Original Article

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Is There a Relationship between the Staining Pattern of Classical Neuroendocrine Markers and Clinicopathological Findings in Neuroendocrine Tumors of the Appendix?

Apendiksin Nöroendokrin Tümörlerinde Klasik Nöroendokrin Belirteçlerin Boyanma Paterni ile Klinikopatolojik Bulgular Arasında Bir İlişki Var Mıdır?

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ABSTRACT

Objective: This study investigates the association between staining patterns of classical neuroendocrine markers, synaptophysin (Snp) and chromogranin-A (Chr), and clinicopathological findings in appendiceal neuroendocrine tumors (NETs). These tumors, often diagnosed incidentally post-appendectomy, pose diagnostic challenges due to their diverse histomorphologic patterns. The study aims to enhance understanding of the relationship between staining patterns and key pathological parameters.

Methods: A retrospective analysis included 28 cases of appendiceal NETs diagnosed over an 8-year period. Histopathological features, including grade, lymphovascular invasion, stage, localization, size, Ki67 proliferation index, and morphological pattern, were reassessed. Immunohistochemical staining of Snp and Chr was examined for extensity and intensity in NET areas.

Results: The study comprised 17 female and 11 male patients, with a mean age of 34 years. Histomorphological patterns included small nests, large nests, and trabecular-palisading patterns. Statistically significant correlations were observed between Snp staining intensity and tumor size, and Chr staining extensity and histomorphologic patterns. Chr staining extensity exceeding 95% was identified in all cases with large nest patterns. Additionally,

ÖZ

Amaç: Bu çalışmada, apendiks nöroendokrin tümörlerinde (NET) klasik nöroendokrin belirteçler olan sinaptofizin (Snp) ve kromogranin-A (Chr) boyanma paternleri ile klinikopatolojik bulgular arasındaki ilişki araştırılmıştır. Genellikle apendektomi sonrası tesadüfen teşhis edilen bu tümörler, çeşitli histomorfolojik paternleri nedeniyle tanısal zorluklar oluşturmaktadır. Bu çalışma, boyanma paternleri ile anahtar patolojik parametreler arasındaki ilişkinin daha iyi anlaşılmasını amaçlamaktadır.

Yöntemler: Retrospektif bir analiz, 8 yıllık bir süre içinde tanı konulan 28 apendiks NET olgusunu içermektedir. Derece, lenfovasküler invazyon, evre, lokalizasyon, boyut, Ki67 proliferasyon indeksi ve morfolojik patern dahil olmak üzere histopatolojik özellikler yeniden değerlendirildi. Snp ve Chr'nin immünohistokimyasal boyanması NET alanlarındaki yaygınlık ve yoğunluk açısından incelendi.

Bulgular: Çalışmaya yaş ortalaması 34 olan 17 kadın ve 11 erkek hasta dahil edildi. Histomorfolojik paternler arasında küçük yuvalar, büyük yuvalar ve trabeküler-palizadlanma vardı. Snp boyanma yoğunluğu ile tümör boyutu ve Chr boyanma yoğunluğu ile histomorfolojik paternler arasında istatistiksel olarak anlamlı korelasyonlar gözlendi. Büyük yuva paterni olan tüm olgularda Chr

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ABSTRACT

Chr staining extensity increased with advancing pathological stage, notably between pT1-pT4 groups.

Conclusion: This study emphasizes the importance of immunohistochemical evaluation, particularly in distinguishing between L and EC cells in appendiceal NETs. The unique trabecular-palisading pattern, associated with L cell histomorphology, demonstrated significant correlations with Chr staining patterns. Snp staining intensity correlated with tumor size, while Chr staining percentage increased with advanced pathological stages. The findings suggest that it will create awareness in pathology practice in terms of both diagnosis and pathological prognostic parameters (tumor size, stage).

Keywords: Appendiceal neuroendocrine tumors, synaptophysin, chromogranin-A, histopathology, immunohistochemistry

ÖZ

boyanma yaygınlığının %95'i aştığı tespit edilmiştir. Ek olarak, Chr boyanma yaygınlığı, özellikle pT1-pT4 grupları arasında olmak üzere, ilerleyen patolojik evre ile artmıştır.

