PHENOTYPIC CHARACTERISTICS OF RELAPSED LEIOMYOMA AND SMOOTH MUSCLE TUMORS OF UNCERTAIN MALIGNANCY POTENTIAL IN REPRODUCTIVE WOMEN

Kiknadze T., Tevdorashvili G., Muzashvili T., Gachechiladze M., Burkadze G.

Tbilisi State Medical University, Georgia

Uterine leiomyoma, represents the most commons pelvic tumor in females. The incidence of leiomyoma represents 20% and 40% in less than 30 and 40 years old females respectively [1]. Leiomyomas are benign monoclonal tumors, which arise from the smooth muscle cells of the myometrium. There are numerous histological subtypes of uterine leiomyoma, including classic, cellular, bizzare/atypical and smooth muscle tumors of uncertain malignancy potential (STUMP) and others [2]. Leiomyosarcomas represent the malignant counterparts of leiomyoma. Although extremely rare, leiomyoma can transform into leiomyosarcoma [3].

In recent years, laparoscopic myomectomy developed as a treatment of choice for leiomyoma, as it represents the less invasive procedure [4]. However, it has been noted that the relapse of leiomyoma can occur after laparoscopic myomectomy [5]. The rate of leiomyoma relapse represents 11.7%, 36.1%, 52.9% and 84.4% after 1, 3, 5 and eight years from laparoscopic myomectomy respectively [6]. Therefore, the investigation of molecular markers, indicating risk of relapse after laparoscopic myomectomy is of high importance.

Smooth muscle tumors with uncertain malignancy potential (STUMP) represent the group of smooth muscle tumors, which cannot be diagnosed surely as benign or malignant [7]. Therefore, the clinical management of this entity is complicated. Mostly, they show relatively non-aggressive behaviour, compared to leiomyosarcomas and survival rates are also relatively higher. However, in 8.7% to 11% of cases the relapse can develop[7]. Therefore, the investigation of molecular markers which indicate the relatively benign or aggressive behaviour of this tumors is of high interest.

In our current study, we investigated the molecular phenotype of different types of leiomyomas, including STUMP, in hysterectomy and laparoscopic myomectomy specimens from patients in reproductive and menopausal age.

Material and methods. *Tissue samples.* Formalin fixed and paraffin embedded tissue material was retrieved from the Research, Diagnostic and Teaching Laboratory of Tbilisi State Medical University, Georgia. Study included altogether 237 tissue specimens distributed in two major groups: group I specimens received by histerectomy (n=155) and specimens received by laparoscopic miomectomy (n=82). Specimens were further subdivided into following categories: from patients in reproductive age and from patients in menopause. Group I included n=102 specimens from reproductive age patients and n=53 specimens from menopausal patients. Group II included n=52 specimens from reproductive age patients and n=30 specimens from menopausal patients. Group I included following histological subtypes: classic leiomyoma (n=69), cellular leiomyoma (n=15), bizzare/atypical leiomyoma (n=22), smooth muscle tumors with uncertain malignancy potential (STUMP) (n=17), leiomyosarcoma (n=12) and control group of normal myometrium (n=20); Group II included following histological subtypes: classic leiomyoma (n=35), bizzare/atypical leiomyoma (n=18) and STUMP (n=29). Cases in group II were further subdivided into relapsed cases and control group.

Immunohistochemistry. 4μ FFPE tissue sections were deparaffinized in xylene, rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Ready to use antibodies against the following antigens were used: Ki67 (K2), cyclin D1 (polyclonal), Bcl2 (bd-2/100/05) cleaved Cas3, ER (6f11) and PR (16) (Novocastra). Staining and visualisation has been performed using Bond polymer refine detection system. The number of positive cells were counted in 20HPF and the following indexes were made: proliferation index – based on Ki67 and cyclin D1 labelling, apoptotic index – based on Bcl2 and Cas3 labelling, ER index and PR index. Proliferation and apoptosis index 0-10% was considered as low and >10% was considered as high. The ER and PR index 0-10% was considered as low, the index 10-50% was considered as moderate and the index >50% was considered as high.

Comparisons between groups were made using Mann-Whitney U and Kruskall-wallis test and correlations were assessed using Spearman's rank correlation. P values <0.05 were considered as significant. All statistical tests were performed using SPSS software V19.00.

