gastrointestinal endocrine neoplasms, and p16 and p53 levels would be useful markers for these malignancies. It is important to explore the role of CD 117 in gastrointestinal tract endocrine tumors with the hope that targeted therapy may be possible for these tumors (10).

Li et al. (10) found similar results for CD 117 expressions in gastrointestinal neuroendocrine tumors. They concluded that CD 117 expression in colorectal endocrine tumors was low and the incidence was probably lower than that of pulmonary neuroendocrine neoplasms. It was still debatable whether CD 117 expression was related to prognosis in pulmonary neuroendocrine carcinomas. Based on the absence of CD 117 expression in the 57 cases in their study, including well and poorly differentiated endocrine tumors in the gastrointestinal tract, they suggested that these tumors were less likely to respond to the currently available tyrosine kinase inhibitors against c-kit (10). Similar to the study of Li et al. (10), we found that CD117 and the type of tumor did not correlate anyway. This correlation was not statistically significant (p>0.05) in our study (Table 2) (chi-square test).

Theodossiou et al. (15) suggested that neuroendocrine malignancies, such as carcinoid, might not express the c-kit protooncogene to the extent that imatinib would have been an effective form of therapy. It was postulated that imatinib, if active in the management of carcinoid tumors, might exert its effect through PDGF or an alternative molecular pathway.

Based on immunohistochemical evaluation of c-kit expression, Koch et al. (16) treated patients with neuroendocrine and other tumors with imatinib (400 mg/d) but without efficacy after 2 months of therapy. Similar results were shown by other investigators. Therefore, monotherapy with imatinib may not be efficacious in patients with neuroendocrine tumors that express c-kit. Tyrosine kinase inhibitors such as Sorafenib that targets several receptors in addition to KIT may be more efficacious in treating patients with neuroendocrine tumors.

The development of novel, targeted agents is of particular interest in neuroendocrine tumors, in which traditional treatment modalities have had only limited success. Promising recent approaches include somatostatin receptor - targeted radiotherapy, inhibition of mTOR, and inhibition of the VEGF signaling pathway. It is possible that many of these agents also have a cytostatic effect. However, the indolent nature of neuroendocrine tumors, while seems to be beneficial from the standpoint of the patient, makes it difficult or impossible to determine from phase II studies whether stable disease reflects drug effect or simply the natural history of the disease. Randomized trials or, alternatively, the development of surrogate endpoints of response, including the validation of biochemical tests may expedite the more definitive evaluation of these potentially promising markers for neuroendocrine tumors (17).

Tezel et al. (18), in their review stated that although neuroendocrine differentiation in non-neuroendocrine tumors was a frequent finding and showed different biological and clinical effects depending on tissue and tumor, the molecular mechanism was not illuminated yet (19).

Even though great progress has been made in our understanding of this rare family of neoplasms, it is still far from adequate, and the clinical management is often difficult. Although surgery appears highly successful in the treatment of tumors smaller than 1cm, guidelines for the treatment of larger tumors and metastatic disease remain unconsolidated. Standard surgical techniques may still provide a chance of cure but must be weighed carefully for each individual patient (20). We reached some of the patients who were still alive. A total of six patients were alive First patient (well-differentiated gastric endocrine neoplasm, WHO Grade I), the second patient (well-differentiated appendiceal endocrine tumor-uncertain malignant potential/well differentiated endocrine neoplasm, Grade I), the third patient (poorly differentiated mixed gastric endocrine and exocrine carcinoma/poorly differentiated endocrine carcinoma, WHO Grade III), the fourth patient (poorly differentiated pancreatic endocrine tumor/poorly differentiated endocrine carcinoma WHO Grade III), the fifth patient (poorly differentiated duodenal endocrine carcinoma/poorly differentiated endocrine carcinoma WHO Grade III) and the sixth patient (well differentiated rectal endocrine tumor/well differentiated endocrine neoplasm WHO Grade I) are still alive postoperatively. The all others who were well differentiated endocrine neoplasm, WHO Grade I were all alive.

Lubensky et al. (3) suggested to perform a molecular genetic analysis in large series of gastrointestinal and pancreatic neuroendocrine tumors of each specific tumor type. Documentation and understanding of specific genetic alterations characteristic for gastrointestinal and pancreatic NET might lead to improvements in diagnosis, morphologic and molecular characterization and treatment of these neoplasms (3,21).

According to Yao et al. (22) everolismus, as compared with placebo, significantly improved progression- free survival among patients with progressively advanced pancreatic neuroendocrine tumors and it was associated with a low rate of severe adverse events.

Klimstra et al. (23) thought that despite the inability to establish a single system of nomenclature, grading and staging for NETs of all sites, common features form the basis of most systems. Documentation of these features would allow greater reliability in the pathology reporting of these neoplasms. They found the proliferative rate (mitotic index or Ki-67 labeling index or Ki-67 labeling rate) was a critical factor from this standpoint.

As a result, we revised and reclassified our neuroendocrine tumor patients according to WHO Classification 2010. We graded them as Grade I, II and III. So, our study, similar to other studies, showed that regardless of the histological type, most neuroendocrine tumors showed immunoreactivity to either synaptophysin or chromogranin A. Ki-67 proliferation index and p53 expressions correlated with the type of tumor and tumor grade, but CD-117 expression did not show any correlation with the type, and grade of the tumor. These markers are not sufficient to predict the prognosis and outcomes of an effective therapy. We still need more genetic and immunohistochemical studies to predict the behaviour and role of these markers among treatment modalities.

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