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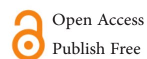

Immunohistochemical Expression of Estrogen Receptors in Pleomorphic Adenoma and Normal Salivary Gland Tissue

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Abstract
Background: The expression of estrogen receptors (ERs) in salivary gland tissue has been observed in some studies. Considering the histological similarity of salivary glands and breast tissue, as well as the prominent role of this receptor in the pathogenesis, treatment, and prognosis of breast tumors, this study aimed to compare the immunohistochemical expression of ERs in pleomorphic adenoma (PA) and normal salivary gland tissue and assess its possible role in salivary gland tumors.
Methods: In this descriptive-analytical study, 26 samples of PA (16 females, and 10 males) and 12 samples of normal salivary gland tissue were selected. Immunohistochemical staining was performed by the standard EnVision method for ERs. The results were evaluated semi-quantitively as the percentage of the nuclear and cytoplasmic staining were analyzed independently by Mann-Whitney, Kruskal-Wallis, and Fisher's exact tests.
Results: All PA samples showed negative nuclear staining for ERs. ER expression was observed in the cytoplasm of the ducts in 27% of tumors and 59% of normal salivary gland tissue samples, but the difference was not statistically significant. In addition, no association was found between ER expression and independent variables, such as age, sex, the type of stroma, or the degree of cellularity in PA, and the location of the specimen.
Conclusion: It seems that the expression of ERs does not play an effective role in the development and progression of PA. However, its occurrence in the normal salivary gland ducts is considerable, and further studies in this field seem to be necessary.
Keywords: Estrogen receptor, Pleomorphic adenoma, Salivary gland, Immunohistochemistry

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Introduction

Salivary gland tumors are relatively common tumors in the head and neck area and account for 5% of these neoplasms. Although many of them are benign, some metastasize to other parts of the body (1). Benign cases can also cause irreparable damage due to their proximity to vital tissues such as the eyes and brain. Therefore, it is possible to propose methods for diagnosis, early treatment, and prognosis of these tumors by investigating their molecular and histopathological characteristics and comparing them with normal salivary gland tissue.

Pleomorphic adenoma (PA) or benign mixed tumor (BMT) is the most common tumor in the major and minor salivary glands, occurring in the parotid gland in 85% of cases (2). This tumor usually manifests in the form of a stiff and painless mass that grows gradually. This tumor may occur at any age, but the highest incidence rate is among the 30- to 60-year-old age group. PA occurs unilaterally in most cases and, in rare cases, bilaterally. The palate is the most commonly affected site in the minor salivary glands. The tumor is mainly limited by capsules in histopathological view (3) and contains a set of myoepithelial cells and a glandular epithelium in a myxoid, chondroid, or hyalinized background (2). The best treatment is surgical removal. Correct surgery will ensure an excellent prognosis and recovery rate (>95%) (3). There is a small risk for malignant mutations, but those may occur only in 5–25% of cases (2,3).

Overall, the physiological activities of the body are controlled by the nervous and endocrine systems in a coordinated manner. The endocrine system exerts its control by secreting hormones. Hormones are chemical messengers that are

secreted in small amounts. They enter the bloodstream and are transported to distant places, where they exert their effects (4).

Estrogen is a steroid hormone that can easily cross the phospholipid membrane, with a receptor located inside the cell nucleus (5). Estrogen is involved in growth, differentiation, and response to inflammation in reproductive and non-reproductive tissues (6). Estrogen acts by binding to the receptor. There are two estrogen

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receptor (ER) subtypes, i.e. ERα and ERβ, each of which is encoded by different genes. ERα and ERβ are widely present in different cell types (7). Studies have shown that salivary gland epithelium with normal histology also produces functional ER proteins, and these proteins seem to play a mediating role in regulating the immune system of this tissue. The salivary gland epithelium is considered a target tissue for this hormone because it has been observed that when the estrogen level changes, the salivary composition and secretion also change (6).

Morphological similarity between salivary gland and breast tumors is a known phenomenon (8). Androgen, estrogen, and progesterone, which act through their specific receptors, play an important role in the growth and development of several tumors, including breast, uterine, and prostate cancers (9). ERs have been used as markers for tumor management for decades and are among the most important biomarkers in breast cancer. Endocrine therapy can be performed if the ER is positive (10).

Immunohistochemistry (IHC) has shown the nuclear distribution of estrogen and progesterone receptors in breast cancer cells (1). After using this technique and identifying estrogen and progesterone receptors, it has been shown that the presence of androgen hormone receptors can improve the prognosis of breast cancer. When both estrogen and progesterone receptors are present in breast cancer tumor cells, the response to treatment is about 80%. When only one of these receptors is present, the prognosis is reduced by about 25 to 45%, and in the absence of both receptors, the prognosis is very poor (< 10%) (11).

