Iranian J Publ Health, 2006, Vol. 35, No. 2, pp.38-41

Immunohistochemical Analysis of Estrogen and Progesterone Receptor Expression in Gingival Lesions

**F Agha-Hosseini [1], F Tirgari [2], S Shaigan [3]*

[1]Dept. of Oral Medicine, Faculty of Dentistry; Tehran University of Medical Science, Iran
[2]Dept of IHC, Imam Khomeini Cancer Institute, Imam Khomeini Hospital, Tehran, Iran
[3] D.D.S

(Received 7 Dec 2005; revised 6 Jul 2005; accepted 4 Jan 2006)

Abstract

Some lesions in the oral cavity and mostly on gingiva have predominant predilection towards females, and mostly occur in the first four decades of life when changes in sex hormone levels in blood are obvious. The present study aimed to investigate the presence and distribution of estrogen and progesterone receptors in peripheral giant cell granuloma (PGCG), pyogenic granuloma (PG) and peripheral ossifying fibroma (POF) on gingiva as an organ target. In a descriptive case series study from March 2002 to April 2003, paraffin blocks from patients with exophitic lesion on gingiva, diagnosed by histopathology as PGCG, PG or POF at Dentistry Faculty of Tehran University of Medical Sciences (TUMS), Iran, were analyzed with Immunohistochemical (IHC) technique. The data analysis was performed by frequency and descriptive statistics. Of 35 patients, 12 estrogen receptors (ERS) and progesterone receptors (PRS) were detected. Nine of them were PRS and three were ERS. Two third of ERS/ PRS were seen in females and one third in males, respectively. In order of decreasing frequency the ERS and PRS were found in PG (n=6), POF (n=4) and PGCG (n=2). In this study, ER/ PR were revealed in three lesions. PR was detected in all of three lesions but we could not see ER in PGCG. Thus, gingiva may be considered as a target organ for sex hormones.

Keywords: *Estrogen receptor, Progesterone receptor, Ossifying fibroma, Pyogenic granuloma, Giant cell granuloma*

Introduction

Gingival tissue is known to be sensitive to changes in the hormonal balance, especially to changes in female sex steroids (1). It has been suggested that changes in hormone levels and types (predominantly estrogen and progesterone) play a great role in the development of some forms of gingival or periodontal disease (2). Support for female sex hormones on gingiva is based upon clinical case reports and a small number of animal studies. Clinical reports of gingival enlargement concurrent with the onset of puberty and during pregnancy, or gingival atrophy and surface desquamation during menopause have led some investigators to regard the gingival as a secondary target organ for the direct action of female sex hormones. Also, human gingiva has been showed to metabolize estrogens (3).

We tried to detect receptors in gingival tissue. Since biopsy of normal tissue is not ethical, we decided to detect those receptors in pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG) and peripheral ossifying fibroma (POF) because these lesions have an obvious predilection for females and occur frequently in specific periods of life such as pre puberty and pregnancy (4).

These lesions may develop in patients undergoing hormone therapy with sex steroids (4). In addition, the majority of these lesions are detected in the first four decades of life, when

38 ***Corresponding author:** Tel: +98 21 66112415, Fax: +98 21 66401132, E-mail: aghahose@sina.tums.ac.ir

hormonal changes are most predominant (5-9). Another reason for selecting these three lesions is that PGCG and POF exclusively develop on the gingival tissue while PG predominantly develops on gingival (6-9).

The aim of this study was detecting the presence and localization of estrogen and progesterone receptors in those three lesions on human gingival as an organ target. All of them have clinical characteristics that may explain a relationship with sex hormones.

Materials and Methods

In a descriptive case-series study from 21st March 2002 to 23rd April 2003, at Dentistry Faculty of Tehran University of Medical Sciences, Iran, patients who had an exophitic lesion on their gingival, clinically similar to one of Peripheral giant cell granuloma, pyogenic granuloma or peripheral ossifying fibroma were biopsied, the samples were stored in formalin 20% and then sent to the pathology laboratory. After confirming clinical diagnosis, immunohistochemical examinations on samples were done. At first 3µm sections from paraffin-embedded block were prepared. After deparaffinization and rehydration antigen retrival was performed in 0.01 mol citrate buffer (pH= 6) for 10 min in microwave oven. After cooling in room temperature and rinsing with PBS (Phosphate buffer saline), the sections were incubated with ER (DAKO Clone 1D5) and PR (DAKO 1A6) antibodies at a 1:100 dilution for an hour. They were then rinsed with PBS and proceed with DAKOLSAB^2 kit and incubated with DAB chromogen (DAB S3000) and H_2O_2, then stained with ethyl green as the background staining. The slides were mounted with entlelane (Merk 1.O7961.0500)

In addition to our samples,we used negative and positive control (ER+ and ER- breast cancer).

