



## Comparison of the Expression Intensity of Estrogen Receptor Marker in Oral and Cutaneous Pemphigus Vulgaris

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### ABSTRACT

**Background and Aim:** Pemphigus vulgaris (PV) is an autoimmune disorder that can clinically manifest as oral mucosal ulcers. Several researchers believe that the level of female sex hormones such as estrogen and progesterone could be effective in the pathogenesis and growth of lesions such as desquamative gingivitis. Studies related to oral ulcers have revealed contradictory results regarding the presence of estrogen in the gingiva and salivary glands. Therefore, the main objective of this research was the evaluation and comparison of estrogen receptor (ER) expression in oral and cutaneous PV.

**Materials and Methods:** In this cross-sectional study, immunohistochemical staining was performed on 40 Paraffin blocks of oral and cutaneous PV. Staining intensity was investigated. Data were analyzed by Mann-Whitney test, Fisher test and T-test using SPSS 18 software.

**Results:** The mean age of the patients equaled  $38.3 \pm 11.8$  and  $42.8 \pm 11.3$  years in oral and cutaneous PV, respectively. The most common sites of ER expression were the buccal mucosa (55%), trunk (21.1%) and scalp (21.1%). Positive ER expression in oral and cutaneous PV was found in 35% and 89.4% of the cases, respectively. Also, there were significant differences in ER expression intensity between cutaneous and mucosal PV ( $P=0.001$ ). There was no significant correlation between sex, age and ER expression in cutaneous and oral PV.

**Conclusion:** The results of the present study showed that there were significant differences in the intensity of ER expression between cutaneous and mucosal PV.

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## Introduction:

Pemphigus vulgaris (PV) belongs to the category of blistering diseases and affects both skin and mucous membranes. It involves the autoantibodies against cell surfaces of keratinocytes, and causes acantholysis and blistering.<sup>(1,2)</sup>

PV is considered an autoimmune disease, based on the production of autoantibodies such as IgG and IgA against intercellular attachments known as desmosomes (desmoglein 1 and 3).<sup>(1,2)</sup>

Thus, according to its etiology, clinical examination of this severe and chronic disease reveals large bullous and/or erosive lesions. Bullas occur as supra-basal clefts in the epidermis and/or epithelium which are susceptible to rupture and erosion. This disease occurs mostly during the fourth and sixth decades of life without any sex predilection.<sup>(3)</sup> Estrogen is a female sex hormone that regulates various physiological processes in reproductive, mammary and other tissues. Estrogen affects different cells through interaction with estrogen receptors (ERs): ER $\alpha$  and ER $\beta$ , which belong to the family of nuclear hormone receptors.<sup>(4)</sup>

In addition to the regulatory effect on human physiology, estrogen also influences diverse pathophysiological processes including cancer.<sup>(4-6)</sup> It has been proposed that female sex hormones indirectly act on the oral cavity and other tissues by up-regulation of VEGF production in macrophages and endothelial cells.<sup>(7)</sup>

Some researchers have reported that ER $\alpha$  is mostly found in classical target tissues of estrogen such as the mammary gland and endometrium, while ER $\beta$  can be found in tissues such as colon and prostate epithelium.<sup>(8)</sup> Valimaa et al. reported that ER $\alpha$  was absent in the buccal mucosa, gingiva and salivary glands, whereas ER $\beta$  was found in keratinocytes and acinar and ductal portion of salivary glands. Therefore, they concluded that estrogen can affect oral tissues via ER $\beta$  (8). On the other hand, Voutsadakis reported that ER $\beta$  shows higher expression levels in benign tumors. Contrariwise, ER $\alpha$  has higher expression levels in malignant tumors.<sup>(9)</sup>

Some authors believe that changes of estrogen levels in blood and saliva leads to changes in oral soft tissues.<sup>(8,10,11)</sup>

It has been suggested that PV may be hormone (estrogen)-mediated and if this hypothesis is confirmed, hormone therapy can be beneficial.<sup>(5)</sup>

Therefore, the present study aimed to evaluate ER expression in patients with oral and cutaneous PV without evaluating specific receptor types due to limited data on the exact location of ERs and the indefinite mechanism of estrogen signaling pathway.

We investigated ER expression in oral and skin lesions of PV, since ERs are present in different tissues such as the gingiva and epithelium, in addition to the classical target tissues, and some researchers believe that change in estrogen levels can cause changes in oral soft tissues.

