The Expression of Estrogen and Progesterone Receptors in the Human Larynx

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Summary: Objectives. The human female voice changes in quality during the menstrual cycle and during pregnancy and menopause. The underlying pathophysiological mechanisms are as yet not known. The aim of this study, therefore, was to evaluate the existence of estrogen receptors (ERs) and progesterone receptors (PRs) in the human vocal fold.

Material and Methods. Biopsies of benign vocal fold lesions from 37 female patients were obtained during surgery. Immunohistochemistry for expression of ERs and PRs was performed and evaluated on a semiquantitative scale by two independent pathologists.

Results. In series 1, immunohistochemical staining showed six sections positive for ER and three sections for PR. One section had positive staining for both receptors. In series 2, immunohistochemical staining showed 10 of the 15 edema biopsies were positive for ER and six for PR. Six biopsies expressed both receptors. Four of the 10 laryngocele biopsies were positive for ER and two for PR. One was positive for both receptors.

Conclusion. Our study demonstrates that ERs and PRs are expressed in the larynx of the female human vocal fold in conjunction with edema. The function of these receptors has to be elucidated in future studies.

Key Words: Estrogen–Progesterone–Receptor–Vocal folds–Laryngeal edema.

INTRODUCTION

Vocal disorders occur in endocrine diseases with dysregulation of the hypopituitary gland, thyroid gland, adrenal glands, testicles, or ovaries. During a severe hypothyroid state, the voice might be hoarse because of a marked edema of the vocal mucosa or a congested vocal muscle. This hoarseness of the voice can also occur during menstruation, pregnancy, and menopause.[1] The vocal harmonics are hormonally dependent. This is illustrated by changes in voice occurring during male and female puberty. The larynx develops in response to the production of sex steroid hormones with significant differences between male and female.[2] As such, it is an androgen-dependent organ.[3,4] Illustrating is that the absence of testosterone before puberty in a castrato will evolve in a man with feminine voice.[5]

The premenstrual and menopausal vocal syndrome is characterized by lowered vocal intensity, vocal fatigue, a decreased range with loss of the high tones, and a loss of vocal quality.[5–9] Hormonal changes associated with the menopause exert effect on the female vocal folds as well as on other target organs. Decrease in serum levels of estrogen and progesterone and increase of androgen dominance during the menopausal transition has been shown to have effect on voice quality.[6] Because voice quality is influenced by hormonal changes, we hypothesize that estrogen receptor (ER) and progesterone receptor (PR) are expressed in the human female larynx.

The ER is a nuclear protein exerting important functions in hormone-dependent tissues.[4] Estrogens have a hypertrophic and proliferative effect on mucosa. One of the functions is the reduction of desquamation of the superficial layers of the squamous epithelium and differentiation and complete maturation of the fat cells. Earlier findings showed ER in the mesenchyme of the vocal folds and lateral to the laryngeal aditus of mice.[10] In baboons, the vocalis muscle and other mesenchymal tissues contain the largest number of receptors.[11] Presence of ER in the human vocal fold has not been shown convincingly.[4,12–15]

Several studies have been performed in cadaver specimen, but ER/PR expression has not been conclusively shown in biopsy material. Schneider et al[13] used specimens of human vocal folds, which were obtained 4–8 hours after death. They could not find any nucleus staining of ER and PR. Newman et al[12] observed the ER and PR expression in the vocal folds by using immunohistochemical staining within 18 hours after the death of 42 donors. Specimens showed positive staining in epithelial cytoplasm and nucleus, glandular cytoplasm and nucleus, and fibroblasts within the lamina propria. Ferguson et al[2] took four laryngeal tissue specimens from autopsy cases within 4–16 hours of death and maintained at −70°C until immunohistochemical analysis could be performed.[2]

One of the reasons that they found no nuclear staining without cytoplasmic staining might be that nuclear stability was lost because of postmortem autolysis and therefore receptor degradation or migration from the nucleus to the cytoplasm can occur. Fodor et al[16] clarified the effect of postmortem delay of sex steroid receptors. They used immunohistochemistry to study the stability of ER, PR, and androgen receptor (AR) in the rat hypothalamus and adjacent structures obtained within 0–24 hours after death after fixating for 20 days. Both AR and ER but not PR immunoreactivity were decreased after

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immersion fixation compared with the perfused sections at time point zero. In brains fixed by immersion, all three receptors decreased gradually with increasing postmortem time, and ER became hardly detectable after 24 hours postmortem. Newman et al[12] found that time interval for receptor degradation was up to 18 hours after death. Ferguson et al[2] found that an interval of 16 hours before degradation of the receptors in four laryngeal tissue specimens.

