Survivin and Ki-67 expression in leiomyomas, leiomyoma variants, leiomyosarcoma, and STUMP : An immunohistochemical and clinical follow-up study

Ayse Filiz Gokmen Karasu[1], Fatma Cavide Sonmez[2], Hasan Turan[3], Cihan Comba[4], Seval Turna[2], Sennur Ilvan[5], Dilek Sema Arici[2]

[1]Bezmialem Vakif University, Faculty of Medicine, Department of Gynecology and Obstetrics, Istanbul, Turkey
[2]Bezmialem Vakif University, Faculty of Medicine, Department of Pathology, Istanbul, Turkey
[3]Istanbul University Cerrahpasa, Faculty of Medicine, Department of Gynecologic Oncology, Istanbul, Turkey
[4]Bakirkoy Research and Training Hospital, Clinic of Gynecologic Oncology, Istanbul, Turkey
[5]Istanbul University Cerrahpasa, Faculty of Medicine, Department of Pathology, Istanbul, Turkey

Abstract

Aim: Survivin is an "inhibitor of apoptosis" protein. Survivin expression is a poor prognostic factor in a variety of solid tumors. In this clinicopathological study, we aimed to investigate survivin immunostaining of leiomyomas, leiomyoma variants, STUMP (Uterine smooth muscle tumor of uncertain malignant potential)'s and LMS (leiomyosarcoma). Our second objective was to investigate whether survivin immunoreactivity in STUMP and LMS may play a role in determining recurrence.

Material and Methods: Consecutive 119 specimens of leiomyoma, leiomyoma variants, STUMP and LMS from the pathology archives of Bezmialem Medical Faculty and Cerrahpasa Medical Faculty were selected. Clinicopathologic characteristics were analyzed and specimens were stained with survivin and Ki-67. The percentage and staining intensity of immunoreactive cells were examined. Additionally, we analyzed whether survivin intensity and expression might be a predictor of LMS recurrence.

Results: The patients in the LMS group were older (p< 0.001). All LMS and all STUMP specimens were stained with survivin. Survivin staining and Ki-67 staining were highest in the LMS and STUMP groups. Survivin staining was 14.2 ± 6.7 % in the LMS group, 11.2± 10.4 % in the STUMP group, 1.85 ± 1.9 % in the leiomyoma group and 1.4 ± 0.2 % in the leiomyoma variant group (p<0.001). Survivin staining intensity was 1.2 ± 0.6 in the LMS group, 0.9 ± 0.2 in the STUMP group, 0.8 ± 0.4 in the leiomyoma group and 0.9 ± 0.3 in the leiomyoma variant group (p=0.025). Both survivin staining percentage and staining intensity correlated with the Ki-67 proliferation index. In the LMS cases that showed recurrence survivin staining was 16% while in the cases that did not reoccur survivin staining was 2% (p<0.001).

Conclusion: The antiapoptotic marker "survivin" has not been studied before for smooth muscle tumors of the uterus. Utilizing survivin in conjuncture with histologic features and Ki-67 can also help to determine malignancy potential and LMS recurrence.

Keywords: Leiomyosarcoma; STUMP; leiomyoma; survivin; immunohistochemistry

INTRODUCTION

Uterine smooth muscle tumors are common neoplasms of the uterus. Leiomyoma is the most common; occurring approximately in 40% of women over the age of 35 (1). Leiomyosarcomas (LMS) are rare aggressive malignant uterine smooth muscle tumors occurring mainly in post-menopausal women (2). They comprise 1.3 % of all uterine neoplasms with an overall incidence of 0.4 % per 100,000 women (3). The histological diagnosis of uterine smooth muscle tumors was revised in 2014 by the WHO (World Health Organization) (4). The diagnosis of LMS involves; hypercellularity, nuclear atypia, increased mitosis and necrosis. Increased mitosis is defined as; over 15 mitotic figures per 10 high-power-field (HPF). Leiomyosarcomas are exceedingly belligerent. The risk of both local and distant metastasis is high even in an early stage of diagnosis. Five-year survival rate ranges between 12-25% (5).