Sonuç: Bu çalışma, apendiks NET'lerinde özellikle L ve EC hücrelerinin ayırt edilmesinde immünohistokimyasal değerlendirmenin önemini vurgulamaktadır. L hücre histomorfolojisi ile ilişkili benzersiz trabeküler-palizatlanma paterni, Chr boyanma paterni ile anlamlı korelasyonlar göstermiştir. Snp boyanma yoğunluğu tümör boyutu ile korelasyon gösterirken, Chr boyanma yüzdesi ileri patolojik evrelerde artmıştır. Bulgular patoloji pratiğinde hem tanı hem de patolojik prognostik parametreler (tümör boyutu, evre) açısından farkındalık yaratacağını düşündürmektedir.

Anahtar Sözcükler: Apendiks nöroendokrin tümörleri, sinaptofizin, kromogranin-A, histopatoloji, immünohistokimya

Introduction

Appendiceal neuroendocrine tumors (NETs) are usually diagnosed incidentally after appendectomies for acute appendicitis (1). They are found in approximately 2% of appendectomies (2). Most tumors are too small to be detected clinically and since the tumor is usually located at the apex, it is unlikely to cause obstruction due to mass effect (2,3). Therefore, the follow-up of the patient after appendectomy depends on the parameters in the pathology report. Appendiceal NETs usually develop from enterochromaffin-like serotonin-secreting EC cells that form insular pattern, solid nests and nodules, and more rarely from glucagon like protein-1 and other proglucagon-related peptide secreting L cells, which usually show trabecular growth pattern (1). It has also been observed that both cell types can cause tumors in a glandular-tubular pattern. Synaptophysin (Snp) and chromogranin-A (Chr), known as classical neuroendocrine markers, are positive in most appendiceal NETs (1). Since Snp can also be positive in tumors other than NETs, its specificity is controversial; therefore, especially Chr positivity is diagnostically important (4). Immunohistochemical and morphologic findings to concretely determine the L cell phenotype are not clear. Glucagon 1, glucagon 2 and peptide YY antibodies have been used to detect L cells and it was found that glucagon 2 was more sensitive (5,6). However, application of these stains in daily use is not suitable in terms of cost and practicality. Appendiceal NETs usually react positively with Snp and Chr, but it is known that L cell types may not stain with Chr as in rectal NETs. This may cause diagnostic difficulties. The aim of this study was to examine the relationship between Snp and Chr staining pattern and clinical findings and to investigate the relationship between staining pattern and histopathologic findings which might cause diagnostic difficulties.

Methods

Material and Case Selection

There were 33 cases of appendiceal NET diagnosed in our pathology clinic within an 8-year period (2015-2023). Five cases were excluded from the study because their slides could not be reached. In our retrospective study, 28 cases were included. Histopathological findings (histological grade, lymphovascular/perineural invasion, stage, tumor localization, tumor size, Ki67 proliferation index, morphological pattern "small-large nest, trabecular-palisading") were re-evaluated on Hematoxylin-Eosin stained slides. Immunohistochemical Chr and Snp stained slides were examined for staining extensity (percentage) and staining intensity (absent, weak, moderate, severe) in NET areas. Clinical findings were obtained from the hospital information system.

Ethical statement: The ethics committee approval of our study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Istanbul Training and Research Hospital with the decision number 357, date: 22.12.2023.

Informed Consent: The archival material of the patients was used and no additional procedures were performed. The study was completely retrospective.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) program version 26.0 was used for statistical analysis of the data in our study. In descriptive statistics, mean value, standard deviation, median, minimum and maximum values for continuous variables and number and percentage values for discrete variables were calculated. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normal distribution as initial analysis. Mann-Whitney U and chi-square tests were used to compare the data between two groups. Pearson correlation test was used for correlation analysis of binary data. The results were evaluated at 95% confidence interval and $p<0.05$ was defined as statistical significance.