One hundred cells of each section were considered as sample and cells with brown nucleus were counted. If at least 5% of cells had turned brown, the result was considered as positive (maximum cell staining is 60-65%). Positive responses were divided to strong and weak based on the number of stained nuclei and brown color intensity. If there was no discoloration to brown in the cell nucleus, the result was registered negative.

The data analysis was processed by frequency and descriptive statistics.

Results

OF 35 patients, 17 (48.5%) cases were pyogenic granulomas, 9 (25.7%) patients had peripheral giant cell granuloma and 9 (25.7%) lesions were diagnosed as peripheral ossifying fibroma. Of 17 PG, 2 (11/8%) had estrogen receptors and in 4 (23/6%) patients progesterone positive were detected. Totally, one third of PG was receptor positive.

None of 9 PGCG showed estrogen receptor but 2 (22.2%) cases, both female, were progesterone positive. Overall one fifth of PGCG were receptor positive. In POF, 1 (11/1%) estrogen positive and 3 (33.3%) progesterone positive cases were found. The estrogen positive case was male and the progesterone receptors were detected in a male and two females. Totally, one third of them were receptor positive.

Eventually two third estrogen positive were female and 7 patients with progesterone receptors were male. Of 17 patients with PG, 15 (88/2%) cases were female. In addition, 5 (55/5%) patients with PGCG and 6 (66/6%) cases with POF were male.

Discussion

In support of the estrogen effects on the gingiva there are clinical observations that it may be enlarged during pregnancy, and may atrophy and desquamate during menopause. These observations have led some investigators to regard the gingiva as another "target organ" for the direct action of estrogen (3).

It is established that the mechanism of steroid hormone action involves the uptake of the hormone by the target cells and its interaction with

the cytoplasmic receptor proteins, forming a hormone receptor complex which is then transported into the nucleus where it activates the genome and, ultimately, stimulate protein synthesis (10).

Steroid hormones including estrogen and progesterone are hydrophobic molecules that bind to intracellular receptor proteins localized within the cytoplasm and the nuclear membrane. These hormones regulate the transcription of specific genes depending on the metabolic condition of the cell. Estrogen may stimulate the proliferation and maturation of gingival connective tissue and epithelium (11).

Some authors concluded through animal study in the oral mucosa and gingiva of rabbits that the gingival may be a target tissue also for the hormone progesterone (10, 12).

The results of a research in detecting estrogen receptors in human gingiva provide the first direct evidence that human gingiva may function as a target organ for estrogens (13). A few investigators studied to demonstrate and localize all sex steroid receptors in healthy oral mucosa using immunohistochemical techniques.

No receptors for estrogen and progesterone were detected by them (1). An attempt was performed to detect estrogen receptor in human gingival by PCR but the results were negative (14).

It has been reported that ten cases of pyogenic granuloma for the detection of estrogen and progesterone receptor proteins (ERs, PRs) were evaluated by immunoperoxidase staining in pregnant women, non-pregnant women and men in 1994. Binding for ER was identified in all patient groups. Although an occasional PR could be noted, this assay was essentially negative in all cases (2). We studied 17 cases of PG to detect ER and PR by immunohistochemical technique. Our results were not similar to them.

Whitakar et al. (2) reported positive answer for ER in one-third of cases in epithelial cells without attention to sex in 1994. Similar to our findings, all ER and three PR were detected in epithelial cells and one PR in endothelial cells. They also evaluated ten cases of PGCG for the detection of ERs/PRs proteins utilizing immunoperoxidase staining in 1994. Staining for ERs proteins was identified in 5 cases (50%), two females and three males. RRs immunoreactivity was essentially negative in all cases (5).

In a study, in fourteen cases, estrogen receptor was found positive but progesterone receptor expression was not detected (11). However, PR was not detected in any cases, while we have identified it in two cases.

