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Diagnostic and Prognostic Significance of p16, p53, and bcl-2 Expressions and Ki-67 Proliferation Index in Benign and Malignant Uterine Smooth Muscle Tumors

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: This study aimed to evaluate diagnostic parameters such as mitotic count and tumor size in; leiomyosarcomas (LMSs) and benign uterine smooth muscle tumors (USMTs) and to define the diagnostic value and prognostic significance of the Ki-67 proliferation index and p16, p53, and bcl-2 expressions by immunohistochemical (IHC) methods.

Materials and Methods: In total, 44 cases diagnosed as LMS, atypical leiomyoma, or cellular leiomyoma at our pathology department from January 2010 to December 2015 were included. IHC staining was performed for bcl-2, p16, p53, and Ki-67 using standard techniques.

Results: Tumor size and mitotic index were significant prognostic factors (p=0.008 and p=0.001, respectively). The rate of diffuse p16 expression was significantly higher in the LMS group than in the other LM group (p=0.001). A Ki-67 positivity rate of >10% (increased proliferation) was statistically significantly higher in the LMS group than in the benign USMT group (p=0.0001). No statistically significant difference was found between the LMS and benign USMT groups with respect to bcl-2 expression (p=0.892). Mitotic count and high Ki-67 expression (>%10) were statistically high in cases with relapse/metastasis (+) (p=0.0001 and p=0.0002, respectively).

Conclusion: In addition to histopathological findings (tumor size and mean mitotic count), diffuse p16 expression and p53 overexpression can be used to distinguish between benign and malignant USMTs. A high mitotic index [≥10/10 (high-power field)] and high Ki-67 expression (>10%) can serve as useful indicators for diagnosing LMS, distinguishing benign tumors, and predicting an aggressive clinical course.

Keywords: Uterine smooth muscle tumor, relapse, metastasis, p16, bcl-2

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INTRODUCTION

Uterine smooth muscle tumors (USMTs) are the most common neoplasms of the female genital tract. They range from typical leiomyomas (TLs) to highly aggressive malignant leiomyosarcomas (LMSs). Benign USMTs are common in the uterus; however, malignant USMTs such as LMS and endometrial stromal sarcomas are rare. LMSs account for approximately 1.3% of all uterine malignancies (1-3). Depending on histopathological features, the subtypes of USMTs include TLs, cellular leiomyoma (CL), atypical leiomyoma (AL), SMTs with unknown malignancy potential (STUMP), and LMS. Uterine LMSs usually have an aggressive clinical course, with high rates of local recurrence and metastasis. Even after surgery for patients with early stage disease (stage I; limited to uterus), the mean 5-year survival rate and recurrence/metastasis rates were reported to be 12%-25% and 53%-71%, respectively (3). The criteria required for histopathological differentiation between LMSs and leiomyomas has recently become clearer. The rate of nuclear atypia, presence of tumor necrosis, and high mitotic activity [mitosis if it is ≥10/high-power field (HPF)] are important criteria for defining malignancy. According to the 2014 criteria of the World Health Organization, moderate–to-significant atypia, high mitotic index, and/or tumor necrosis are required for diagnosing LMS (2). In some cases, the histopathological criteria may not be sufficient or may be unclear for diagnosing LMS. The histological differentiation between LMS and CL or AL may be problematic. Some immunohistochemical indicators are useful to pathologists for diagnosis of such cases. In addition to their usefulness in diagnostics, further analysis of some indicators is also required to determine the clinical course and he biological potential of a disease.

p16 is a cyclin-dependent kinase (CDK) inhibitor that specifically binds to the cyclin-dependent kinase CDK-4, inhibits the catalytic activity of the CDK-4–cyclin D complex, and acts as a negative cell cycle regulator (1, 4, 5). The immunohistochemical overexpression of p16 is more frequently observed in LMSs than in benign leiomyomas, and p16 overexpression may be useful for distinguishing between benign and malignant USMTs (2, 3, 6).