Results and discussion. The results of Ki67, Cyclin D1, BCL2, Cas3, ER and PR analysis in myomectomy specimens showed the following results: in normal myometrium mean Ki67 expression was 1.7 in reproductive age and 0.4 in menopause; mean Cyclin D1 expression level was 1.2±0.3 in reproductive age and 0.2±0.05 in menopause; mean Bcl2 expression level was 0.9±0.1 in reproductive age and 2.1±0.7 in menopause; mean Cas3 expression level was 0.7±0.3 in reproductive age and 1.8 in menopause; mean ER expression level was 80 in reproductive age and 40 in menopause and mean PR expression level was 89.6±6.9 in reproductive age and 42±7.1 in menopause. In classic leiomyoma, the mean Ki67 expression was 3±1.1 in reproductive age and 1.2±0.8 in menopause; mean Cyclin D1 expression level was 2.4±1.2 in reproductive age and 0.9±0.5 in menopause; mean Bcl2 expression level was 6.7±2.1 in reproductive age and 8.3±3.2 in menopause; mean Cas3 expression level was 5.4±2.4 in reproductive age and 7.9±3.3 in menopause; mean ER expression level was 70.2±10.3 in reproductive age and 34.3±5.6 in menopause and mean PR expression level was 75.7±9.7 in reproductive age and 36.3±6.9 in menopause. In cellular leiomyoma the mean Ki67 expression was 8.9±3.6 in reproductive age and 3.4±1.4 in menopause; mean Cyclin D1 expression level was 7.6±3.4 in reproductive age and 3.1±1.1 in menopause; mean Bcl2 expression level was 9.6±3.9 in reproductive age and 10.9±4.1 in menopause; mean Cas3 expression level was 8.5±2.9 in reproductive age and 9.9±3.2 in menopause; mean ER expression level was 60.5±9.4 in reproductive age and 29.1±4.8 in menopause and mean PR expression level was 63.2±7.1 in reproductive age and 30.2±5.5 in menopause. In bizzare/atypical leiomyoma the mean Ki67 expression was 12.5±3.8 in reproductive age and 4.7±1.1 in menopause; mean Cyclin D1 expression level was 10.5±4.4 in reproductive age and 3.5±0.9 in menopause; mean Bcl2 expression level was 13.3±3.2 in reproductive age and 15.7±4.5

Table 1. Distribution of Ki67, Cyclin D1, Bcl2, Cas3, ER and PR in myomectomy tissue specimens

Normal Miometrium	Repr. Ag.	10	1,7	1,2	81,3	0,7	80	89,6
	Menop. Ag.	10	0,4	0,2	89,2	1,8	40	42
Classic LM	Repr. Ag.	45	3	2,4	67,6	5,4	70,2	75,7
	Menop. Ag.	24	1,2	0,9	71,2	7,9	34,3	36,3
Cellular LM	Repr. Ag.	10	8,9	7,6	59,7	8,5	60,5	63,4
	Menop. Ag.	5	3,4	3,1	62,3	9,9	29,1	30,2
Bizzare/Atypical LM	Repr. Ag.	14	12,5	10,5	13,3	11,2	50,6	52,1
	Menop. Ag.	8	4,7	3,5	15,7	13,8	23,8	23,9
STUMP	Repr. Ag.	13	20,8	18,6	15,6	7,6	43,9	46,8
	Menop. Ag.	4	6,3	5,2	17,3	9,3	17,6	18,7
LMS	Repr. Ag.	10	42,1	40,2	3,8	2,9	35,7	40,7
	Menop. Ag.	2	10,9	8,7	4,2	3,7	12,4	13,5

in menopause; mean Cas3 expression level was 11.2±2.2 in reproductive age and 13.8±3.6 in menopause; mean ER expression level was 50.6±8.4 in reproductive age and 23.8±6.7 in menopause and mean PR expression level was 52.1±7.1 in reproductive age and 23.9±3.7 in menopause. In STUMP the mean Ki67 expression was 20.8±11.4 in reproductive age and 6.3±1.5 in menopause; mean Cyclin D1 expression level was 18.6±10.8 in reproductive age and 5.2±1.3 in menopause; mean Bcl2 expression level was 8.7±2.1 in reproductive age and 10.6±3.5 in menopause; mean Cas3 expression level was 7.6±2.5 in reproductive age and 9.3±2.9 in menopause; mean ER expression level was 43.9±5.6 in reproductive age and 17.6±4.8 in menopause and mean PR expression level was 46.8 in reproductive age and 18.7±5.6 in menopause.

