Sex Hormone Receptor Expression in the Human Vocal Fold Subunits

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Summary: Objective. The study aimed to evaluate the existence of sex hormone receptors in the subunits of vocal fold.

Study Design. This is a cadaver study.

Methods. The androgen, estrogen, and progesterone receptors were examined in the epithelium (EP), superficial layer of the lamina propria (SLP), vocal ligament (VL), and macula flava (MF) of the vocal folds from 42 human cadavers (21 male, 21 female) by immunohistochemical methods. Their staining ratios were scored and statistically compared.

Results. The androgen receptor score was significantly higher for the MF than for the EP and SLP ($P < 0.001$ and $P = 0.001$, respectively). The androgen receptor score was significantly higher for the VL than for the EP and SLP ($P < 0.001$ and $P = 0.001$, respectively). No significant difference was noted in the androgen receptor scores between the MF and VL and between the EP and SLP. The estrogen receptor score showed no significant difference between the MF and VL, whereas all other areas showed statistically significant differences ($P < 0.001$). No significant differences were observed between the EP and SLP in terms of progesterone receptor scores, but statistically significant differences were detected among the other areas.

Conclusion. Sex hormone receptors exist within several subunits of the vocal fold, mostly in the MF and VLs.

Key Words: Vocal fold subunits–Macula flava–Sex hormone receptor–Vocal ligament–Vocal fold epithelium.

INTRODUCTION

From its surface to its depths, the membranous vocal fold is composed of the epithelium (EP), superficial layer of the lamina propria (SLP), vocal ligament (VL), and vocalis muscle[1,2] (Figure 1). The distribution of the extracellular matrix of the lamina propria varies, as it is necessary for vocal fold vibration.[1] The maculae flavae (MF) are located at the anterior and posterior edges of the membranous parts of the bilateral vocal folds. The VL stretches between the anterior and posterior maculae flavae.[3–5] Hirano[1] defined the maculae flavae as dense elastic masses of fiber located at the anterior and posterior edges of the vocal fold: the anterior (nodulus elasticus) and posterior maculae flavae, respectively. These cellular structures seem to have an important role in the development of the fibrous structures of the vocal fold, and they contain stellate cells.[6] The maculae flavae play a necessary role in the extracellular matrix to provide the viscoelastic characteristics of an adult's vocal fold lamina propria. Additionally, they affect the aging of the vocal fold mucosa.[5] However, their roles have not been precisely explained.

Hormonal changes affect the human voice, as confirmed by several studies. The differences among the levels of androgen, estrogen, and progesterone throughout the lifetime mean that the structure and the functional characteristics of the larynx also constantly vary.[7–10] The hormonal changes during menstrual cycle and menopause are considered to be the reasons for the change in women's voice quality.[7,11,12] In addition, several studies have confirmed that people receiving hormone treatments may also encounter certain changes in their voices.[13,14] Understanding the local effects of these hormones on the larynx requires knowledge of the distribution of their receptors, or other theories that explain the mechanism of how sex hormones affect the larynx must be considered. However, only a few studies have been conducted on this subject, with varying results.

In this study, our aim was to identify specific clues regarding the structure and functions of maculae flavae by revealing the distribution of the receptors located in the different areas of the layered structure of the human vocal fold.

METHODS

The study was conducted on 42 fresh human cadavers. Laryngeal specimens were obtained during routine autopsies, within 24 hours from the time of death. Cadavers with a history of intubation involving a laryngeal surgery, with a larynx pathology or malformation, with a history of burns or asphyxia, or with a history of a trauma or operation in the neck region were excluded from the study. The Ministry of Justice, Institution of Forensics Ethics Committee, approved the study.

In total, 84 vocal folds were obtained from 42 cadavers with an age average of 44.6 ± 19.3 years and an age interval of 1 month to 80 years. The larynx was removed and then the posterior cricoid lamina was vertically incised through the middle line to reveal the endolaryngeal structures. An experienced clinician macroscopically evaluated these specimens. A middle ear elevator was used to elevate the thyroid cartilage's internal perichondrium, and the vocal folds and ventricles were extracted. Specimens were fixed in 10% buffered neutral formaldehyde solution for 24 hours. Following the fixation, the vocal folds were incised as a full layer in the middle line coronal plane and sliced through the membranous cord in the anterior and posterior positions at 3–4 mm intervals. These tissues were placed in follow-up cassettes and embedded in paraffin blocks following a routine 16-hour tissue