Ki-67 is a nuclear protein that is detected using mindbomb E3 ubiquitin protein ligase 1, which is a monoclonal antibody that is associated with RNA transcription and cell cycle progression (7, 8). In addition, numerous studies have demonstrated that the Ki-67 proliferation index (PI) in LMSs is significantly higher than that in benign leiomyomas (4, 7, 9).

p53 functions as a negative regulator of cell growth. The p53 proto-oncogene is associated with apoptosis and cell cycle regulation. p53 is reportedly overexpressed and/or mutated in LMS (10-12). Mutations in or overexpression of p53 have been determined to be significant in LMSs (25%-47%) but are rarely observed in benign leiomyomas (1-3, 5).

The expression of bcl-2, which is a protein involved in the cell cycle and in regulating apoptosis, was shown to be associated with improved prognosis. Several studies of LMS also reported that bcl-2 overexpression was associated with less lymphovascular invasion and prolonged survival (1, 3).

This study aimed to evaluate diagnostic parameters such as mitotic count and tumor size in malignant USMTs (LMSs) and benign USMTs (TL, AL, and SL), which exhibit benign features but require a diagnosis different from LMS. The study also aimed to define the diagnostic value and clinical and prognostic significance of Ki-67 PI and p16, p53, and bcl-2 expressions by immunohistochemical methods.

MATERIALS and METHODS

Case selection and histological evaluation

A retrospective analysis of the medical record of the departments of surgery and pathology at Bağcılar Education and Training Hospital from January 2010 to December 2015 was performed for LMS, AL, and CL. In total, 44 cases were included. This study was approved by our institutional ethics committee. Data from clinical follow-ups, including those regarding patient age at diagnosis and date of recurrence/metastasis, were obtained from the medical records. Data regarding histological type, tumor size, and mitotic index were sourced from the pathology reports. Hematoxylin and eosin-stained slides were evaluated and reviewed on the basis of microscopic features and the Bell criteria. The Bell criteria for LMS include at least two of the following criterion: diffuse moderate-to-severe atypia, a mitotic count of at least 10 mitotic figures (MFs)/10 HPFs, and tumor cell necrosis (13, 14). Mitotic activity was assessed by counting MFs in five different areas in 10 HPFs in the most cellular areas; the cases were then divided into the following two groups: ≥10/10 BBA and <10/10 HPF. All mitotic counts were evaluated using Olympus BX53 microscope (Olympus, Tokyo, Japan) with a standard 22-mm-diameter eyepiece at HPF 400× magnification (0.237-mm^2 field of view).

Immunohistochemistry

After hematoxylin and eosin-stained slides were reviewed, tissue blocks with the most representative tumor features of minimum necrosis, hemorrhage, or artifacts were sectioned; 2-3-micron-thick sections were collected on positively charged slides for immunohistochemistry using antibodies against p16, p53, bcl-2, and Ki-67. The sections were incubated for 60 min in an oven at 60°C. After a 10-min incubation at room temperature, the sections were stained in an autostainer using a Ventana BenchMark XT model device according to the XT DAB V3 protocol based on multimer technology. The sections were immunostained for the following selected proteins: p16, bcl-2, p53, and Ki-67.

While evaluating p16 and bcl-2 expressions by immunohistochemistry, moderate-to-strong nuclear and cytoplasmic staining and/or a combination of nuclear and cytoplasmic staining were considered positive. While nuclear and/or cytoplasmic staining in <5% of cells was considered negative, staining in 5%-50% of cells and >50% of cells were considered focal positive and diffuse positive, respectively. However, for statistical analysis, staining in >50% of cells was considered positive, whereas staining in <50% of cells was considered negative (1).

Moderate and strong nuclear staining for p53 and Ki-67 PI was assumed to be positive staining. On the basis of the total staining rates of tumor cells, cases were categorized with respect to p53 positivity as follows: 0%-5% as negative, 5%-50% as focal positive, and >50% as diffuse positive. However, for statistical analysis, staining in >50% of tumor cells was considered positive, whereas that in <50% of tumor cells was considered negative (1).

Cases were evaluated for Ki-67 PI by dividing them into two groups on the basis of whether >10% or <10% staining was observed (15). For quantitatively evaluating Ki-67 staining, the five most intensively stained areas were selected while screening HPF at 40× magnification, and the average of these values was used (16).