Various studies have examined a correlation between several prognostic factors such as; patient age, clinical stage, tumor size, presence of necrosis, mitotic rate, degree of nuclear pleomorphism, and vascular invasion (3,5-6). Immunohistochemistry has also been used to evaluate uterine smooth muscle neoplasms for pathologic classification. One of the most studied markers is Ki- 67; which is a marker for proliferative activity. It was found in LMS to be highly expressed compared to leiomyomas or STUMP (Smooth Muscle Tumors of Uncertain Malignant Potential) that did not recur (6-8). O'Neill et al. determined that Ki-67 staining greater than 30% was indicative of LMS (9). A small number of apparently benign smooth muscle tumors pose difficult diagnostic challenges because they can mimic malignancy in certain criteria. According to the current WHO classification if a tumor shows any unusual combinations of histologic features that do not meet all the criteria for LMS and there is compelling doubt regarding a malignancy the term STUMP is appropriate (4). The clinical behavior of STUMP is not clarified.The majority of cases follow a benign course, however there are some case reports describing subsequent recurrences as a leiomyosarcoma (10).

Survivin is an inhibitor of apoptosis (IAP) protein. IAP proteins play a role in cell cycle regulation by binding caspases. IAP overexpression is a poor prognostic marker in a variety of solid tumors and has been studied in various human malignancies (11,12). In this study; we aimed to investigate survivin immunostaining of leiomyosarcomas. Our second objective was to investigate whether survivin expression in patients with leiomyosarcomas can play a role in determining LMS recurrence.

MATERIAL and METHODS

Cases

This was a retrospective experimental study. After an institutional review board approval from Bezmialem Vakif University (number 2/35-7/2016) consecutive cases were selected from the archives of the pathology departments of Bezmialem Vakif University Medical Faculty and Istanbul University Cerrahpasa Medical Faculty Hospital in Istanbul. The study was in accordance with the ethical standards described in the Declaration of Helsinki, The diagnosis of the cases was confirmed by 2 pathologists (F.C.S and D. S.A). Selected specimens comprised of 119 cases. They were; 35 leiomyomas, 56 leiomyoma variants, 16 STUMP and 12 leiomyosarcomas. The leiomyoma variants were as follows; 7 vascular leiomyomas, 8 lipoleiomyomas, 5 symplastic leiomyomas, 14 cellular leiomyomas, 2 mitotic active leiomyomas, 20 leiomyomas with enfarctoid necrosis. All the LMS cases were initial primary cases. Patients with diagnosis of LMS and STUMP were put on a clinical follow-up schedule,with pelvic examination and abdominopelvic ultrasound every 6 months and chest X-ray and pelvic computed tomography scans every year.

Statistical analysis

A single block from each case was selected for immunohistochemistry. STUMP was diagnosed if the tumor contained an ambiguous morphology that could not be placed in either the benign or malignant categories. LMS was diagnosed if there was the presence of at least 2 of the 3 criteria that include moderate-to-severe cytologic atypia, high mitotic index and tumor cell necrosis (4,13-14).

Immunohistochemical analysis

The blocks were selected for immunohistochemical staining. 4-μm-thick sections of formalin-fixed and paraffin-embedded blocks were used for staining. The sections were stained with survivin (RB-9245-R7, rabbit polyclonal antibody) on an automatic immunohistochemistry device (Ventana, Benchmark XT). Nuclear stainings of tumoral smooth muscle cells for survivin were considered as positive. Section of gastric carcinoma was used as a positive control for survivin. The stained sections were examined by a Nikon light microscope (Nikon-Eclipse-Ci). The percentage of positive cells was determined by counting 100 tumoral cells. The intensity of staining was examined as mild (score 1), moderate (score 2) and severe (score 3). Monoclonal rabbit Ki-67 (Thermo Scientific, Cheshire, UK) was applied to all STUMP and LMS cases. Additionally, Ki-67 was applied to selected leiomyoma variants that showed a diagnostic dilemma. The correlation of survivin staining and Ki-67 proliferation score was investigated in selected cases. The photographs were captured with a digital camera (Nikon-Eclipse-80i-DS-Ri1).

