Original Article

Evaluation of estrogen receptor *alpha*, estrogen receptor *beta*, progesterone receptor, and cKIT expression in desmoids tumors and their role in determining treatment options

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Summary **The present study evaluates the protein expression of estrogen receptor *alpha* (ERα), estrogen receptor *beta* (ERβ), progesterone receptor (PR) and cKIT in a wide number of desmoids tumors and their role in determining treatment options. Fifty-nine cases classified as muscle aponeurotic fibromatosis were selected. Samples were grouped by tumor location in: head and neck, extremity and abdominal/trunk; type of resection of the primary tumor (complete resection with adequate margins, marginal resection and resection with inadequate margins); type of treatment (exclusive surgery, surgery followed by radiation therapy and surgery followed by tamoxifen or cyclooxygenase inhibitor). A tissue microarray (TMA) was built and the immunohistochemical reactions were performed against ERα, ERβ, PR, and c-kit. All cases were negative for ERα, PR and c-KIT. 53/59 cases were positive for ERβ. No significant difference was observed among clinical variables and the ERβ status. The estimated 5 and 10 year local recurrence free survival (LRFS) for the patients with complete or marginal resection was 75% and 75%, respectively. Tumor location ($p = 0.006$) and type of resection ($p = 0.001$) were predictive of local relapse in the univariate analysis. All patients treated with post-operative tamoxifen were LRFS ($p = 0.035$). Head and neck and extremities lesions showed higher recurrence rates compared to abdominal/trunk lesions. Marginal resection was associated with local recurrence. In conclusion, although this is a retrospective study, the results presented can contribute to better understanding of the mechanisms under desmoid tumor development and can propose tamoxifen as a therapeutic option to be tested in prospective trials.**

Keywords: Immunohistochemistry, hormone receptors, desmoids tumors

1. Introduction

Desmoid tumors, also known as muscle-aponeurotic fibromatosis, present locally agressive fibroblastic proliferation. It arises in deep soft tissues and is characterized by local invasion and high rates of local recurrence. However, no metastatical potential is observed. The incidence of desmoid tumors is 2 to 4 cases/1,000,000, and it is more frequent in female gender (*1*). Complete surgical resection of primary tumor is associated with best outcomes and death is a late event in the course of the disease and is the result of local destruction and complications.

Most of patients with desmoids tumors have long term survival even with recurrent disease. Unresectable disease is usually treated with radiation therapy, chemotherapy or hormone therapy. The results of such treatments are disappointing due to low response rates. Hormone therapy has been explored as a strategy to disease control showing null to satisfactory

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response rates (*2-5*). The inhibition of cKIT by imatinib represents another therapeutic option which can be employed in the control of muscle aponeurotic fibromatosis (*4*). Data related to estrogen receptor expression as well as expression of other molecules such as progesterone receptor (PR) and cKIT and its relation to clinical outcome and treatment response is controversial (*6,7*).

The aim of the present study was to analyze the protein expression of estrogen receptor *alpha* (ERα), estrogen receptor *beta* (ERβ), PR and cKIT in a wide number of desmoids tumors treated in a same institution and their role in determining treatment options.

2. Methods

2.1. *Patients and samples*

Fifty-nine cases classified as muscle aponeurotic fibromatosis based on the WHO classification were selected (*8*). Samples were grouped by tumor location in: head and neck, extremity and abdominal/trunk; type of resection of the primary tumor (complete resection with adequate margins, marginal resection and resection with inadequate margins); type of treatment in: exclusive surgery, surgery followed by radiation therapy and surgery followed by tamoxifen or cyclooxygenase (COX) inhibitor.

2.2. *TMA construction and immunohistochemistry*

A tissue microarray containing 59 cases spotted in duplicate was built for the immunohistochemical study (*9*). The immunohistochemical reactions were performed using a polymeric biotin-free detection system (Advance, DAKO™) according to the company guideline and standard protocols using the following antibodies: α-estrogen (clone SP1, NeoMarkers®) working dilution 1/5,000, β-estrogen receptor (polyclonal, Chemicon®) working dilution 1/400, PR (clone 636, DAKO®) working dilution 1/2,000, and c-kit (polyclonal, DAKO®) working dilution 1/200. Cases were considered positive when at least 1% of tumor cells showed moderate nuclear staining (Allred scoring system).