The results of Ki67, Cyclin D1, BCL2, Cas3, ER and PR analysis in laparoscopic surgical specimens showed the following results: in classic leiomyoma the mean Ki67 labelling index was 6.9±2.2 in relapsed and 3.1±1.3 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 3.7±1.4 and 1.4±0.6 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 5.3±2.4 in relapsed and 2.7±1.8 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 2.1±0.4 and 1±0.3 in relapsed and control groups respectively. Mean Bcl2 labelling index was 3.2±0.9 in relapsed and 5.9±1.7 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 3.9±1.9 and 8.8±2.7 in relapsed and in control cases respectively. Mean Cas3 labelling index was 2.9±0.8 in relapsed and 5.1±1.9 in control cases from patients in reproductive age and 3.1±0.8 and 7.2±2.9 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 85.8±9.6 in relapsed and 79.6±7.9 in control group in reproductive age patients, whilst it was 48.2±8.2 and 42.9±6.3 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 90.6±9.1 in relapsed group and 85.7±7.8 in control group in reproductive age patients, whilst it was 50.7±5.5 and 46.2±4.9 in menopausal patients in relapsed and control groups respectively; In bizzare/atypical leiomyoma the mean Ki67 labelling index was 26±3.9 in relapsed and 12.3±2.7 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 11.4±3.3 and 4.9±1.8 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 24.5±4.6 in relapsed and 11.2±3.7 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 10.6±2.9 and 3.8±1.1 in relapsed and control groups respectively. Mean Bcl2 labelling index was 5.7±2.1 in relapsed and 13.9±4.1 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 7.9±3.9 and 16.2±4.5 in relapsed and in control cases respectively. Mean Cas3 labelling index was 4.9±2.1 in relapsed and 12.3±3.8 in control cases from patients in reproductive age and 6.8±2.3 and 14.1±3.5 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 65.6±10.3 in relapsed and 47.5±8.5 in control group in reproductive age patients, whilst it was 40.1±5.5 and 30.2±6.3 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 68.9±9.2 in relapsed group and 50.3±8.7 in control group in reproductive age patients, whilst it was 47.2±4.3 and 35.1±2.3 in menopausal patients in relapsed and control groups respectively; In STUMP the mean Ki67 labelling index was 61.4±8.6 in relapsed and 21.2±3.3 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 21.8±6.9 and 7.3±2.1 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 52.7±7.6 in relapsed and 19.1±4.7 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 18.6±3.3 and 5.7±2.2 in relapsed and control groups respectively. Mean Bcl2 labelling index was 3.3±0.9 in relapsed and 9.2±3.3 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 4.8±1.8 and 11.3±3.4 in relapsed and in control cases respectively. Mean Cas3 labelling index was 2.6±0.9 in relapsed and 8.1±2.6 in control cases from patients in reproductive age and 3.7±1.3 and 9.8±3.3 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 53.7±9.4 in relapsed and 42.2±7.5 in control group in reproductive age patients, whilst it was 30.9±6.9 and 20.8±5.8 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 57.1±8.8 in relapsed group and 44.3±5.4 in control group in reproductive age patients, whilst it was 36.3±6.7 and 23.6±7.9 in menopausal patients in relapsed and control groups respectively.

Table 2. Distribution of Ki67, Cyclin D1, Bcl2, Cas3 in laparoscopy tissue specimens

		Rel.	Cont.	Rel.	Cont.	Rel.	Cont.	Rel.	Cont.	Rel.	Cont.
Classic LM	Repr. Ag.	13	9	6,9	3,1	5,3	2,7	69,3	65,2	2,9	5,1
	Menopause	7	6	3,7	1,4	2,1	1	71,6	68,7	3,1	7,2
Bizzare/ Atypical LM	Repr. Ag.	6	5	26	12,3	24,5	11,2	17,2	14,3	4,9	12,3
	Menopause	4	3	11,2	4,9	10,6	3,8	18,1	16,2	6,8	14,1
STUMP	Repr. Ag.	12	7	61,4	21,2	52,7	19,1	15,1	13,4	2,6	8,1
	Menopause	6	4	21,8	7,3	18,6	5,7	15,8	12,9	3,7	9,8

Table 3. Distribution of ER and PR in laparoscopy tissue specimens

				ER		PR	
		Rel.	Cont.	Rel.	Cont.	Rel.	Cont.
Classic LM	Repr. Ag.	13	9	85,8	79,6	90,6	85,7
	Menopause	7	6	48,2	42,9	50,7	46,2
Bizzare/Atypical LM	Repr. Ag.	6	5	65,6	47,5	68,9	50,3
	Menopause	4	3	40,1	30,2	47,2	35,1
STUMP	Repr. Ag.	12	7	53,7	42,2	57,1	44,3
	Menopause	6	4	30,9	20,8	36,3	23,6

Further investigation results of proliferative and apoptotic markers in both groups showed the following results: in group I (myomectomy specimens) in reproductive age patients cellular leiomyomas, bizzare/atypical leiomyoma and leiomyosarcomas were divided into two major groups based on the expression of Ki67, cyclin D1, Bcl2 and Cas3. Particularly, in cellular leiomyomas low Ki67 labelling index (<=10) were present in 6/10 cases (60%), whilst high Ki67 labelling index (>10) was present in 4/10 (40%) cases. Low cyclin D1 labelling index was also presented in 6/10 (60%) cases and high cyclin D1 labelling index was presented in 4/10 (40%) cases. Low Bcl2 labelling index was presented in 7/10 (70%) cases and high Bcl2 labelling index was presented in 3/10 (30%) cases, similar to Cas3 labelling index, which was also presented as low in 7/10 (70%) and high in 3/10 (30%) cases. In bizzare/atypical leiomyomas low Ki67 index was presented in 8/14 (57.1%) cases and high Ki67 labelling index was presented in 6/14 (42.9%) cases similar to cyclin D1, which was also presented as low in 8/14 (57.1%) and as high in 6/14 (42.9%) cases. Low Bcl2 and Cas3 was presented in 9/14 (64.3%) cases and high Bcl2 and Cas3 was presented in 5/14 (35.7%) cases. In leiomyosarcoma low Ki67 and cyclin D1 index was presented in 2/10 (20%) cases and high Ki67 and cyclin D1 was presented in 8/10 (80%) cases. Low Bcl2 and Cas3 was presented in 9/10 (90%) cases, whilst high Bcl2 and Cas3 was presented in 1/10 (10%) case.