Various studies have investigated ER expression in kidney (12), prostate (13), colon (14), liver (15), lung (16), breast (17), and salivary gland cancers (18-20).

Considering the known similarity between salivary glands and the breast in terms of structure and function in serological pathology, the significant advances in breast cancer treatment (8), and inconsistencies in the results of previous studies, the present study aimed to investigate the IHS expression of ER in benign PA and normal salivary gland tissue and the possible use of this marker in future salivary gland tumors. Although ERs have been examined in previous studies, as the results have been contradictory and the receptor has been observed in some studies (1,21) but not in others (9,20), we decided to further investigate the expression of ERs.

Materials and Methods

This experimental-interventional study was performed in vitro on samples taken from the Pathology Department of Imam Khomeini Hospital (between the years 2005 to 2009) and Apadana Hospital in Ahvaz (during the years 2009 and 2010).

In addition to demographic characteristics such as age and sex, lesion site was also obtained from the medical records. A number of samples were excluded from the study due to incomplete medical records or inappropriate blocks. When histopathological slides were being prepared to confirm the diagnosis, a number of other samples were also excluded due to non-definitive diagnoses or insufficient samples. Finally, 26 PA samples were selected. To investigate ER expression in normal salivary tissues, we used normal tissues that were obtained from 12 mucosal samples selected by the same method.

IHC staining was performed using the standard EnVision method. In short, after being sliced, the samples were placed on Poly-L-Lysin coated slides and dried at 37 °C for 24 hours. The samples were then deparaffinized in xylene and rehydrated in various ethanol grades. Subsequently, to stop endogenous peroxidase activity, the samples were placed in 0.3% peroxide methanol (H_2O_2) for 30 minutes at room temperature and then washed with phosphate-buffered saline (PBS) solution (pH = 7.2). IHC staining (Dako, Denmark, lot number 102809XY) of ER was performed based on the manufacturer's instructions. After being incubated with the initial antibody, the samples were incubated with (anti-mouse) polymer solution for 30 minutes and washed with PBS. Afterward, diaminobenzidine hydrochloride (DAB) (3, 3′-Diaminobenzidine) was used, which gives the antigen-antibody complex a brown color. The samples were then counterstained using hematoxylin, and after dehydration, lamellas were placed on them.

Finally, the IHC staining status assessment was performed by two pathologists using a 40x light microscope (Japan, Olympus CX21) based on the staining extent of the samples and by randomly counting 1000 tumor cells. A lymphoma sample was used as a positive external control, and the lymphocytes in the studied samples were used as internal positive controls. Staining was also performed to evaluate the negative control status by removing the initial antibody and using PBS instead. Positive controls showed a strong reaction but no staining was observed in negative controls. Finally, the staining extent was rated as semi-quantitative according to similar articles, including the study by Nasser et al (20) (Table 1).

Mann-Whitney and Kruskal-Wallis tests were used to compare the results between

two or more groups, respectively. Also, Fisher's exact test was used in order to

compare the positive cytoplasmic staining by age, sex, and lesion site, and their relationship in benign PA and

Table 1. Rating based on the staining percentage of tumor cells

Rating (score)	Nuclear staining percentage of ER
0	0–5
1	6–25
2	26–50
3	51–100

ER, Estrogen receptor.

normal salivary gland tissue. The tests were performed at a 95% confidence interval (error rate = 0.05), and a *P* value < 0.05 was considered as the significance level in all tests.

Results

The present study was performed on 26 benign PAs. The number of male and female participants, was 10 and 16, respectively. Their age range and mean age were 16–75 years and 45.5 years, respectively. The number of PAs in the parotid and submandibular glands and the palate was 19, 4, and 3, respectively. Nuclear IHS staining of ER was reported to be negative in all PAs (Figure 1). Cytoplasmic staining was reported to be positive in 7 of the 26 cases (27%) (Figure 2). Normal salivary tissues with confirmed mucosal diagnosis were used to evaluate ER presence in normal salivary glands. The staining results showed no staining in mucosal acini (Figure 3), and positive ER staining was reported in only 7 cases (59%) of ductal and epithelial cells, which constitute a small part of the whole parenchyma (Figure 4). There was a significant difference between tumor and normal tissues in terms of cytoplasmic staining ($P > 0.05$) (Table 2).