Olivera et al. studied using 88 cases of giant cell tissues (GCTs) immunohistochemistry and found that 51% of the samples expressed ER (15). On the contrary, we could not identify ER in any case of PGCG but could detect PR in 2 cases.

In the present study, the presence of ERs or PRs was identified in about one third of those three lesions on the gingiva but not all cases. Perhaps the negative lesions did not contain ERs or PRs and their development was not contingent on hormone levels. It was possible that ERs or PRs were present in these lesions, but in concentrations too low to be detected. In addition, perhaps ERs or PRs were present, but the antibodies and reagents used were not sensitive enough to identify them (2).

Immunohistochemical demonstration of hormone receptor expression is only a rough measure of hormone responsively. The sensitivity, variety and tissue distribution of receptors are more important than their mere presence (7).

With attention to our results, the gingiva may be considered a target organ for ovarian hormones, estrogen and progesterone.

Acknowledgements

The authors wish to thank all the corresponding personnel of Immunohistochemical Dept. of Imam Khomeini Hospital, Tehran, Iran.

References

1. Ojanotko A, Porssell H, Laine M, Hurttia H, Blauer M, Tuohimaa P (1992). Immu-

nohistochemical detection of androgen receptor in human oral mocusa. *Archs Oral Biol*, 37(6): 511-14.
2. Whitaker SB, Bouquot JE, Augusta Ga, Morgantown W (1994). Identification and semiquantification of estrogen and progesterone receptors in pyogenic granuloma of pregnancy. *Oral Surg Oral Med Oral Pathol*, 78(6): 755-60.
3. Yung Yih W, Richardson L, Krotochvil FJ, Avera SP, Zieper MB (2000). Expression of estrogen receptors in desquamative gingivitis. *J periodontal*, 71(3): 482- 87.
4. Whitaker SB, Bouquot JE, Augusta Ga, Morgantown W (1994). Estrogen and progesterone receptor status of central giant cell lesions of the jaws. *Oral Surg Oral Med Oral Pathol*, 77(6): 641-44.
5. Whitaker SB, Bouquot JE, Alimario AE, Whitaker TJ, Morgantown W, Cedarvill A (1994). Identification and semiquantification of estrogen and progesterone receptors in peripheral giant cell lesions of the jaws. *J Periodontal*, 65(3): 280-3.
6. Neville BW, Damm DD, Allen CM, Bouquot JE (2002). *Oral and Maxillofacial Pathology*. 2nded. Saunders, Philadephia, *Pennsylvania*, pp.: 544-47.
7. Wood NK, Gauz PW (1997). *Differential diagnosis of Oral and Maxillofacial Lesions*.5th ed. Mosby, *St Louis*, pp.:340-42.
8. Martin S, Greenberg, Michael G (2003). *Burket's oral medicine diagnosis and treatment.* 10th ed. BC Decker Inc, *Ontario*, pp.: 141-43.
9. Regezi JA, Sciubba J (1999). *Oral pathology and clinical pathology correlations.* 2nd ed. Saunders, Philadelphia, *Pennsylvania*, pp.: 398-401.
10. Vittek J, Gordon GG, Rappaprot SC, Munnangi PR, Southern AL (1982). Specific progesterone receptors in rabbit ginigiva. *J clin Endocrinol Metab*, 54(3): 608-12.
11. Gunhan M, Gunhan O, Celasun B, Multa M, Bostanic H (1998). Estrogen and progesterone receptors in the peripheral giant cell granulomas of the oral cavity. *Journal of Oral Science*, 40(2):57-60.
12. Mohamed AH (1974). The localization of H3 progesterone in the oral mucosa of rabbits. *J Periodontal*, 45(12):844- 52.
13. Vittek J, Hernandez MR, wenk EJ. Rappaport SC, southern Al (1982). Specific estrogen receptors in human gingiva. *J clin endocrinol metab*, 54(3):608-12.
14. Parkar MH, Newman HN, Olsen. (1982). Polymerase chain reaction analysis of oestrogen androgen receptor expression in human ginigival and periodontal tissue. *Archs oral Biol*, 41 (10): 979-83.
15. Olivera P, Perez E, Ortega A, Terual R,Gomes C, Moreno LF, Duen A, DeLa Garza J, Melendez-zajgala J, Maldanado V (2002). Estrogen receptor expression in giant cell tumors of the bone. *Hum Pathol*, 33(2): 165-69.