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Laryngeal cytological aspects in women with surgically induced menopause who were treated with transdermal estrogen replacement therapy

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Objective: To investigate the effects of estrogen replacement therapy (ERT) on laryngeal cytology in postmenopausal women.

Design: Prospective open clinical trial.

Setting: Outpatient menopausal clinic in the Department of Gynecology, University of Catania, Catania, Italy.

Patient(s): Eighty-four healthy, surgically postmenopausal women, of whom 48 were treated with ERT and 36 were considered as a control group.

Intervention(s): Transdermal E_2 treatment by patches or gel, evaluation of laryngeal cytology with cytobrush by indirect laryngoscopy, and questionnaire for the voice history.

Main Outcome Measure(s): Changes in cytologic aspects of laryngeal cells with respect to vaginal cytology by hematoxylin and eosin staining; subjective voice changes.

Result(s): Sixty-seven women completed the study. Ten women from the ERT group and five from the control group dropped out because of the invasive laryngoscope method; two subjects in the control group were excluded because of pathologies of the vocal cord. Hematoxylin and eosin staining confirmed similar superficial-intermediate aspects of the cells between the laryngeal and the vaginal smears in ERT-treated women. In the control group, both smears showed aspects of atrophy-dystrophy. The ERT group had a subjectively better quality of voice than the control group.

Conclusion(s): Our study confirms that the larynx is an estrogen target, as are vaginal cells. ERT may provide prevention and treatment of dystrophic pathologies of the vocal cords in postmenopausal women. (Fertil Steril® 2000;74:1073–79. ©2000 by American Society for Reproductive Medicine.)

Key Words: ERT, larynx, cytology, postmenopause

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After natural and surgically induced menopause, the subjective and objective complaints, as well as organic and metabolic organ damage, depend on estrogen deficiency syndrome (1). Estrogen deficiency affects some topographically remote organs and is manifested by clinically heterogeneous symptoms. The syndrome involves, therefore, all medical disciplines. The common origin of all pathologic conditions ensuing from estrogen deficiency calls for interdisciplinary collaboration in a new discipline: climacteric medicine (2).

Studies on a population survey reported that more postmenopausal women reported deepening of the voice than premenopausal women (3). This observation may confirm that the female voice undergoes particular changes at about the time of menopause, which are not seen in men of similar age. The human larynx is a hormonal steroid target organ. Voice mutation during maturation is well known. Disorders of the female voice occur during menstruation, pregnancy, and menopause; and in some endocrine diseases of the hypophysis, thyroid gland, adrenal glands, and ovaries. Female voice performers may have problems during the premenstrual phase. There may be a strong correlation between premenstrual dysphonia and luteal insufficiency (4). The vocal quality changes in women after hormonal therapy for

TABLE 1

Types of ERT used for surgically postmenopausal women.

No. of users	E_2 patch	E_2 hemihydrate patch	E_2 hemihydrate gel
13	Clinara 50 (3.9 mg)[a]	—	—
3	Estraderm TTS 50 (4 mg)[b]	—	—
8	Dermestril 50 (4 mg)[c]	—	—
7	Epiestrol (4 mg)[d]	—	—
9	—	FemSeven 50 (1.5 mg)[e]	—
8	—	—	Sandrena (1 g)[f]

[a] Schering, Milan, Italy.
[b] Novartis Farma, Origgio-Varese, Italy.
[c] Rottapharm, Monza-Milan, Italy.
[d] Pfizer, Latina, Italy.
[e] Bracco, Milan, Italy.
[f] Organon, Rome, Italy.

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the management of conditions such as endometriosis, hormonal imbalances, fibrocystic breast disease, and menstrual dysfunction (5), with adverse side effects such as huskiness, weakness, hoarseness, and virilization of the voice (6). Similar aspects may be observed after the treatment of postmenopausal women with hormone replacement therapy (HRT) (7) or with anabolic steroids (8). Therefore, the larynx may be considered as a secondary sex organ.

Menopause is associated with many alterations of functional and organic well-being, such as insomnia, hot flushes, emotional and psychological changes, genital atrophy, osteoporosis, cardiovascular disease, decrease in sexual performance, decreased concentration, and other alterations such as laryngeal mucosa and functional changes (9). Studies of postmenopausal singers have shown cracking of the voice (10). In addition to the hormonal changes, postmenopausal women have to deal with age-related factors that affect the voice, such as atrophy of the laryngeal muscles, stiffening of laryngeal cartilage, and virilization of the voice due to increases in ovarian androgen (11).

