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Immunohistochemical Expression of Estrogen and Progesterone Receptors in Epulis Fissuratum

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Abstract

***Background*:** Epulis Fissuratum (Epulis Fissuratum (EF) or Denture Epulis or inflammatory fibrous hyperplasia) is a common hyperplastic tumor-like lesion with reactive nature, related to loose and ill-fitting, full or partial removable dentures and it is more common in women than men. For this reason, hormonal influences may also play role in its creation. The effect of steroid hormones especially sex hormones (Estrogen and progesterone) on oral mucosa is identified in some studies. In the present study, the distribution pattern and presence of estrogen and progesterone receptors in epithelial, stromal, endothelial and inflammatory cells in Epulis Fissuratum was investigated.

***Materials and Methods*:** This cross-sectional study was carried out on 30 samples of paraffin blocks with Epulis Fissuratum diagnosis and 30 samples of normal mucosal tissues as a control group who have had surgery as a margin beside the above lesions and had been obtained from the oral and maxillofacial pathology departement of Babol Dental School since 2003 up to 2010. Intensity of staining and immunoreactivity were evaluated using subjective index and considering the positive control group (breast carcinoma).

***Results*:** Epithelial, stromal, endothelial and inflammatory cells didn't show reaction with monoclonal antibodies against estrogen and progesterone in none of the samples.

***Conclusion*:** It seems that the hypothesis of the existence of estrogen and progesterone receptors in epulis fissuratum and normal oral mucosa is ruled out. The possibility of direct effect of estrogen and progesterone in occurring of epulis fissuratum is rejected.

Introduction

Epulis Fissuratum (Inflammatory Fibrous Hyperplasia (IFH) or Denture Epulis is a common hyperplastic tumor-like lesion with reactive nature, related to loose and ill-fitting, full or partial removable dentures. Usually, two fold of tissue is created and the edge of the denture fits in a groove between the two folds. The excess (extra) tissue is firm and fibrous; however, some lesions have an erythematosus and ulcerated appearance similar to pyogenic granuloma. Its size varies from smaller local hyperplasia (1 cm) to extensive lesions which involve the entire length of the vestibule [1, 2]. EF is usually created in the anterior portion of the jaws and the facial aspect of the alveolar ridge and is more commonly found in women. Like other denture-related lesions, EF often occurs in middle-aged and older adults [1, 2].

In microscopic examination, it shows hyperplasia of fibrous connective tissue and where the denture presses on the tissue, there are several folds and grooves. Overlying epithelium is often hyperkeratotic and irregular hyperplastic rete ridges are often seen. Sometimes epithelium shows inflammatory papillary hyperplasia or Pseudoepitheliomatous hyperplasia. Chronic inflammatory infiltration also exists. Treatment of Epulis Fissuratum consists of surgical excision of the lesion and microscopic examination of the tissue. To preventing of recurrence of the lesion, an appropriate denture should be made for the patient or the old denture should be relined [1, 2].

According to some researchs, the incidence of this lesion is due to hormonal changes after menopause which makes the overlying mucosa susceptible to such hyperplastic reactions [3]. Physiological and pathological response of the tissue to hormone depends on the reaction between hormone and its special receptors in the tissue because for direct response to hormone, the tissue needs to have specific receptors of that hormone [3, 4].

Steroid hormone receptors are like intracellular proteins binding to DNA which play the role of regulators of cell growth. Hormone binding will result in the receptor deformation and following hormone receptor complex will transported to the nucleus. In the nucleus, this complex will bind to special sequences of nucleotides leading to regulation in transcription of target genes

including the adhesion molecule, cytokines, growth factors, maturity, metabolism, degrading enzymes and extracellular matrix components -related genes [3, 5-7].

The conducted studies indicate that oral soft tissues are sensitive to changes in serum levels of steroids in female sex. Some cases such as desquamative gingivitis show a predilection for women and samples from these lesions appear to be ER positive, supporting a role for estrogen in etiology of this disease [8]. Similarly, during pregnancy, the severity of gingivitis is increased and there is a higher risk for development of gingival pyogenic granuloma [9]. Estrogen receptors (ER) and progesterone receptors (PR) have been investigated during several studies in some oral normal tissues such as human and rabbit gingival tissue, tooth pulp, salivary glands, reactive lesions such as pyogenic granuloma, peripheral giant cell granuloma as well as neoplastic tissues. But Garcia couldn't indicate the existence of these receptors in peripheral ossifying fibroma [10-16].

On the other hand, estrogens are known as regulator of epithelial maturation in target organs and so it is thought that high levels of estrogen during menopause has effects on the maturation process of oral epithelial and leads in appearing of thin, atrophic epithelium predisposed to inflammatory changes [13].