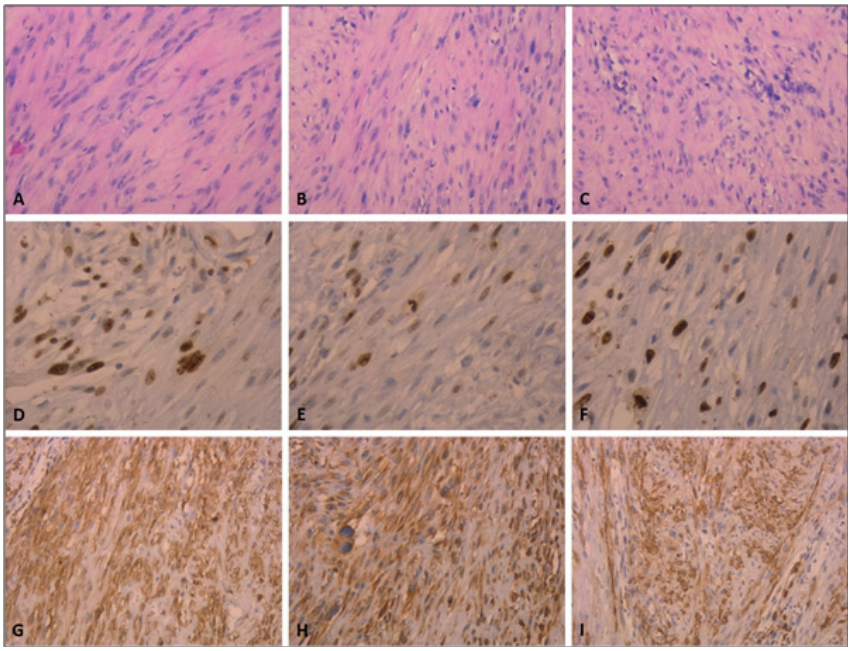
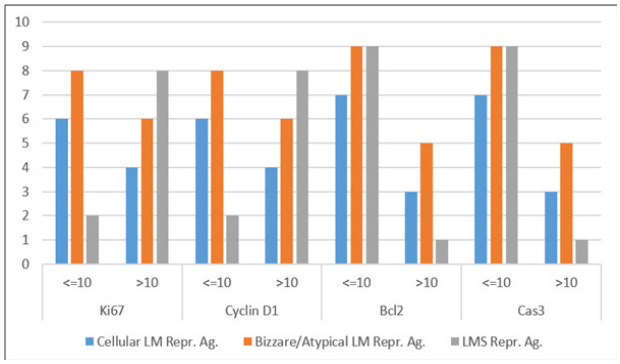
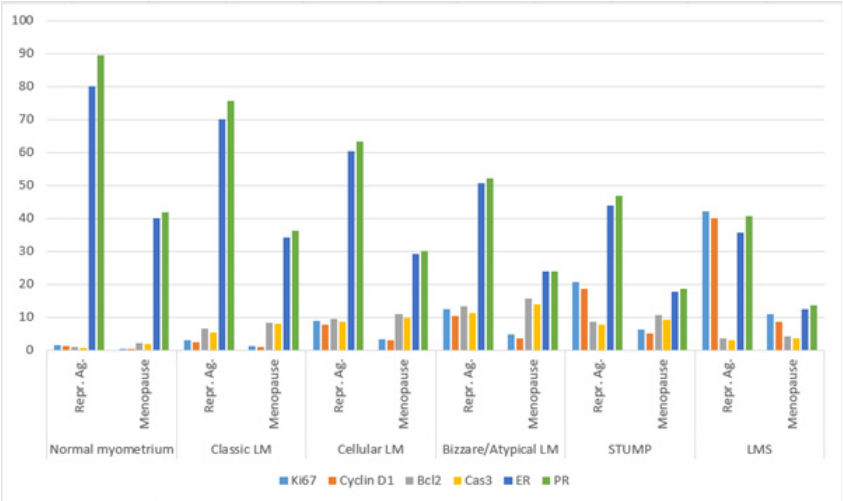


Fig. 1. A. Classic leiomyoma, H&E, x200 B. Bizzare/atypical leiomyoma, H&E, x200 C. STUMP, H&E, x200, D. Ki67 in bizzare/atypical leiomyoma, IHC, x200, E. Ki67 in STUMP, IHC, x200, F. Ki67 in leiomyosarcoma, IHC, x200, G. Bcl2 in classic leiomyoma, IHC, x200, G. Bcl2 in STUMP, IHC, x200, I. Bcl2 in , IHC, x200

