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Pathologic features, Ki-67 indices and Melan-A expression in adrenal neoplasms

Adrenal neoplazmlarda patolojik özellikler, Ki-67 proliferasyon indeksi ve Melan-A ekspresyonu

Durak MG[1] Akın MM[1] Canda MŞ[1] Koçdor MA[2] Çömlekçi A[3] Birlik B[1]

[1]Dokuz Eylül Üniversitesi Tıp Fakültesi, Patoloji Anabilim Dalı, İzmir, Türkiye
[2]Dokuz Eylül Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, İzmir, Türkiye
[3]Dokuz Eylül Üniversitesi Tıp Fakültesi, Endokrinoloji Bilim Dalı, İzmir, Türkiye

Summary

Aim: Differential diagnosis of adrenocortical neoplasms and pheochromocytoma may occasionally be very difficult. Although recently developed molecular techniques are encouraging, histopathologic features and immunohistochemical markers are still the mainstay of diagnosis. The aim of this study is to re-evaluate the clinicopathologic features of our cases diagnosed as adrenocortical adenoma/carcinoma and pheochromocytoma.

Material and Methods: Sixteen cases of adrenocortical neoplasm and 7 cases of pheochromocytoma, 3 of which were bilateral, diagnosed between 1995 and 2010 at Dokuz Eylül University Pathology Department were reviewed. The immunohistochemical staining of Ki-67 and melan-A were examined.

Results: The mean age of the adrenocortical neoplasm cases was 45 years (range, 25-60). Fifteen of 16 adrenocortical neoplasms were cortical adenoma and 1 was cortical carcinoma. The mean Ki-67 proliferation index in the cortical adenoma cases was 1.7% (range, 0.2-6.0%). Four of the 15 adenoma cases (27%) were melan-A positive. In one cortical carcinoma case, the Ki-67 proliferation index was 20% and melan-A was negative. The mean age of the pheochromocytoma cases was 46 years (range, 28-63). Four pheochromocytoma cases were benign, whereas 3 cases, 2 of which were bilateral, had histopathologic features suggesting malignant behavior. The mean Ki-67 proliferation index of 10 pheochromocytomas from 7 cases was 3.3% (range, 0.2-20.0%), and none of these cases showed melan-A immunoreactivity.

Conclusion: Although the number of patients is limited in this study, our findings suggest that Ki-67 immunohistochemical stain can be used in routine practice to determine tumor biology in adrenal neoplasms, and melan-A positivity may favor cortical origin in differential diagnosis of adrenocortical tumors and pheochromocytoma.

Key Words: Adrenocortical adenoma, adrenocortical carcinoma, pheochromocytoma, Ki-67, melan-A.

Özet

Amaç: *Adrenokortikal neoplazm ile feokromositom ayırıcı tanısı zaman zaman oldukça güçlük göstermektedir. Son dönemde gelişen moleküler yöntemler umut verici olsa da, tanı verirken hala temel olarak tümörün histopatolojik ve immunhistokimyasal boyanma özellikleri dikkate alınmaktadır. Bu çalışmanın amacı, bölümümüzde adrenokortikal adenom/karsinom ve feokromositom olarak tanı almış olguların klinikopatolojik özelliklerini yeniden gözden geçirmektir.*

Yöntem ve Gereç: *Dokuz Eylül Üniversitesi Patoloji Bölümü'nde, 1995 ile 2010 yılları arasında tanı alan 16 adrenokortikal neoplazm ile 3'ü bilateral yerleşimli olmak üzere 7 feokromositom olgusunun klinikopatolojik özellikleri yeniden gözden geçirildi. Tümörlü bloklara uygulanan Ki-67 ve melan-A immunhistokimyasal boyaları değerlendirildi.*

Yazışma Adresi: Merih Güray DURAK
Dokuz Eylül Üniversitesi Tıp Fakültesi Patoloji Anabilim Dalı, İZMİR

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Bulgular: *Adrenokortikal neoplazm olguları 25 ile 60 yaş arasında olup ortalama yaş 45'dir. Onaltı adrenokortikal neoplazm olgusunun 15'i adenom, 1'i karsinomdur. Adenom olgularının Ki-67 proliferasyon indeksi %0.2 ile %6.0 arasında olup ortalama Ki-67 proliferasyon indeksi %1.7'dir. Onbeş adenom olgusunun 4'ünde (%27) melan-A pozitifliği saptanmıştır. Tek kortikal karsinom olgusunda Ki-67 proliferasyon indeksi %20 olup melan-A negatif olarak izlenmiştir. Feokromositom olguları 28 ile 63 yaş arasında olup ortalama yaş 46'dır. Dört feokromositom olgusu benign, 2'si bilateral yerleşimli olan 3 olgu ise malign özelliktedir. Yedi feokromositom olgusuna ait toplam 10 tümörde Ki-67 proliferasyon indeksi %0.2 ile %20 arasında olup ortalama Ki-67 proliferasyon indeksi %3.3'tür, ve bu olguların hiçbirinde melan-A immunreaktivitesi görülmemiştir.*