Statistical analysis

Continuous variables were expressed as mean± SD or median (interquartile range) when appropriate. Categorical variables were expressed as percentages. To compare nonparametric continuous variables, the Kruskal Wallis-test was used. To compare categorical variables, the Chi-square test was used. The Pearson or Spearman correlation coefficient was used to determine parametric and nonparametric measurements of statistical dependence between two variables when appropriate. A two-tailed P-value of less than 0.05 was considered to indicate statistical significance. The statistical analyses were performed using software (SPSS 18.0, SPSS Inc, Chicago, Ill).

RESULTS

Clinical and pathologic characteristics of patients

Thirty-three patients underwent myomectomy, 41 patients had a hysterectomy, 45 patients had a hysterectomy and bilateral salpingo-oophorectomy. The mean age of the patients was; 43 ± 7 in the leiomyoma group, 50 ±9 in the STUMP group, 58 ± 9 in the LMS group and 45 ± 6 in the leiomyoma variant group. The patients in the LMS group were significantly older (p< 0. 0001). The tumor characteristics are given in Table 1. Mitosis count was significantly greater in the LMS group (p< 0. 0001).

Expression of Survivin and Ki-67

Survivin staining was applied to all 119 specimens (Figure 1). All LMS and all STUMP specimens were stained. Only

9 of the leiomyomas and 8 of the leiomyoma variants did not exhibit any survivin staining. Survivin staining profile; both as stained cell percentage and intensity was highest in the LMS group followed by the STUMP group. Survivin staining was 14.2±6.7 % in the LMS group, 11.2±10.4 in the STUMP group, 1.85±1.9 in the leiomyoma group and 1.4±0.2 in the leiomyoma variant group (p<0.001). Survivin staining intensity was 1.2±0.6 in the LMS group, 0.9±0.2 in the STUMP group, 0.8±0.4 in the leiomyoma group and

0.9±0.3 in the leiomyoma variant group (p=0.025) (Table 1). Leiomyomas and leiomyoma variants showed minimal and mild staining.

Ki-67 staining was applied to 47 specimens. All LMS and all STUMP specimens were stained with Ki-67. Additionally, 19 out of the 56 leiomyoma variants (33.9 %) were stained with Ki-67. Both survivin staining intensity and staining percent of positive cells correlated with Ki-67 (p.2773) staining and mitosis (Table 2).

Table 1. Clinopathologic characteristics of uterin smooth muscle neoplasms

	Leiomyoma n= 35	STUMP n= 16	LMS n= 12	Leiomyoma variant n= 56	P-value
Tumor size (cm)	8.3 ± 1.9	9 ± 3.7	10.9 ± 4.6	9 ± 4.1	0.249
Patient Age	43 ± 7	50 ± 9	58 ± 9	45 ± 6	0.01
Menopausal Patients n/%	10 (28.6 %)	10 (62.5 %)	11 (91.7 %)	19 (33.9%)	< 0.001
Tumor number	4 ± 2	3.6 ± 2.7	1.5 ± 1.3	2.1 ± 2.4	0.009
Mitosis (MF/10 HPF)	1.2 ± 0.9	4.1 ± 2.4	21.4 ± 8.5	1.7 ± 1.7	< 0.001
Survivin staining (%)	1.85 ± 1.9	11.2 ± 10.4	14.2 ± 6.7	1.4 ± 0.2	< 0.001
Survivin staining intensity	0.8 ± 0.4	0.9 ± 0.2	1.2 ± 0.6	0.9 ± 0.3	0.025
Ki-67 staining percentage(%)	-	12.4 ± 3.4	29 ± 2	5.7 ± 3.8	< 0.001