RESULTS

Of 44 cases, 13 were diagnosed as LMS (Figures 1 and 2), 18 as AL, and 13 as CL. When the cases were grouped as either LMS or other LM, the mean age of the cases in the LMS group (49.54±9.67; n=13) was statistically significantly higher than that of the cases in the other LM group (40.1±7.7; n=31; p=0.001).

The mean tumor size was 11.88±7.79 cm in the LMS group and 7.54±2.7 cm in the other LM group; this difference was statistically significant (p=0.008).

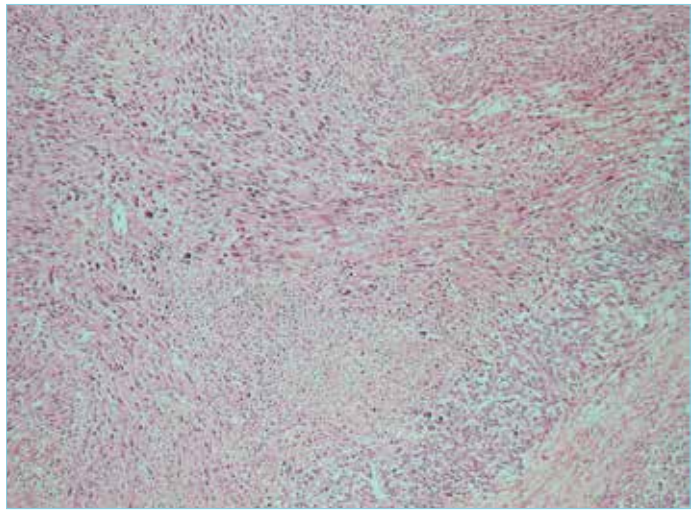

Figure 1. Nuclear pleomorphism, atypia, and necrosis in LMS; H&E 20×