Results

Seventeen of the patients were female and 11 were male. The mean age was 34 years (range 14 to 70 years). The mean tumor

size was 1.07 cm and ranged between 0.2-5 cm. Seventeen cases were located in the distal (68%), 4 cases in the proximal (16%), 2 cases in the middle part (8%) of the appendix, and 2 cases involved the appendix diffusely (8%). Since three cases were fragmented, no comment could be made about the localization. The number of cases with histologic grade 1 was 21 and grade 2 was 7. In terms of the predominant histomorphologic pattern, 19 cases showed small nest pattern, 7 cases showed large nest pattern and 2 cases showed trabecular - palisading pattern. There were 2 cases with tubular pattern. One of these cases had a predominant pattern of small nest while the other had a predominant pattern of large nest and was included in these groups. Lymphovascular invasion was detected in six cases and 4 of them showed small nest pattern. There were 10 cases in T1 stage, 13 cases in T3 stage, 5 cases in T4 stage and no cases in T2 stage. Both of the cases with trabecular-palisading pattern were in T1 stage and the tumor diameter was 0.5 cm (Table 1). In all cases, Snp showed moderate to strong staining in more than 90% of the tumor, with no weak staining. Chr staining with an extensity of more than 95% was detected in all cases with large nest pattern (Figure 1). There were 6 cases with Chr staining in less than 90% of the tumor area. In 4 of them, less than 50% and weak staining was seen. Two of these 4 cases showed trabecular-palisading pattern and two of them showed small nest pattern (Figure 2).

A statistically significant relationship was found between Snp staining intensity and tumor size (p=0.042). The staining intensity increased as the tumor size increased. When histomorphologic patterns were evaluated in terms of Chr staining extensity (SE), a statistically significant correlation was found (p=0.029). In addition, Chr SE increased with increasing pathologic stage, which was statistically significant especially between pT1-pT4 groups (p=0.038). No significant correlation was found between other parameters and staining patterns.

Discussion

Appendiceal NETs are frequently encountered incidentally as small-sized tumors (1). For pathologic diagnosis, immunohistochemical findings are indispensable for both diagnosis and differential diagnosis (4). EC and L cells are important components reflecting the histopathologic and phenotypic diversity of NETs of the appendix. Although the prognostic significance of hormonal differences in NETs originating from L or EC cells has not been demonstrated, the differences of these cell types play an important role in determining the histopathologic diagnosis (7). They may present diagnostic difficulties due to factors such as patchy-poor staining with Chr. L cells may generally be less reactive to neuroendocrine markers than EC cells (1). In a study conducted by Sohn et al. (6) in rectal NET cases, L cells were detected mostly in G1, G2 tumors located in the mucosa and submucosa below 1 cm. In our study, similarly, tumors showing trabecular-palisading feature (L cell histomorphology) were found to be at early stage (T1), and have small tumor size (0.5 cm) and G1-G2 grades. Although the number of cases showing trabecular - palisading pattern was small in our study, the findings were statistically significant in terms of Chr SE in these cases. In this pattern, Chr expression is highly reduced (especially staining prevalence is between 5-10%) while Snp is positive in these areas (moderate or strong

Table 1. Synaptophysin and chromogranin-A staining patterns according to histopathologic findings

	Synaptophysin staining extensity		Chromogranin-A staining extensity		Synaptophysin staining intensity			Chromogranin-A staining intensity		
	≤90%	>90%	≤90%	>90%	Weak	Moderate	Strong	Weak	Moderate	Strong
Pathologic stage										
T1 (n=10) n (%)	4 (40)	6 (60)	6 (60)	4 (40)	0	8 (80)	2 (20)	4 (40)	2 (20)	4 (40)
T3 (n=13) n (%)	2 (15)	11 (85)	1 (7)	12 (93)	0	6 (46)	7 (54)	0	3 (23)	10 (77)
T4 (n=5) n (%)	0	5 (100)	0	5 (100)	0	2 (40)	3 (60)	0	1 (20)	4 (80)
p-value	0.16		**0.016**		0.19			0.06		
Histologic grade										
Grade 1 (n=21) n (%)	6 (28)	15 (72)	6 (28)	15 (72)	0	12 (58)	9 (42)	4 (20)	3 (14)	14 (66)
Grade 2 (n=7) n (%)	0	7 (100)	1 (15)	6 (85)	0	4 (57)	3 (43)	0	3 (43)	4 (57)
p-value	0.27		0.87		1			0.95		
Histologic patterns										
Small nest (n=19) n (%)	6 (31)	13 (69)	5 (27)	14 (73)	0	12 (63)	7 (37)	3 (16)	4 (21)	12 (63)
Large nest (n=7) n (%)	0	7 (100)	0	7 (100)	0	2 (28)	5 (72)	0	1 (15)	6 (85)
Trabecular-palisading (n=2) n (%)	0	2 (100)	2 (100)	0	0	2 (100)	0	1 (50)	1 (50)	0
p-value	0.17		**0.029**		0.13			0.072		