2.3. *Statistical analysis*

Statistical analysis was performed using SPSS program for Windows (version 8, SSPS Inc., Chicago, IL, USA). The Kaplan-Meier method was used for actuarial survival estimates. The multivariate analysis was performed using the proportional COX model of regression by the method of stepwise-forward. Local Recurrence Free Survival (LRFS) was calculated from the time of complete or marginal resection until detection of recurrence. Patients with inadequate surgery were excluded from the analysis.

3. Results

The median age for the 78 patients was 30. Forty-eight patients were female and 30 male. Sixty-six patients had no previous treatment and 12 had previous biopsy or incomplete surgery. According to anatomical distribution, 31 patients had lesion located in abdominal/trunk region, 37 in extremities and 10 in head and neck region. Considering surgical procedures 40 patients had adequate surgery with disease free margins, 21 patients had marginal resection of the tumors and 17 patients had inadequate surgery with tumors achieving margins. After admission at our institution, 57 patients were treated with exclusive surgery and 21 received combined treatment consisting of surgery followed by tamoxifen (15 patients), and surgery followed by radiotherapy (6 patients) (Table 1). The recurrence rate for the 61 patients with complete and marginal resection was 24.6% (Table 1).

Immunohistochemical study was conducted in 59 cases adequately represented in TMA spots. All cases were negative for ERα, PR and c-KIT expression. Fifty three out of 59 cases were positive for ERβ. No significant difference was observed among clinical variables and the ERβ status as shown in Table 2.

The estimated 5 and 10 year LRFS for the patients with complete or marginal resection was 75% and 75%, respectively (Figure 1). Tumor location ($p = 0.006$) and type of resection ($p = 0.001$) were predictive of local relapse in the univariate analysis. LRFS rates for each clinical variable are shown in Table 3. The actuarial

Table 1. Distribution of clinical variables for the 78 patients with fibromatosis

Variable	Category	Number of cases
Age (years)	< 50	38
	> 50	40
Median (years)	30	
Sex	Male	30
	Female	48
Situation at admission	Not treated	66
	Biopsy or tncomplete Surgery	12
Tumor site	Abdomen /visceral	31
	Extremities	37
	Head and neck	10
Type of ressection	Adequate	40
	Marginal	21
	Inadequte	17
Type of treatment	Exclusive surgery	57
	Combined Treatment	
	Surgery + radiotherapy	6
	Surgery + tamoxifen	15
Local recurrence*	No	46
	Yes	15

* Patients with complete and marginal resection ($n = 61$).

Table 2. ERβ status versus clinical variables for 59 patients with fibromatosis

Variable	Category	ERβ		*p*
		Negative *n* (%)	Positive *n* (%)	
Age (years)	< 50	3 (10)	27 (90)	1.00
	> 50	3 (10.3)	26 (89.7)	
Sex	Male	3 (13)	20 (87)	0.669
	Female	3 (8.3)	33 (91.7)	
Admission situation	Not treated	5 (10)	45 (90)	1.00
	Biopsy or incomplete surgery	1 (11.1)	8 (88.9)	
Tumor site	Abdomen/cavity	3 (13)	20 (87)	–*
	Extremities	2 (7.4)	25 (92.6)	
	Head and neck	1 (11.1)	8 (88.9)	
Type of resection	Adequate	3 (9.4)	29 (90.6)	–*
	Marginal	2 (12.5)	14 (87.5)	
	Inadequate	1 (10)	10 (90)	
Type of treatment	Exclusive surgery	3 (7.1)	39 (92.9)	–*
	Surgery + tamoxifen	3 (27.3)	8 (72.7)	
	Surgery + tamoxifen + COX inhibitor	0 (0)	1 (100)	
	Surgery + radiotherapy	0 (0)	5 (100)	
Local recurrence	No	4 (9.3)	39 (90.7)	0.658
	Yes	2 (12.5)	14 (87.5)	
Post-operative tamoxifen	No	3 (6.4)	44 (93.6)	0.092
	Yes	3 (25)	9 (75)	
Post-operative radiotherapy	No	6 (11.1)	48 (88.9)	1.00
	Yes	0 (0)	5 (100)	

* Pearson or Chi-square test could not be applied.

