ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/medsci.2020-79897

Clinical Significance of Ki-67 Proliferation Index and p53 Gene Expression in the Evaluation of Hormone Profiles, Invasiveness and Pre- and Post-operative Assessment of Pituitary Macroadenomas

Ki-67 Proliferasyon İndeksi ve p53 Gen Ekspresyonunun Hipofiz Makroadenomalarının Hormon Profilleri, İnvazyon Dereceleri ve Operasyon Öncesi ve Sonrası Değerlendirilmesindeki Klinik Önemi

Ahmet Mesrur HALEFOĞLU[a], Canan TANIK[b], Mustafa KARABİNA[a]

[a]Department of Radiology, University of Health Sciences Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, TURKEY
[b]Department of Pathology, University of Health Sciences Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, TURKEY

ABSTRACT Objective: The aim of this study was to investigate the clinical significance of Ki-67 proliferation index and p53 gene expression in the pre-operative and post-operative evaluation of pituitary macroadenomas. **Material and Methods:** We retrospectively evaluated 81 pituitary macroadenoma patients, where pre-operative, six-month and one-year follow-up post-operative dynamic contrast-enhanced magnetic resonance imaging examinations were present in our picture archiving and data system. All patients underwent surgical tumor removal and were pathologically diagnosed as having macroadenomas. **Results:** In Ki-67 positive macroadenoma cases, maximal tumor diameter was found significantly higher than in Ki-67 negative adenoma cases. When tumor invasiveness was compared based on Hardy's classification with the Ki-67 proliferation index values of macroadenomas, no statistically significant correlation was found between tumor invasiveness and Ki-67 values. We can state that there is a strong correlation between Ki-67 positiveness and increase in residual tumor dimensions. In p53 negative and positive macroadenoma cases, there was no significant correlation between p53 gene expression and maximal tumor diameter. When we compared the tumor invasiveness was compared based on Hardy's classification with the p53 gene expression of macroadenomas, no statistically significant correlation was found between tumor invasiveness and p53 values. We can say that there is a strong correlation between p53 gene expression positiveness and increase in residual tumor dimensions. **Conclusion:** Ki-67 proliferation index and p53 gene expression level determination of macroadenomas and their association with the hormone profiles, invasiveness, preoperative primary tumor and post-operative residual tumor dimension parameters can be combined in order to predict early tumor progression and patient prognosis.

Keywords: Immunohistochemistry; magnetic resonance imaging; pituitary adenoma; recurrence

ÖZET Amaç: Hipofiz makroadenomlarının, operasyon öncesi ve sonrası değerlendirilmesinde Ki-67 proliferasyon indeksi ve p53 gen ekspresyonunun klinik önemini araştırmak. **Gereç ve Yöntemler:** Çalışmamızda, operasyon öncesi ve sonrası 6. ay ve 1. yıl takip dinamik kontrastlı manyetik rezonans görüntüleme incelemeleri resim arşiv ve data sistemi içerisinde bulunan 81 hipofiz makroadenoma hastasını retrospektif olarak değerlendirdik. Tüm hastalarda, cerrahi olarak tümör çıkarıldı ve patolojik olarak makroadenoma tanısı konuldu. **Bulgular:** Ki-67 pozitif olan makroadenoma olgularında maksimal tümör boyutları, Ki-67 negatif olan adenoma olgularına göre belirgin derecede yüksek bulundu. Hardy'nin klasifikasyonuna göre tümör invazyonu ile makroadenomaların Ki-67 proliferasyon indeks değerleri karşılaştırıldığında, tümör invazyonu ile Ki-67 proliferasyon indeks değerleri arasında istatistiksel olarak anlamlı bir korelasyon saptanmadı. Ki-67 pozitifliği ile rezidüel tümör boyutlarındaki artış arasında güçlü bir korelasyon bulunduğunu söyleyebiliriz. Rezidüel tümör boyut artışı ile Hardy'nin klasifikasyonu arasında istatistiksel olarak önemli bir korelasyon mevcut değildir. p53 negatif ve pozitif makroadenoma olgularında, p53 gen ekspresyonu ile maksimal tümör boyutu arasında önemli bir korelasyon bulunmadı. Makroadenomaların, p53 gen ekspresyonu ile Hardy'nin klasifikasyonuna göre tümör invazyonu karşılaştırıldığında, p53 değerleri ile tümör invazyonu arasında istatistiksel olarak önemli bir korelasyon saptanmadı. Operasyon sonrası rezidüel tümör değerlendirilmesinde, p53 gen ekspresyon pozitifliği ile rezidüel tümör boyutları artışı arasında kuvvetli bir korelasyon bulunduğunu söyleyebiliriz. **Sonuç:** Makroadenomaların Ki-67 proliferasyon indeksi ve p53 gen ekspresyon düzeylerinin saptanması ve bunların hormon profilleri, invazyon, operasyon öncesi primer tümör ve operasyon sonrası rezidüel tümör boyutu parametleri ile olan ilişkileri erken tümör progresyonunun ve hasta prognozunun tahmininde kombine olarak kullanılabilir.

Anahtar Kelimeler: İmmünohistokimyasal; manyetik rezonans görüntüleme; hipofizer adenoma; nüks

Correspondence: Ahmet Mesrur HALEFOĞLU
Department of Radiology, University of Health Sciences Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, TURKEY/TÜRKİYE
E-mail: halefoglu@ hotmail.com

Peer review under responsibility of Turkiye Klinikleri Journal of Medical Sciences.

Received: 03 Nov 2020 ***Accepted:*** 21 Dec 2020 ***Available online:*** 14 Jan 2021

Pituitary adenomas constitute about 10-15% of all intracranial tumors.[1] Although they are usually benign and have a tendency to slow growth, in some cases adenomas may present with signs of mass effect like visual loss, hypopituitarism and hormonal problems. In approximately one third of the cases, infiltration of the surrounding structures including the cavernous sinus can be seen and this can partly explain why relapsing might occur frequently even after total removal of the tumor tissue.[2,3] These tumors are usually huge in size and have an invasive nature, therefore complete surgical removal is almost impossible in most of the cases. Large tumor size, invasion and inadequate resection of the tumor are important risk factors in terms of progression or recurrence.[4] Some histologic predictors have been used to evaluate tumor progression or recurrence. Ki-67 is known as a nuclear antigen and its labeling index is regarded as a marker for normal and abnormal cellular proliferation (growh fraction). Immunohistochemical p53 gene expression has also been used as another histologic marker in daily practice. The aim of our study was to investigate the utility of these two histologic markers in the prediction of clinical and radiologic findings of macroadenomas. These include hormone profiles, invasiveness, preoperative primary and postoperative residual tumor dimensions. We sought a correlation between these two histologic markers through obtained clinical and radiological findings.

MATERIAL AND METHODS

PATIENT SELECTION

This study was approved by our Instutitional Research Ethics Committee and informed consents were obtained from all of the patients (Ethics Committee Approval: Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital, decision no: 1429, date: 25.2.2020). The study was carried out according to Helsinki Declaration principles. Beetween March 2015 and April 2019, retrospective evaluation of 81 pituitary macroadenomas was performed where preoperative, six-month and one-year follow-up postoperative dynamic contrast-enhanced magnetic resonance imaging (MRI) examinations were available in our picture archiving and data system. In all patients, the tumors were removed surgically and pathology diagnoses were consistent with macroadenoma. Patient cohort was of 39 males (48.2%) and 42 females (51.8%). Their mean age was 45.6±12.55 years. Mean tumor diameter was calculated as 27.1±10.8 mm (12-62 mm). They were classified as 17 null cell adenoma, 16 pluriform adenoma, 16 adrenocorticotrop hormone (ACTH)-releasing adenoma, 19 growth hormone (GH)-releasing adenoma and 13 gonadotropine-releasing adenoma based on hormonal profiles.