ER staining in PA with myxoid, chondroid, hyalinized, and mixed backgrounds based on cellularity rate (hypercellular, cellular, and hypocellular) and lesion site (parotid, submandibular, and palate) in PA and normal salivary gland tissue (parotid, submandibular, sublingual, and lower lip) were investigated, and the results showed no statistically significant difference between them.

ER staining was also compared considering age and sex in PA and normal salivary gland tissue and no significant difference was observed.

Discussion

In the present study, all 26 PAs (100%) showed negative nuclear IHS staining for ER. Cytoplasmic IHC staining of ER was positive in 27% of cases. In normal salivary gland tissue, all cases showed negative nuclear IHC staining for ER, and ER cytoplasmic staining was positive in 7 cases (59%). The distribution pattern of ER staining included cytoplasm of ductal cells in PAs and ductal cytoplasm and

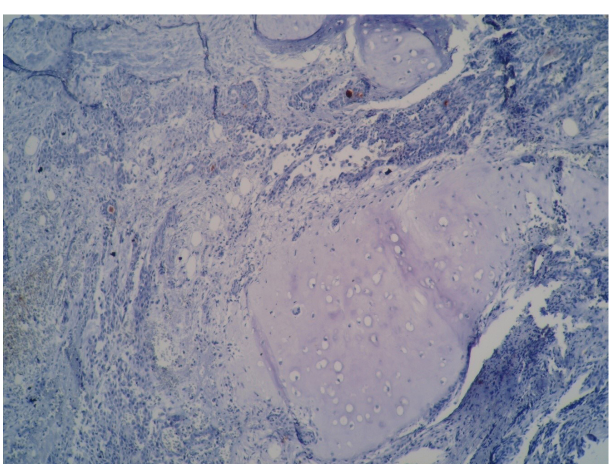

Figure 1. No immunohistochemistry staining of estrogen receptors in benign pleomorphic adenoma at 10x magnification

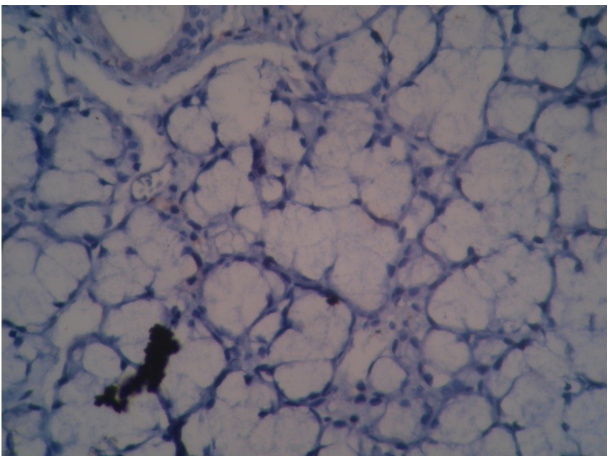

Figure 3. No immunohistochemistry staining of estrogen receptors in normal salivary gland tissue at 40x magnification

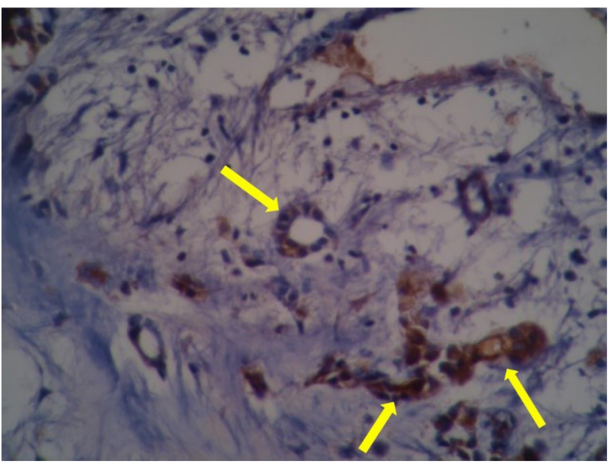

Figure 2. Cytoplasmic immunohistochemistry staining of estrogen receptors in ductal epithelial cells of benign pleomorphic adenoma (arrows) at 40x magnification