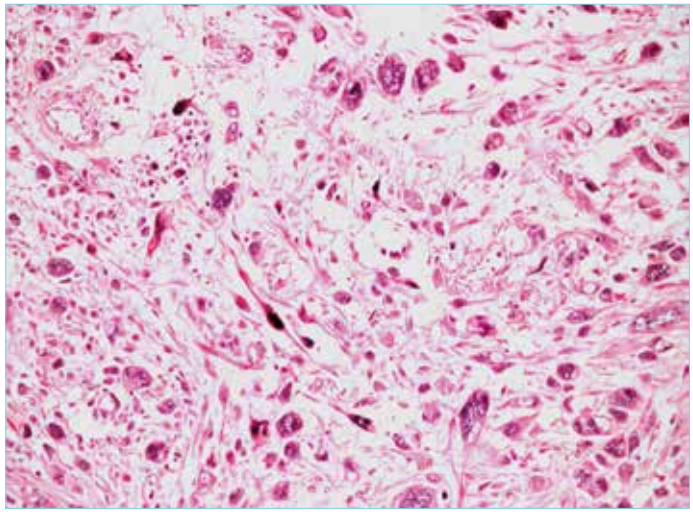

Figure 2. Nuclear pleomorphism and atypia in LMS; H&E 40×

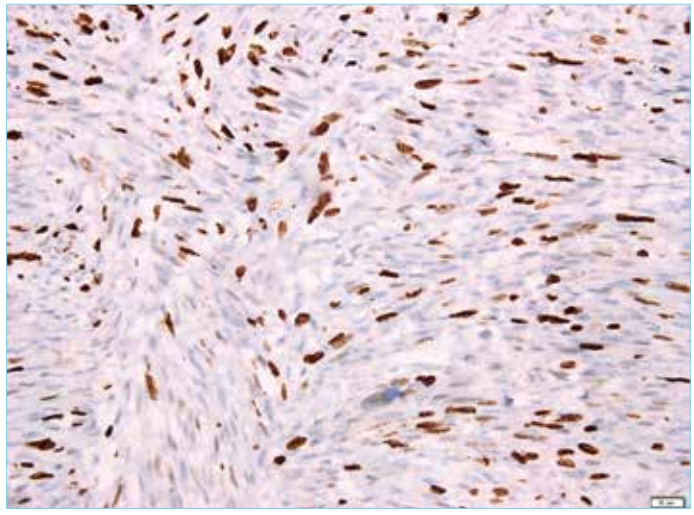

Figure 5. High Ki-67 PI in LMS; 40×

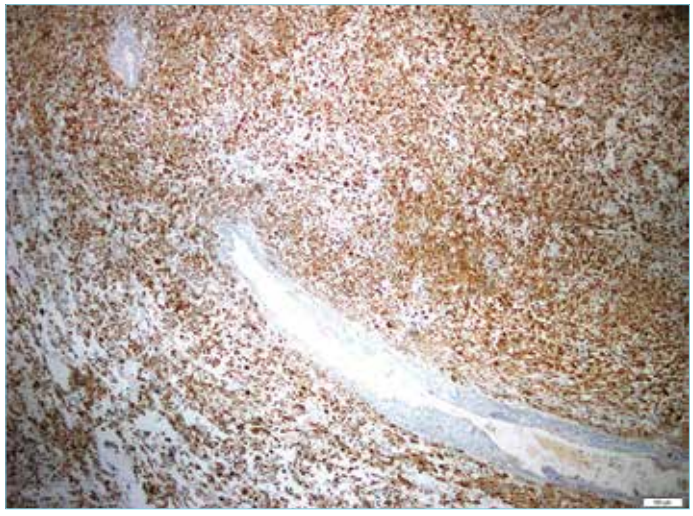

Figure 3. Diffuse p16 positivity in LMS; 110×

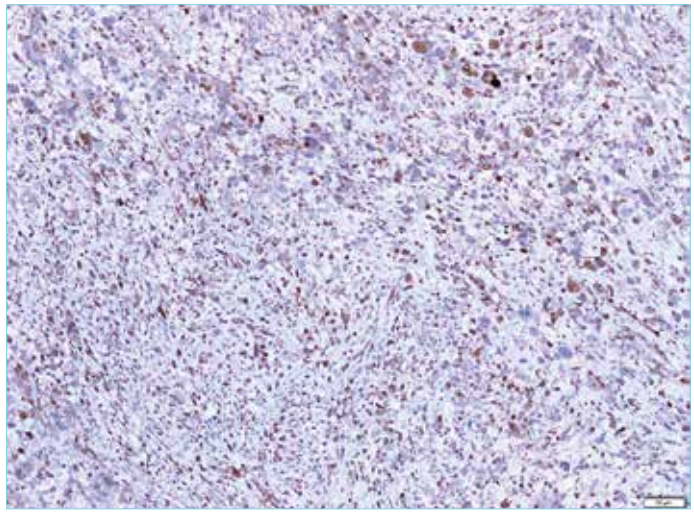

Figure 6. Focal bcl-2 positivity in LMS; 20×

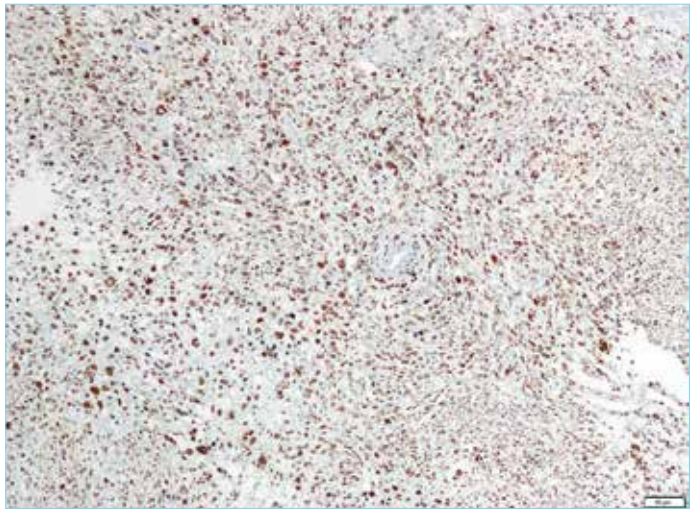

Figure 4. p53 overexpression in LMS; 20×

The mean mitotic count was statistically significantly higher in the LMS group (13.62±11.18) than in the other LM group (0.39±0.72; p=0.001; Table 1).