MAGNETIC RESONANCE IMAGING ANALYSIS

MR examination was performed by using a 12-channel phased-array head coil on a 1.5 tesla clinical scanner (Avanto- SQ Engine, Siemens, Erlangen, Germany). MRI parameters were as follows: First, non-contrast Turbo spin echo (TSE) sagittal T2 weighted [time to repeat (TR): 2700 msec, time to echo (TE): 96 msec, slice thickness 3 mm, field of view (FOV): 180x180 mm, nex: 2, matrix: 256x320], TSE coronal T2 weighted (TR: 2630 msec, TE: 96 msec, slice thickness 3 mm, FOV: 180x180 mm, nex: 2, matrix: 224x320), TSE sagittal T1 weighted (TR: 500 msec, TE: 17 msec, slice thickness 3 mm, FOV: 180x180 mm, nex: 2, matrix: 205x256), TSE coronal T1 weighted (TR: 500 msec, TE: 17 msec, slice thickness 3 mm, FOV: 180x180 mm, nex: 2, matrix: 205x256) and axial TSE T1 weighted (TR: 410 msec, TE: 8 msec, slice thickness 5 mm, FOV: 230x230 mm, nex: 2, matrix: 205x256) images were obtained. Then, following 0.1 mmol/kg iv gadolinium administration, sagittal and dynamic coronal plane TSE T1 weighted (TR: 300 msec, TE: 12 msec, slice thickness 3 mm, FOV: 180x180 mm, nex: 2, matrix: 205x256) images were also provided. All patients had pre-operative, six-month and one-year follow-up post-operative dynamic contrast-enhanced MRI examinations.

The images were interpreted by two experienced neuroradiologists by consensus who were blinded to the patients' pathology results. In all cases, maximal primary tumor and residual tumor diameters on pre-operative and post-operative MRI scans were calculated, respectively. Hardy's classification system was used to evaluate invasiveness of macroadenomas and categorized into five types: A) tumor bulging into the chiasmatic cistern, B) tumor reaching the anterior third

ventricle, C) huge suprasellar extension filling the third ventricle entirely, D) parasellar extension into the frontal, temporal, or posterior fossa, E) lateral expansion towards the cavernous sinus.[5]

PATHOLOGIC EVALUATION

Pathology and immunohistochemistry (IHC): The surgical specimens used in this study were obtained from neurosurgery clinics. Formalin-fixed and paraffin-embedded sections of 4 µm from samples were used for IHC staining. Paraffin-embedded tissue Ki-67 and p53 re-evaluation was scored by a specialized pathologist. The biological markers of proteins were analysed based on the immunohistochemical analysis protocol in paraffin sections by using the staining procedure. Leica bond system dewaxed paraffin by using leica reagent. Leia cell conditioning solution (pH 8.4, -100 °C, 60 min) was applied in order to restore antigenic epitopes. Those sections were incubated by using monoclonal antibodies during 32 min at 37 °C and identified with leica DAB detection kit. At the end of IHC reaction, these sections were contrasted with hematoxylin solution, then coloured with the reagent of an aqueous solution of buffered lithium carbonate, and finally covered with glass slides. All IHC results were independently reviewed by the neuropathologist who was blinded to the clinical data. They were expressed in the nucleus with the color of the red.

STATISTICAL ANALYSIS

For statistical analysis, the software named SSPS 15.0 for Windows was applied. Descriptive statistics were determined as follows: For categorical variables, number and percentage, for numerical variables mean, standard deviation, minimum, and maximum were used. The ones in groups were compared using the chi-square test. Since numerical variables showed a normal distribution pattern, the Student's t-test was used for comparison. The intersection value was analyzed using ROC curve analysis. Statistical significance level alpha was determined as $p<0.05$.

RESULTS

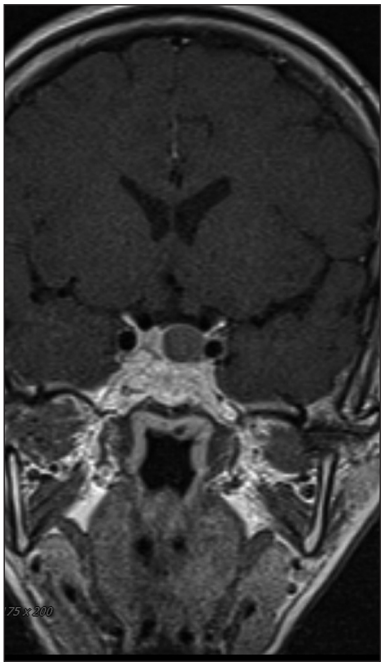
Our patient cohort's clinic, radiology and pathology features were summarized on Table 1. In this study, we considered Ki-67 proliferation index as positive at a 2-3% and above levels. Thus, 63 patients (77.8%) were found to be Ki-67 negative and 18 patients (22.2%) were positive. Regarding p53 gene expression, 76 patients (93.8%) were negative and only 5 patients (6.2%) were positive. In comparison of Ki-67 negative and positive macroadenoma cases in terms of tumor dimensions, maximal tumor diameter was found as 12-44 mm (mean diameter: 25.4±8.8 mm) and 13-62 mm (mean diameter: 32.9±15 mm) in Ki-67 negative and positive cases, respectively. Hence, in Ki-67 positive macroadenoma cases, maximal tumor diameter was found significantly higher than in

TABLE 1: General features of patient population.

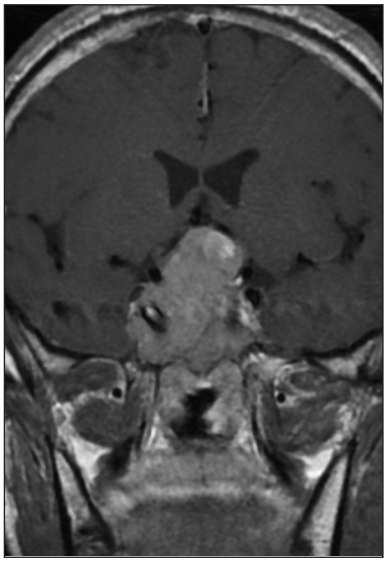
		Frequency	Percent
Suprasellar invasion	Negative	17	21.0
	Positive	64	79.0
3rd ventricle recess invasion	Negative	29	35.8
	Positive	52	64.2
Cavernous sinus invasion	Negative	17	21.0
	Positive	64	79.0
Hardy's classification	A	11	13.6
	B	2	2.5
	D	3	3.7
	E	65	80.2
Size [Mean±SD (Minimum-Maximum)]		27.1±10.8 (12-62)	
Hormone	Null cell	17	21.0
	Pluriform	16	19.8
	ACTH	16	19.8
	GH	19	23.5
	Gonadotrope	13	16.0
Residual increase in size	Negative	66	81.5
	Positive	15	18.5
p53	Negative	76	93.8
	Positive	5	6.2
Kİ-67	1%	11	13.6
	1-2%	42	51.9
	2%	10	12.3
	2-3%	6	7.4
	3%	3	3.7
	3-4%	4	4.9
	4-5%	1	1.2
	5%	1	1.2
	5-6%	1	1.2
	10%	1	1.2
	15%	1	1.2
Ki-67	Negative	63	77.8
	Positive	18	22.2

SD: Standard deviation; ACTH: Adrenocorticotrop hormone; GH: Growth hormone.