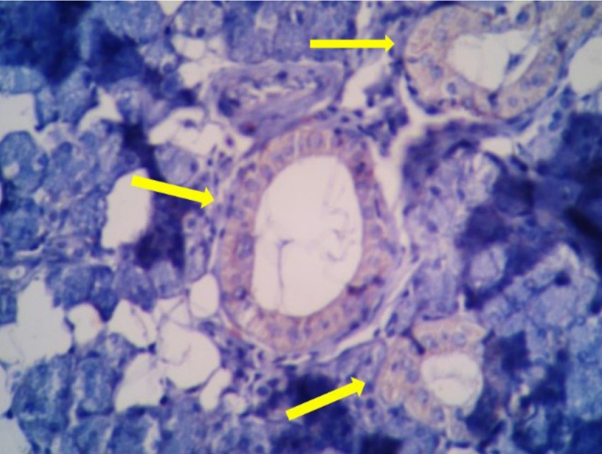

Figure 4. Cytoplasmic immunohistochemistry staining of estrogen receptors in ductal epithelial cells of normal salivary gland tissue (arrows) at 40x magnification

Table 2. Results of cytoplasmic immunohistochemistry staining of estrogen receptors in pleomorphic adenoma and normal salivary gland tissue

Type of tissue	Numbe	Positive cytoplasmic staining	Mean rank
Pleomorphic adenoma	26	7	18.5
Normal salivary gland	12	7	21.67

epithelium in normal salivary gland tissue. The findings of the present study were consistent with the results of many studies on salivary glands (1,21,22). Desouza A. et al. showed that ER expression was negative in PAs, recurrent PAs, and carcinoma ex pleomorphic adenoma (CXPA) (22). Teymoortash et al also found negative nuclear staining for ER in all PAs and normal salivary gland tissue samples, but ER expression was observed in the cytoplasm of the salivary ducts of normal parotid gland tissue and the epithelial components of PA (21).

The absence of nuclear IHS staining for ER in PA is also consistent with the results of studies by Tarakji et al. They also reported negative nuclear staining in all PAs, but 63% showed positive cytoplasmic staining for ER (1). Ito et al also reported negative ER expression in all PAs (9). However, their study did not perform nuclear and cytoplasmic IHS staining for ER in PA separately, and it was not clear whether the negative result was related to cytoplasmic or nuclear staining of ER. Nasser et al also reported negative ER expression in all benign salivary gland tumors. However, poor positive nuclear staining (score 1) was reported in 3% of the studied malignant tumors (20).

Glas et al also reported positive nuclear staining in 19% of samples with recurrent PA and 17% of the control group (23). They did not report a significant difference between primary PA and recurrent PA groups in terms of ER expression. Similar to our study, the ER expression in men and women was investigated in their study, and results showed no relationship between sex and ER expression. Considering changes in the salivary glands with age, and also due to upregulation of ER in women, the present study compared the probable effect of variables such as patient age and sex and even the lesion site on ER expression. The results showed no statistically significant relationship between ER incidence and age, lesion site, and sex in PA and normal salivary gland tissue groups.

On the other hand, some researchers have reported contradictory results. For example, Liang et al observed ERβ in 66% of salivary duct carcinomas and 28% of adenocarcinomas (24). Also, Barrera et al reported positive ER expression in 17% of cystic adenoid carcinomas (19). It is possible that the ER expression in these studies was due to the malignancy of the tumor under study.

Tsinti et al also reported ERα and ERβ staining in more than 85% of epithelial cells of all minor normal salivary ducts, but the cells showed weak nuclear and cytoplasmic staining for ERα and ERβ (6). The difference in cytoplasmic staining may be due to differences in the antibody concentration. In a study, Wong et al reported that the nuclear staining for ERα in PA was 49% in epithelial cells and 37% in normal salivary duct cells (18). However, in the present study, nuclear staining for ERα was not observed in PA, which may be due to differences in the type of antibodies.

Conclusion

It seems that ER expression does not play an effective role in the development and progression of PA. However, its occurrence in ductal cells of normal salivary glands is worth considering, and further studies on this subject seem to be necessary.

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Authors' Contribution

Conceptualization: Fatemeh Asareh, Farzaneh Mehranfar.
Data curation: Fatemeh Asareh, Farzaneh Mehranfar.
Formal analysis: Fatemeh Asareh.
Funding acquisition: Farzaneh Mehranfar.
Investigation: Farzaneh Mehranfar.
Methodology: Fatemeh Asareh.
Project administration: Fatemeh Asareh.
Resources: Farzaneh Mehranfar.
Software: Farzaneh Mehranfar.
Supervision: Fatemeh Asareh.
Validation: Farzaneh Mehranfar.
Visualization: Fatemeh Asareh.
Writing–original draft: Farzaneh Mehranfar.
Writing–review & editing: Fatemeh Asareh.

Competing Interests

None declared.

Ethical Approval

This study was approved by the Ethics Committee of Jundishapur University of Medical Sciences (1748/D/B/8P, dated 7/17/2023).

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