The rate of diffuse p16 expression in >50% of cells was statistically significantly higher in the LMS group than in the other LM group (p=0.001). The rate of diffuse p16 expression during the follow-up was 13.44 (2.46-73.22)-times higher in the LMS group than in the other LM group (Figure 3).

The rate of p53 overexpression was statistically significantly higher in the LMS group than in the other LM group (p=0.006). The rate of p53 overexpression in the LMS group was 21 (0.99-44.27)-times higher than that in the other LM group (Figure 4).

The Ki-67 positivity rate of >10% expression in cells (increased proliferation) was statistically significantly higher in the LMS group than in the other LM group (p=0.0001). The rate of Ki-67 PI of >10% expression in cells in the LMS group was 36 (2.77-62.4)-times higher than that in the other LM group. No statistically significant difference was found between the LMS and other LM groups with respect to bcl-2 expression (p=0.892; Figure 5).

When the cases were evaluated with regard to relapse/metastasis, six of 13 LMS cases developed metastasis. In addition, the follow-up period for LMS cases ranged from 3 to 60 months (Table 2, 3).

Table 1. Clinical and immunochemical features of LMSs and benign USMTs

		LMS (n=13)		SL and AL (n=310)		p	OR (95% GA)
Age (years)		49.54±9.67		40.1±7.7		0.001	1.15 (1.03–1.28)
Tumor size (cm)		11.88±7.79		7.54±2.7		0.008	1.12 (1.01–1.49)
Mitotic count (number)		13.62±11.18		0.39±0.72		0.0001	
p16	>%50	11	84.62%	9	29.03%	0.001	13.44 (2.46–73.22)
	<%50	2	15.38%	22	70.97%		
bcl-2	>%50	7	53.85%	16	51.61%	0.892	3.28 (0.59–18.37)
	<%50	6	46.15%	15	48.39%		
Ki-67	>%10	12	92.31%	1	3.23%	0.0001	36 (2.77–62.4)
	<%10	1	7.69%	30	96.77%		
p53	>%50	3	23.08%	0	0.00%	0.006	21 (0.99–44.27)
	<%50	10	76.92%	31	100.00%		

LMS: leiomyosarcoma; USMTs: uterine smooth muscle tumors; AL: atypical leimyoma; OR: odds ratio

Table 2. Clinical, histopathological, and immunohistochemical features of LMS cases with relapse/metastasis or without relapse/metastasis

		Relapse/metastasis (+) n=6		Relapse/metastasis (–) n=38		p	OR (95% GA)
Age (years)		51±13.48		41.61±7.98		0.02	1.27 (1.04–1.54)
Tumor size (cm)		16.58±8.85		7.59±2.89		0.0001	1.52 (1.04–2.23)
Mitotic count (number)		17.33±15.58		2.24±4.43		0.0001	1.24 (1.07–1.46)
p16	>%50	4	66.67%	16	42.11%	0.261	2.75 (0.45–16.90)
	<%50	2	33.33%	22	57.89%		
bcl-2	>%50	2	33.33%	21	55.26%	0.318	0.44 (0.06–2.48)
	<%50	4	66.67%	17	44.74%		
Ki-67	>%10	5	83.33%	8	21.05%	0.002	18.75 (1.9–84.21)
	<%10	1	16.67%	30	78.95%		
p53	(–)	0	0.00%	3	7.89%	0.476	0.78 (0.03–16.97)
	(+)	6	100.00%	35	92.11%		

LMS: leiomyosarcoma; OR: odds ratio

The mean age of cases with relapse/metastasis was statistically significantly higher than that without relapse/metastasis (p=0.02).

The mean mitotic count of cases with relapse/metastasis was statistically significantly higher than that of cases without relapse/metastasis (p=0.0001).

The rate of Ki-67 expression in >10% of cells was statistically significantly higher in cases with relapse/metastasis than in those without relapse/metastasis (p=0.002). The risk of relapse/metastasis was 18.75 (1.9-84.21)-times higher in cases with Ki-67 expression in >10% of cells than in those with Ki-67 expression in <10% of cells.

No statistically significant difference was found between cases with or without relapse/metastasis with respect to the distribution of bcl-2 expression (p=0.318; Figure 6).