Hormone receptors can be identified using different methods including Ligand Bonding, autoradiography, reverse transcriptase polymerase chain reaction and immunohistochemistry. Of course, it seems that evaluation of estrogen and progesterone receptors genes and using the two methods of immunohistochemistry and PCR or In situ hybridization (ISH) together may be more accurate than other methods in the evaluation of hormone receptors. Due to the conducted studies about oral reactive lesions and the effect of estrogen and progesterone hormones on the oral mucosa and the effects of hormones on the initiation of EF and its high clinical incidence on women, the present study has been conducted to investigate the distribution of estrogen and progesterone receptors in Epulis Fissuratum.

Materials and Methods

After Institutional Ethics Committee approval, 30 formalin-fixed paraffin-embedded archival specimens of EF (Inflammatory Fibrous Hyperplasia) were obtained from the department of oral pathology of the Babol University of Medical Sciences during 2003-2010. The microscopic slides stained with hematoxylin and eosin for confirming the diagnosis then appropriate blocks were selected. The samples were not related to pregnant women or contraceptive and hormone drugs users. All of the selected samples had enough epithelium and connective tissues and many foci of chronic inflammatory infiltration and obvious blood vessels in the connective tissue (at least with 5 HPF widths). Also, 30 samples of the normal mucosa tissue which had surgery as a margin beside other lesions were considered as the control group. For immunohistochemical staining, sections of the samples were prepared using poly-l-lysine-coated slides. Since all the tissues were formalin-fixed, the slides were placed in a microwave (650 watts) and sodium citrate buffer for 10 minutes until the tissue antigens appeared; then, the slides container was put aside for 20 min. in order to be cooled. After being rinsed with distilled water, all the slides were stained by monoclonal antibodies (Dako, Cytomation, Denmark) in order to disclose the estrogen (Clone:1D5) and progesterone (Clone: PgR 636) receptors. Breast carcinoma was also stained with this method as a positive control group. Primary antibodies were used and kept in refrigerator for 16 hours; Avidin-biotin peroxidase method was used along with Tris Buffered Saline (TBS).

Amino Ethyl Carbazol (AEC) was selected as chromogen and all sections were counterstained by Mayer's hematoxylin. It should be mentioned that in order to obtain the negative control group in each case, mouse serum was used by omission of the primary antibody.

Intensity of staining and immunoreactivity was evaluated by two pathologists regarding the positive (breast carcinoma) and negative control groups which was based on nucleus staining of estrogen and progesterone receptors. The cells were studied in terms of immunoreactivity in qualitative and ranking way. Grading was as follows: 0: Absent (no staining); 1: Weak (focal or slight scattered staining); 2: Moderate (slight diffuse or prominent scattered staining); 3: Strong (prominent diffuse staining) [13]. The under study cells in each case were epithelial, connective, inflammatory and endothelial cells and at least in 5 HPF width.

Results

26 out of 30 EF samples of the study were female and 4 cases were male. The patients were at the age range of 39-80 years with average age of 58. In terms of anatomic location, 18 samples were mandibular and 12 were maxillary.

Immunohistochemical findings: Epithelial cells showed no staining for estrogen (ER) and progesterone receptors (PR) (Fig. 1, A & B).

In the study of stromal cells, all the tissue samples showed a negative reaction to receptors (Fig. 2, A & B).

Inflammatory cells didn't show reaction with monoclonal antibodies against estrogen and progesterone in any of the samples (Fig. 3, A & B).

Staining of vascular endothelial cells for estrogen and progesterone receptors had negative results in all the samples (Fig. 2, A & B).

But, positive reaction for estrogen and progesterone receptors was observed in the positive control sample (breast carcinoma) (Fig. 4, A & B).

Discussion

The present study indicated the lack of staining in 30 selected samples of Epulis Fissuratum for presence of estrogen and progesterone receptors. In the present study, estrogen and progesterone receptors were not found in the epithelial cells of all the samples.

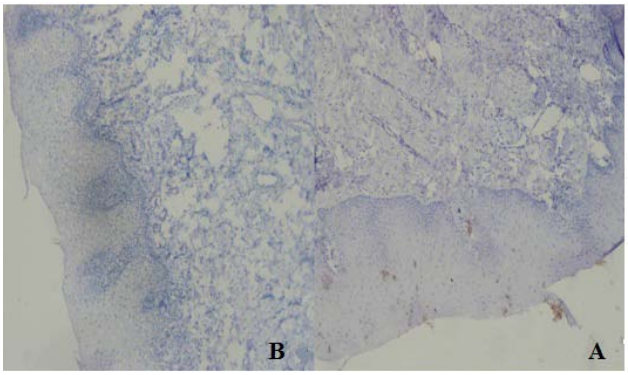

Figure 1. Epulis Fissuratum .Immunohistochemical staining for ER (A) and PR (B) in epithelial cells 100x.No cell is stained.