Sonuç: *Çalışmadaki olgu sayısı sınırlı olmakla birlikte, bulgular adrenal neoplazmlarda tümör biyolojisini saptamak için Ki-67 immunhistokimyasal boyasının rutin uygulamalarda yararlı olabileceğini göstermektedir. Ayrıca adrenokortikal tümörler ile feokromositom ayırıcı tanısında, immunhistokimyasal olarak melan-A pozitifliği kortikal orijini destekleyen bir bulgu olarak kullanılabilir.*

Anahtar Kelimeler: *Adrenokortikal adenom, adrenokortikal karsinom, feokromositom, Ki-67, melan-A.*

Introduction

Histologic distinction between adrenal cortical and medullary tumors can be challenging from time to time. Cortical tumors are mostly adenomas that are considered to be a functionally heterogeneous group of neoplasms arising from any of the cortical layers. The majority of cortical adenomas are unilateral. Clinical features of these tumors are associated with the hormonal secretory status of the tumor, and most tumors are associated with either hyperaldosteronism or Cushing syndrome (1). Adrenal cortical carcinomas (ACCs) on the other hand, are rare neoplasms with an incidence of one per million population per year. They are more likely to be functional than adenomas, and are often associated with virilism (1-3).

Neoplasms of the adrenal medulla include pheochromocytoma/paraganglioma of the adrenal medulla that are composed of chromaffin cells, and neuronal neoplasms (1,4). Pheochromocytomas can be sporadic or familial, with those associated with familial syndromes more commonly bilateral. Although most pheochromocytomas are benign, metastases have been defined in approximately 10% of cases. The clinical presentation of most patients is hypertension (1, 2, 4).

Recently, especially with the aid of computerized tomography and magnetic resonance imaging techniques, adrenal neoplasms can be detected earlier and frequently with smaller sizes. This shows the necessity of more accurate criteria in histologic diagnosis. Immunohistochemistry is frequently used in the differential diagnosis of adrenal neoplasms, since molecular techniques are mostly limited to research laboratories. The number of immunohistochemical markers that are used in routine practice have increased recently with the addition of some novel markers, such as inhibin, calretinin and melan-A (5-8). Immunohistochemical assessment of cell proliferation by the analysis of cell cycle-associated antigens, such as MIB-1 (Ki-67) also helps in determining the biology of the tumor (9).

The aim of this study is to re-evaluate the clinicopathologic features of cases diagnosed as adrenocortical adenoma/carcinoma and pheochromocytoma, and to investigate the importance of Ki-67 and melan-A immunohistochemical markers in the differential diagnosis of adrenal neoplasms.

Materials and Method

Sixteen cases of adrenocortical neoplasm and 7 cases of pheochromocytoma diagnosed at Dokuz Eylül University Faculty of Medicine Department of Pathology between 1995 and 2010 were re-examined histologically, and clinical data were reviewed. The age and sex of the patients as well as weight, size and lateralization of the tumor were determined. Hematoxylin-eosin (H-E) stained slides of adrenocortical neoplasm cases were reviewed according to the malignancy criteria outlined by Weiss et al. (10) and modified by Aubert et al. (11). The histologic features of the adrenal medullary cases were re-examined as described by Thompson et al. and were given a score (12).

Immunohistochemical stains were performed on paraffin sections using antibodies against Ki-67 (Clone MIB-1, 1:50; Zymed) and melan-A (clone A103, 1:100; Neomarkers).

Ki-67 positivity index was obtained by counting 500 cells of lesions and calculating the percentage of cells with nuclear immunoreactivity (13). For melan-A, granular, cytoplasmic staining of at least 10 % of the tumor cells with moderate or greater intensity was required for a positive reaction (5).

Table 1. Clinicopathologic features of cases with the immunostaining results of Ki-67 and melan-A.