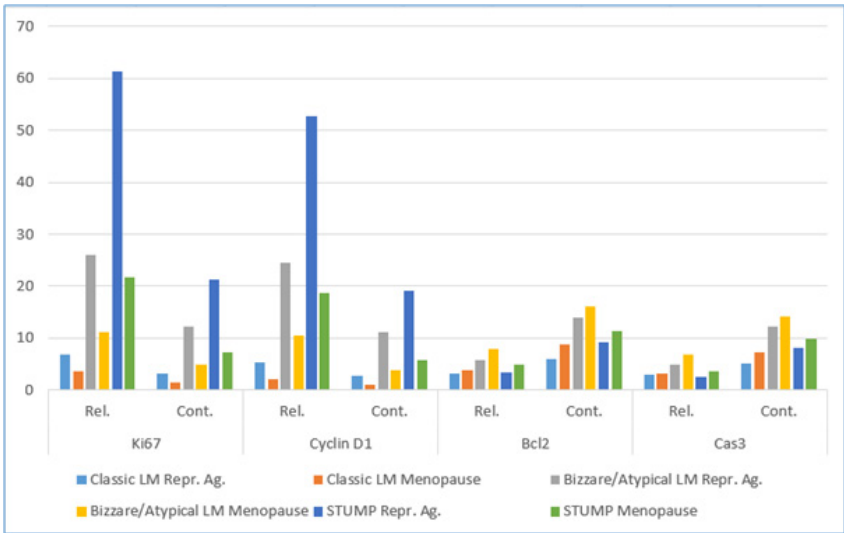


Graph 1. Distribution of cellular LM, bizzare/atypical LM and LMS cases in high and low proliferation and apoptotic groups in myomectomy specimens of reproductive women

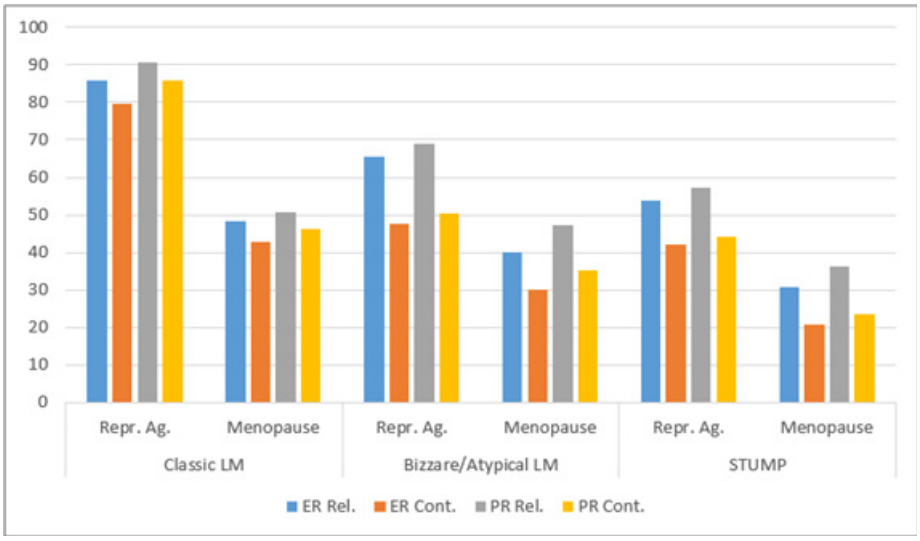
In laparoscopic surgical specimens, similar trend has been seen in groups with classic leiomyoma and bizzare/atypical leiomyomas. In classic leiomyoma in control group, 6/9 (66.7%) cases were characterised with low and 3/9 (33.3%) cases were characterised with high Ki67 and cyclin D1 labelling index. In relapsed group, 5/13 (38.5%) cases were characterised with low and 8/13 (61.5%) cases were characterised with high Ki67 and cyclin D1 labelling index. On the other hand, in control group 7/9 (77.8%) cases were characterised with low Bcl2 and Cas3 labelling index, whilst 2/9 (22.2%) cases were characterised with high Bcl2 and Cas3 labelling index. In relapsed group, 9/13 (69.2%) cases were characterised with low Bcl2 and Cas3 labelling index and 4/13 (30.8%) cases were characterised with high Bcl2 and Cas3 labelling index. In bizzare/atypical leiomyomas in control group 2/5 (40%) cases were characterised with low Ki67 and Cyclin D1 labelling index and 3/5 (60%) cases were characterised with high Ki67 and cyclin D1 labelling index. In relapsed group, 1/6 (16.6%) case were characterised with low Ki67 and cyclin D1 labelling index and 5/6 (83.4) cases were characterised with high Ki67 and cyclin D1 labelling index. With regard to apoptotic index, in control group 3/5 (60%) cases were characterised with low Bcl2 and Cas3 index and 2/5 (40%) cases were characterised with high Bcl2 and Cas3 index. In relapsed group, 3/6 (50%) cases were characterised with low Bcl2 and Cas3 index and 3/6 (50%) cases were characterised with high Bcl2 and Cas3 index.