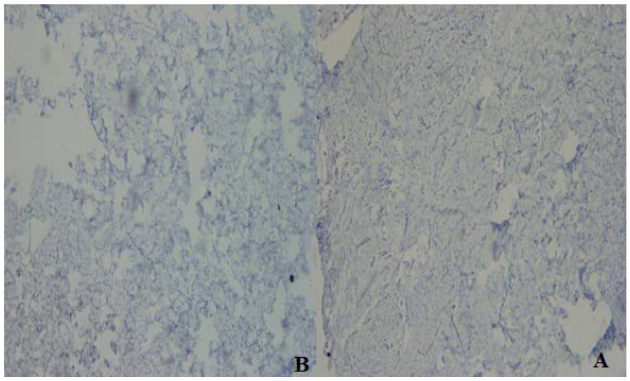

Figure 2. Epulis Fissuratum . Immunohistochemical staining for ER (A) and PR (B) in stromal and endothelial cells 100x. No cell is stained.

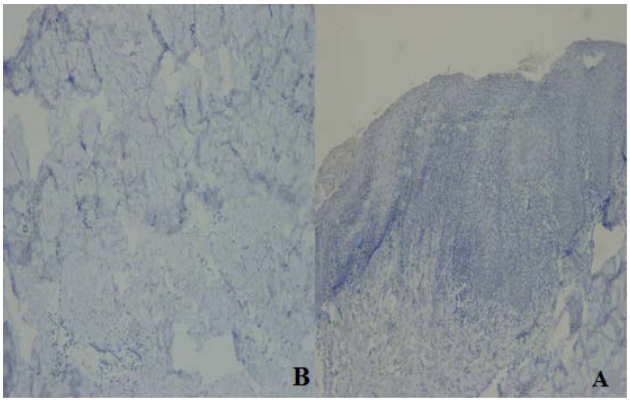

Figure 3. Epulis Fissuratum . Immunohistochemical staining for ER (A) and PR (B) in inflammatory cells 100x. No cell is stained.

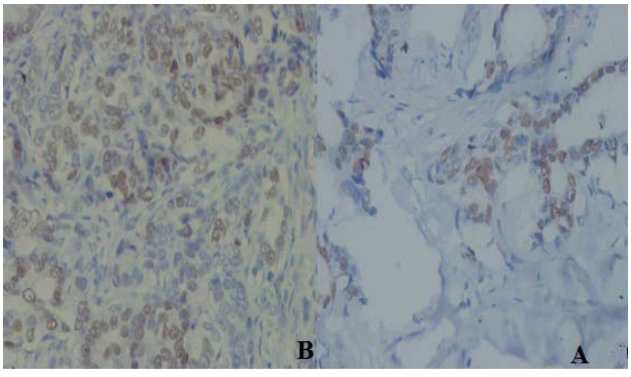

Figure 4. Breast carcinoma (positive control), Immunohistochemical staining for ER (A) and PR (B) 400x.

The result of the present study is consistent with the study of Whitaker et al. which they found no positive reaction for these receptors in epithelial cell of lesions with pulpal origin, but other studies justified the presence of estrogen and progesterone receptors in the epithelial cells of oral cavity, skin and breast which all are derivatives of ectoderm due to their embryonic origin [17].

Leimola-Virtanen et al. in evaluating of the effect of estrogen on the cytology of buccal mucosa during menstruation cycle and menopause phase and the presence of ER in the buccal mucosa concluded that estrogen is not the only effective factor on maturity changes in epithelial cells of buccal mucosa. Used antibodies in immunohistochemistry methods couldn't also show the presence of ER in the buccal mucosa [18]. Likewise, in the study of Ojanotko et al. estrogen and progesterone receptors were not observed in the oral mucosal tissues being consistent with the results of the present study [5].

In the current study, mesenchymal (stromal) cells didn't show staining for estrogen and progesterone markers in any sample. While Ojanotko et al. reported that estrogen can induce proliferation of gingival fibroblasts leading to the maturity of its connective tissue mainly through the effect on collagen turnover which is inconsistent with the present study.

The reaction of estrogen and progesterone receptors in inflammatory and vascular endothelial cells indicated the lack of staining in the present study, but other studies indicated that metabolism of androgen and estrogen increase in the inflamed gingiva compared to the healthy gingiva. Progesterone also increases the permeability of gingival blood vessels [19].