Epithelial smears from the larynx and vagina are similar at various times during the ovarian cycle (4). Estrogen target cells have been found in the larynx (12). The presence of laryngeal receptors for gonadal steroids emphasizes a specific functional role for hormones in laryngeal development and disease. Little attention has been given to the larynx as a site for the hormonally mediated effects relating to vocalization changes or to lesions of the larynx, where hormonally mediated changes may also occur (13).

The presence of high-affinity binding sites for 17β-E_2 was confirmed in membranes from normal human laryngeal epithelium, laryngeal papillomas (14), and laryngeal carcinoma (15, 16). Both 17β-E_2 with high affinity to such sites and the number of receptors for this estrogen are comparable to the density of such receptors identified in breast tissue (17,

TABLE 2

Cytologic smears of vocal cord epithelium and vaginal epithelium in ERT-treated surgically postmenopausal women.

Age (y)	Time from menopause	Type of continuous ERT	Time of therapy	Laryngeal cells	Vaginal cells
56	7 y	Dermestril 50	6 y	S/I	S/I
56	8 y	Dermestril 50	5 y	Dropout	S/I
57	7 y	Dermestril 50	5 y	S/I	S/I
56	6 y	Dermestril 50	4 y	S/I	S/I
57	8 y	Dermestril 50	4 y	Dropout	S/I
57	7 y	Dermestril 50	4 y	S/I	S/I
57	9 y	Dermestril 50	3 y	S/I	S/I
56	8 y	Dermestril 50	4 y	S/I	S/I
56	8 y	Climara 50	4 y	S/I	S/I
56	7 y	Climara 50	3 y	S/I	S/I
55	6 y	Climara 50	4 y	S/I	S/I
49	10 mo	Climara 50	9 mo	S/I	S/I
50	9 mo	Climara 50	8 mo	S/I	S/I
55	6 y	Climara 50	3 y	S/I	S/I
53	4 y	Climara 50	3 y	S/I	S/I
55	4 y	Climara 50	2 y	S/I	S/I
55	5 y	Climara 50	3 y	S/I	S/I
56	4 y	Climara 50	3 y	Dropout	S/I
49	4 y	Climara 50	4 y	Dropout	S/I
55	6 y	Climara 50	1 y	Dropout	S/I
54	4 y	Climara 50	2 y	S/I	S/I

Note: S/I = superficial-intermediate cells >2.

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TABLE 3

Cytologic smears of vocal cord epithelium and vaginal epithelium in ERT-treated surgically postmenopausal women.

Age (y)	Time from menopause	Type of continuous ERT	Time of therapy	Laryngeal cells	Vaginal cells
60	11 y	Estraderm TTS 50	10 y	S/I	S/I
58	9 y	Estraderm TTS 50	8 y	Dropout	S/I
58	8 y	Estraderm TTS 50	8 y	S/I	S/I
50	11 mo	Epiestrol 50	8 mo	Dropout	S/I
53	2 y	Epiestrol 50	18 mo	S/I	S/I
55	4 y	Epiestrol 50	2 y	S/I	S/I
49	1 y	Epiestrol 50	8 mo	S/I	S/I
54	5 y	Epiestrol 50	2 y	S/I	S/I
51	2 y	Epiestrol 50	15 mo	Dropout	S/I
52	2 y	Epiestrol 50	1 y	S/I	S/I
50	14 mo	FemSeven 50	9 mo	S/I	S/I
52	2 yr	FemSeven 50	8 mo	S/I	S/I
54	4 y	FemSeven 50	8 mo	S/I	S/I
56	5 y	FemSeven 50	10 mo	S/I	S/I
51	2 y	FemSeven 50	9 mo	S/I	S/I
48	11 mo	FemSeven 50	8 mo	Dropout	S/I
49	1 y	FemSeven 50	8 mo	S/I	S/I
50	2 y	FemSeven 50	9 mo	S/I	S/I
49	1 y	FemSeven 50	8 mo	S/I	S/I
46	1 y	Sandrena 1 mg	9 mo	S/I	S/I
50	2 y	Sandrena 1 mg	1 y	S/I	S/I
50	3 y	Sandrena 1 mg	1 yr	S/I	S/I
51	1 y	Sandrena 1 mg	9 mo	S/I	S/I
51	2 y	Sandrena 1 mg	8 mo	Dropout	S/I
50	2 y	Sandrena 1 mg	11 mo	S/I	S/I
48	11 mo	Sandrena 1 mg	8 mo	S/I	S/I
51	2 y	Sandrena 1 mg	1 y	S/I	S/I

Note: S/I = superficial-intermediate cells >2.