Cases (n)	Age/sex	Tm size (cm)	Tm weight (gram)	Lat	Pathologic diagnosis	Ki-67 (%)	Melan-A	Clinical feature
1	31/F	3	10	Left	ACA	2	+	Primary HT
2	45/F	17	100	Right	ACC	20	-	Incidental
3	54/M	2	7.5	Left	ACA	1	-	Conn synd.
4	40/F	4.5	15	Right	ACA	1	-	Incidental
5	49/F	2.5	10	Right	ACA	2.5	-	Incidental
6	42/F	6.5	50	Right	ACA	0.2	-	Incidental
7	42/F	2.4	15	Left	ACA	1	-	Incidental
8	25/M	2.4	20	Left	ACA	2	-	Conn synd.
9	60/F	4.5	20	Right	ACA	2	-	Primary HT
10	51/F	4	25	Right	ACA	4	+	Cushing synd.
11	58/M	7.5	80	Right	ACA	6	+	Incidental
12	49/F	1.4	5	Left	ACA	1.4	-	Incidental
13	38/F	2.5	12	Left	ACA	0.6	+	Conn synd.
14	48/F	4	20	Left	ACA	0.8	-	Cushing synd.
15	33/F	1.5	4	Left	ACA	1	-	Conn synd.
16	60/F	1.8	10	Left	ACA	0.6	-	Conn synd.
17	44/F	5.5	70	Left	pheo	1	-	MEN IIA
		3.2	15	Right	pheo	1	-	
18	28/F	5	50	Left	pheo	1	-	Primary HT
19	63/F	8	200	Left	pheo	6	-	Incidental
20	56/M	9	175	Left	pheo*	20	-	Incidental
21	33/M	7	60	Left	pheo*	2.2	-	MEN IIA
		3.4	20	Right	pheo*	0.2	-	
22	47/M	5	60	Left	pheo*	0.6	-	MEN IIA
		5.2	60	Right	pheo*	0.8	-	
23	54/M	2.7	6	Right	pheo	0.6	-	Primary HT

Abbreviation: Lat: lateralization, ACA: adrenal cortical adenoma, ACC: adrenal cortical carcinoma, pheo: pheochromocytoma, HT: hypertension, MEN: multiple endocrine neoplasia

* pheochromocytoma cases with a histologic score of ≥4

Results

Clinicopathological features of the patients are summarized in (Table-1). Fifteen of the 16 adrenocortical neoplasms were diagnosed as adenoma, and one as carcinoma. Twelve adenoma cases were in women. The median age of the adenoma cases was 48 years (range, 25-60 years). The median weight of the tumors was 15 gr (range, 4-80 gr), and the median size was 2 cm (range, 1.4-7.5 cm). Nine tumors (60%) were located in the left adrenal and 6 (40%) were in the right. The only ACC case was a 45 year-old woman, in whom the weight of the tumor was 100 gr and the size was 17 cm.

Four of the 7 pheochromocytoma cases were women. The median age of the pheochromocytoma cases was 47 years (range, 28-63 years). Three patients had bilateral pheochromocytoma, all of whom had multiple endocrine neoplasia (MEN) IIA syndrome. The median weight of ten tumors in 7 patients was 60 gr (range, 6-200 gr), and the median size of the tumors was 5.1 cm (range, 2.7-9 cm). Four patients were diagnosed as benign pheochromocytoma, whereas 3 patients had histologic features consistent with malignant behavior.

In H-E stained sections of adrenocortical adenoma cases, relatively well-circumscribed lesions usually had thin fibrous capsule histologically. Some of the lesions

had a lobular appearance owing to the presence of fibrovascular septa between adenoma cells. These cells had centrally localized round to oval bland nuclei with abundant vacuolated or eosinophilic cytoplasm. Ki-67 positivity index of the cases was in the range of 0.2% to 6%, and in 4 cases (27%) melan-A positivity was present (Figure-1). In the ACC case, there was prominent pleomorphism in the cells, increased mitoses, necrosis and hemorrhage. Ki-67 positivity index of this case was 20%, and melan-A was negative.

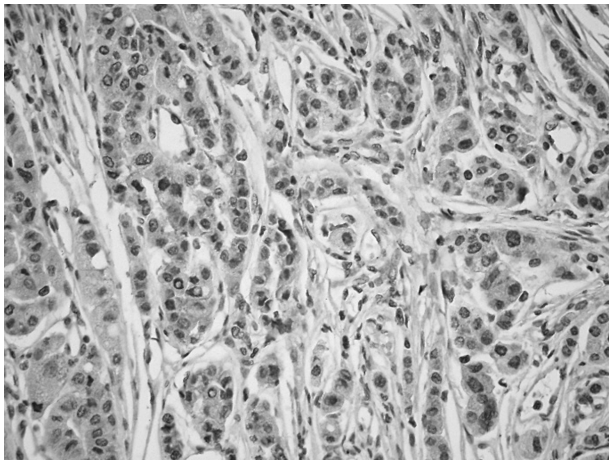

Figure 1. Granular, cytoplasmic staining of melan-A immunohistochemical stain in an adrenal adenoma case (Case no 13, Melan-A, X400).