Shahrabi et al. evaluated estrogen and progesterone receptors in 15 cases of EF. ER was found in 93.3% and PR in 80% in of stromal cells. Estrogen in epithelial cells was seen in most of the cases while progesterone expression was lower than it. Positive immunoreactivity for ER in 20% and PR in 40% of the cases was found in inflammatory cells. Also, immmunoreactivity of estrogen and progesterone receptors in endothelial cells was found in 4 or 5 cases and it was concluded that although chronic irritation may be considered as an initiator factor in developing of EF, but some of the constitutive cells of the lesion could be a potential target for estrogen and progesterone hormones but in the present study, none of the epithelial, stromal, inflammatory and endothelial cells indicated a immunoreactivity for the antibodies for estrogen and progesterone receptors.

Razavi et al. did not fonud any immunoreactivity for estrogen and progesterone receptors in 25 cases of peripheral and central giant cell granuloma (PGCG) of the jaws. They rejected the possible direct effect of the hormone in developing of these lesions. They also suggested that conducting more research in this regard using more sensitive methods seems to be necessary[20]. There are some evidences indicating that inflammatory cells can metabolize progesterone and other studies also

indicated that androgen and estrogen metabolism will be increased in the inflamed gingiva more than healthy gingiva [21]. On the other hand, steroid hormone interaction with its receptors and the formation of hormone-receptor complex and its transfer to the nucleus can result in DNA and RNA synthesis and finally leading to hypertrophy and hyperplasia [9].

Previous studies indicated that in patients with Phenytoin-induced gingival hyperplasia, compared to healthy tissues, there is more interaction to hormone and/or the intramuscular injection of radioactive progesterone will result in the accumulation of this substance in the stromal cells of gingival connective tissue, but in the present study, the lesion which is a reactive lesion to chronic irritation resulting from an ill-fitting denture and in fact it is a inflammatory fibrous hyperplasia, the lesion didn't show the incidence of estrogen and progesterone receptors. Generally, it seems that regarding the more incidence of EF in females, the effect of estrogen and progesterone in developing of the lesion may be indirect [21, 22]. Perhaps, one of the reasons for the difference in difference researches about hormone receptors is due to the application of different methods such as ligand bonding, autoradiography, reverse transcriptase polymerase chain reaction and immunohisto-chemistry [3, 5, 6, 23].

In the present study, estrogen and progesterone receptors may exist in these lesions but their concentration may be so low and less than the threshold to be discovered by the immunohistochemistry staining method; with performing more accurate methods and gene evaluation of estrogen and progesterone receptors and simultaneous application of the two methods-immunohistochemistry and polymerase chain reaction or in situ hybridization- immunoreactivity may be detected in relation to these markers. Of course, the sensitivity of the antibody reagents being used in immunohisto-chemistry staining and the primary time of tissue fixation in evaluation of the presence of the above markers are among the effective factors on the results of the research [22, 24].

Some of the researchers propose that age and gender of the patient, specific histology of lesion, the way of doing immunohistochemistry technique, lack of appropriate criteria and index for the evaluation of stained cells and the insufficient sensitivity of the antibodies used in immunohistochemistry method are the reasons of disagreement in the results of immunohistochemistry method. Tissue fixation time is also important because longtime fixation can affect the immunoreactivity of the receptors [24, 25].

Other studies also indicated that estrogen and progesterone receptors are sensitive to heat and proteolytic enzymes; therefore, lack of staining in some cases, may be due to the receptors destruction during laboratorial stages. According to most of the studies, the reason of negative results is the problem existing in immunohistochemistry technique and the way of performing immunohistochemistry technique; so, in the present study it has been tried to do the technique with maximum accuracy and minimum error and by repeating the process for reducing the technical mistakes as much as possible.

According to the present study, the hypothesis of the existence of estrogen and progesterone receptors in all of case and control samples- Epulis Fissuratum and normal mucosa tissue- is rejected. Considering that hormone receptor molecule in the present study could not be detected by IHC method, it seems that IHC method is not appropriate for evaluating the expression of estrogen and progesterone receptors in oral lesions.

So, it is suggested that the study be done with more sample size and using more accurate methods such as gene evaluation of estrogen and progesterone receptors and simultaneous application of the two methods-immunohistochemistry and PCR or In situ hybridization-to detection the expression of the estrogen and progesterone receptors.

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Authors' Contributions

Maryam Seyedmajidi: Gathering data, review, writing and editing
Shahryar Shafaee: Review
Mohammad Azhdari: writing
Sorayya Khafri: Analysis
Sepideh Siadati:Gathering data
Mohammad Mehdizadeh: Gathering data

Conflict of Interest:

The authors declare no conflict of interest.

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