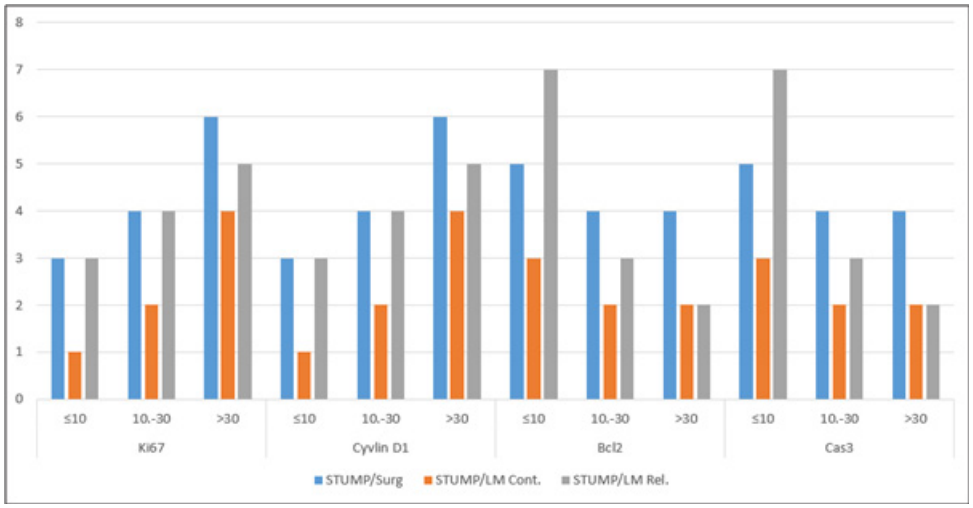
Graph 2. Distribution of cellular LM and bizzare/atypical LM cases in high and low proliferation and apoptotic groups in laparoscopic specimens of reproductive women. LM, leiomyoma, Repr.Ag., reproductive age



Graph 3.The comparative analysis of Ki67, cyclin D1, Bcl2 and Cas3 in laparoscopic myomectomy specimens. LM, leiomyoma, Repr. Ag. reproductive age.



Graph 4. Comparative analysis of ER and PR in laparoscopic surgical specimens. LM, leiomyoma, Cont., control specimens, Rel., relapsed specimens



Graph 5. Distribution of STUMP cases in different proliferative and apoptotic groups, Surg., surgical specimen, LM, laparoscopic myomectomy, Rel., relapsed cases

In cases of STUMP in both group I and group II three groups were identifiable based on proliferation and apoptotic index. Particularly in group I 3/13 (23%) cases were characterised with low Ki67 and cyclin D1 labelling index (<=10), 4/13 (30.8%) cases were characterised with moderate Ki67 and cyclin D1 labelling index (10-30) and 6/13 (46.2%) cases were characterised with high Ki67 and cyclin D1 labelling index (>30). In addition, 5/13 (38.4%) cases were characterised with low Bcl2 and Cas3 labelling index, 4/13 (30.8%) cases were characterised with moderate Bcl2 and Cas3 labelling index and 4/13 (30.8%) cases were characterised with high Bcl2 and Cas3 labelling index. In group II, in control specimens 1/7 (14.3%) cases were characterised with low proliferative index, 2/7 (28.6%) cases were characterised with moderate proliferative index and 4/7 (57.1%) cases were characterised with high proliferative index. On the opposite side, 3/7 (42.9%) cases were characterised with low Bcl2 and Cas3 index, 2/7 (28.6%) cases were characterised with moderate Bcl2 and Cas3 index and 2/7 (28.6%) cases were characterised with high Bcl2 and Cas3 index. In relapsed group 3/12 (25%) cases were characterised with low proliferation index, 4/12 (20%) cases were characterised with moderate proliferation index and 5/12 (55%) cases were characterised with high proliferative index. In addition, 7/12 (58.3%) cases were characterised with low apoptotic index, 3/12 (25%) cases were characterised with moderate apoptotic index and 2/12 (16.7%) cases were characterised with high apoptotic index.

Comparative analysis of studied markers in group I indicated that maximal expression of ER and PR is seen in control group (normal myometrium) and it gradually decreased in leiomyomas, reaching the minimal expression levels in leiomyosarcomas. Ki67 and Cyclin D1 labelling index is higher in all histological subgroups of reproductive age patients, compared to specimens from patients with menopause, whilst the opposite has been seen in cases of Bcl2 and Cas3 expression, which is higher in all histological groups of menopausal patients compared to reproductive patients. Higher expression of proliferation markers was seen in cases with bizzare/atypical leiomioma, STUMP and leiomyosarcoma, compared to classic and cellular leiomyomas. On the opposite the lowest expression apoptotic markers have been seen in cases with bizzare/atypical leiomioma, STUMP and leiomyosarcoma compared to classic and cellular leiomyomas.