Results are expressed as mean ±SD or frequency (with in group percentage)

Table 2. Correlation between mitosis and immunohistochemical markers

|	Age	Ki67 staining percentage (%)	Survivin staining (%)	Survivin staining intensity	Mitosis (MF/10 HPF)
Age	-	0.263 (p=0.074)	0.241 (p=0.008)	0.077 (p=0.404)	0.297(p=0.001)
Ki -67 staining percentage (%)	0.263 (p=0.074)	-	0.693 (p< 0.001)	0.383 (p< 0.001)	0.808 (p< 0.001)
Survivin staining (%)	0.241 (p=0.008)	0.693 (p< 0.001)	-	0.641 (p< 0.001)	0.522 (p< 0.001)
Survivin staining intensity	0.077 (p=0.404)	0.383 (p = 0.008)	0.641	-	0.229 (p = 0.012)
Mitosis (MF/10 HPF)	0.297 (p=0.001)	0.808 (p< 0.001)	0.522	0.229	-

Spearman coleration test is used
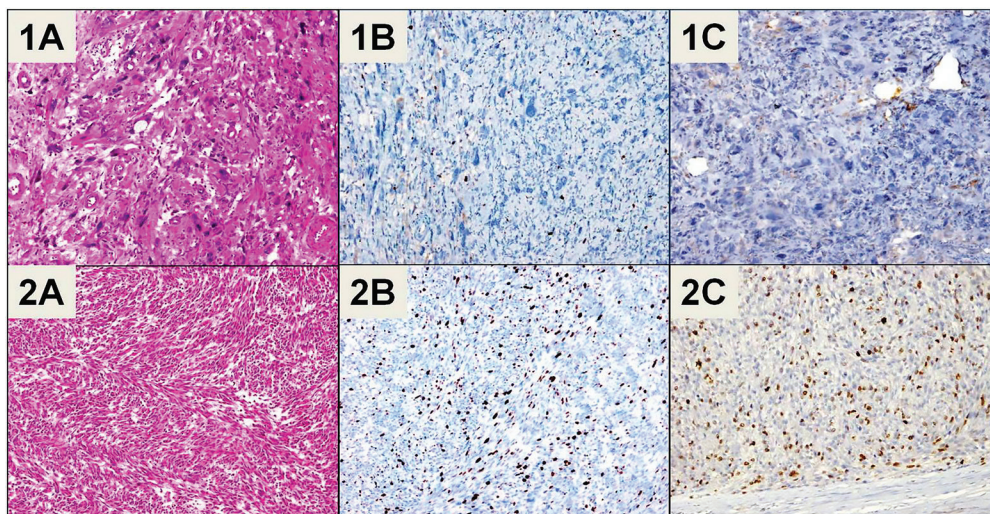
Figure 1. included Survivin and Ki-67 expression in leiomyomas, leiomyoma variants, leiomyosarcoma, and STUMP : An immunohistochemical and clinical follow-up stud

Relationship between survivin staining and clinical course of leiomyosarcomas

Of the 12 LMS cases, 7 showed recurrence. None of the STUMP cases showed recurrence. When we analyzed immunostaining characteristics of these cases, we found that in the recurrence group the percentage of survivin positive cells was 16 %; which was significantly higher compared to the non-recurrence group; 2 %. This difference was statistically significant (p<0.001). Survivin staining intensity was not an indicator of recurrence. (Table 3). The percentage of Ki-67 positive cells was 35%; which was also significantly higher compared to the non-recurrence group; 1%. This result also reached statistical significance. (P =0.004).

