

Receptor modifications in vulvar dystrophies before and after treatment with topical hormones: comparison between the dextran-charcoal technique and immunohistochemical evaluation

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Summary

Purpose of investigation: The objective of the study was first to quantify estrogen receptors (ERs) and progesterone receptors (PRs) in dystrophic vulvar tissue before and after topical hormone treatment in an attempt to evaluate whether receptor modifications occurred. Second we compared quantitative analysis with immunohistochemical staining of the vulvar specimens.

Methods: We studied 115 vulvar specimens obtained from 75 consenting women ranging from 21 to 78 years of age. Of the patients, 12 had histologically normal vulvar skin, 45 had vulvar dystrophies that were not treated by topical steroid therapy, 28 patients had vulvar dystrophies that were treated by testosterone propionate (TP) 2%, 12 patients had vulvar dystrophies that were treated by progesterone in hydroalcoholic gel and 18 patients had vulvar malignant tumors. For immunohistochemical analysis we considered 25 cases of vulvar dystrophies: 11 cases of squamous hyperplasia (SH) and 14 cases of lichen sclerosus (LS). Among these 25 cases, 15 (5 SH and 10 LS) were treated with TP 2%.

Results: After treatment of the vulvar dystrophies with progesterone, the positivity of ERs decreased (58.3% vs 77.8%). After treatment of the vulvar dystrophies with TP 2%, the positivity of PRs significantly decreased (14.3% vs 68.9%) whereas after treatment with progesterone the positivity of PRs increased (83.3%). The immunohistochemical study showed some differences in comparison to the quantitative study. In fact we found low basal positivity especially for PRs (16% vs 68.9% of the quantitative study). This finding was due to the use of a cutoff of at least ++ in order to increase the specificity. After treatment with TP 2%, we observed an increase of immunohistochemical positivity for ERs even in cases that were negative before treatment and a lack of PRs even in cases that were positive before treatment.

Conclusions: These data demonstrate the efficacy of androgen therapy with TP 2% in vulvar dystrophies with increased trophism due to the increase of ERs.

Key words: Vulvar dystrophies; Squamous hyperplasia; Lichen sclerosus; Receptor modifications.

Introduction

Vulvar dystrophies such as squamous hyperplasia (SH), lichen sclerosus (LS) and LS with areas of hyperplasia are preneoplastic lesions that could develop into cancer. The risk of progression to cancer of these lesions is 1.8%-4.1% for HS [1] and 4-5% for LS [2]. According to our experience [1], 2.08% of SH had progression to cancer (2 cases of cancer out of 96 with a follow-up ranging from 6 to 12 years) whereas no cases of LS that were treated developed into cancer (162 patients who were followed up for 3-12 years). Thus it is mandatory to select high-risk cases such as SH with atypia that should be treated by surgery. On the other hand, topical medical therapy with corticosteroids for SH and testosterone propionate (TP) 2% or gel progesterone for the other forms could be indicated. TP 2% ameliorated subjective symptomatology in about 72% of vulvar dystrophies. Regression of symptomatology has been achieved in LS by 1% hydroalcoholic progesterone [1].

Studies of receptors in the human vulva have already been reported [3-6]. Vulvar tissue contains specific sites for estrogen receptors (ERs) [3], progesterone receptors (PRs) [4-5] and androgen receptors (ARs) [6].

However the relationship between sexual steroids and human vulvar tissue has not been clearly explained. The vulva responds to the physiological hormonal modifications related to puberty, fertile age, pregnancy and menopause. In dystrophic lesions of the vulva PRs seemed to have the highest concentrations [7]. The administration of topical progesterone induced an increase of vulvar receptors such as ARs and PRs [7].

Since 1982 laboratory assays for androgen, estrogen and progesterone receptors have been performed with the dextran-charcoal technique [8].

In recent years immunohistochemical studies with monoclonal antibodies have been widespread. Hodgins *et al.* [9], in an immunohistochemical study with monoclonal antibodies for androgen, estrogen and progesterone receptors, demonstrated a different topographical distribution of these receptors in the normal human vulva.

In fact ARs were more represented in the transitional zone from the vagina to vulva, and near vulvar skin, with the highest concentration in the keratinocytes of vulvar epidermis, in the sebaceous glands, hair follicles and in the epidermis of the labia majora and minora. In contrast, ERs were highest in the vaginal epithelium and stroma, and lowest in the suprapubic skin, whereas PRs were seen in the vaginal epithelium, fibroblasts and smooth muscle but not in the vulva. The authors [9] concluded that the transitional zone from the vagina to vulva is marked by an increase in ARs and a decrease in ERs and PRs.