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18). Stratified squamous epithelium of the vocal fold includes three cellular layers: a basal layer, an intermediate layer, and a superficial layer. The stratified squamous epithelium undergoes modifications in both structure and function depending on the hormonal stimulation it receives (19, 20).

During the menopausal transition, serum levels of estrogen decrease and subsequently remain low (21). HRT is widely used to treat various aspects of the menopausal syndrome, such as hot flushes and sweating, and to protect postmenopausal women against bone loss, cardiovascular disease, and Alzheimer's disease. HRT has been used to forestall menopause-associated voice changes, especially among professional singers. There is, however, a lack of objective voice measurements to support this treatment (22).

In the present study, we determined the morphologic aspects of the cytologic layers of the larynx in postmenopausal women treated or not treated with HRT.

MATERIALS AND METHODS

The study was performed at the Menopausal Clinic of the Department of Gynecological Science and at the Service of Phonology of the Department of Otorhinolaryngology, School of Medicine, University of Catania, Catania, Italy. It was approved by the Institutional Review Board of the research committees of both departments. All subjects gave written informed consent.

Subjects

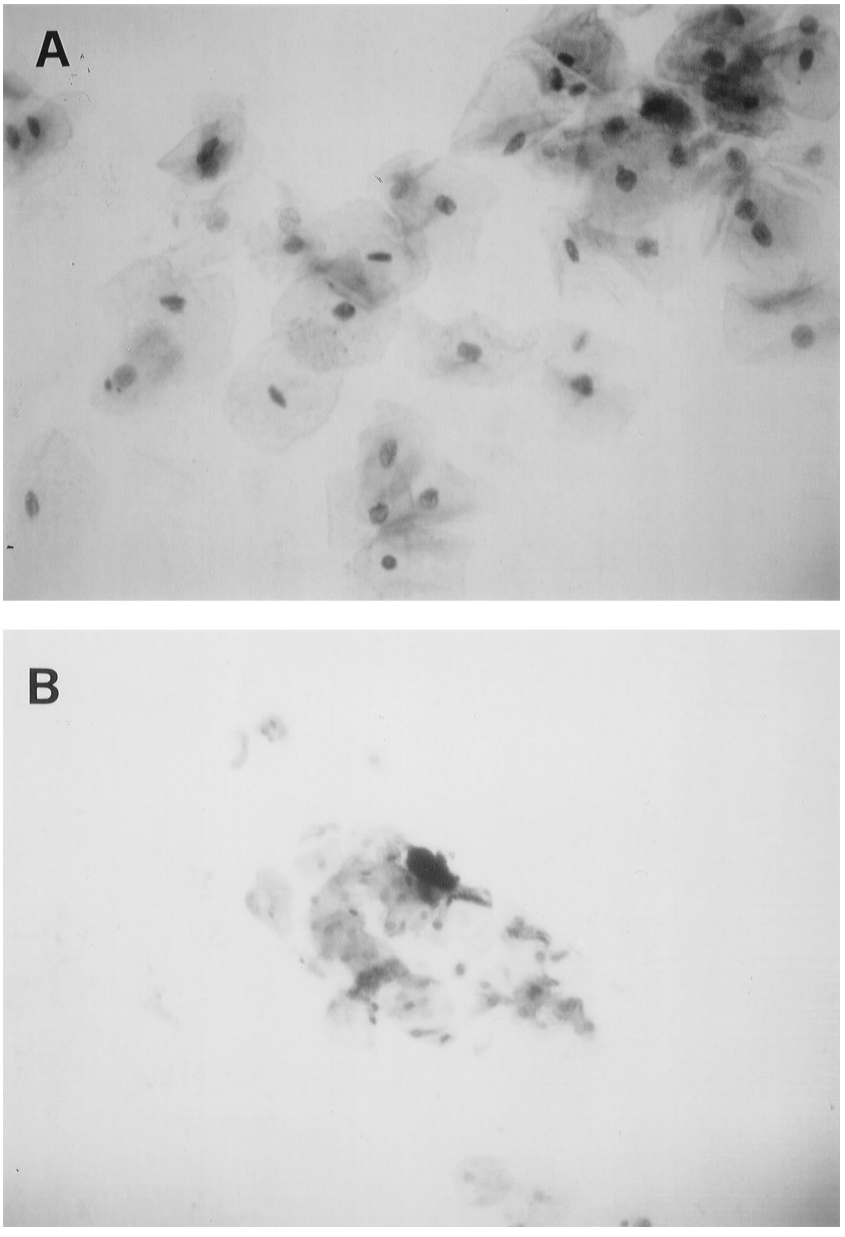
Forty-eight healthy, surgically postmenopausal women ranging in age from 46 to 60 years (mean age, 53.1 years) were treated with estrogen replacement therapy (ERT) for 8 months to 10 years for climacteric symptoms. Thirty-six healthy, surgically postmenopausal subjects ranging in age from 43 to 58 years (mean age, 51.9 years) requiring treatment for climacteric symptoms were used as a control group before starting their first HRT treatment.