positivity of 90% or more). This reveals a unique relationship between immunohistochemical and histomorphologic findings. Considering the staining pattern of Chr, we think that it may be useful to enlist the help of other neuroendocrine cell markers such as CD56, INSM1 in addition to Snp in NET suspicious cases with small tumor size.

Although NETs originating mostly from L cells are known to show a tubular-pseudoglandular growth pattern, tumors originating from EC cells may also show this pattern (1,8). Although neuroendocrine marker positivity of EC cells provides an easy solution, L cells may cause diagnostic confusion with conventional adenocarcinomas and especially goblet cell adenocarcinoma showing tubular-glandular growth with Chr negativity. As known in goblet cell adenocarcinomas, varying amounts of neuroendocrine cells are among the tumor components. In addition, L cells may lose CDX2 expression as well as Chr (9). It has also been reported that L cells express markers such as prostatic acid phosphatase and PAX8 (9,10). With these striking findings, NETs arising from L cells can easily be confused with metastatic disease. Recent studies have reported that SATB2 is positive in both rectal and appendiceal NETs and is useful in differentiation from pancreatic and duodenal NETs (10).

In our study, Snp staining intensity increased with increasing tumor size and Chr SE increased with increasing pathological stage. Specific prognostic data for NET are very limited and stage seems to be the only relevant parameter to determine aggressive disease (1). In one study, tumor size greater than 15 mm, presence of lymphovascular invasion and G2 grade were identified as independent indicators to determine the presence of lymph node metastasis (11). Other studies have not associated some of these parameters with disease-related survival (12-14). All these inconsistencies reflect the high heterogeneity of study planning and case selection, which are fundamental biases of retrospective studies.

