Effects of Ovariectomy and Estrogen Replacement Therapy on Laryngeal Tissue: A Histopathological Experimental Animal Study

Arzu Tatlipinar, Pembegül Günes, Dilek Özbeyli, Burak Çimen and Tanju Gökçeer

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What is This?
Abstract

Objective. To determine the histopathological effect of estrogen deficiency and hormone replacement treatment on laryngeal tissue in ovariectomized rats.

Study Design. Animal study.

Setting. The study was conducted at the animal experiment laboratory of Marmara University School of Medicine, Istanbul, Turkey.

Subjects and Methods. Six-month-old female Wistar albino rats were divided into the following 3 groups (n = 8 per group): sham-operated control, ovariectomized, and ovariectomized with estrogen replacement. Rats in the ovariectomized with estrogen replacement group received 17β-estradiol valerate (200 µg/kg, subcutaneously) once a week. Animals were killed after 8 weeks of intervention.

Results. Significant changes were observed in the ovariectomized group when edema in lamina propria, inflammation in squamous, respiratory epithelia and lamina propria, pseudostratification, and cilia loss were assessed. Except cilia loss, there were no significant differences in the assessments between the sham-operated control and ovariectomized with estrogen replacement groups.

Conclusions. On the basis of histopathological evaluations, it was shown that estrogen replacement helped to improve laryngeal changes due to experimentally induced menopause.

Keywords

menopause, larynx, estrogen replacement

Menopause is characterized by a permanent cessation of menstruation resulting from the loss of ovarian follicular activity.1 Well-known phenomena accompanying menopause are vasomotor symptoms, sleep disturbances, urogenital symptoms, loss of bone density (osteoporosis), and an increased risk for cardiovascular disease. However, the larynx also can be a source of complaint.2 A significant number of sex steroid receptors have been detected in human larynx tissues,3-5 suggesting a probable effect of these hormones on phonatory function and justifying their specific role in the development of the larynx.

Abitbol et al6 demonstrated a striking parallel between smears from vocal cords and cervical smears, showing a relative mucosal atrophy in menopause. Other studies have documented changes in the vocal quality of menopausal women, such as lack of intensity, vocal fatigue, low voice and hoarse voice,7-10 and reduction of fundamental frequency (F0)7,11 and have suggested probable beneficial effects of estrogen replacement therapy (ERT) on the larynx for the prevention of changes in voice associated with menopause.12,13 However, all of these studies concern dynamic (vocal quality), morphologic (endoscopic examination), and cytologic aspects of the larynx.

This study was designed to show the histopathological effect of estrogen deficiency and hormone replacement therapy (HRT) on the laryngeal tissue experimentally.

Materials and Methods

Study Design and Setting

The study was conducted at the animal experiment laboratory of Marmara University School of Medicine. Six-month-old female Wistar albino rats (n = 24) weighing 160 to 230 g were

Arzu Tatlıpınar, MD1, Pembegül Güneş, MD2, Dilek Özbeyli, DVM3, Burak Çimen, MD4, and Tanju Gökçeer, MD1

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1Haydarpaşa Numune Research and Training Hospital, 1st Ear Nose and Throat Clinic, Uskudar, Istanbul, Turkey
2Haydarpaşa Numune Research and Training Hospital, Pathology Clinic, Uskudar, Istanbul, Turkey
3Marmara University School of Medicine, Experimental Animal Laboratory, Uskudar, Istanbul, Turkey
4Haydarpaşa Numune Research and Training Hospital, Biochemistry Clinic, Uskudar, Istanbul, Turkey

Corresponding Author:
Arzu Tatlıpınar, MD, Haydarpaşa Numune Research and Training Hospital, 1st ENT Clinic, Uskudar, Istanbul, Turkey
Email: arzutatli@yahoo.com

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housed 4 per cage and fed standard pellet rat chow (1% calcium, 0.5% phosphate, and 100 IU vitamin D3) and had free access to tap water. The 12:12 hour light cycle started at 0700 hours, and the room temperature was maintained at 21°C to 24°C. The experiments described in this report were conducted according to the approval of Marmara University School of Medicine animal experiment ethical committee.

The rats were randomly assigned to 3 groups (n = 8 rats per group) as follows: sham-operated control (SHAM), ovariectomized (OVX) without treatment, or OVX with estrogen replacement (OVX-ER). The rats in the third group (OVX-ER) were subcutaneously injected with a depot preparation of estradiol valerate (200 µg/kg, Estradiol Depot 10 mg, Jenapharm, Germany) once a week for 8 weeks, beginning 1 week after OVX.

**Surgical Procedure**

To induce menopause in rats, ovariectomy operations were performed. Ovariectomies were done under ketamine HCl (100 mg/kg) and largactil (3 mg/kg) anesthesia. The fur over the surgical area was shaved. Bilateral ovariectomies were performed using a midline vertical 2- to 3-cm incision through the inferior abdomen. The ovaries and surrounding fat tissue were removed. The incision was closed in a layered fashion. In the SHAM group, only an abdominal incision and suturation were done; there was no removal of the ovaries.