In reproductive age patients lowest Ki67 and Cyclin D1 labelling index has been seen in normal myometrium, followed by classic leiomyomas. The expression of Ki67 and Cyclin D1 is almost three times higher in cellular leiomyoma compared to classic leiomyoma and four times higher in bizzare/atypical leiomyoma compared to classic leiomyoma. Whilst in STUMP and leiomyosarcoma the expression of Ki67 and Cyclin D1 is seven times and 14 times higher, respectively. With regard to apoptotic index, the highest apoptotic index has been seen in bizzare/atipycal leiomioma and lowest apoptotic index has been seen in normal myometrium, followed by leiomyosarcoma. Highest ER and PR expression has been seen in normal myometrium and lowest ER and PR expression has been seen in leiomyosarcomas.

Comparative analysis of proliferation and apoptotic proteins in laparoscopic surgical specimens showed that in all histological subtypes of leiomyomas proliferation markers Ki67 and cyclin D1 are expressed at nearly twice as much higher levels in relapsed group, compared to control group in both reproductive and menopausal age women. On the other hand, in the apoptotic markers Bcl2 and Cas3 are expressed almost twice as less levels compared to control group in both reproductive and menopausal patients.

The comparative analysis of ER and PR in laparoscopic surgery group indicated that in all histological subtypes, ER expression was much higher in relapsed group compared to control group. Whilst the progesterone showed the opposite trend.

The analysis of STUMP cases showed that three groups these histopathological entity is identifiable based on proliferation and apoptotic indexes. It has been shown that the majority of STUMP cases which were relapsed, belong to the high proliferative and low apoptotic groups. Whilst in control group or in surgical specimens STUMP cases are relatively equally distributed in different proliferative and apoptotic groups.

It is known that sex steroid hormone oestrogen plays and important role in the pathogenesis of leiomyoma [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs. Oestrogen is involved in the upregulation of several genes which cause the leiomyoma formation [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs, including growth factors, collagens and oestrogen and progesterone receptors [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs. In our study we showed that the expression of ER is significantly higher in laparoscopic myomectomy specimens after relapse, compared to control group or hysterectomy specimens. To the best of our knowledge we are first who demonstrated such a finding. With regard to cell proliferation and apoptotic markers, it is indicated that the balance between these two plays an important role in the development of virtually all types of tumors, including leiomyomas [9]. In our study we demonstrated that the balance between proliferation and apoptotic markers is markedly altered in relapsed leiomyomas. Particularly the expression of cell proliferation markers, particularly Ki67 and cyclin D1 is significantly higher in relapsed cases compared to control group. Whilst the expression of apoptotic markers Bcl2 and Cas3 is significantly decreased. In addition, there is the possibility to divide STUMP cases into three molecular subtypes, based on proliferation and apoptotic indexes. Particularly, these groups include cases with low proliferation and high apoptotic potential, cases with moderate proliferation and apoptotic potential and cases with high proliferation and low apoptotic potential. Later is more similar to leiomyosarcomas, whilst first group is more similar to classic leiomyomas. We are sure, that it is the first demonstration of such a finding.

Conclusions

ER expression is markedly higher in relapsed leiomyomas, compared to control group. Whilst PR shows the opposite trend. This finding can be used as a potential marker for leiomyoma relapse after laparoscopic myomectomy.

The relapsed leiomyomas after laparoscopic myomectomy are characterised with high proliferation and low apoptotic potential, which can also be used as a potential marker for leiomyoma relapse after laparoscopic myomectomy.

STUMP represents the heterogeneous group of smooth muscle tumors, with three different molecular subtype. Particularly, cases with with low proliferation and high apoptotic potential, resembling more to classic leiomyomas, cases with moderate proliferation and apoptotic potential and cases with high proliferation and low apoptotic potential, resembling more to leiomyosarcomas. This finding should be considered in clinical management of these tumors.

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SUMMARY

PHENOTYPIC CHARACTERISTICS OF RELAPSED LEIOMYOMA AND SMOOTH MUSCLE TUMORS OF UNCERTAIN MALIGNANCY POTENTIAL IN REPRODUCTIVE WOMEN

Kiknadze T., Tevdorashvili G., Muzashvili T., Gachechiladze M., Burkadze G.

Tbilisi State Medical University, Georgia

Uterine leiomyoma represents the most common pelvic tumor in females, including numerous histological subtypes, from which smooth muscle tumors of uncertain malignancy potential (STUMP) represents the diagnostic challenge. On the other hand, the study of the relapse risk markers after laparoscopic myomectomy is of high interest. We investigated the molecular phenotype of different types of leiomyoma after hysterectomy or laparoscopic surgery in reproductive and menopausal women. Standard immunohistochemistry was used to detect proliferation markers Ki67 and cyclin D1, apoptotic markers Bcl2 and Cas3, and ER and PR. The results of our study indicated that ER expression is significantly higher in relapsed leiomyoma, compared to control group. In addition, relapsed leiomyomas are characterised with high proliferation and apoptotic index. With regard to STUMP despite histological homogeneity, this entity is characterised with the presence of three distinct molecular subtypes, based on proliferation and apoptotic marker expression, which should be used as diagnostic aid in these tumors.