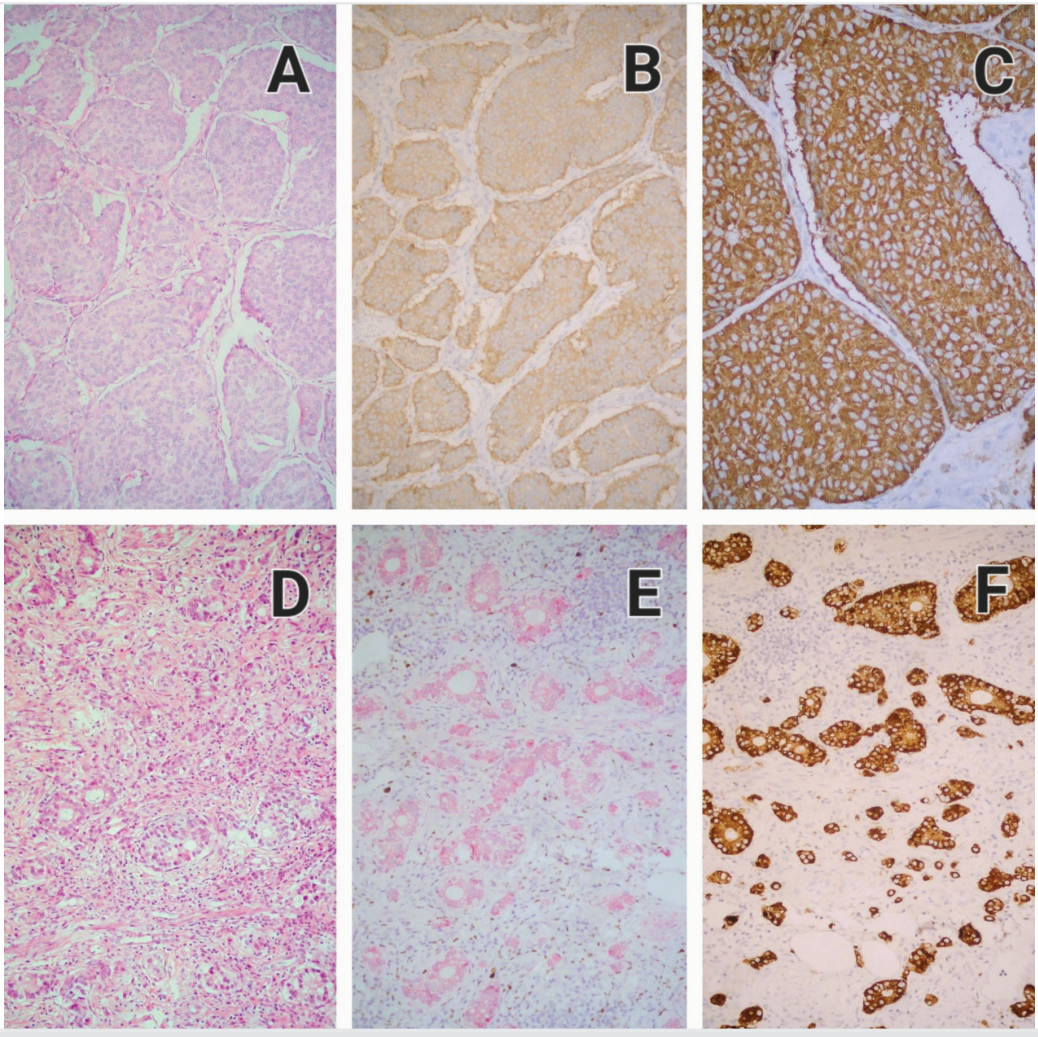

Figure 1. Appendiceal neuroendocrine tumor showing large nest pattern (A: Hematoxylin-Eosin x200. B: Synaptophysin x200, extensity: 100%, intensity: moderate C: Chromogranin-A x200 extensity: 100%, intensity: severe). Appendiceal neuroendocrine tumor showing tubular and predominantly (not in figure) small nest pattern (D: Hematoxylin-Eosin x100. E: Synaptophysin+Ki67 (double staining) x100, extensity: 100%, intensity: moderate F: Chromogranin-A x100, extensity: 100%, intensity: severe)