At the end of 8 weeks, rats were anesthetized with Na-Penthotal (20 mg/kg) and decapitated. The larynges of the rats were dissected, excised, and placed in 10% formaldehyde solution for histopathological evaluation.

**Plasma Estradiol Assays**

Before decapitation of the rats, intracardiac blood samples for assessment of serum hormone levels were obtained. Blood samples were centrifuged and then frozen until the time of assay. Samples were analyzed using the enzyme-linked immunosorbent assay method.

A Calbiotech mouse/rat estradiol kit (catalog No. ES 180S-100; Spring Valley, California) was used to measure estrogen levels.

**Pathologic Evaluation**

The larynx specimens were sliced to 10-µm thickness and stained with hematoxylin and eosin. All tissue sections were examined in blinded fashion by an experienced pathologist.

Edema, vascular dilatation, inflammation in lamina propria, inflammation in squamous epithelium, and inflammation, cilia loss, and pseudostratification in the respiratory epithelium were evaluated histopathologically under light microscopy. When fewer than the normal number of cilia was observed in areas where they should normally be seen, a grade of mild (+) was assigned. If there was a severe loss of cilia, the grade was moderate (++); if a total loss was observed, the grade was severe (+++). The reference point for all of these evaluations was normal histology. For statistical scoring, the following values were assigned to these grades: no change (0), 1 (+), 2 (++), and 3 (++++).

**Statistical Analysis**

Data were processed using NCSS 2007 and PASS 2008 Statistical Software (Kaysville, Utah). In addition to standard descriptive statistical calculations (mean, median, and standard deviation), a Kruskal-Wallis test was used in the assessment of parameters according to groups. A Mann-Whitney U test was used for the evaluation of differences. The statistical significance level was established at P < .05.

**Results**

Because of surgical infection, 1 rat in the OVX group was lost in the follow-up period. Intrarater reliability of histopathological evaluation was 88.3%.

**Serum Estrogen Levels**

The mean serum estrogen levels were 12.38 ± 3.76 pg/mL, 6.21 ± 3.76 pg/mL, and 58.43 ± 15.92 pg/mL in the SHAM, OVX, and OVX-ER groups, respectively. The differences in the mean serum estrogen levels between the groups were statistically significant (P < .01; Table 1).

**Edema in Lamina Propria**

The comparison of edema in lamina propria in the SHAM and OVX groups revealed a significant difference (P = .009). Similarly, when OVX and OVX-ER were compared, there was a significant difference (P = .040). Edema in lamina propria in the OVX group was detected at a higher level than in the SHAM and OVX-ER groups (Table 2).

**Vascular Dilation**

The comparison of vascular dilation in the larynx mucosa in the OVX and SHAM groups revealed a significant difference (P = .014). Similarly, a significant difference was detected between OVX and OVX-ER (P = .029). Vascular dilation was more common in OVX than in OVX-ER (Table 2).
Inflammation

When inflammation was evaluated in the lamina propria, squamous epithelium, and respiratory epithelium, statistically significant differences were found between the OVX and SHAM groups \((P = .045, P = .029,\) and \(P = .014,\) respectively). Inflammation was a more common finding in OVX than in other groups. There was no statistically significant difference between the SHAM and OVX-ER groups \((P = .019,\) Table 2, Figure 1).

Pseudostratification in Respiratory Epithelium

When the groups were compared according to pseudostratification, a statistically significant difference was found \((P = .005)\). Pseudostratification in the OVX group was higher than in the SHAM \((P = .045, P = .029,\) and \(P = .045,\) respectively). Pseudostratification in the OVX-ER group was higher than in the SHAM \((P = .040,\) Table 2).

Cilia Loss

A statistically significant difference was found when the groups were compared according to cilia loss \((P = .001)\). Cilia loss in the OVX group was higher than in the SHAM \((P = .001)\) and OVX-ER \((P = .009)\) groups (Figure 1). On the other hand, cilia loss was higher in the OVX-ER group than in the SHAM group \((P = .038;\) Table 2).

Discussion

Loss of ovarian function after menopause decreases the trophic action on several organs and biologic functions, usually because of estrogen deficiency. As an example, the endometrium undergoes a gradual atrophy, starting with an inactive phase in which neither proliferation nor secretion is present and ending up as a thin layer, often riddled with cystic cavities.
lined by a cuboidal or flat epithelium, and stroma becomes fibrotic.\textsuperscript{15} The voice undergoes changes due to estrogen deficiency,\textsuperscript{2} as do several other extragenital targets. To investigate the mechanism to explain the impact of the sex hormones on the voice, several studies have been carried out regarding sex hormone receptors in the larynx.\textsuperscript{3,4,16,17} In an animal study, Wu et al\textsuperscript{18} were able to confirm the presence of estrogen receptors in the laryngeal muscles of the \textit{Xenopus laevis} (African clawed frog). In another study, Newman et al\textsuperscript{13} showed the presence of hormone receptors for androgens, estrogens, and progesterone in the donor larynx of 26 men and 16 women by using immunohistochemical methods.