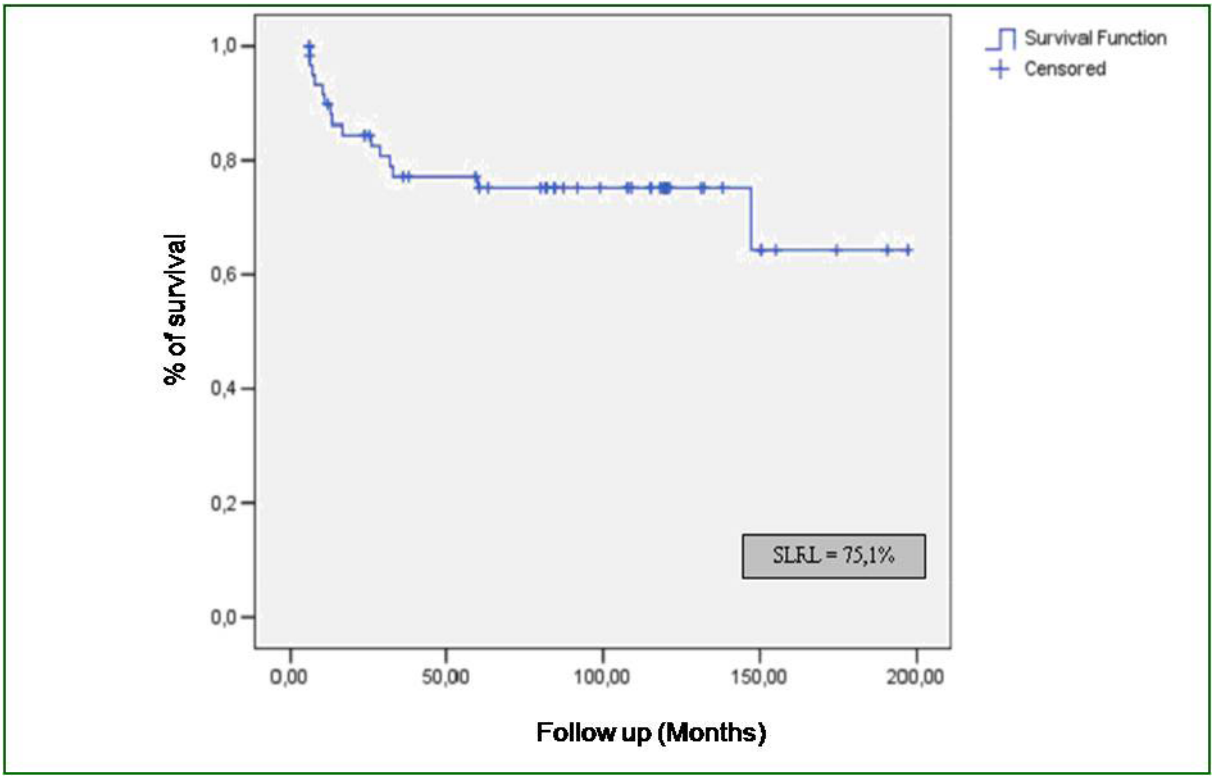

Figure 1. Local recurrence free survival in months for 61 patients with fibromatosis.

LRFS curves for variables with statistical significance are shown in Figures 2 and 3. LRFS was 100% for the group of patients treated with post-operative tamoxifen ($p = 0.035$) (Figure 4).

In the multivariate analysis, head and neck and extremities lesions had higher recurrences rates compared to abdominal/trunk lesions. Marginal resection was associated with local recurrence (Table 4).