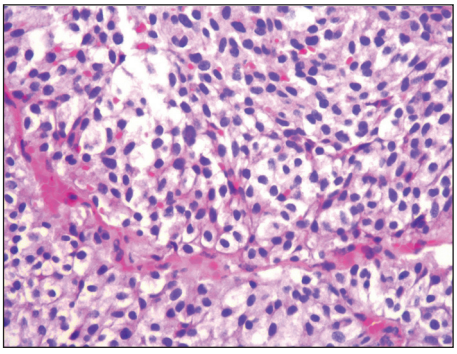
Ki-67 negative adenoma cases (p=0.009) (Picture 1, Figure 1). A receiver operating characteristic (ROC) curve analysis was carried out in order to predict Ki-67 positivity using maximal tumor diameter and a cutoff value of ≥26.5 mm yielded a 66.7% sensitivity and a 57.1% specificity (Figure 2). When we compared tumor invasiveness based on Hardy's classification with the Ki-67 proliferation index values of macroadenomas, there was no statistically significant correlation between tumor invasiveness and Ki-67 values (p=0.652) (Picture 2, Table 2). Regarding the hormone profiles of macroadenomas in correlation with Ki-67 values, we observed that in Ki-67 positive tumors, although the number of ACTH-releasing adenoma cases were significantly lower, go-



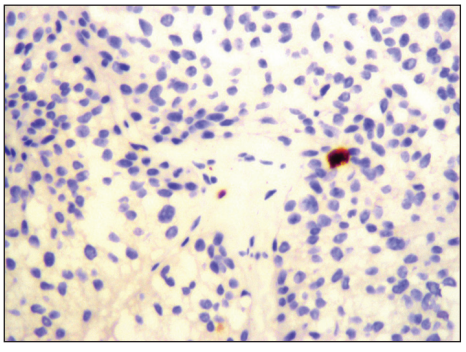
PICTURE 1a: Coronal T1 weighted contrast-enhanced magnetic resonance imaging (MRI) of a 35-year-old woman. A homogenous, hypointense, well-demarcated, moderate size, intrasellar mass lesion is present. Ki-67 was found negative.



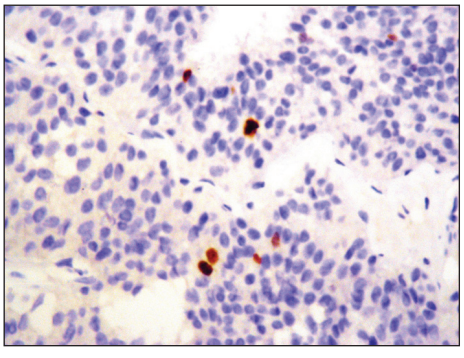
PICTURE 1b: Coronal T1 weighted contrast-enhanced MRI of a 51-year-old man. A huge intrasellar mass lesion extending to the suprasellar cistern and third ventricule also encasing both cavernous carotid arteries is present. Ki-67 was found positive.



PICTURE 1c: Adenoma tissue containing round, oval shaped nuclei.



PICTURE 1d: Pituitary adenoma containing less than 1% Ki-67 proliferation marker.



PICTURE 1e: Pituitary adenoma containing more than 3% Ki-67 proliferation marker.

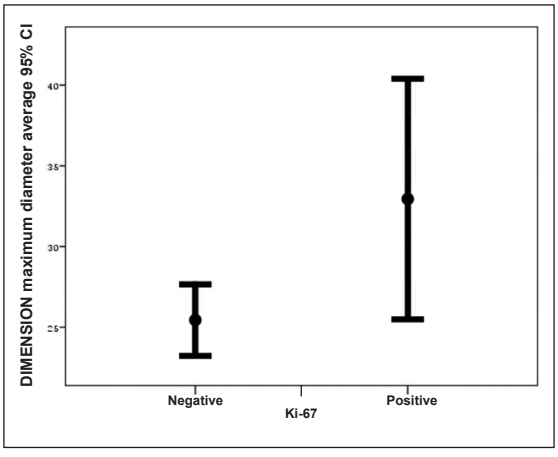
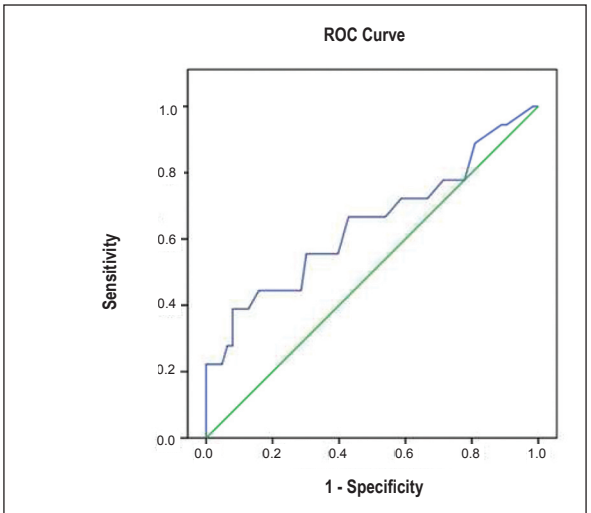


FIGURE 1: Comparison of Ki-67 positive and Ki-67 negative cases in terms of maximal tumor diameter.

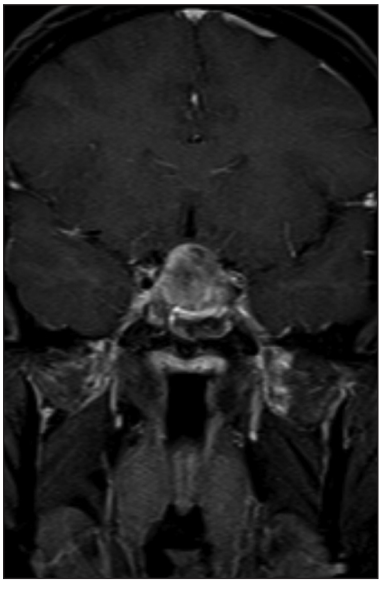


AUC: 0.644 (95% CI 0.483-0.806)

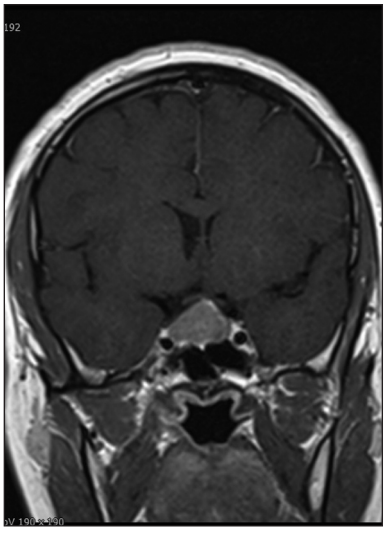
Coordinates of the curve Test result variable (s): SIZEmax Positive if greater than or equal to (a)	Sensitivity	Specificity
21.5	0.722	0.381
22.5	0.722	0.413
23.5	0.667	0.460
24.5	0.667	0.476
25.5	0.667	0.492
26.5	**0.667**	**0.571**
27.5	0.611	0.587
28.5	0.556	0.603
29.5	0.556	0.635
30.5	0.556	0.698

FIGURE 2: ROC curve analysis for maximal tumor diameter in terms of predicting Ki-67 positiveness.

