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Effect of Pregnancy on Vocal Cord Histology: An Animal Experiment

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Background: Voice may be affected during the period of pregnancy, especially in the third trimester. However, the exact mechanisms leading to the phonatory changes have not yet uncovered.
Aims: The aim of this study is to investigate the possible histological changes in the vocal cords of the pregnant rats in three separate trimesters.
Study Design: Animal experiment.
Methods: Twenty-five Wistar-Albino female rats were divided into four groups: control group, pregnancy day 7 (Group 1), pregnancy day 14 (Group 2) and pregnancy day 20 (Group 3). The laryngeal specimens were obtained under general anesthesia. Histological assessment was performed using Hematoxylin-eosin and toluidine blue. A stereological analysis of vocal cord tissue was performed using a NIS-Elements D32 Imaging Software.
Results: Lamina propria was observed to be edematous, and the lamina propria area was thickened starting from the second trimester. Glycosaminoglycans were observed to increase in the second trimester. Although none was encountered in the control, mast cells were observed in the lamina propria layer of the vocal cord starting in the muscular layer in the first trimester proceed to the subepithelial region as degranulated just before term. The covering epithelium remained unchanged throughout pregnancy.
Conclusion: Lamina propria thickening may be attributed to both edema and increased glycosaminoglycans. The presence of mast cells in the cordal tissue may induce edema during pregnancy in rats.
Keywords: Pregnancy, voice, mast cell, glycosaminoglycans

Voice is characterized by its frequency, intensity and harmonics. It is a unique, complex phenomenon with a great impact on communication, social interaction, personality and artistic impression. There are many factors that may affect voice such as sex, age, vocal misuse, smoking, and reflux.

Pregnancy is a period of hormonal changes. Progesterone and estrogen are the leading hormones whose maternal serum levels keep increasing throughout pregnancy. There are many physiological changes occurring throughout this period. As well as weight gain, increased fluid load, nausea, vomiting, generalized edema and reflux are some of the changes that may cause different clinical outcomes. Regarding otolaryngological manifestations, the most common and well-known is the pregnancy-induced rhinitis (1).

Phonatory changes secondary to hormonal alterations as in puberty, menstrual periods, pregnancy and menopause are known (2). During the first and second trimesters of gestation, voice is usually unaffected because of the perfect lubrication of the vocal cords. However, in the third trimester, it is markedly affected, which is attributed to breathiness, laryngeal reflux and increased generalized edema (3-7). Another study showed that although there was no significant change in symptoms related to voice, vocal fatigue was more prevalent in pregnant women (8). Nevertheless, there are many other factors that may influence voice changes during pregnancy. The exact etiology of voice changes in pregnancy, in fact, has not been assessed in detail to date.

The purpose of this study is the evaluation of possible histological changes in the vocal cords at different stages of pregnancy. Because such a study would not be applicable to human subjects due to ethical issues, this study used rats.

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MATERIALS AND METHODS

Following approval of the local ethical committee, this study was conducted on 25 Wistar-Albino female rats in the proestrus period obtained from the institutional experimental animal laboratory. The rats were kept at 22±2°C, in the dark for 12 hours and in the light for another 12 hours. They were fed ad libitum. They were kept in cages with male rats at the ratio of 4/1 for overnight. On the following morning, the male rats were removed from cages and vaginal smears of the female rats were obtained. In the event that sperm was observed on light microscopy of the smear, the rat was accepted as being at day 0 of the gestational period. On the 7th (Group 1, n=7), 14th (Group 2, n=7), and 20th days (Group 3, n=7), the rats were sacrificed under general anesthesia, and after observation of the fetuses, the larynx was dissected. Verification of pregnancy was performed via examination and direct examination of the fetuses during the sacrifice procedure. The Control Group was formed of the non-pregnant rats (n=4).

The laryngeal specimens were fixed in 10% neutral formalin solution. Paraffin blocks were dissected at a 5 μm thickness, beginning from anterior to posterior in the coronal axis. The sections were dyed with Hematoxylin-Eosin for general examination, with Toluidine Blue for mast cell and extracellular matrix identification. The analysis was performed with a Nikon Eclipse 80i photomicroscope (Nikon; Tokyo, Japan). Anterior commissure was the starting point, and ten serial sections were obtained from each vocal cord which were used for stereological analysis with NIS-Elements D32 Imaging Software (Nikon; Tokyo, Japan). The area of the lamina propria (LP) of the cords was calculated. In order to obtain objective results, histological examination was performed blinded by one of the authors who was unaware of the groups.

Statistical analysis

Statistical Package for the Social Sciences for Windows version 17.0 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. One-way ANOVA test was performed and Post Hoc Tukey test was used to evaluate the post-test differences among the groups. $P<0.05$ was accepted to be significant.

RESULTS

The results (mean±SE) of LP area were as follows; Control Group=75760.06±6505.29 μm^2, Group 1=93322.36±6753.05 μm^2, Group 2=104062.86±5210.36 μm^2, Group 3=104842.77±5971.26 μm^2. The differences between the Control group and Group 2 and 3 were found to be significant (p=0.005 and p=0.021, respectively), while it was insignificant between the Control group and Group 1. Although an increase in the area of LP was observed in the second and third trimester, the difference between them was insignificant (p=1.00). It is likely that the differences between Group 1 and 2 and Group 1 and 3 were not significant (p=0.596, p=0.667 respectively).