4. Discussion

Muscle aponeurotic fibromatosis or desmoids tumors are rare neoplasms that arise in soft tissues. The pathogenesis of these tumors is not well understood and the hormone sensitivity of desmoids still controversial. The expression of hormone receptors and cKIT has been extensively studied in desmoids-type fibromatosis due to the fact that these molecules could be used as therapeutic targets.

Immunohistochemical studies on desmoids tumors have shown contradictory findings on hormone receptor status. Our study detected high rate of ER *beta* receptor expression (89%) but no ER *alpha* (0%). More interestingly we found that patients treated with tamoxifen had a much better outcome than those treated by surgery alone or radiation, with LRFS of 100%

Table 3. Local recurrence free survival at 5 and 10 years according to clinical variables for the 78 patients with fibromatosis

Variable	Category	*n*	LRFS in 5 years (%)*	LRFS in 10 years (%)*	*p***
Age (years)	< 50	38	73.8	73.8	0.726
	> 50	40	77.4	77.4	
Sex	Male	30	66.7	66.7	0.123
	Female	48	81.6	81.6	
Admission situation	Not treated	66	72.9	72.9	0.236
	Biopsy or incomplete surgery	12	62.5	62.5	
Location	Abdomen/cavity	31	95.8	95.8	0.006
	Extremities	37	59.0	59.0	
	Head and neck	10	66.7	66.7	
Type of ressection	Adequate	40	88.9	88.9	0.001
	Marginal	21	49.6	49.6	
	Inadequate	17	–***	–***	
Type of treatment	Exclusive surgery	57	71.3	71.3	0.345
	Combined treatment	21	83.3	83.3	

* Only patients with adequate and marginal resection ($n = 61$); ** p value by Log-rank test; *** Not applicable to patients with inadequate resection.

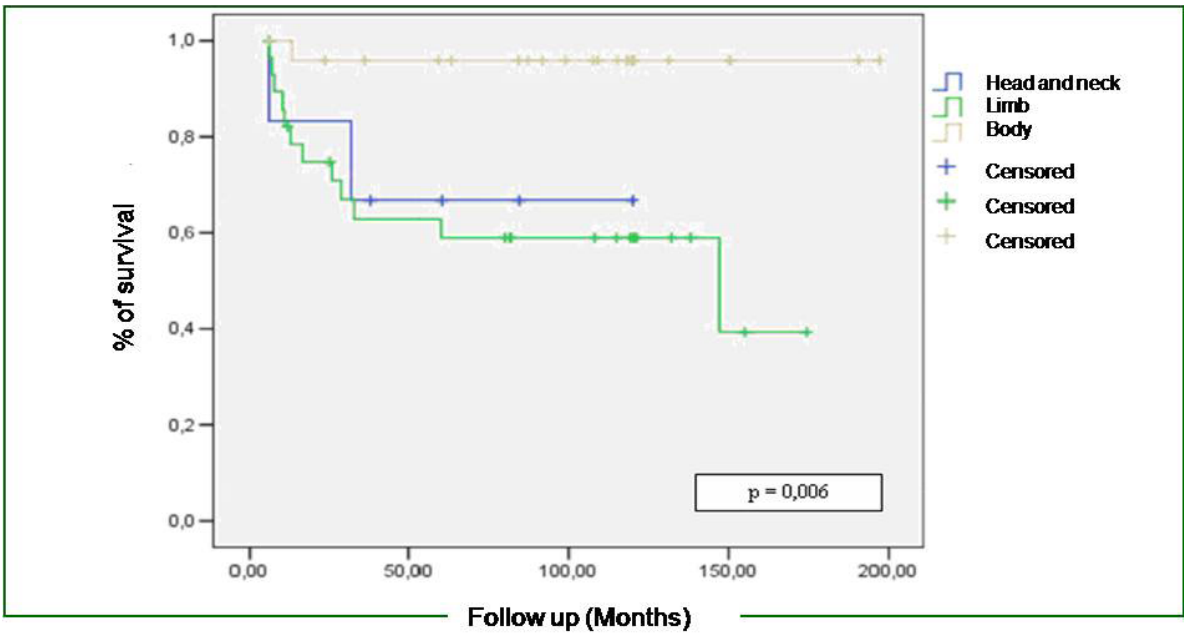

Figure 2. Local recurrence free survival in months for 61 patients with fibromatosis considering type of resection.