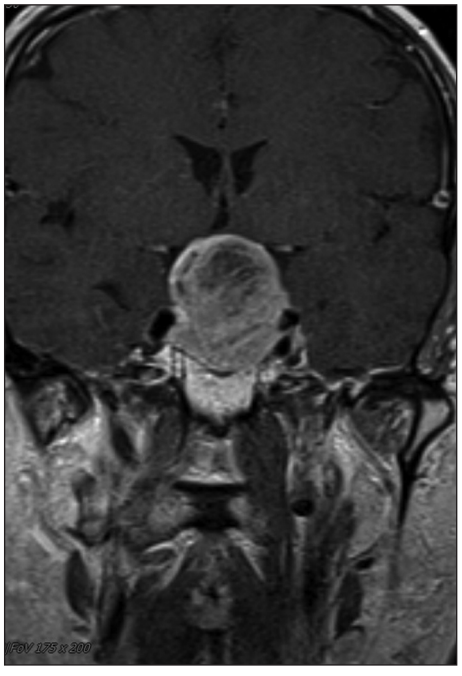
nadotropine-releasing adenoma cases were found significantly higher compared to Ki-67 negative macroadenoma cases (p=0.008) (Picture 3, Table 2, Figure 3). Post-operative residual tumor evaluation showed that in Ki-67 negative cases, in 60 out of the total 63 patients (95.2%), no increase in residual mass was detected, and in only 3 cases (4.8%) an increase in residual mass dimension was found. However, in Ki-67 positive macroadenoma cases, 12 out of 18 (66.7%) patients showed an increase in their residual tumor size and in only 6 cases (33.3%) there was no increase in residual tumor. These findings showed that there is a strong correlation between Ki-67 positiveness and an increase in residual tumor dimension



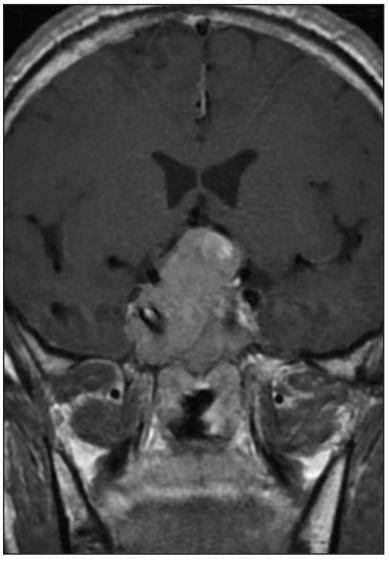
PICTURE 2a: Coronal T1 weighted contrast-enhanced magnetic resonance imaging (MRI) of a 46-year-old man. A Hardy's D suprasellar mass lesion is detected. Patient was Ki-67 negative.



PICTURE 2b: Coronal T1 weighted contrast-enhanced MRI of a 35-year-old woman. Another Hardy's D suprasellar mass lesion is present, but the patient is Ki-67 positive.



PICTURE 2c: Coronal T1 weighted contrast-enhanced MRI of a 43-year-old man. Hardy's E suprasellar mass lesion is present. Patient was Ki-67 negative.



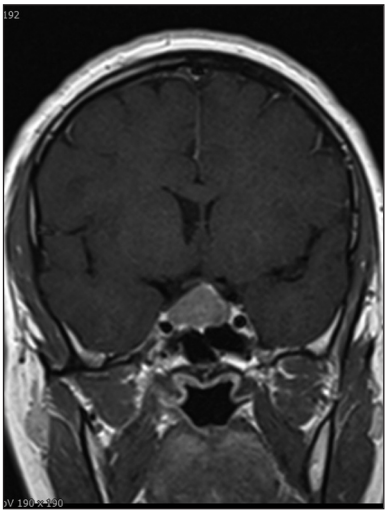
PICTURE 2d: Coronal T1 weighted contrast-enhanced MRI of a 51-year-old man. Another Hardy's E suprasellar mass lesion is present, but the patient is Ki-67 positive.

(p=0.001) (Picture 4, Table 2, Figure 4). When we compared the residual tumor mass diameters with the pre-operative primary tumor, we observed that in increased residual tumor size cases, there was a strong correlation with pre-operative higher tumor dimension (Picture 5, Table 3) (p<0.001). On the other hand, there was no statistically significant correlation between residual tumor increase and Hardy's classification (p=0.253) (Table 3). In comparison of p53 negative and positive macroadenoma cases, maximal tumor diameter was found to be 12-56 mm (mean diameter: 26.6±10.3 mm) and 20-62 mm (mean diameter: 34.2±17.1 mm) in p53 negative and positive cases, respectively. In p53 negative and positive macroadenoma cases, there was no significant correlation between p53 gene expression and maximal tumor diameter (p=0.132). When we compared tumor invasiveness based on Hardy's classification with the p53 gene expression of macroadenomas, there was again no statistically significant correlation between tumor invasiveness and p53 values (p=1.000) (Table 4). Regarding the hormone profiles of p53 negative and p53 positive macroadenomas, we found no significant correlation between the p53 gene expression and the hormone profiles of macroadenomas (p=0.232) (Table 4). Post-operative residual tumor evaluation revealed that in p53 negative cases, in 64 cases from a total of 76 patients (84.2%) no residual mass was found, but in 12 cases (15.8%) there was an increase in residual mass dimension. However, in

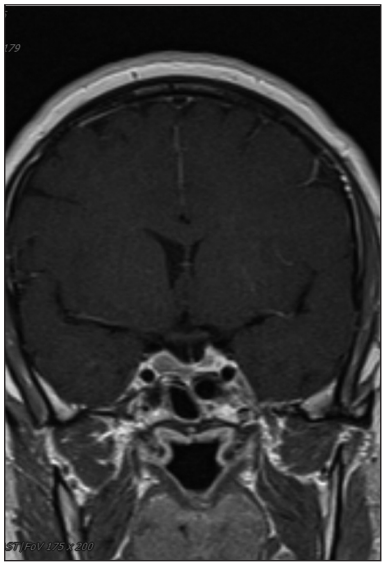
TABLE 2: Comparison of Ki-67 positive and Ki-67 negative cases in terms of Hardy's classification, hormone profiles and post-op residual tumor increase.

	Ki-67				
	Negative		Positive		
Hardy's classification	n	%	n	%	p value
A	10	15.9	1	5.6	0.652
B	2	3.2	0	0.0	
D	2	3.2	1	5.6	
E	49	77.8	16	88.9	
Size [Mean±SD (Minimum-Maximum)] hormone	25.4±8.8	(12-44/26)	32.9±15.0	(13-62/31)	0.009
Null cell	15	23.8	2	11.1	0.008
Pluriform	13	20.6	3	16.7	
ACTH	15	23.8	1	5.6	
GH	15	23.8	4	22.2	
Gonadotrope	5	7.9	8	44.4	
Residual increase in size					
Negative	60	95.2	6	33.3	<0.001
Positive	3	4.8	12	66.7	

SD: Standard deviation; ACTH: Adrenocorticotrop hormone; GH: Growth hormone.

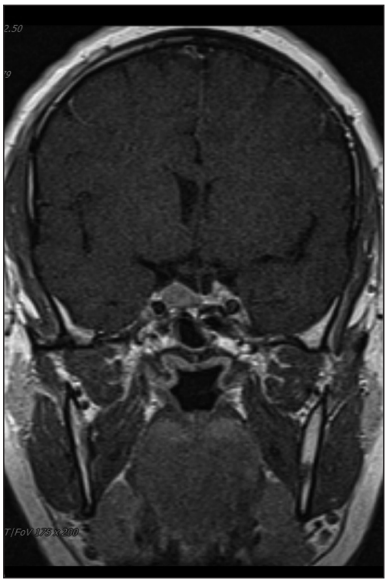


PICTURE 3a: Coronal T1 weighted contrast-enhanced magnetic resonance imaging (MRI) of a 35-year-old woman. An intrasellar mass lesion extending superiorly to the optic-chiasmatic region is present. The patient has gonadotropin hormone releasing macroadenoma and was Ki-67 positive.