Histological investigation of the vocal cords in the Control Group revealed a regular, undisturbed stratified squamous epithelium together with normal glycosaminoglycan (GAG) staining. Mast cells were lacking both in the epithelium and LP.

In Group 1 (pregnant day 7), a light edema together with scattered mast cells located among muscular fibrils were observed. GAG staining intensity was similar to in non-pregnant rats (Figure 1).

In Group 2 (Day 14), edema in the LP was increased compared with Group 1 (Day 7), as shown in Figure 2a. Mast cells were observed to be among the muscular fibrils and on the border of LP. GAG staining was seen to be overt (Figure 2b).

On the 20th day of pregnancy (Group 3), edema in the LP was persistent with an increased number of capillaries and enlargement in the subepithelium; Reinke's edema was remarkable. GAG staining was similar to Group 2, with mast cells only observed in the subepithelial region (Figure 3a, b).

Histologically, the epithelium was not observed to go through any change during pregnancy. Moreover, no inflammatory cells or fibrosis were encountered.

DISCUSSION

Pregnancy is a period in which many hormonal, physiological, anatomical and mechanical changes occur; estrogen and progesterone levels increase, the level of the diaphragm rises, chest circumference enlarges and subcostal angle widens. Fluid load increase is one of the most remarkable changes, and may increase by 6.5-8.5 L, with maternal blood volume increasing by 1600-1800 mL. Voice, being a product of many systems, can be expected to be disturbed with those changes (8).

Our study is the first to focus on the histological changes at different stages of pregnancy in rats. We observed an overt increase in LP area in the second and third trimesters. As well as edema, GAG staining intensity was also increased in these trimesters compared with the control and first trimester. Observation of Reinke's edema and increased capillaries in the last trimester together with the migration of the mast cells into LP enable us think about the resulting LP thickening as a result of both edema and increased GAG. In light of these data, we can say that the reason behind the voice changes taking place in pregnancy must not be attributed solely to bodily changes but to the changes in vocal cords themselves. LP thickening

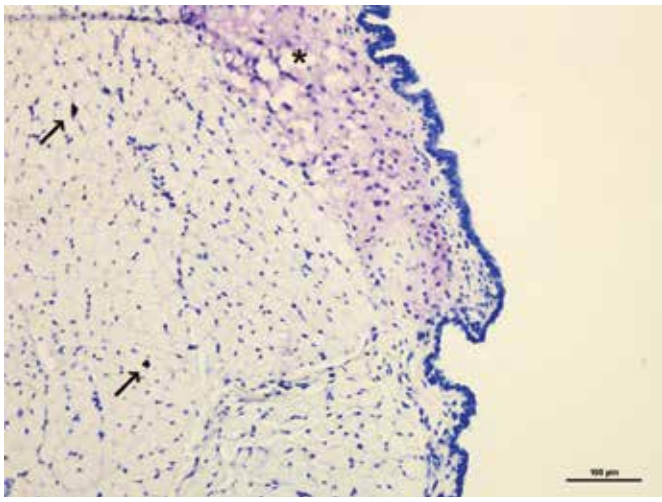

FIG. 1. Toluidine Blue staining ×10 revealed minimal edema in lamina propria (*) together with mast cells scattered in the muscle (arrows), and GAG staining similar to the Control Group.

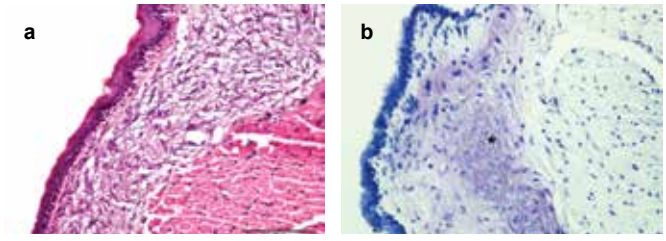

FIG. 2. a, b. Pregnancy day 14. Edema in the lamina propria was observed to increase comparing to day 7 (H&E ×20) (a), GAG staining was seen to be more intense (*) compared to the control and pregnancy day 7 (Toluidine Blue ×20) (b)

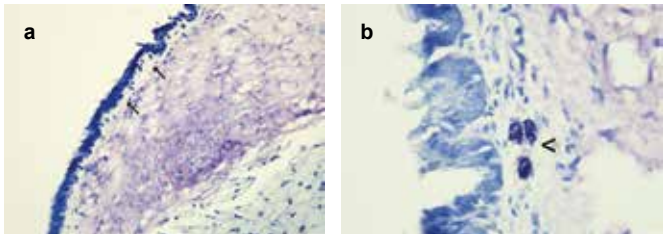

FIG. 3. a, b. Pregnancy day 14. Edema in the lamina propria was observed to increase comparing to day 7 (H&E ×20) (a), GAG staining was seen to be more intense (*) compared to the control and pregnancy day 7 (Toluidine Blue ×20) (b)

resulting in an increase in mass of the cords may reflect as vocal changes.