Furthermore, no statistically significant difference was observed between cases with or without relapse/metastasis with respect to p53 positivity (p=0.476).

No statistically significant correlation was observed between metastasis and p53 in cases of LMS (p=0.118). However, the risk for metastasis was found to be 7 (0.29-17.19)-times higher in p53-negative cases than in p53-positive cases.

Table 3. Metastatic and nonmetastatic LMS cases and specific properties

No.	Age (years)	Tumor size (cm)	Mitotic count (per 10 HPF)	p16	bcl-2	Ki-67	p53	Metastasis
1	50	9	7-8	<50%	<50%	>10%	Negative	None
2	57	10.5	5–10	5%–50% focal	Negative	>10%	Negative	Bone metastasis (after 51 months)
3	70	30	46	>50%	<50%	>10%	Negative	Multiple metastasis (after 8 months)
4	34	2.5	11	>50%	>50%	>10%	>50%	None
5	45	4.5	19	>50%	5%–50%	>10%	>50%	None
6	57	8	4-5	>50%	Negative	>10%	<50%	None
7	48	9	15	>50%	>50%	>10%	Negative	Lymph node metastasis (after 13 months)
8	54	13	2-3	>50%	>50%	>10%	>50%	None
9	48	15	>20	>50%	5%–50%	>10%	Negative	Lung metastasis
10	43	8	5-6	5%–50%	Negative	>10%	5%–50%	None
11	49	7	9	>50%	>50%	>10%	Negative	Colonic serosal metastasis (at diagnosis)
12	34	13	15	>50%	>50%	>10%	Negative	None
13	54	25	>15	>50%	>50%	>10%	Negative	Lymph node metastasis (at diagnosis)

LMS: leiomyosarcoma; HPF: high-power field

Statistical analysis

All statistical analyses were performed using the Number Cruncher Statistical System Software Package (NCSS, 2007, Utah, USA). All values were expressed as mean±SD. Independent samples t-test was used to compare the groups. Quantitative data were compared using the chi-square test, Fisher's exact test, and odds ratios (ORs). A p value of <0.05 was considered to be statistically significant.

DISCUSSION

The prognosis of uterine sarcomas varies depending on the histological type (2, 9). LMSs have the most unpredictable clinical behavior, and uterine LMSs generally have an aggressive clinical course. Therefore, accurate diagnosis and tumor qualification are important for surgery and treatment management.

No consensus has been reached regarding the correlation between the clinical course of LMS and possible prognostic factors such as age, disease stage, tumor size, necrosis, vascular invasion, and mitotic index (2, 9, 15-17). Most studies reported that tumor size and mitotic index are the most significant parameters for prognosis (2-4). In the study by Abeler et al., which included 245 uterine sarcomas, mitotic index and tumor size were found to be the only significant prognostic factors (9, 18). In our series, a high mitotic index (≥10/10 HPF) was found to be statistically significant for the diagnosis and prognosis of LMSs (p=0.001 and p=0.006, respectively).

According to the 2009 FIGO classification and staining system, myometrial invasion and cervical involvement were replaced by tumor size (2, 9). In recent studies, tumor size was found to be a prognostic factor for stage I disease, and both 5 and 10-cm thresholds were proposed (9, 19-21). Moreover, in our study, the mean tumor size was statistically significantly larger in the LMS group (11.88±7.79) than in the AL and CL group (p=0.008). In terms of metastasis of LMS, the mean tumor size was statistically significantly larger in cases with metastasis than in those without metastasis (p=0.0001).

Despite the well-established histopathological criteria, the distinction of LMS from certain variants of benign leiomyoma, particularly AL, can be challenging in some cases. Several studies have reported that p16 is overexpressed in LMS; therefore, p16 may be useful for distinguishing between benign and malignant USMTs (3, 6, 7, 22). Chen and Yang (7) compared among LMS, STUMP, and other LMs and reported that a strong/moderate-to-significant staining pattern was observed in all LMS and STUMP cases that were positive for p16, whereas weak focal staining was observed in some UL and CL cases. Moreover, because 80% of AL cases exhibited a strong diffuse p16 expression, the authors suggested that p16 overexpression along with p53 and Ki-67 expressions plays a limited role in the distinction of LMS from benign leiomyomas (7). Atkins et al. (5) also reported a diffuse staining pattern in many LMS and STUMP cases, whereas a weak focal staining pattern was observed in some TL cases. They suggested that p16 is useful for diagnosing LMS; even if STUMP cases exhibit a certain p16 staining pattern, if they are strongly stained for p16, they should be reported as LMSs. In our study, the rate of diffuse (>50%) p16

expression was also found to be higher in the LMS group than in the AL and CL group (p=0.001).