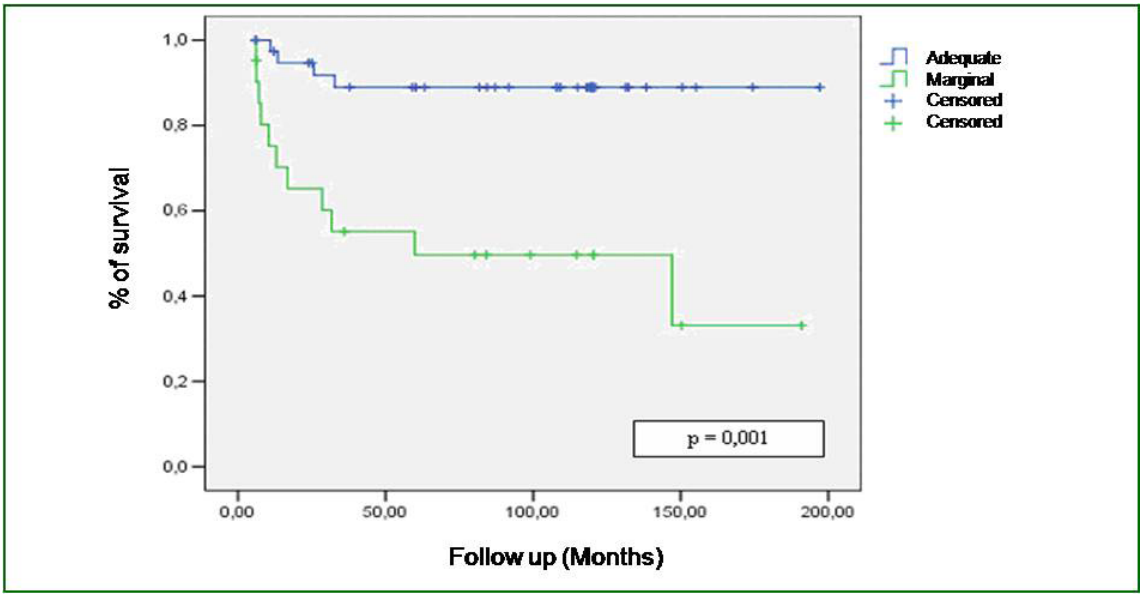

Figure 3. Local recurrence free survival in months for 61 patients with fibromatosis considering topography.