The objective of this study was first to quantify ERs and PRs in dystrophic vulvar tissue before and after topical hormone treatment in an attempt to evaluate whether receptor modifications occurred. Second we compared quantitative analysis with immunohistochemical staining of the vulvar specimens.

Materials and Methods

After obtaining approval from the local ethics committee, we studied 115 vulvar specimens obtained from 75 consenting women ranging in age from 21 to 78 years over a 10-year period (1996-2005); 27 tissue specimens were from premenopausal women and 48 from postmenopausal women. Of the patients, 12 had histologically normal vulvar skin, 45 had vulvar dystrophies not treated by topical steroid therapy (16 cases of SH and 24 cases of LS and 5 cases of LS with low grade atypia), 28 patients had vulvar dystrophies treated by testosterone propionate 2% in vaseline or in extra virgin olive oil, 12 patients were treated by progesterone in hydroalcoholic gel and 18 patients had vulvar malignant tumors.

At the General Pathology Institute of Sassari University quantitative laboratory assays for ERs and PRs were performed with the dextran-charcoal technique [8]. The results were expressed in fmol/mg of cytosolic proteins. The minimum concentration considered positive was 3 fmol/mg of protein.

The immunohistochemical study was performed at the Pathologic Anatomic Institute: 25 cases of vulvar dystrophies were considered (11 of SH and 14 cases of LS). SH patients had a mean age of 71 whereas LS patients had a mean age of 62. Among these 25 cases, 15 (5 SH and 10 LS) were treated with TP 2% in vaseline or extra virgin olive oil.

Patients underwent vulvar biopsy during vulvoscopy and specimens were fixed in paraffin. Immunostaining was carried out by a standard avidin-biotin technique with diaminobenzidine chromogen. After incubation in an appropriate blocking serum, sequential sections were incubated with primary antibody against either estrogen or progesterone receptors for 20-24 hours at 4°C, washed in PBS and visualized by appropriate biotinylated antimouse or anti-rat immunoglobulins followed by avidin peroxidase conjugate and diaminobenzidine. Positive nuclei stained red whereas negative nuclei stained green. The percentage of positively stained nuclei was estimated and expressed on a scale +, ++, +++. The cutoff that we considered positive was ++ in order to increase the specificity.

Results

Dextrane-charcoal technique

In normal vulvar tissue the positivity of ERs and PRs were respectively, 41.7% and 75%, with low positivity (33.3%) for both hormonal receptors. Receptor concen-

trations were not high, with a mean \pm SD of 5.1 ± 1.3 and 24.3 ± 5.8 fmol/mg for ERs and PRs, respectively (Table 1).

Table 1. — Normal vulvar tissue (no. 12) - (dextrane-charcoal technique)

	No.	%	fmol/mg (mean \pm SD)
ER+	5/12	41.7	5.1 ± 1.3
PR+	9/12	75.0	24.3 ± 5.8
ER+ PR+	4/12	33.3	

Before treatment the positivity of ERs and PRs in dystrophic tissue was 77.8% and 68.9%, respectively, with a prevalence in SH in comparison to LS because of the highest cellularity. The concentrations of PRs were significantly higher than those in normal vulvar tissue (56.0 ± 8.4 and 41.7 ± 7.3 in SH and LS vs 24.3 ± 5.8 fmol/mg in normal vulvar tissue) (Table 2).

Table 2. — Vulvar dystrophies before treatment (no. 45) - (dextrane-charcoal technique)

	Squamous hyperplasia (no. 16)			Lichen sclerosis (no. 29)			
	No.	%	fmol/mg (mean \pm SD)	No.	%	fmol/mg (mean \pm SD)	
ER+	35/45	77.8	$13/16$ 81.2	11.2 ± 3.1	22/29	75.9	9.5 ± 2.1
PR+	31/45	68.9	14/16 87.5	56.0 ± 8.4	17/29	58.6	41.7 ± 7.3
ER+ PR+	26/45	57.8	11/16 68.7		15/29	51.7	

After treatment of the vulvar dystrophies with TP 2%, the positivity of PRs significantly decreased (14.3% vs 68.9%) whereas after treatment with progesterone the positivity of PRs increased (83.3% with concentration of 52.7 ± 9.3 fmol/mg). After treatment of the vulvar dystrophies with progesterone, the positivity of ERs decreased (58.3% vs 77.8%) but not significantly (Table 3). These data confirm that PRs could represent an important parameter in an attempt to evaluate the efficacy of topical hormone therapy.

