Immunohistochemical profile of intravenous leiomyomatosis

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Summary

To determine the immunohistochemical staining profile of intravenous leiomyomatosis (IVL), we analysed six IVLs and 12 ordinary leiomyomas (LM) for immunoreactivity with a panel of 11 antibodies.

All IVLs and LMs reacted with antibodies to α-smooth muscle actin (αsm), h caldesmon, vimentin and progesterone receptor (PR). Five of six IVLs and all LMs reacted with desmin. All IVLs were negative for CD-10. Only one LM exhibited focal CD-10 positivity. Three of six IVLs and nine of 12 LMs showed estrogen receptor expression. All IVLs and LMs showed immunnegativity with MIB-1 and inhibin.

There were not any significant differences between immunoreactivity patterns of IVL and LM for asm, desmin, h caldesmon, CD-10, MIB-1 and PR. We conclude that, although they appear to be useful markers in differentiating IVL from ESS and LMS, a larger study also including ESS and LMS would be necessary to confirm their validity.

Key words: Endometrial stromal sarcoma; MIB-1; Estrogen receptor; Progesterone receptor.

Introduction

Intravenous leiomyomatosis (IVL) is an uncommon uterine tumor characterized by grossly visible intravascular proliferation of benign smooth muscle [1]. Occasional cases of IVL have an unusual appearance that may create problems in histologic interpretation [1]. The differential diagnosis of IVL includes endometrial stromal sarcoma (ESS), leiomyosarcoma (LMS) and rarely vascular tumors [1]. However reports which analyzed the immunohistochemical profile of IVL were restricted to only a few number of case reports [2-7]. Over the last years immunohistochemistry has proven to be valuable in the diagnosis of these tumors. The new development in this area is the suggestion that CD 10 may be a spesific marker for endometrial stromal tumors [8]. Additionally h-caldesmon has been shown by some authors to be more spesific than desmin in recognising smooth muscle differentiation [8]. The present study was undertaken in order to characterize the immunohistochemical profile of IVL.

Material and Method

Six cases of IVL and 12 cases of LM from the Pathology Department of Zeynep Kamil Maternity Hospital were obtained for this study. The clinical features of IVL cases are summarized in Table 1. The tumors were evaluated as IVL conforming to the Norris and Palmley [1] criteria. One representative paraffin block was chosen for immunohistochemical study. Five micron-thick representative tissue sections were immunohistochemically stained for MIB-1 and ER/PR using the combination of the avidin-biotin complex peroxidase method with microwave antigen retrival (15 min-0.001 N citrate buffer, pH 6). The tissue sections were incubated with monoclonal mouse antibodies. (All Neo Markers, Fremont, CA, ready to use). The sections were incubated with biotinylated goat anti-polyvalent and then with peroxidase conjugated streptavidin. They were stained with diaminobenzidine and counterstained with hematoxylin. Appropriate positive controls were performed at the same time while tissue sections in which the primary antibody was omitted were used as negative controls. The evaluation of ER and PR was performed according to the method described by Carcangiu *et al.* [10] based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells was graded as follows: 1) 0 to 25% of the nuclei stained; 2) 26 to 75 of the nuclei stained; 3) more than 75% of the nuclei stained. The staining intensity was scored as follows: 1) absent or weak; 2) strong; and 3) very strong. The sum of both parameters gave the immunohistochemical score. Tumors were divided into three categories depending on the immunohistochemical score. Category I corresponded to a score of 2, category II to a score of 3 or 4, and category III to a score of 5 or 6. Category I tumors were considered as immunonegative whereas category II and III tumors were considered as immunopositive. Those cases with only scattered rare nuclei stained with MIB-1 and other monoclonal antibodies were recorded as less than 1% staining and reactivity score of < 5% evaluated as immunonegative [11]. The differences between the variables were measured by Fisher's exact test and χ-square analysis, where appropriate. A p value of less than 0.05 was considered statistically significant. The analyses were performed using the Microst statistical program for Windows.

Results

Cases with a diagnosis of IVL were all characterized by the presence of endothelium covered proliferations of cytologically benign smooth muscle within the lumens of myometrial vessels. Sections stained for endothelial antigens were avaible in all cases.

Immunohistochemistry

The immunohistochemical results are summarized in Table 2.

Keratin: Only one of 12 LMs was positive with antikeratin antibody. The staining pattern was focal and cytoplasmic. None of the IVLs showed expression.

Revised manuscript accepted for publication December 10, 2003

Table 1. — *Clinical features of cases with intravenous leiomyomatosis.*

Case	Age	Presentation	Therapy	Follow-up
1	27	Menometrorrhagia, pelvic pain	Myomectomy	Due to pelvic recurrence, 12 months later TAH&BSO
2	21	Pelvic pain, pressure, menorrhagia	Myomectomy, removal of br.lg.tm + GnRH agonist	NED but 6 months later TAH&BSO
3	46	Pelvic pain menorrhagia	TAH&BSO	NED, 18 months
4	39	Menorrhagia, pelvic pressure	TAH&BSO, removal of br. lg. tm	NED, 6 months
5	47	Menorrhagia, pelvic pain	TAH&BSO	NED, 24 months
6	42	Pelvic pain, pressure	TAH&BSO	None

NED: No evidence of disease, br.lg.tm.: Broad ligament tumor.