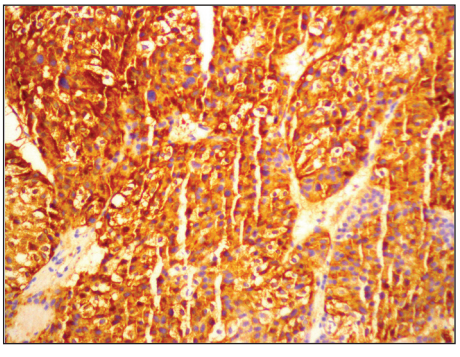


PICTURE 3b: Coronal T1 weighted post-operative six-month follow-up contrast-enhanced MRI of the same patient shows residual mass lesion on the right lobe.

p53 positive macroadenoma cases, 3 out of 5 (60%) patients showed an increase in their residual tumor size and 2 cases (40%) had no increase of the residual tumor. Therefore, there is a strong correlation between p53 gene expression positiveness and increase in residual tumor dimension (p=0.042) (Picture 6, Table 4). In this study, the maximal diameter of ACTH-releasing adenomas was found significantly lower compared to those of null cell adenomas (p=0.002) and pluriform adenomas (p=0.004) (Table 5, Figure 5). When we compared the hormone profiles of macroadenomas with increased residual tumor size, there was no statistically significant cor-



PICTURE 3c: Coronal T1 weighted post-operative one-year follow-up contrast-enhanced MRI reveals increase in size of the residual mass.



PICTURE 3d: Gonadotropin-releasing macroadenoma shows immunohistochemical positivity for FSH.

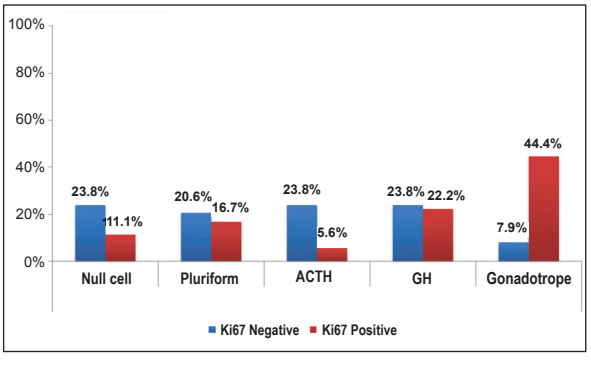
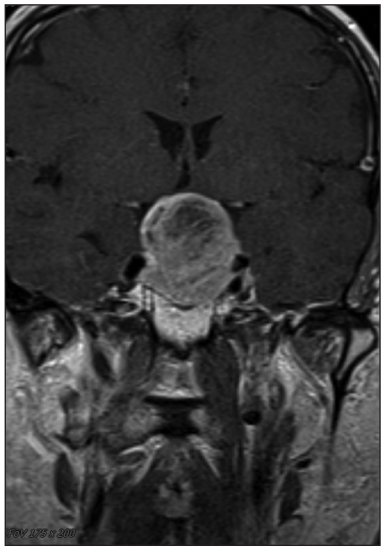
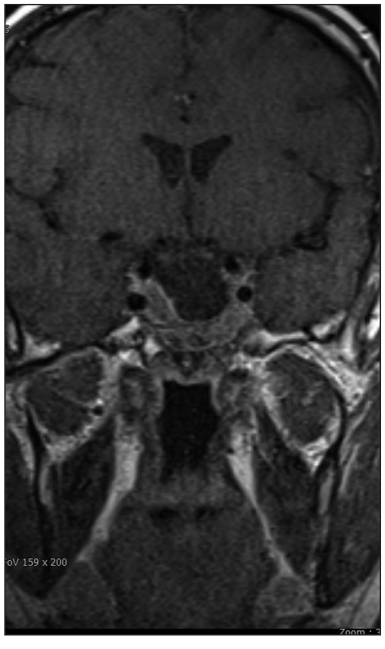


FIGURE 3: Hormon profiles of Ki-67 positive and Ki-67 negative cases.
ACTH: Adrenocorticotrop hormone; GH: Growth hormone.



PICTURE 4a: Coronal T1 weighted pre-operative contrast-enhanced magnetic resonance imaging (MRI) of a 43-year-old man. A huge intrasellar mass lesion extending to the third ventricle is present in a Ki-67 negative patient.

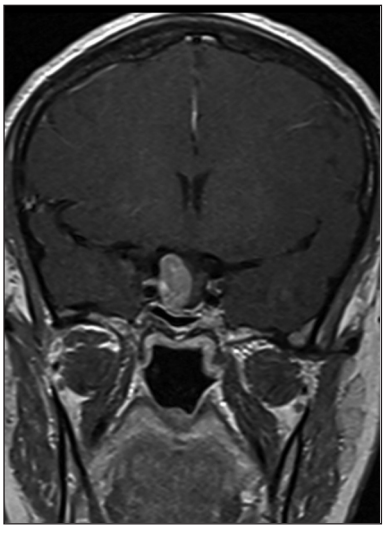


PICTURE 4b: Coronal T1 weighted post-operative one-year follow-up contrast-enhanced MRI of the same patient shows no residual mass lesion.

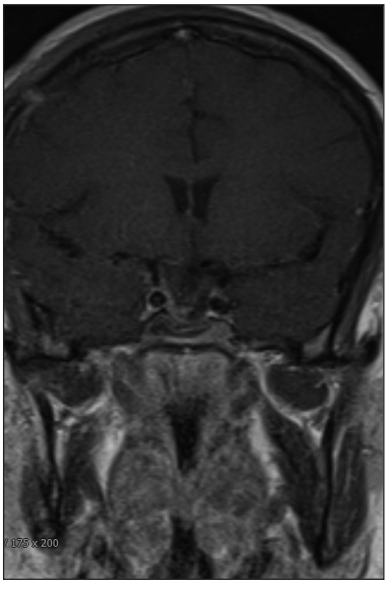
relation between the hormone profile of macroadenomas and increased residual tumor size (p=0.101) (Table 6).

DISCUSSION

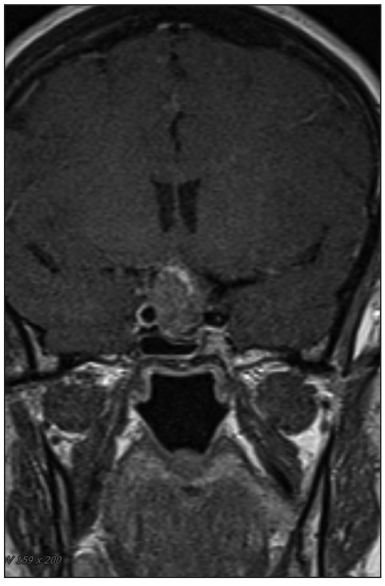
Computed tomography (CT) and MRI are widely being used in the evaluation of pituitary macroadenomas. While MRI enables comprehensive delineation of the intrasellar and parasellar soft tissue structures with excellent contrast resolution, CT provides a detailed anatomical assessment of the sellar bony structures. Pituitary invasive macroadenomas have high recurrence rates. They are not considered to be malignant tumors and their biological behaviour is presumed to be between non-infiltrative adenomas and carcinomas. Radiological signs of tumor invasion



PICTURE 4c: Coronal T1 weighted contrast-enhanced MRI of a 45-year-old woman. An intrasellar mass lesion located in the right lobe of the gland is present. The patient was Ki 67 positive.



PICTURE 4d: Coronal T1 weighted post-operative six-month follow-up contrast-enhanced MRI of the same patient shows no residual mass.



PICTURE 4e: Coronal T1 weighted post-operative one-year follow-up contrast-enhanced MRI of the same patient reveals recurrent mass lesion.