Both groups of subjects had surgical menopause by hysterectomy and ovariectomy for benign gynecologic disease (i.e., myoma). The body mass index (BMI) of each woman was within the normal range (mean ± SD, 23.6 ± 1.2 kg/m^2).

Climacteric symptoms of the women requiring HRT were hot flushes, night sweating, sleep problems, vaginal dryness,

FIGURE 1

Superficial-intermediate cells in cytologic smears of the vagina (**A**) and in the larynx (**B**) epithelium in surgically postmenopausal women treated with transdermal estrogen therapy.



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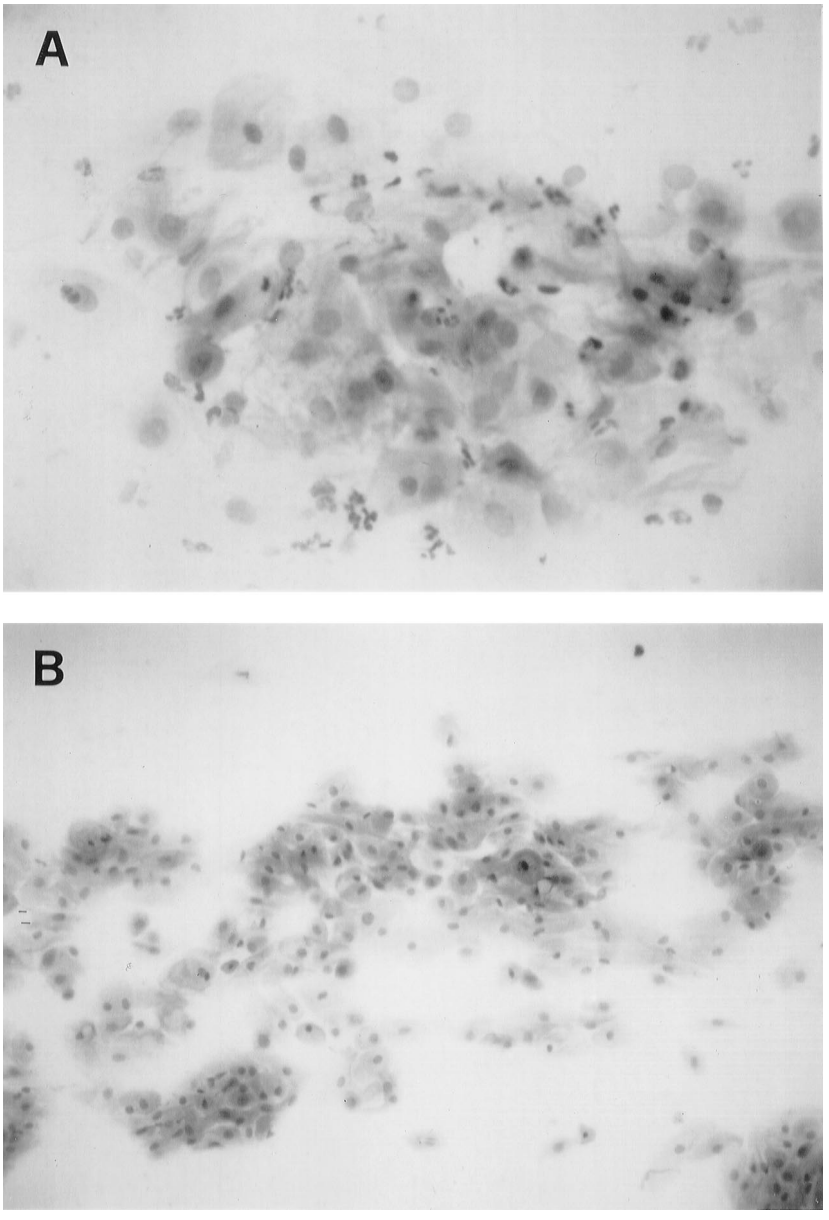
and fatigue. Before inclusion in the study, a general medical and gynecologic examination was performed. Any significant medical problem such as extragenital hormonal diseases, smoking, or the use of drugs and alcohol excluded subjects of both postmenopausal groups from participation.