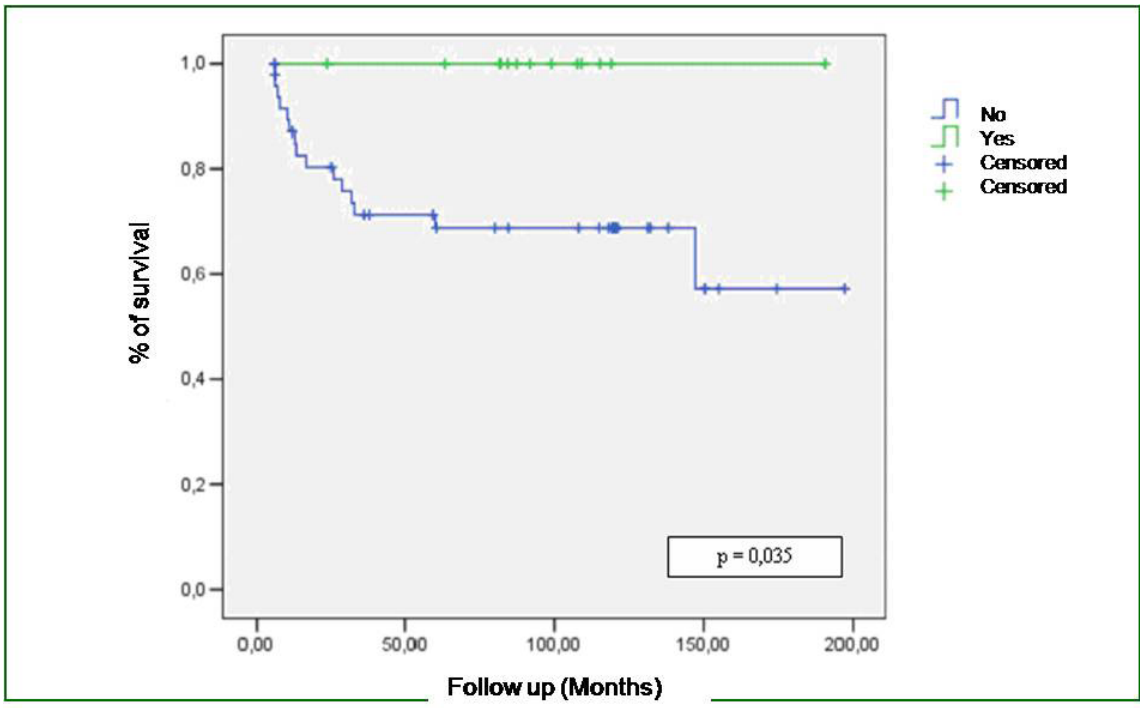

Figure 4. Local recurrence free survival in months for 61 patients with fibromatosis considering pos-operative tamoxifen.

Table 4. Risk factors for local recurrence in patients with fibromatosis

Variable	Category	*p**	Hazard ratio (HR)	95% CI
Location	Abdomen/cavity (reference)	–	1.00	–
	Superior extremity	0.043	10.49	1.07 – 102.84
	Inferior extremity	0.036	9.22	1.15 – 73.72
	Head and neck	0.049	11.32	1.00 – 127.19
Type of resection	Adequate (reference)	–	1.00	–
	Marginal	0.003	6.33	1.88 – 21.22

*COX regression.

at 5 and 10 years (p = 0.035), although only a small group of patients received tamoxifen an most of these patients had abdominal/trunk tumors. Nine out of the 12 patients treated with tamoxifen were ERβ positive (75%) and 3 were ERβ negative (25%) (p = 0.092). ERβ expression was not associated with better outcome neither with response to tamoxifen in our study, but we should emphasize that the characteristic and the size of the sample of patients were underpowered to show an improvement in outcomes for this low grade disease.

Tamoxifen is the most commonly agent used in hormone manipulation for breast cancer and other estrogen sensitive tumors (*3,7*). Tamoxifen is a selective estrogen receptor (ER) modulator that acts *via* binding on ER *alpha* and inhibits cell proliferation (*3*). Most of the studies have shown that desmoids tumor are ERα negative. Few studies have analyzed ERβ. Leithner A *et al.* (*6*) reported immunehistochemical analysis on 80 desmoid tumors and all cases were negative for ERα and PR and less than 10% (7/80) of cases were positive for ERβ. Deyrup *et al.* (*7*) showed 100% positivity of ERβ in 40 cases of extra-abdominal fibromatosis and no expression of ERα. Our results could contribute to explain or better understand the hormone sensitivity of these tumors in the scenario of negative ERα. The mechanisms responsible for tumor response to therapy with imatinib must be identified (*4*). In accordance with previous data, the present did not detect immunohistochemical expression of cKIT.

In summary, we detected high percentage of ERβ expression in desmoids and, in agreement with the literature, no expression of ERα and cKIT was observed. The observation, in this study, that adjuvant tamoxifen therapy is protective after adequate or marginal resection of desmoids tumor is also relevant. Although this is a retrospective study, the results presented can contribute to better understanding of the mechanisms under desmoid tumor development and can propose tamoxifen as a therapeutic option to be tested in prospective trials.

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