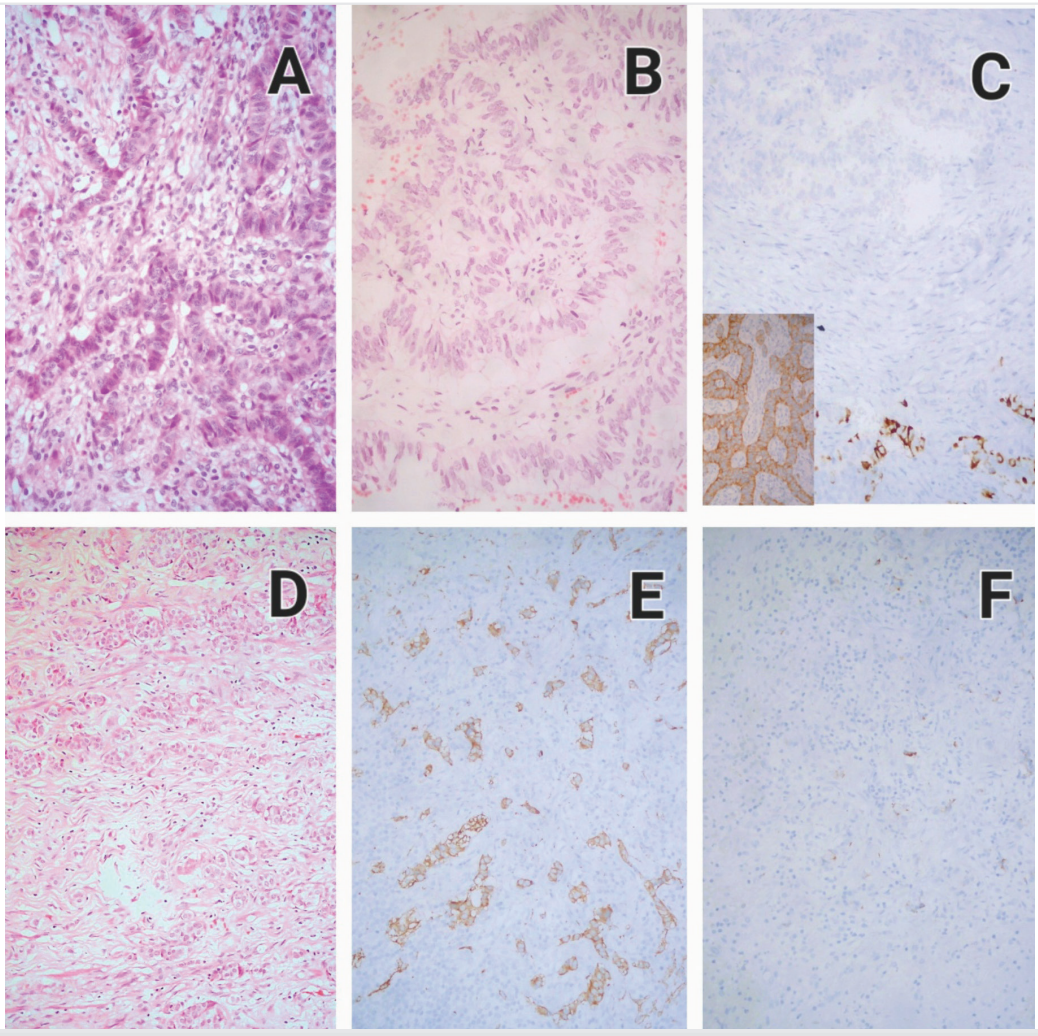

Figure 2. Appendiceal neuroendocrine tumor showing trabecular-palisading pattern [A, B: Hematoxylin-Eosin x200, x400. C: Chromogranin-A "extensity: 10%, intensity: moderate" and Synaptophysin "extensity: 100%, intensity: severe" (inset)]. Appendiceal neuroendocrine tumor showing small nest pattern (D: Hematoxylin-Eosin x100. E: Synaptophysin x100, extensity: 90%, intensity: severe, F: Chromogranin-A x100, extensity: 5%, intensity: weak)

Although right hemicolectomy is strongly recommended especially in tumors larger than 2 cm, some parameters, especially location at the base of the appendix, R1 resection status, lymphovascular invasion, mesoappendiceal invasion (extension over 3 mm) and G2 tumor grade in tumors smaller than 2 cm emphasize that right hemicolectomy should be discussed in an appropriate multidisciplinary setting after appendectomy (1). Since the treatment and follow-up protocol for each patient may differ, all histopathologic parameters should be specified in the pathology report to determine the clinical decision.

Study Limitations

The most important limitation of our study is the small number of cases showing trabecular-palisading pattern. In addition, not including prognostic data (such as recurrence, metastasis, life expectancy, disease-related death) may be another limitation. However, since the aim of our study was to compare immunohistochemical findings with other pathologic parameters, we think that these findings can be ignored.

Conclusion

The NETs of the appendix pose diagnostic challenges and highlight the importance of immunohistochemical evaluations to distinguish between L and EC cells. This study highlights the unique histomorphologic and immunohistochemical features of NETs, such as the trabecular-palisading pattern. Our study also demonstrates that prognostically, Snp staining intensity correlates with tumor size, while Chr staining percentage increases in advanced pathological stages.

Ethics

Ethics Committee Approval: The ethics committee approval of our study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Istanbul Training and Research Hospital with the decision number 357, date: 22.12.2023.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.C., Concept: M.C., Design: M.C., Ö.G., Data Collection or Processing: M.C., E.Y., S.C., Ö.G., Analysis or Interpretation: M.C., E.Y., S.C., Ö.G., Literature Search: M.C., S.C., Ö.G., Writing: M.C., Ö.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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