Table 3. Relationship Between Survivin Staining and Recurrence

	Recurrence (+) (n=7)	Recurrence (-) (n=5)	P value
Survivin staining (%)	16 (8-30)	2 (1-3)	<0.001
Survivin staining intensity	1 (1-2)	1(1-1)	0.179
Ki -67 staining percentage (%)	35 (25-60)	12 (5-15)	0.04

Datas are presented as median and interquantil range
p= Kruskal Wallis-test is used

DISCUSSION

Although most uterine smooth muscle tumors can be classified as benign or malignant; difficulties in discrimination may arise. A precise diagnosis for leiomyoma versus leiomyosarcoma would be beneficial both for the pathologist and the clinician as LMS are very aggressive. In this study, our main findings were that survivin was highly expressed in both LMS and STUMP. The expression was highest in LMS cases. In leiomyoma and leiomyoma variants there was a minimal expression and weak staining intensity. In the literature; several markers have been studied for LMS immunohistochemistry. The most frequently studied biomarkers are hormone receptors such as estrogen, progesterone and androgen receptors (15). These have shown a moderate frequency of expression in uterine leiomyosarcomas. Progesterone receptor positivity seems to be associated with a lower risk of recurrence and better survival in women with leiomyosarcoma (16-18).

O'Neill and colleagues have studied p16, p53 and MIB1 staining in a series of uterine leiomyomas, leiomyoma variants, STUMPs, and leiomyosarcomas. In their series, a combination of high p16, p53 and MIB1 expression was found only in leiomyosarcomas and they concluded that this may be of diagnostic value. LDH-D is another marker and it was shown to be expressed in patients with uterine sarcoma (19). Additionally LMS exhibit VEGF, FGF-2 receptor positivity. Arita et al reported that VEGF was stained significantly stronger in uterine sarcomas than in normal myometrium (20). In all the aforementioned studies similar observations were made regarding the fact that there is not one specific marker for LMS diagnosis that is identifiable.

Survivin is a member of an antiapoptotic protein family. Survivin is undetectable in normal tissues (21) whereas survivin upregulation is correlated with poor prognosis and recurrence in solid tumors, including neuroblastoma (22), gliomas (23), stomach cancer (24), non–small cell lung cancer (25), breast cancer (26), pancreatic cancer (27), esophageal cancer (28), and melanoma (29).Survivin has been a popular marker in cancer research not only because it is often upregulated in malignant lesions but also because of the potential exploitation of intracellular survivin pathways in cancer diagnosis and therapy (30).

In gynecologic tumors, survivin expression has been studied with cervical cancer (31), ovarian cancer (32-34), carcinomatous endometrium (35-37) and tamoxifen associated endometrial lesions (38). We demonstrated that survivin is expressed in all smooth muscle tumors to a varying extent with LMS showing the most striking immunostaining. Our data has shown that all 12 LMS cases showed survivin expression. Our results suggest that when used in combination with histologic criteria and Ki-67; survivin staining can be utilized as a useful tool.

There are a few limitations to our study. Our main limitation was that the number of cases in our study, was limited to make an ideal statistical analysis and thus our findings are hard to extrapolate. This fact arose from the rare incidence of LMS (0. 4 % per 100,000 women). The advantages of our study include; being the first study to examine survivin immunohistochemistry in uterine smooth muscle tumors. Additionally, all pathology specimens were re-evaluated by the same pathologists.

CONCLUSION

In summary; the antiapoptotic marker "survivin" was not studied before for smooth muscle tumors of the uterus. Survivin positive immunostaining may increase the level of confidence in deciding a malignancy potential diagnosis when utilized in conjunction with histologic features and Ki-67.

Ayse Filiz Gokmen Karasu ORCID: 0000-0001-7480-4691
Fatma Cavide Sonmez ORCID: 0000-0003-0406-9198
Cihan Comba ORCID: 0000-0003-1902-8014
Hasan Turan ORCID:0000-0002-3161-2689
Seval Turna ORCID:0000-0003-3951-0369
Dilek Sema Arıcı ORCID:0000-0003-2104-1349
Sennur Ilvan ORCID: 0000-0002-6746-6599

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