The relationship between the voice and circulating sex steroid hormone levels also has been established in the literature; Amir et al\textsuperscript{18} identified subjective perceptions of vocal changes in postmenopausal women.

Abitbol et al\textsuperscript{6} performed an observational study and examined 100 menopausal women. They performed videolaryngostroboscopy, electrolaryngography, and an analysis of vocal smears, and they found dysphonia in 17% of menopausal women. Unilateral and bilateral muscular atrophy, thinning of the vocal fold mucosa with a reduction in amplitude during phonation, asymmetry between the right and left vocal folds, loss of the white appearance of the mucosa, microarborices, and a reduction of the motion of the cricoarytenoid joints were detected in this group. In a study from Schneider et al,\textsuperscript{19} videolaryngoscopy and videostroboscopy were performed in 24 menopausal women. A more viscous mucosa, a mild swelling of the vocal folds, and edema in the subepithelial Reinke space were the findings in patients with vocal complaints (n = 16).

Potential functional or anatomical changes in the larynx caused by menopause can affect vocal performance and the acoustic characteristics of the voice. In a study from Raj et al,\textsuperscript{11} an acoustic analysis was performed in 20 menopausal women and 35 reproductive age women. Statistically significant variations were seen in F0, the frequency range, the S/Z ratio (the ratio between the phonation time of “s” and “z”), and the maximum phonation duration in postmenopausal women. In Boulet and Oddens’s study,\textsuperscript{2} 48 elite vocal performers reported their experience with huskiness, loss of top notes, changes in vocal timbre, and reduced flexibility and stability of their voice around the age of 50 years.

The preservation of an optimal vocal quality is important for many women, especially for those who use their voice professionally. Hence, some studies searched the influence of HRT on laryngeal function. Abitbol et al\textsuperscript{6} noted that in 73% of 42 menopausal women, vocal quality improved after HRT. Caruso et al\textsuperscript{12} investigated the effects of ERT on the laryngeal cytology of 38 women with surgically induced menopause. In their study, hematoxylin and eosin staining confirmed similar superficial-intermediate aspects of the cells between the laryngeal and vaginal smears in ERT-treated women. However, in the control group without treatment, both smears showed aspects of atrophy-dystrophy. Based on these results, they concluded that ERT may provide prevention and treatment of dystrophy of the vocal cords in postmenopausal women.

Although there is controversy in the literature regarding the voice characteristics of postmenopausal women,\textsuperscript{17,29} most of the literature reports agree on the effect of estrogen deficiency on vocal quality and morphology of the larynx and the positive effect of HRT on laryngeal function and cytology. However,
there is still a lack of information regarding histopathological changes due to menopause. The effect of HRT on laryngeal tissue is another topic that, to our knowledge, has not been studied histopathologically in the literature. Our study focused on the histopathological effect of menopause and HRT on laryngeal tissue. The impact of vocal aging is another component that should be taken into account when discussing menopausal voice changes. Since menopause was induced surgically, it was also possible to eliminate the effects of aging in our experimental study. In our study, we found that ovariectomy leads to inflammation, vascular dilation, edema, pseudostratification, and cilia loss in laryngeal tissue and that ERT results in a significant improvement in inflammation, vascular dilation, edema, and pseudostratification. Although ERT had some effect on cilia loss, it was not significant. In our opinion, estrogen deficiency may cause atrophy and dryness of laryngeal mucosa similar to genital organs. During laryngeal motions (phonation and swallowing), trauma may occur due to friction in the larynx, which may trigger inflammation.

**Conclusion**

The voice shows different characteristics in reproductive-age women and in postmenopausal women. Based on this, many studies in the literature have researched the relationship between sex hormone changes and laryngeal changes. Possible histopathological changes of the larynx due to menopause and the effect of HRT on laryngeal tissue are important subjects to fully understand the effect of estrogen on laryngeal function. In our study, we observed inflammation, edema, vascular dilation, pseudostratification, and cilia loss in laryngeal tissue due to ovariectomy. Except for cilia loss, ERT significantly improved all of these changes. Based on these facts, our study results may support the use of HRT in postmenopausal women, particularly for those who use their voice professionally.

**Author Contributions**

Arzu Tatlıpınar, corresponding author, conception and design, writer, data collection and analysis; Pembeğül Güneş, design, revision; Dilek Özbeyli, design, data collection; Burak Çimen, data collection and analysis; Tanju Gökceer, design, revision.

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