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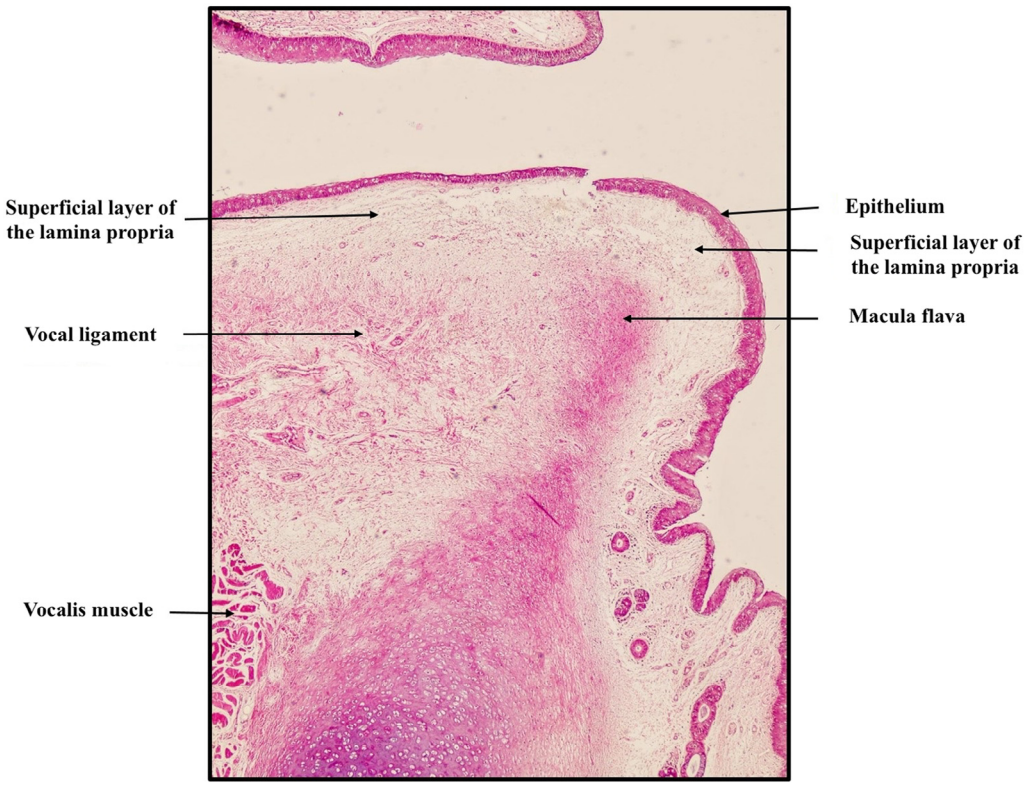

FIGURE 1. Subunits of the vocal fold: nonkeratinized squamous vocal fold epithelia; superficial layer of the lamina propria; macula flava showing intense eosinophilic staining as a separate area; vocal ligament; and vocal muscle fibers (×40, hematoxylin-eosin).

follow-up process. After the follow-up, the tissues were sectioned with a microtome into 3 mm sections, which were then stained with hematoxylin-eosin for microscopy analysis. All specimens were evaluated by the same pathologist (Author 4). During the microscopy analysis, three cross sections were prepared from each specimen, which comprised the vocal fold EP, SLP, MF, and VL, for immunohistochemical study.

The immunohistochemical study was conducted using the standard CC1 antigen retrieval protocol with a BenchMark ULTRA staining device (Ventana Medical Systems, Oro Valley, AZ) and the ultraView Universal DAB Detection Kit (Ventana Medical Systems). The primary antibodies were for the androgen receptor (anti-AR, clone SP107; undiluted ready-to-use, Ventana Medical Systems), estrogen receptor (anti-ER, clone SP107; undiluted ready-to-use, Ventana Medical Systems), and progesterone receptor (anti-PR, clone 1E2, undiluted ready-to-use, Ventana Medical Systems). The staining activities were conducted by the same pathologist and evaluated with a trinocular microscope (Olympus BX51TF; Olympus Corp., Tokyo, Japan). Regardless of its severity, nuclear staining was considered positive. The staining prevalence was scored by evaluation of the positive cell percentage in the most intensely stained areas in the related anatomic region. Nuclear staining in cells was considered as positive staining, whereas cytoplasmic staining was considered as false-positive staining. Receptor staining below 1% was considered negative.[15] The scoring was made on the basis of the College of American Pathologists reporting guidelines for biomarker tests on breast cancer and was scaled as score 0: <1%, score 1: 1%–10%, score 2: 11%–50%, and score 3: 51%–100%.[16]

The distributions of the androgen, estrogen, and progesterone receptors in the vocal fold were evaluated in substructural areas that included the vocal fold EP, SLP, VL, and MF. For each cadaver, the staining ratios were evaluated for the hormone receptors in each area.

Statistical analysis

The statistical analyses were run using the *SPSS* 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA) program. The following were the definitive statistics: the numbers and percentages for categorical variables, the average for numerical variables, and the standard deviation. The numerical variables were ordinal, so comparisons between two independent groups were conducted using the Mann-Whitney *U* test. The variables were ordinal, so the Friedman test was used for comparison of more than two dependent groups. Subgroup analyses were conducted using the Wilcoxon test and interpreted with the Bonferroni correction. The relationships between numerical variables and ordinal variables were examined with the Spearman correlation. The statistical alpha significance level was considered as $P < 0.05$.

RESULTS

The score ratios for the sex hormone receptors in the subunits of the vocal fold are shown in Table 1. Statistically significant