Microscopic examination of most of the pheochromocytoma cases revealed the typical "Zellballen" configuration. The cells that varied considerably in size and shape had round or oval nuclei with prominent nucleoli and abundant granular amphophilic cytoplasm (Figure-2). Ki-67 positivity index of the cases was in the range of 0.2% to 20% (Figure-3), and all of the cases were melan-A negative.

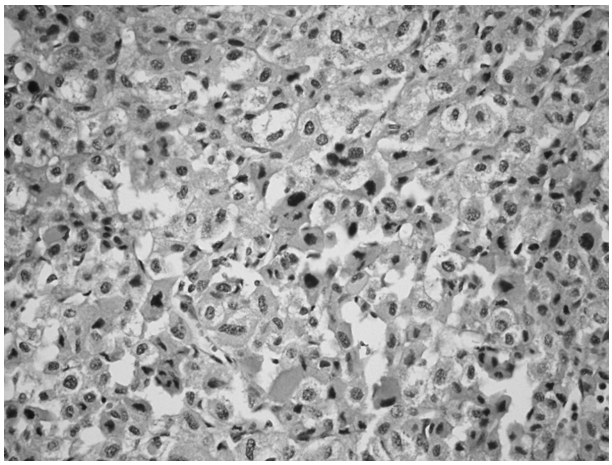

Figure 2. A diffuse growth pattern in a malignant pheochromocytoma, with nuclear pleomorphism, hyperchromasia and occasional mitosis (Case no 20, X200).

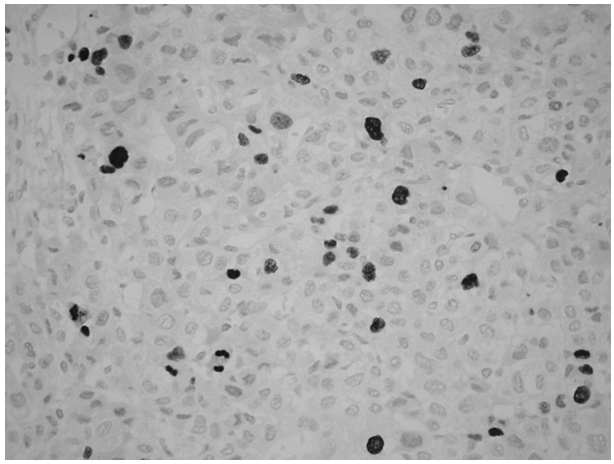

Figure 3. Immunohistochemical staining of Ki-67 shows high Ki-67 proliferation index in the same case as in figure 2 (Ki-67, X400).

Discussion

The great majority of adrenocortical neoplasms are adenomas. Adenomas are usually found incidentally at autopsies, or as a result of radiographic imaging techniques performed for other reasons. They can occur in any age and in both sexes. Some cases are detected clinically due to hormonal hyperfunction of glucocorticoids (Cushing syndrome), androgenic/estrogenic steroids (adrenogenital syndrome), or mineralocorticoids (Conn syndrome) (4). In our study, 5 patients with adrenocortical adenoma had primary aldosteronism (Conn syndrome), 2 patients had cortisol hypersecretion (Cushing syndrome), and 2 patients had primary hypertension. Six of the 15 adenoma patients were nonfunctional.

ACCs are rather rare neoplasms which make up nearly 0.2% of all cancer deaths in the United States (3). Although they can develop at any age and in both sexes, the peak incidence is around age 70 with a secondary smaller peak in the first two years of life. They are more frequently seen in women and children when they are functional (3, 4). Cortical carcinomas are typically large tumors weighing more than 100 gr. However, it should be noted that some tumors weighing less than 50 gr can metastasize, while a small proportion of tumors weighing more than 1000 gr may not (4). The only carcinoma case in our study was nonfunctional.

A variety of parameters have been proposed to determine malignancy in adrenocortical neoplasms (14). Some use only histologic criteria (10, 15), whereas others use both histologic and clinical features to differentiate benign from malignant cortical neoplasms. The most accepted one today is the one described by Weiss et al. and modified by Aubert et al. (10, 11). The histopathologic criteria proposed by Weiss et al. are the presence of high

nuclear grade and pleomorphism, more than 5 mitoses per 50 high-power fields, atypical mitotic figures, eosinophilic cytoplasm in more than 75% of the tumor cells, diffuse growth pattern, necrosis, venous, sinusoidal, and capsular invasion. According to their study, the presence of three or more of the above criteria highly correlates with subsequent malignant behavior (10). Our cases were re-evaluated according to these criteria. All adenoma cases showed 2 or fewer of these 9 malignancy criteria, whereas in one ACC case 6 criteria, including capsular, venous, sinusoidal invasion, necrosis, nuclear pleomorphism of tumor cells, and more than 5 mitosis per 50 high-power fields, were present.