Keywords: relapse risk markers, laparoscopic myomectomy, smooth muscle tumors of uncertain malignancy potential, STUMP.

РЕЗЮМЕ

РЕЦИДИВИРУЮЩИЕ ЛЕЙОМИОМЫ И ГЛАДКОМЫШЕЧНЫЕ ОПУХОЛИ С НЕОПРЕДЕЛЁННЫМ ЗЛОКАЧЕСТВЕННЫМ ПОТЕНЦИАЛОМ – ФЕНОТИПИЧЕСКИЕ ОСОБЕННОСТИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА

Кикнадзе Т.Г., Тевдорашвили Г.Г., Музашвили Т.З., Гачечиладзе М.Г., Буркадзе Г.М.

Тбилисский государственный медицинский университет, Грузия

Лейомиомы матки - частые опухоли тазовой полости у женщин, из различных подтипов которых особенную диагностическую проблему представляют гладкомышечные опухоли с неопределённым злокачественным потенциалом. На сегодняшний день весьма актуально определить риск развития рецидива после лапароскопических миомэктомий. Изучены фенотипические особенности различных типов лейомиом у женщин репродуктивного и менопаузального возраста, которые получены путём гистерэктомии или лапароскопических миомэктомий. Стандартным иммуногистохимическим методом изучены молекулярные маркеры: пролиферативные маркеры Ki67 и cyclin D1, маркеры апоптоза Bcl2 и Cas3, ER и PR. Результаты исследования показали, что экспрессия ER значительно выше в рецидивирующих лейомиомах в сравнении с контрольной группой, а экспрессия PR - ниже. Рецидивирующие лейомиомы характеризуются высокой пролиферативной и низкой апоптозной активностью. Что касается гладкомышечных опухолей с неопределённым злокачественным потенциалом, несмотря на гистологическую однородность, в этой нозологии возможно выделение трёх молекулярных подтипов по характеру экспрессии пролиферативных и апоптозных маркеров, что необходимо учитывать при диагностике этого заболевания.

რეზიუმე

მორეციდივე ლეიომიომები და გლუვკუნთოვანი სიმსივნეები გაურკვეველი ავთვისებიანობის პოტენციალით - ფენოტიპური თავისებურებები რეპროდუქციული ასაკის ქალებში

თ.კიკნაძე, გ.თევდორაშვილი, თ.მუზაშვილი, მ.გაჩეჩილაძე, გ.ბურკაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, საქართველო

საშვილონოს ლეიომიომები წარმოადგენს მენჯის ღრუში განვითარებულ ხშირ სიმსივნეებს ქალებში, რომელთა მრავალი ჰისტოლოგიური ქვეტიპიდან, განსაკუთრებულ დიაგნოსტიკურ პრობლემას წარმოადგენს გლუვკუნთოვანი სიმსივნეები ავთვისებიანობის გაურკვეველი პოტენციალით. სადღეისოდ აქტუალურია ლეიომიომების რეციდივის განვითარების რისკის განსაზღვრა ლაპაროსკოპიული მიომექტომიის შემდეგ.

შესწავლილია ჰისტერექტომიით და ლაპაროსკოპიული მიომექტომიით მიღებული სხვადასხვა ტიპის ლეიომიომების ფენოტიპური მახასიათებლები რეპროდუქციული და მენოპაუზური ასაკის ქალებში. სტანდარტული იმუნოჰისტოქიმიური მეთოდით გამოკვლეულია ისეთი მარკერების ექსპრესია, როგორებიცაა პროლიფერაციული მარკერები Ki67 და cyclin D1, აპოპტოზური მარკერები Bcl2 და Cas3 და ER, PR. კვლევის შედეგებმა აჩვენა, რომ მორეციდივე ლეიომიომებში გაცილებით უფრო მაღალია ER-ის ექსპრესია შედარებით საკონტროლო ჯგუფთან, მაშინ როდესაც PR-ის ექსპრესია, პირიქით, დაბალია. მორეციდივე ლეიომიომები ხასიათდებიან მაღალი პროლიფერაციული და დაბალი აპოპტოზური აქტივობით. რაც შეეხება გლუვკუნთოვან სიმსივნეებს გაურკვეველი ავთვისებიანობის პოტენციალით, მიუხედავად ჰისტოლოგიური ერთგვაროვნებისა, ამ ნოზოლოგიაში შესაძლებელია სამი სახის მოლეკულური ქვეჯგუფის გამოყოფა პროლიფერაციული და აპოპტოზური მარკერების ექსპრესიის მიხედვით, რაც აუცილებელია გათვალისწინებული იყოს ამ დაზიანების დიაგნოსტიკაში.