Hormone Replacement Therapy

Thirty-one women were treated with transdermal E_2 patches for 8 months to 10 years. Nine women were treated with transdermal E_2 hemihydrate patches for 8 months to 1 year. Finally, eight women were treated with E_2 hemihydrate gel for 8 months to 15 months. The patches were changed once a week (Climara, Schering, Milan, Italy; and Fem-Seven, Bracco, Milan, Italy) or twice a week (Estraderm TTS, Novartis Farma, Origgio-Varese, Italy; Dermestril, Rottapharm, Monza-Milan, Italy; and Epiestrol, Pfizer, Latina, Italy), and the gel (Sandrena; Organon, Rome, Italy)

FIGURE 2

Dystrophic-atrophic cells in cytologic smears of the vagina (**A**) and in the larynx (**B**) epithelium in the control group.



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was used once a day. ERT was used as a continuous regimen. Table 1 shows the types of treatment used.

Cytologic Smears

All women in both groups underwent a vocal epithelium smear and a Papanicolaou test for vaginal cytology. Both cytologic smears were performed on the same day for each woman. Before the laryngeal samples, each woman was given local anesthesia with sodium benoxinate; then, the vocal folds were brushed by a cytobrush (SCS International, Genova, Italy) connected to a curved metallic arm by indirect laryngoscopy with a laryngotelescope (Stuckrad 90°; R. Wolf, Knittlingen, Germany) using a video system (Panasonic WV-KS152; Matsushita Electric Industrial Co., Osaka, Japan). Finally, each sample of the laryngeal mucosa was smeared and fixed onto slides. Each cytologic smear was used for routine hematoxylin and eosin staining.

Questionnaire for the Voice History

The voice history was directed toward unspecified and specified subjective voice complaints. The questionnaire in-

cluded open questions about any voice changes, either positive or negative, observed over the 6 months before the study. Each woman was asked to describe the nature of the changes and the circumstances under which they occurred, and whether she believed that the voice changes were due to her menopausal state. Unspecified voice complaints such as change in timbre, voice breaks, voice instability, voice lowering, rapid voice fatigue, and hoarseness were scored as 0 (normal or equal) or 1 (abnormal or unequal).

RESULTS

Each woman in the ERT group tolerated the hormone treatment well, without adverse effects such as local irritation at the application site of the patches or gel. Laryngeal brushing was performed by indirect laryngoscopy under local anesthesia. This is a simple procedure, and substantially is without risks. Therefore, it is better than direct laryngoscopy with general anesthesia.

However, there were dropouts because of the invasive method for cytologic smears of the larynx, both in the treated group (10/48, 20.8%) and in the control group (5/36, 13.8%). Moreover, two women in the control group had a laryngoscopic diagnosis of Reinke disease (edema) and polyps in one woman, and polyps alone in the other, of the vocal cord. Therefore, cytologic smears were analyzed from 38 ERT-treated women and 29 subjects in the control group.

Tables 2 and 3 show both the laryngeal and vaginal cytologic aspects of the ERT-treated group and the number of dropouts.

Table 4 shows the cytologic aspects of the vocal cords and vaginal epithelium in the control group.