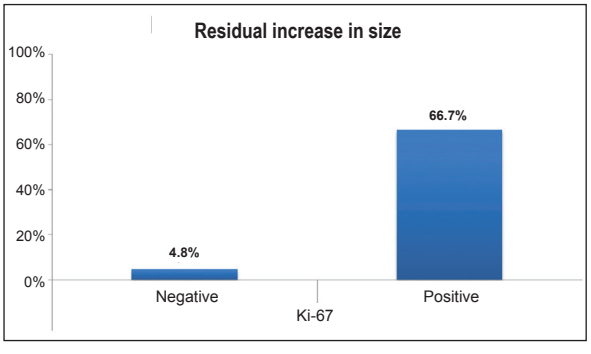
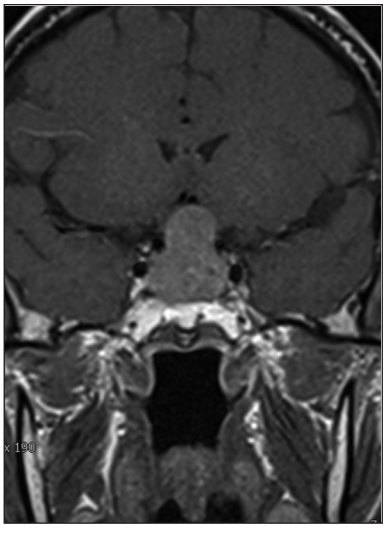


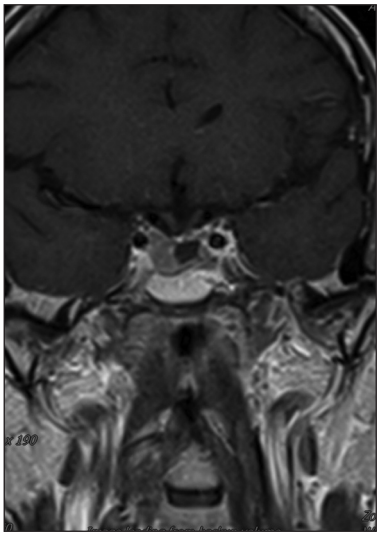
FIGURE 4: Comparison of Ki-67 positive and Ki-67 negative cases for residual tumor increase.

should be considered with the presence of those findings: complete erosion of the clivus, tumor elongation inferiorly to the sphenoid sinus and tumor extension beyond to the lateral boundary of the cavernous carotid artery or complete encircling of the artery.

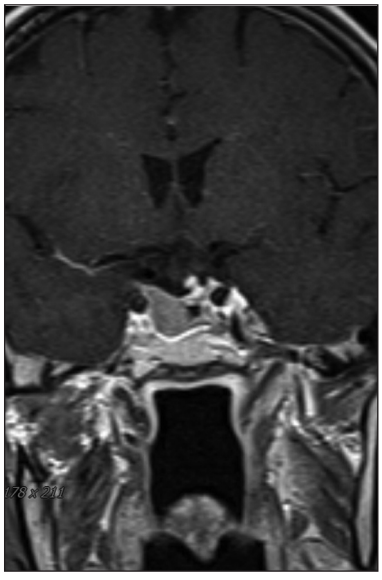
In order to predict tumor behaviour of macroadenomas, some histological markers have been used. In this regard, Ki-67 labeling index and p53 gene expression have been considered as useful histologic predictors in relevance to the progression or recurrence of macroadenomas. Ki-67 is regarded as a cell cycle specific nuclear antigen and can be easily recognised by various immunohistochemical methods like monoclonal antibody MIB-1.[6] It is typically expressed in proliferating cells during the G1, S, G2 and M phases of the cell cycle except G0 phase.[7] MIB-1 antibody can provide detection of Ki-67 in formalin-fixed and paraffin-embedded tissues. It provides knowledge about cellular proliferation rate or tumor growth fraction and hence can be used in the routine histologic evaluation of brain tumors including pituitary macroadenomas. But in pituitary macroadenomas Ki-67 labeling index levels have usually been found to be relatively low compared to other brain tumors.[8] A Ki-67 proliferation index level



PICTURE 5a: Coronal T1 weighted contrast-enhanced MRI of a 60-year-old man. A huge intrasellar mass lesion extending to the third ventricular region is present.



PICTURE 5b: Coronal T1 weighted post-operative six-month follow-up contrast-enhanced MRI of the same patient reveals residual mass in the right lobe.



PICTURE 5c: Coronal T1 weighted post-operative one-year follow-up contrast-enhanced MRI. There is increase in residual mass size.

TABLE 3: Comparison of residual tumor increase according to preoperative tumor diameter and Hardy's classification.

	Residual increase in size				
	Negative		Positive		
Hardy's classification	n	%	n	%	p value
A	11	16.7	0	0.0	0.253
B	2	3.0	0	0.0	
D	2	3.0	1	6.7	
E	51	77.3	14	93.3	
Maximal tumor diameter	24.9±8.7 cm	12-44 (24.5)	36.7±14.2 cm	15-62 (35)	<0.001

of 2-3% and above was considered as positive in this study. p53 expression of the tumors can also be detected by using immunohistochemical methods. p53 gene product is a nuclear phosphoprotein whose immunohistochemical accumulation has been regarded as an unfavorable prognostic factor for a broad range of human tumors. It can also be used as a histologic predictor in the evaluation of tumor behaviour of macroadenoma cases. Although overall expression rate of p53 has been found to be low in many studies, its overexpression could correlate with progression or recurrence of macroadenomas. Thapar et al. in their study found that a Ki-67 labeling index cutoff value of 3% and higher had a 73% sensitivity and 97% specificity in the discrimination of noninvasive versus invasive macroadenoma cases.[9] Pizarro et al. measured Ki-67 antigen levels of 159 pituitary adenomas using MIB-1 monoclonal antibody test. They found that tumoral proliferative activity detected by the level of Ki-67 antigen is significantly higher in invasive adenomas compared to those of noninvasive ones.[1] Zhao et al. in a 57 clinically verified invasive pituitary adenoma cases found a significant association between Ki-67 labeling index and the invasiveness of the tumor. They concluded that Ki-67 labeling index detected by MIB-1 could be an important marker in the evaluation of potential invasiveness of pituitary adenomas.[10] Paek et al. investigated the correlation between Ki-67 labeling index with clinical and radiological characteristics of pituitary macroadenomas in 44 patients. They found that clinical findings such as visual field defect and recurrence were correlated with the high Ki-67 labeling index. But there was no statistically significant correlation between Ki-67 labeling index and sex, age, type of tumor, maximal tumor diameter, Hardy's clas-

TABLE 4: Comparison of p-53 positive and p-53 negative cases in terms of Hardy's classification, pre-operative maximal tumor diameter, hormone profiles and post-operative residual tumor increase.

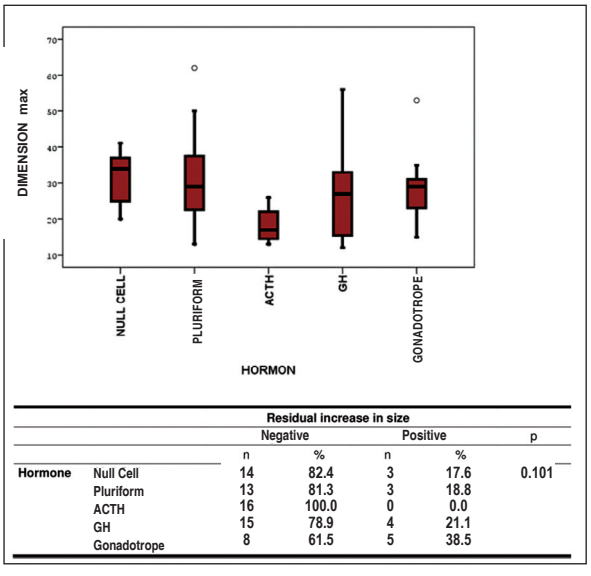
	p53				
	Negative		Positive		
Hardy's classification	n	%	n	%	p value
A	11	14.5	0	0.0	1.000
B	2	2.6	0	0.0	
D	3	3.9	0	0.0	
E	60	78.9	5	100	
Size [Mean±SD (Minimum-Maximum)]	26.6±10.3	(12-56/26)	34.2±17.1	(20-62/29)	0.132
hormone					
Null cell	17	22.4	0	0.0	0.232
Pluriform	14	18.4	2	40.0	
ACTH	16	21.1	0	0.0	
GH	18	23.7	1	20.0	
Gonadotrope	11	14.5	2	40.0	
Residual increase in size					
Negative	64	84.2	2	40.0	0.042
Positive	12	15.8	3	60.0	

SD: Standard deviation; ACTH: Adrenocorticotrop hormone; GH: Growth hormone.