Table 3. — Vulvar dystrophies after treatment (no. 40) - (dextrane-charcoal technique)

	Testosterone propionate 2% (no. 28)			Progesterone (no. 12)		
	No.	%	fmol/mg (mean \pm SD)	No.	%	fmol/mg (mean \pm SD)
ER+	14/28	50.0	4.2 ± 1.1	7/12	58.3	4.5 ± 1.1
PR+	4/28	14.3	4.8 ± 1.4	10/12	83.3	52.7 ± 9.3
ER+ PR+	4/28	14.3		6/12	50.0	

The positivity of ERs in cases of vulvar carcinoma (18) was higher than that of normal vulvar tissue (66.7% vs 41.7%) but lower than that of vulvar dystrophies (77.8%). In contrast, the positivity of PRs in cases of vulvar carcinoma was lower than that of normal vulvar tissue (44.4% vs 75.0%). The concentration of PRs was relatively lower than that of vulvar dystrophies (28.1 ± 5.9 vs 56.0 ± 8.4 and 41.7 ± 7.3 fmol/mg of SH and LS, respectively) (Table 4). The reduction of PRs seemed to be directly proportional to the decrease in tumor differentiation thus demonstrating that tumor tissue became free from endocrine control during its growth.

Table 4. — Vulvar carcinoma (no. 18) - (dextrane-charcoal technique)

	No.	%	fmol/mg (mean ± SD)
ER+	12/18	66.7	7.2 ± 1.5
PR+	8/18	44.4	28.1 ± 5.9
ER+ PR+	6/18	33.3	

Immunohistochemical study

With regard to the immunohistochemical study, 13/25 were completely negative for ERs and 8/25 (32.0%) were positive (with ++ considered the positive cutoff). PRs were completely negative in 19/25 cases, with only 4/25 (16%) positive cases. Two out of 25 (8.0%) cases were positive for both ERs and PRs (Table 5).

Table 5. — Immunohistochemical staining

	Negative	+	++	+++	Positive (%)
ER	13/25	4	5	3	8/25 (32.0)
PR	19/25	2	3	1	4/25 (16.0)
ER - PR	20/25	3	1	1	2/25 (8.0)

With regard to vulvar dystrophies, ERs were more positive in 5/11 (45.4%) SH than 3/14 (21.4%) LS, but there was no statistically significant difference for PRs between SH and LS (18.2% vs 14.3%). We did not report cases positive for both ERs and PRs in LS but 2/11 (18.2%) cases were positive for both ERs and PRs in SH (Table 6).

Table 6. — Immunohistochemical staining in lichen sclerosus and squamous hyperplasia

	Squamous hyperplasia (no. 11)		Lichen sclerosus (no. 14)
ER+	8	5/11 (45.4%)	3/14 (21.4%)
PR+	4	2/11 (18.2%)	2/14 (14.3%)
ER+ PR+	2	2/11 (18.2%)	—

After treatment with TP 2% three cases of SH that were positive for ERs before treatment remained positive after treatment whereas among two cases negative for ERs before treatment one case resulted positive after treatment with a total incidence of 80% (4 cases out of 5); after treatment all cases were negative for PRs. Three cases of LS that were positive for ERs before treatment remained positive after treatment; among seven cases negative for ERs before treatment four cases became positive after treatment with a total incidence of 70% (7 cases out of 10). Two cases positive for PRs before treatment were negative after treatment; eight cases negative before treatment remained negative after treatment (Table 7).

Table 7. — Immunohistochemical staining before and after treatment with testosterone propionate 2%

	Squamous hyperplasia (no. 5)			Lichen sclerosus (no. 10)		
	Before	After	Total	Before	After	Total
ER+	3	4	4/5 (80.0%)	3	7	7/10 (70%)
ER-	2	1	—	7	3	—
PR+	2	—	—	2	—	—
PR-	3	5	—	8	10	—

Discussion and Conclusions

Vulvar tissue presents ERs [3], PRs [4, 5] and ARs [6]. In dystrophic lesions quantitative assay of cytoplasmic receptors demonstrated an increase of ERs and PRs. Treatment with TP 2% reduced the concentrations of these receptors and especially of PRs. On the contrary, PRs increased after progesterone therapy.

The immunohistochemical study showed some difference in comparison to the quantitative study. In fact we found low basal positivity especially for PRs (16.0% vs 68.9% of the quantitative study). This finding was due to the use of a cutoff of at least ++ in order to increase the specificity. After treatment with TP 2%, an increase of immunohistochemical positivity was observed for ERs even in cases that were negative before treatment and a lack of PRs even in cases that were positive before treatment. These data demonstrate the efficacy of androgen therapy in vulvar dystrophies with an increase of trophism due to the increase of ERs.

Further studies with a large series are necessary to draw definitive conclusions in order to identify the cases that could have the highest risk of neoplastic transformation on the basis of hormone receptor status.

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