As shown in Figure 1, hematoxylin and eosin staining confirmed the similar cytologic aspects of the vaginal smear and the laryngeal smear in the subjects treated with ERT. Both samples emphasized the superficial-intermediate type of cells, with a ratio index of >2. No variation was observed as a result of different ERT regimens. On the other hand, Figure 2 shows the similar cytologic aspects of both the vaginal and the laryngeal smears in the control group, with frequent aspects of atrophy-dystrophy in both smears.

Table 5 shows the results of the voice history. The ERT group seemed to have a subjectively better quality of voice than the control group. Moreover, within the ERT group, the percentage of women with unspecified and specified voice complaints had decreased significantly.

DISCUSSION

Menopause is the permanent cessation of menstruation resulting from the loss of ovarian follicular activity (1). Loss of ovarian function, which occurs after menopause, decreases the trophic action on several organs and biologic functions, usually because of estrogen deficiency. The voice undergoes changes due to estrogen deficiency (13) such as several other extragenital targets.

Our study, using a case-control design, investigated changes in the cytologic aspects of the vagina and larynx in two groups of postmenopausal women who had undergone bilateral ovariectomy with hysterectomy for benign diseases

TABLE 4

Cytologic smears of vocal cord and vaginal epithelium in the control group.

Age (y)	Time from menopause	Laryngeal cells	Vaginal cells
50	3 y	Dystrophy	Atrophy
47	6 mo	Dystrophy	Dystrophy
52	5 y	Dystrophy	Atrophy
50	6 mo	Dystrophy	Dystrophy
49	1 y	Dystrophy	Dystrophy
50	8 mo	Dystrophy	Dystrophy
53	2 y	Dystrophy	Dystrophy
53	2 y	Dystrophy	Atrophy
52	1 y	Dystrophy	Dystrophy
58	9 y	Dystrophy	Atrophy
50	11 mo	Dystrophy	Dystrophy
53	2 y	Dystrophy	Dystrophy
55	6 y	Dystrophy	Atrophy
53	4 y	Dystrophy	Atrophy
50	10 mo	Dystrophy	Dystrophy
50	6 mo	Normotrophy	Dystrophy
51	3 y	Dystrophy	Dystrophy
58	7 y	Dystrophy	Atrophy
50	1 y	Dystrophy	Dystrophy
49	1 y	Normotrophy	Normotrophy
52	3 y	Dystrophy	Dystrophy
48	7 mo	Normotrophy	Normotrophy
55	6 y	Dystrophy	Atrophy
57	8 y	Dystrophy	Atrophy
55	5 y	Dystrophy	Dystrophy
56	5 y	Dystrophy	Atrophy
51	2 y	Dystrophy	Dystrophy
51	3 y	Dystrophy	Dystrophy
50	10 mo	Dystrophy	Dystrophy

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TABLE 5

Subjective voice changes evaluated by the voice history.

Voice evaluation	ERT group	Control group	*P*
Voice complaints	16	62	<.01
Changed timbre	0	37	<.01
Voice breaks	0	5	NS
Instability	0	16	<.05
Voice lowering	11	31	<.05
Voice fatigue	3	46	<.01
Hoarseness	13	27	<.05

Note: Values are percentages of subjects. NS = not significant.

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(i.e., myoma). Because the ovary continues to secrete androgen after menopause, we wanted to investigate women who had surgical menopause to eliminate any effects of ovarian androgens on cytologic smears. Androgens produce unsteadiness of the voice, rapid changes of timbre, and lowering of fundamental voice frequency (11), which may cause relationship problems. Moreover, we considered women with normal BMI so that any production of estrogen, by aromatase activity on the adrenal androgens in adipose tissue, was elicited. Consequently, we treated surgically postmenopausal women with continuous ERT, without the addition of progesterone.

Our study demonstrates that the larynx is an estrogen target organ in the same way that vaginal mucosa is. In fact, the cytologic aspects of epithelial smears from both organs are undistinguishable from one another. This occurs in postmenopausal women whether treated with ERT or not. The pyknotic index constituted a quantitative measurement of estrogen stimulation in both groups. In the subjects treated with ERT, the pyknotic nuclei reached an eosinophilic superficial-intermediate appearance.

Our data show that the larynx possesses receptors for ovarian hormones and that estrogen plays an important role in laryngeal trophism. The evidence thus suggests that ERT may provide prevention, and possibly treatment, of pathophysiology such as vocal cord dystrophy that can occur in postmenopausal women.

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