TABLE 5: Comparison of the maximal diameter of ACTH-releasing adenomas with that of other adenomas.

		SIZEmax			
		Mean±SD	Minimum-Maximum	Median	p value
Hormone	Null cell	31.5±7.2	20-41	34	<0.001
	Pluriform	31.1±12.7	13-62	29	
	ACTH	18.1±4.6	13-26	17	
	GH	26.3±12.5	12-56	27	
	Gonadotrope	28.8±9.5	15-53	29	

SD: Standard deviation; ACTH: Adrenocorticotrop hormone; GH: Growth hormone.



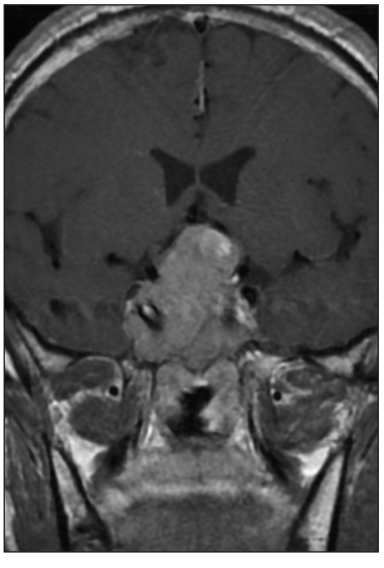
		Residual increase in size				
		Negative		Positive		p
		n	%	n	%	
Hormone	Null Cell	14	82.4	3	17.6	0.101
	Pluriform	13	81.3	3	18.8	
	ACTH	16	100.0	0	0.0	
	GH	15	78.9	4	21.1	
	Gonadotrope	8	61.5	5	38.5	

FIGURE 5: Comparison of hormone profiles and maximal tumor diameters. ACTH: Adrenocorticotrop hormone; GH: Growth hormone.

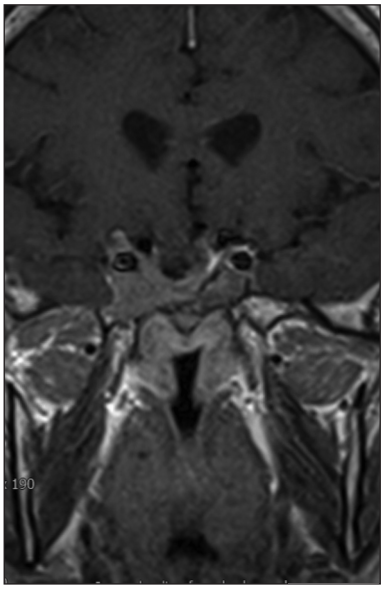
sification and invasiveness of tumor into the sphenoid sinus or cavernous sinus.[11] In our study, we evaluated tumor invasiveness according to Hardy's classification and compared these radiologic findings with the Ki-67 proliferation index values of macroadenomas. However, we did not find a statistically significant correlation between tumor invasiveness and Ki-67 values. The tumor size, indicated as the maximal diameter of the tumor on MRI and its relationship to the Ki-67 labeling index was investigated in some studies and there was no significant correlation.[11,12] In our study, maximal tumor diameter was found to be significantly higher in Ki-67 positive macroadenoma cases than those of Ki-67 negative ones. Jaffrain-Rea et al. assessed the proliferative index in a prospective series of 132 pituitary tumors and found that Ki-67 values showed escalation with increased tumor volume and invasiveness.[13]

Although there is a benign nature and slow growth rate in adenomas, their recurrence rate could be as high as 10% to 35%.[4] The most reasonable explanation for this situation might be inadequate removal of the tumor. Paek et al. in their patient cohort of a total 44 pituitary macroadenomas found that in 14 cases tumor recurrence occured. In this group, macrodenomas with recurrence had a significantly higher mean Ki-67 labeling index values (1.27%) than those of without recurrence (0.56%).[11]

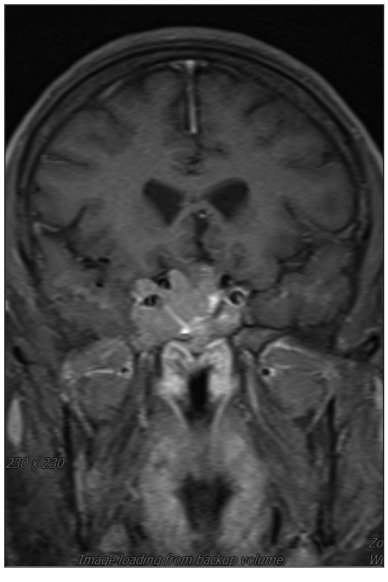
Filippella et al. in their study including 45 pituitary adenomas determined a cut off value of 2.9% for Ki-67 proliferation index and emphasized this value as the most reliable biologic marker in terms of predicting recurrence status of pituitary macroadenomas.[14] Shibuya et al. showed that recurrent pituitary adenomas exhibited higher Ki-67 labeling index val-



PICTURE 6a: Coronal T1 weighted contrast-enhanced magnetic resonance imaging (MRI) of a 51-year-old man. A huge intrasellar mass lesion extending to the third ventricular region and encasing both cavernous carotid arteries is present. The patient was p53 positive.



PICTURE 6b: Coronal T1 weighted post-operative six-month follow-up contrast-enhanced MRI of the same patient shows residual mass on the right.



PICTURE 6c: Coronal T1 weighted post-operative one-year follow-up contrast-enhanced MRI. There is an obvious increase in residual mass.

ues (3.6%) than those of primary adenomas (0.8%).[15] Ekramullah et al. in their study demonstrated significantly higher Ki-67 proliferation index values in the regrowing pituitary adenoma group compared to the cured adenoma patients (0.86% and 0.23%, respectively).[16] Contrary to these results, there are some studies in the literature which do not support a relationship with Ki-67 proliferation index and recurrence. Scheithauer et al. studied 176 pituitary tumors and did not find an association between the Ki-67 proliferation index and recurrence. They had only 7 patients with recurrence in a total of 78 cases whose Ki-67 proliferation index values were found to be more than 3%.[17] In our study, we found a strong association between Ki-67 positiveness and increase in residual tumor dimensions.

In the literature, there are studies that investigated whether the association could be between pituitary tumor Ki-67 labeling index levels with their hormone profiles. Thapar et al. showed significantly higher mean growth fraction levels in hormonally active pituitary adenomas (3.25%) compared to those of non-functioning adenomas (2.06%).[9] Wolfsberger et al. in their 150 functioning adenoma series found that silent ACTH-cell and prolactin-releasing adenomas had the highest, but null-cell adenomas and gonadotropinomas had the lowest proliferation index values.[18] On the other hand, Pizarro et al. in a 139 pituitary adenoma cases did not find a correlation between tumoral proliferative activity expressed by Ki-67 antigen and hormonal status of adenomas.[1]

In our study, we found that gonadotropine-releasing adenoma cases were significantly higher, whereas ACTH-releasing adenoma cases were significantly lower in Ki-67 labeling index positive adenoma cases. Besides, maximal diameter of ACTH-releasing adenomas was found to be significantly lower than those of null cell adenomas and pluriform adenomas. On the other hand, there was no statistically significant correlation between the hormone profiles and increased residual tumor size.

TABLE 6: Comparison of hormone profiles and post-operative residuel mass increase.