In this study, we investigated the presence of ER and PR expression in benign laryngeal tissue obtained from female patients operated for benign vocal fold lesions. The specimens were fixated immediately after the removal.

MATERIAL AND METHODS

Patient material

Histological material was obtained from two groups of patients. Group 1 consisted of archived data (period 2006) from 25 female patients treated for benign vocal fold lesions. This group provided 34 vocal fold samples. The samples contained polyps, cysts, laryngeal (Reinke space) edema, granulomas, nodules, and laryngoceles. Age range was 18–74 years. Group 2 consisted of archived data (period 2003–2006) from 12 female patients, providing vocal fold tissue reflecting edema and laryngocele. This group provided 25 vocal fold samples. Age range was 35–78 years.

Immunohistochemistry

Specimens were, after operation, immediately fixated in formaldehyde and embedded in paraffin after processing. For this study, 3-μ thick sections were cut and placed overnight in a 37°C stove followed by deparaffinization in xylene and rehydration in graded ethanol (100% and 70%). In between the ethanol steps, the sections were placed for 15 minutes in 5% H_2O_2 in methanol for endogenous blockage. The sections were then separated for antigen retrieval. The ER sections and controls were placed in boiling Tris-ethylenediaminetetraacetic acid solution (Klinipath, Duiven, The Netherlands) and pretreated for 10 minutes at 750 W in a microwave. The PR sections and controls were placed in citrate buffer (Dakocytomation; DAKO, Heverlee, Belgium) in the same procedure. Benign breast tissue served as a positive control.

After cooling down for 30 minutes, sections were washed with Tris buffer 0.005 mol/L and 120 μL antibodies (ER 1:100, PR 1:50) were applied for 45 minutes. After washing with Tris buffer, 120 μL Powervision (Poly-HRP-GAM/R/R IgG; Immunologic, Duiven, The Netherlands) was applied for 30 minutes. After washing with Tris buffer, 200 μL diaminobenzidine (DAB) (liquid DAB + substrate chromogen system [Dakocytomation] was applied for 20 minutes. After washing with Tris buffer, staining with hematoxylin was performed followed by a bath in cold tap water. Dehydration in graded ethanol (70–100%) and xylene. All procedures were performed at room temperature.

Histological evaluation

Slides were evaluated independently by two senior pathologists. The pathologists were blinded for patient characteristics and the slides were scored in randomized order. Histological sections were scored for ER and/or PR positivity in different tissue compartments. Staining of surface epithelium, fibrous stroma, and gland epithelium were scored on a semiquantitative scale; 0 was scored as no staining and 3 as intense staining in the whole tissue compartment. Staining with positivity of more than 2 was judged as positive staining and thus as ER/PR positivity in the patient. Before interpreting the study outcome, the interrater variability was determined. To obtain the interrater variability, the measurements of each rater were compared using descriptive statistics and the percentage of the total amount. The reliability between raters was expressed as percent agreement. A difference in scoring by the pathologists of more than 1 was judged as not consistent. These sections were reevaluated by both pathologists together in a separate session, and staining intensity was scored together. This way of semiquantitative hormone receptor scoring is a widely used method.[17,18]

Statistics

Descriptive statistics were used. The sample sizes were too small to perform statistical analysis as Kappa tests. Numerical descriptions are given in Tables 1 and 2. In Table 2, the sample size, the subgroups, and demography distribution are showed.

RESULTS

Benign breast tissue served as a positive control. All laryngeal slides showed less intense staining than these positive controls. The ER and PR staining was mainly in fibroblasts aligning small vessels and more prominent in specimen from laryngeal edema (Figure 1). In Table 1, the disagreement percentage between the two raters is given.

TABLE 1.
Disagreement Percentage Between the Raters

	Rater 1 (n)	Rater 2 (n)	Disagreement, n and % of Total n	Result After Conference
Series 1, n = 34				
ER positivity	7	7	5 (14.7)	6
PR positivity	1	10	9 (26.5)	4
Series 2, n = 25				
ER positivity	19	16	10 (40)	14
PR positivity	6	11	11 (44)	8