The most important overall finding associated with malignancy is the weight of the tumor (>100 gr). Other nonhistologic criteria proposed by Hough et al. (16) include clinical features, such as the presence of 17-ketogenic steroids in urine, response to adrenocorticotropin, and weight loss. Although the number of cases are limited in our study, the weight of the tumor in adenoma cases (the mean weight:20 gr) was less than that of the carcinoma case (100gr).

The differentiation between benign and malignant pheochromocytomas relies on the presence of recurrence or clinically detectable metastases. A number of histologic criteria have been evaluated in adrenal medullary tumors, including capsular, vascular invasion, growth pattern, cellularity, tumor cell spindling, nuclear pleomorphism, hyperchromasia, presence of necrosis and mitosis (Table-2) (12). However, no single histologic feature can adequately identify malignancy in these tumors. Thompson has proposed a scoring system for the identification of biologic behavior in pheochromocytomas. According to his study, capsular, vascular invasion, profound nuclear pleomorphism and hyperchromasia were weighted with one point, the rest of the histologic features were weighted with two points. A total score of ≥ 4 points suggested malignant clinical behavior in his series (12). We re-examined our cases according to the scoring system proposed by Thompson et al. Three patients, two of whom had bilateral pheochromocytoma with MEN IIA syndrome, had a score ≥ 4. The score of the remaining patients was <4.

Sasano et al (17) have emphasized the importance of a multivariate scoring system in discerning the biologic behavior of adrenocortical tumors, that no single parameter can be effective in the differential diagnosis of adenoma and carcinoma. They have proposed that immunohistochemical assessment of cell proliferation, by the analysis of the cell cycle-associated antigens, such as Ki-67, is very helpful in evaluating malignancy in the detection of adrenocortical neoplasms. Likewise, Bernini et al (13) have reported that p53 and Ki-67 immunohistochemical stains are overexpressed in carcinoma cases, and therefore, could be used in the diagnosis of malignancy in adrenocortical tumors. The median Ki-67 proliferation index of our adenoma cases was 1% (range, 0.2-6%), whereas in one carcinoma case it was 20%. Our results are in consistent with the results of Bernini et al (13), who have reported a positivity index of ≤1% in cortical adenomas, and 3-40% in carcinomas.

Table 2. The histologic criteria evaluated in pheochromocytoma patients.

Histologic feature	Score
Large nests or diffuse growth pattern	2
Central or confluent necrosis	2
High cellularity	2
Cellular monotony	2
Tumor cell spindling	2
Mitosis (>3/10 HPF)	2
Atypical mitotic figures	2
Extension into adipose tissue	2
Vascular invasion	1
Capsular invasion	1
Profound nuclear pleomorphism	1
Nuclear hyperchromasia	1

Abbreviation: HPF: high power fields.

Recent literature emphasizes the effectiveness of melan-A (A103) immunohistochemical stain in differentiating adrenocortical neoplasms from non-adrenal carcinomas and pheochromocytoma (5, 6, 18). The monoclonal antibody A103 recognizes an antigen on melanoma cells known as Melan-A or MART-1 (5). Nevertheless, Busam et al. (18) have reported that A103 immunoreactivity can also be seen in adrenal cortical tumors and other steroid-producing cells. Melan-A positivity was present in 4 of our 15 adenoma cases (27%), whereas it was negative in the only carcinoma case. Loy et al (5) have reported that all of their cortical adenoma and carcinoma cases showed melan-A positivity. Similarly, Renshaw and Granter (19) have reported 65% melan-A positivity in their series of adrenocortical tumors. We believe that decreased A103 staining sensitivity may be due to the staining method. Melan-A immunohistochemical stain

can be used in determining steroid-hormone-producing tumors, i.e., in differentiating adrenocortical neoplasms from other carcinomas and adrenal medullary tumors. However, it does not separate benign from malignant adrenocortical tumors (5,6).

In conclusion, adrenal neoplasms are rare tumors, in which both clinical and histopathologic evaluation should be carefully made for appropriate patient management. Although the number of patients is limited in this study, our findings suggest that in determining tumor biology, Ki-67 positivity index can be used in routine practice. Melan-A immunohistochemical marker on the other hand, can be helpful in the differential diagnosis of adrenal cortical and medullary tumors.

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