There are a few studies focusing on vocal changes during the period of pregnancy. Cassiraga et al. (9) reported abnormal parameters of auditory perceptual evaluation, a higher incidence of reflux, a predominance of clavicular breathing and reduced phonation time in most pregnant women in their third trimester, concluding that voice quality was affected by the physiological and bodily changes produced during pregnancy. Hamdan et al. (8) could not detect any differences in vocal symptoms between the pregnant presented for delivery and control groups; only vocal fatigue was shown to be more prevalent in the pregnant group. They showed a decreased MPT in an acoustic analysis that significantly increased after delivery. Baptista et al. (10) noted reduced vocal fold motility and increased glottal adduction in pregnant professional singers at the third trimester. They attributed these changes to elevated concentrations of estrogen and progesterone with their expected effect on tissue viscosity and water retention. Although all of these studies mentioned above concentrated on the vocal changes at the third trimester, we demonstrated that the histological changes in the vocal cords started in the second trimester of pregnancy.

Regarding the location of mast cells in the larynx, the number of studies is limited. Mast cells are reported to locate in the epiglottis and subglottic area. Although one study reported occasional mast cells in the cordal tissue, the other reported none. In this study, we could not find mast cells in the control animals, but found them in pregnant rats. The presence and migration of mast cells into LP as the pregnancy progresses may be of importance with respect to the explanation of the edema taking place in the cords (11,12). However, by this study itself, we cannot say directly that mast cells cause edema as there were no degranulated cells.

The vocal cord is a hormonal target organ. The female voice evolves from childhood to menopause under the influences of sex hormones. It is sensitive to sexual hormonal variations such as adolescence, menstruation, pregnancy and menopause. There are many reports in the literature about voice changes during periods of hormonal changes (13-15).

Newman et al. (3) demonstrated the hormone receptors androgen, estrogen and progesterone in human vocal fold tissue obtained from fresh cadavers. The receptors were found in the cytoplasm and the nucleus of the vocal fold tissue with statistically significant differences according to age and gender distributions. Brunings et al. (16) found estrogen and progesterone receptors in the biopsy specimens of the benign vocal fold lesions to be expressed in conjunction with edema. On the other hand, Nacci et al. (17) showed that sex hormone receptors were absent in the vocal cords and concluded that the different expression of some growth factors in the laryngeal tissue influenced by hormonal variations might result in vocal changes.

One of our interesting findings is the increased GAG content in vocal cords at the second and third trimesters. GAGs are interstitial proteins responsible for the viscosity of vocal folds. Hyaluronic acid (HA), one of the main GAGs in LP, is fundamentally important for the vocal fold mucosal wave laxity and for optimal vocal quality (18). Numerous types of disorders can change the amount of HA in the vocal folds and compromise phonation quality, with aging being the most prevalent.

However, the exact mechanism behind this increase in GAG content in pregnancy needs further investigation. It may be attributed to the altered sex hormone status of the subjects. Pedroso et al. (19) studied the hyaluronic acid concentration of the vocal cords and reported an increase in the puerperal period. Glucocorticoids were reported to regulate extracellular matrix metabolism in human vocal fold fibroblasts (20). In the light of this knowledge, it may be hypothesized that the change in sex hormone levels may have a direct effect on the pregnant voice together with the metabolic, physiological and anatomical changes during that period.

One more point is worth mentioning. Although there are similarities between human and rat vocal cords, our results may give an opinion but it cannot be applied directly to pregnant women. In humans, there are two known factors that may affect the vocal cord histology during pregnancy. One is reflux and the other is pregnancy-induced rhinitis. Reflux has a very high incidence in pregnancy and has the potential to affect vocal cord histology. However, to our knowledge there has been no report in the literature about laryngeal reflux in rats. Additionally, rats are accepted to be non-emetic. This inability of rats to vomit is a result of particular anatomophysiological characteristics (21,22). Pregnancy-induced rhinitis, which is defined as hyperemic and edematous mucosa with hypersecretion of mucous in the nasal cavity, and nasopharynx, should be kept in mind when regarding the voice changes in pregnancy. It was reported to be as high as 22% of pregnant women, putting some strain on the larynx with a potential to cause those findings (23). The associated symptom, mouth breathing, may additionally cause drying of the laryngeal mucosa leading to histological changes in the cords. To our knowledge, this also has not been documented in rats yet. These facts lead us to interpret our vocal cord histological findings to result from the pregnancy itself.

Our restriction in this study is the lack of immunohistochemical analysis, in which hyaluronic acid and collagen subtypes in addition to sex hormone receptors would have been identified.

In this study, we clearly demonstrated thickened LP starting in the second trimester in pregnant rats. Increased edema together with the increased GAG content might have led to that thickening. Edema is thought to result from capillary congestion and the presence of mast cells in LP. The covering epithelium remained unchanged throughout the pregnancy, and no inflammatory cells were noted in the lamina propria.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Abant İzzet Baysal University School of Medicine.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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