Table 2. — *Immunohistochemistry results.*

Antibody	IVL cases		LM cases		p
	#	%	#	%	
Sitokeratin	–		1/12	8.3	0.6
EMA	–		1/1	8.3	0.6
CD-10	–		1/12	8.3	0.6
MIB-1	–		–		
Inhibin	–		–		
αsm	6/6		12/12	100	1
Desmin	5/6		12/12	100	0.9
h-caldesmon	6/6		12/12	100	1
PR	6/6		12/12	100	1
ER	3/6		9/12	75	0.9
Vimentin	6/6		12/12	100	1

Epithelial membrane Antigen (EMA): The single case of LM was reactive with EMA. The staining pattern was focal and cytoplasmic. None of the IVL cases showed EMA expression.

CD-10: One of the 12 LMs showed focal, weak and cytoplasmic CD 10 expression. All six IVLs were negative.

Inhibin: All IVLs and LMs were negative.

Desmin: Five of six IVLs and all of 12 LMs showed diffuse cytoplasmic expression of desmin.

h-caldesmon, asm, and vimentin: All IVLs and LMs were positive for h-caldesmon, asm, and vimentin. The staining pattern with all markers was diffuse and cytoplasmic.

MIB-1: All six IVLs and 12 LMs were negative.

PR: All cases with a diagnosis of IVL and LM showed diffuse and intense nuclear PR expression (Figure 1).

ER: Three of six IVLs and nine of 12 LMs were reactive for ER. The staining pattern was diffuse and nuclear.

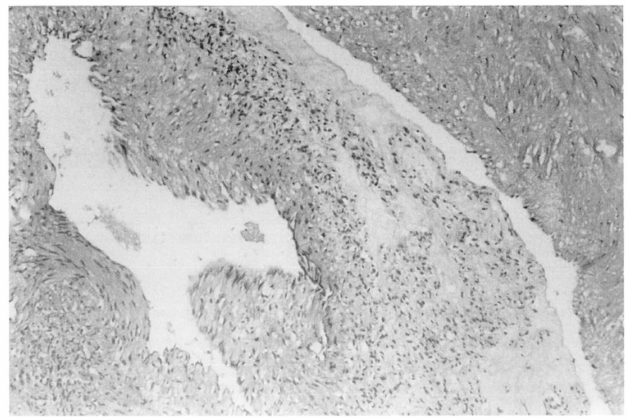

Figure 1. — IVL showed diffuse and intense nuclear PR expression.

Discussion

Occasional cases of IVL have an unusual appearance that may create problems in histologic interpretation [1]. These variants of IVL represent the intravascular counterparts of cellular leiomyomas, myxoid leiomyomas, lipoleiomyomas and leiomyomas with bizarre nuclei [1]. Some IVLs may have an angiomatoid pattern with thick-walled blood vessels within the intravascular tumor. Thrombosis of these thick-walled vessels may occur [1]. Rarely are the vessels thin-walled and ectatic; the appearance of the intravascular tumor in such cases resembles that of a cavernous hemangioma [1]. Thus the differential diagnosis of IVL includes vascular tumors, ESS and LMS. The cellular variant of IVL may be confused with ESS. There were some reported cases of IVL accessioned as ESS which were classified upon review as IVL [12]. The term cellular variant was restricted to densely cellular tumors with fusiform cells and scant cytoplasm [12]. ESS usually has a more uniform histologic appearance than IVL and IVL typically has thick-walled vessels.

In the present study no significant difference was observed between the immunoreactivity pattern of IVL and LM. The practical significance of asm, desmin, h-caldesmon expression (incidatives of smooth muscle neoplasm) and CD-10 (incidative of endometrial stromal neoplasm) negativity in all IVLs is that IVL can be distinguished from ESS by the aid of immunohistochemistry. Examples of cellular IVL with unusual degrees of mitotic activity and cytologic atypia should be distinguished from LMS. LMS almost never exhibits grossly visible vascular involvement and is characterized by significant degrees of nuclear pleomorphism and high mitotic rates [13]. In many studies the MIB-1 index was helpful in distinguishing between LMS and benign smooth muscle tumors [14-17]. MIB-1 expression of ≥ 15% was seen in 11 of 12 LMSs [17]. Zhai *et al.* [16] and Mittal *et al.* [17] also showed that compared to benign smooth muscle tumors the expression of both ER nad PR was markedly reduced in LMSs. PR was absent in ten of 12 LMSs but present in 14 of 15 cellular leiomyomas. In the present study all six IVLs were negative for MIB-1.

However all IVLs showed diffuse, intense expression particularly for PR.

In gynecological pathology inhibin is diagnostically the most useful antibody and is a sensitive immunohistochemical marker of most ovarian sex cord-stromal tumors [18]. All six IVLs and 12 LMs were negative for inhibin. Oliva *et al.* [8] showed that inhibin was negative in all LMs. In the present study one of the 12 LMs was reactive for keratin and another one was reactive for EMA. Oliva *et al.* [8] noted that two of ten LMs showed 2+ and 3+ positivity with keratin in 15% and 25% of tumor cells, respectively.

In conclusion there were not any statistically significant differences between the immunoreactivity patterns of IVL and LM for αsm, desmin, h caldesmon, CD-10, MIB-1 and PR. Thus these immunohistochemical results may raise the question as to whether immunohistochemistry has validity in differentiating IVL with unusual histologic features from ESS and LMS, as an ancillary method like in the differential diagnosis of ordinary leiomyomas. Performing this study in a larger series also including ESS and LMS cases will be helpful in enlightening this issue.

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