In the literature, many studies have reported that overexpression of and mutations in p53 are observed in uterine LMS; however, no genetic changes in p53 have been described in benign leiomyomas. Zhai et al. (1) reported very weak or negative staining for p53 in benign leiomyomas with no p53 mutation and a strong positive correlation between p53 staining and p53 mutation in LMSs. In their study, partial or diffuse p53 expression was observed by immunohistochemical staining in 76% of 21 LMS cases, but 24% of the cases were negative for p53. In the same study, nonconformance between p53 staining and p53 mutation was detected in some cases, and mutations in wild-type p53 were reportedly associated with weak or negative p53 staining. In addition, positive staining of p53 may not demonstrate p53 mutations (1). In our study, all TL, CL, and AL cases exhibited weak/focal staining for p53, whereas three LMS cases exhibited strong diffuse staining, one LMS case exhibited strong focal staining, and nine LMS cases had no staining.

The interesting finding in our study was that no p53 staining was observed in metastatic LMS cases. p53 mutations are frequently observed in serous ovarian tumors, which characteristically show either 100% staining or no staining (2). Mota et al. (23) investigated the presence of p53 mutations in high-grade serous ovarian carcinomas and reported that the most common p53 mutations were missense mutations; tumors with these mutations had strong and diffuse immunohistochemical p53 positivity. However, the authors defined these tumors as TP53 null cell tumors in the presence of somatic (nonsense, frameshift, and splice junction) p53 mutations, accounting for 30% of all cases, and reported the complete absence of p53 expression by immunohistochemistry in these tumors (23, 24); moreover, cases with p53 null mutations had a worse clinical course. In our study, p53 overexpression was found to be statistically significant in the distinction of LMS from benign tumors (p=0.006). No statistically significant correlation was observed between LMS metastasis and p53 (p=0.118). However, metastasis was higher in p53-negative cases than in p53-positive cases, and the risk for metastasis was 7 (0.29-17.19)-times higher in p53-negative cases than in p53-positive cases. The prognostic value of this finding may be clarified by studying a large series with p53 mutations. Clinicopathological studies of bcl-2 expression in cervical, breast, and colon carcinomas suggest that bcl-2 is a good prognostic factor. In addition to USMTs, a positive correlation between bcl-2 expression and overall survival was reported in several studies (1, 9, 10). In our study, no statistically significant difference was observed between LMS cases with or without relapse/metastasis with regard to bcl-2 expression (p=0.318). In addition, no statistically significant difference was found between benign and malign USMTs with respect to bcl-2 expression (p=0.892).

The limitations of is study include the limited number of LMS cases and short clinical follow-up periods for some cases.

CONCLUSION

Compared with LM, LMS are often observed in cases with advanced ages. The mean tumor size in LMS is generally >10 cm and is larger than that in LM. Bcl-2 expression cannot be used for diagnosing LMS and the prognostic prediction of malignant USMT's prognosis. In addition to histopathological findings, the presence of diffuse p16 expression and p53 overexpression can be used as evidence to distinguish between benign and malignant USMT. Close monitoring of p53-negative LMS cases should be recommended to detect metastasis. High mitotic index (≥10/10 HPF) and high Ki-67 PI (>10%) can be useful indicators for diagnosing LMS, distinguishing benign tumors, and predicting an aggressive clinical course.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Bağcılar Training and Research Hospital.

Informed Consent: Informed consent is not necessary due to the restorsrective nature of the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: AKA., ÜST., YK., ATU. Performed the experiments or case: AKA., ÜST., YK., ATU. Analyzed the data: AKA., ÜST., YK., ATU. Wrote the paper: AKA., ÜST., YK., ATU. All authors have read and approved the final manuscript.

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