	Residual increase in size				
	Negative		Positive		
Hormone	n	%	n	%	p value
Null cell	14	82.4	3	17.6	0.101
Pluriform	13	81.3	3	18.8	
ACTH	16	100.0	0	0.0	
GH	15	78.9	4	21.1	
Gonadotrope	8	61.5	5	38.5	

ACTH: Adrenocorticotrop hormone; GH: Growth hormone.

Gejman et al. in their study indicated that a threshold value of 1.3% for the Ki-67 labeling index could be considered as a very suggestive indicator for post-operative tumor progression, but they did not find a significant association between p53 overexpression and tumor progression or recurrence.[19] Thapar et al. in their study consisting of 70 pituitary adenomas and 7 carcinomas demonstrated a highly significant correlation between p53 expression and biologic behaviour of pituitary tumors. They found immunopositivity for p53 in a total of 12 cases in their patient cohort in which all of them were either invasive adenoma or pituitary carcinoma cases. They concluded that p53 expression might have diagnostic utility in the prediction of aggressive tumor behaviour.[20] We also found a significant correlation between p53 gene expression positiveness and increase in residual tumor dimensions.

CONCLUSION

In conclusion, determination of Ki-67 proliferation index and p53 gene expression levels in macroadenomas and their relationship to the hormone profiles, invasiveness, preoperative primary tumor and postoperative residual tumor dimension parameters can be combined in the prediction of early tumor progression and patient prognosis. Thus, active surveliance and treatment protocols can be developed more specifically with these combined protocols and may be individualized for each patient. Further comprehensive studies are required to ratify our findings.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ahmet Mesrur Halefoğlu, Canan Tanık;* ***Design:*** *Ahmet Mesrur Halefoğlu;* ***Control/Supervision:*** *Ahmet Mesrur Halefoğlu, Mustafa Karabina;* ***Data Collection and/or Processing:*** *Canan Tanık, Mustafa Karabina;* ***Analysis and/or Interpretation:*** *Ahmet Mesrur Halefoğlu;* ***Literature Review:*** *Ahmet Mesrur Halefoğlu, Mustafa Karabina;* ***Writing the Article:*** *Ahmet Mesrur Halefoğlu;* ***Critical Review:*** *Ahmet Mesrur Halefoğlu;* ***References and Fundings:*** *Ahmet Mesrur Halefoğlu, Canan Tanık;* ***Materials:*** *Ahmet Mesrur Halefoğlu, Mustafa Karabina.*

REFERENCES

1. Pizarro CB, Oliveira MC, Coutinho LB, Ferreira NP. Measurement of Ki-67 antigen in 159 pituitary adenomas using the MIB-1 monoclonal antibody. Braz J Med Biol Res. 2004;37(2):235-43.[Crossref] [PubMed]
2. Mastronardi L, Guiducci A, Puzzilli F. Lack of correlation between Ki-67 labelling index and tumor size of anterior pituitary adenomas. BMC Cancer. 2001;1:12.[Crossref] [PubMed] [PMC]
3. Atkin SL, Green VL, Hipkin LJ, Landolt AM, Foy PM, Jeffreys RV, et al. A comparison of proliferation indices in human anterior pituitary adenomas using formalin-fixed tissue and in vitro cell culture. J Neurosurg. 1997;87(1):85-8. [Crossref] [PubMed]
4. Hsu DW, Hakim F, Biller BM, de la Monte S, Zervas NT, Klibanski A, et al. Significance of proliferating cell nuclear antigen index in predicting pituitary adenoma recurrence. J Neurosurg. 1993;78(5):753-61. [Crossref] [PubMed]
5. Hardy J. Transsphenoidal surgery of hypersecreting pituitary tumors. In: Kohler PO, Ross GT, eds. Diagnosis and Treatment of Pituitary Tumors. New York: American Elsevier: Excerpta Medica; 1973. p.179-94.
6. McCormick D, Chong H, Hobbs C, Datta C, Hall PA. Detection of the Ki-67 antigen in fixed and wax-embedded sections with the monoclonal antibody MIB1. Histopathology. 1993;22(4):355-60.[Crossref] [PubMed]
7. Knosp E, Kitz K, Perneczky A. Proliferation activity in pituitary adenomas: measurement by monoclonal antibody Ki-67. Neurosurgery. 1989;25(6):927-30.[Crossref] [PubMed]
8. Kitz K, Knosp E, Koos WT, Korn A. Proliferation in pituitary adenomas: measurement by MAb KI 67. Acta Neurochir Suppl (Wien). 1991;53:60-4.[Crossref] [PubMed]
9. Thapar K, Kovacs K, Scheithauer BW, Stefaneanu L, Horvath E, Pernicone PJ, et al. Proliferative activity and invasiveness among pituitary adenomas and carcinomas: an analysis using the MIB-1 antibody. Neurosurgery. 1996;38(1):99-106; discussion 106-7.[Crossref] [PubMed]
10. Zhao D, Tomono Y, Nose T. Expression of P27kip1 and Ki-67 in pituitary adenomas: an investigation of marker of adenoma invasiveness. Acta Neurochir (Wien). 1999;141(2):187-92. [Crossref] [PubMed]

11. Paek KI, Kim SH, Song SH, Choi SW, Koh HS, Youm JY, et al. Clinical significance of Ki-67 labeling index in pituitary macroadenoma. J Korean Med Sci. 2005;20(3):489-94.[Crossref] [PubMed] [PMC]
12. Carboni P Jr, Detta A, Hitchcock ER, Postans R. Pituitary adenoma proliferative indices and risk of recurrence. Br J Neurosurg. 1992;6(1): 33-40.[Crossref] [PubMed]
13. Jaffrain-Rea ML, Di Stefano D, Minniti G, Esposito V, Bultrini A, Ferretti E, et al. A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas. Endocr Relat Cancer. 2002;9(2):103-13. [Crossref] [PubMed]
14. Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, Colao A, Meduri G, Chanson P. Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf). 2006 Oct;65(4):536-43.[Crossref] [PubMed]
15. Shibuya M, Saito F, Miwa T, Davis RL, Wilson CB, Hoshino T. Histochemical study of pituitary adenomas with Ki-67 and anti-DNA polymerase alpha monoclonal antibodies, bromodeoxyuridine labeling, and nucleolar organizer region counts. Acta Neuropathol. 1992;84(2):178-83.[Crossref] [PubMed]
16. Ekramullah SM, Saitoh Y, Arita N, Ohnishi T, Hayakawa T. The correlation of Ki-67 staining indices with tumour doubling times in regrowing non-functioning pituitary adenomas. Acta Neurochir (Wien). 1996;138(12):1449-55. [Crossref] [PubMed]
17. Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, et al. Pathobiology of pituitary adenomas and carcinomas. Neurosurgery. 2006;59(2):341-53; discussion 341-53.[Crossref] [PubMed]
18. Wolfsberger S, Wunderer J, Zachenhofer I, Czech T, Böcher-Schwarz HG, Hainfellner J, et al. Expression of cell proliferation markers in pituitary adenomas--correlation and clinical relevance of MIB-1 and anti-topoisomerase-IIalpha. Acta Neurochir (Wien). 2004;146(8): 831-9. [Crossref] [PubMed]
19. Gejman R, Swearingen B, Hedley-Whyte ET. Role of Ki-67 proliferation index and p53 expression in predicting progression of pituitary adenomas. Hum Pathol. 2008;39(5):758-66.[Crossref] [PubMed]
20. Thapar K, Scheithauer BW, Kovacs K, Pernicone PJ, Laws ER Jr. p53 expression in pituitary adenomas and carcinomas: correlation with invasiveness and tumor growth fractions. Neurosurgery. 1996;38(4):765-70; discussion 770-1.[Crossref] [PubMed]