## Materials and methods:

In this analytical cross-sectional study, 20 Paraffin blocks of oral PV were retrieved from the archives of the oral and maxillofacial pathology department of Qazvin University of Medical Sciences, Iran, by census method. Also, 20 Paraffin blocks of cutaneous PV were retrieved from the archives of Sina hospital, Tabriz, Iran. Hematoxiline & Eosin (H&E)-stained tissue sections of each block were reviewed to confirm the diagnosis. Only the samples with suitable and adequate tissue for sectioning and staining and with complete information regarding age, sex and the anatomical site of the lesion were included in the study. First, 4  $\mu$ m-thick sections were prepared for immunohistochemical staining. Immunohistochemical technique was performed using Streptavidin-Biotin Complex and ER antibody (DAKO, Carpinteria, USA). Afterwards, the slides were examined under a light microscope and the expression intensity of the cells was determined following the method used by Yih et al, as follows:<sup>(5)</sup>

-: negative

+: weak

++: moderate

+++ : strong

Cells were examined at x400 magnification. Normal testicular tissue with +++ staining intensity was used as a positive control.<sup>(12)</sup> Finally, the data were entered into SPSS 18 software and were analyzed by Mann-Whitney test, Fisher test and T-test.

**Result:**

In the present study, 40 paraffin blocks were used as samples, although one of the cutaneous blocks was excluded from the study due to inappropriate processing and loss of texture. Among the 39 samples, 16 cases (41%) were retrieved from males and 23 cases (59%) were retrieved from females. Males and females were almost equally affected by PV. (Table 1) There was no significant correlation between sex and type of PV (oral and cutaneous) (P=0.975).

**Table 1- Frequency distribution of PV based on gender**

	Gender	Type of Pemphigus Vulgaris (PV)		Total
		Mucosal (Number, %)	Cutaneous (Number, %)	
	Male (N=16)	8 20.5%	8 20.5%	16 41%
	Female (N=23)	12 30.8%	11 28.2%	23 59%
	<b>Total</b>	20 51.3%	19 48.7%	39 100%

The mean age of the patients affected by oral PV was 38.3±11.8 years. Female (12 cases) to male (8 cases) ratio was 1.5/1. Positive ER expression was found in 7 samples (35%). The most common sites of ER expression were the buccal mucosa (55%) and tongue (20%), respectively. The results revealed no significant correlation between sex and frequency distribution of PV in different parts of the oral cavity (P=0.48).

The mean age of the patients affected by cutaneous PV was 42.8±11.3 years. Female (11 cases) to male (8 cases) ratio was 1.3/1. Positive ER expression was found in 13 samples (68.4%). The most common sites of ER expression were the trunk and scalp, with similar frequency (21.1%). The results revealed no significant correlation between sex and frequency distribution of PV in different parts of the skin (P=0.09).

Finally, positive ER expression in cutaneous and oral PV equaled 89.4% and 35%, respectively. There were significant differences in the expression intensity of ER between cutaneous and mucosal PV (P=0.001). Moderate (++)

and strong (+++) expression intensities were observed only in cutaneous PV. (Table 2)

**Table 2- Marker expression intensity based on PV type**

Lesion type	Intensity of marker expression				Total
	-	+	++	+++	
Mucosal PV	13(33.3%)	7(17.9%)	0	0	20(51.3%)
Cutaneous PV	2(5.1%)	13(33.3%)	3(7.7%)	1(2.6%)	19(48.7%)
Total	15(38.5%)	20(51.3%)	3(7.7%)	1(2.6%)	39(100%)

**Discussion:**

PV is a chronic vesiculobullous autoimmune disease, which can involve both mucous membranes and skin. Occurrence is most common during the fifth and sixth decades of life. In most cases, PV initially manifests as oral lesions.<sup>(13)</sup> This can explain the lower mean age of the patients with oral PV compared with that of the patients affected by cutaneous PV in the present study.

The pathogenesis of PV can be explained by the presence of circulating and tissue-bound autoantibodies against desmosomal molecules on the cell surfaces of keratinocytes: desmoglein 1 and desmoglein 3. Desmogleins belong to the superfamily involved in cell–cell adhesion. These autoantibodies cause loss of cell–cell adhesion in the epithelium, which results in suprabasilar intraepithelial vesicle formation.<sup>(13)</sup>

As mentioned earlier, our results showed that the mean age of the patients with oral PV was 38.3±11.8 years with the female-to-male ratio equal to 1.5/1. These findings are consistent with the results of the study by Shamim et al.<sup>(14)</sup>

The average age of the patients in the mentioned study was 42.73 years with the female-to-male ratio of 1.73/1. Also, they found no significant correlation between sex and frequency distribution of PV in different parts of the oral cavity, which was consistent with our findings. Furthermore, the most common sites of ER localization in the mentioned study were the buccal mucosa and palate in both genders, similar to our results which showed that the buccal mucosa was the most common site of ER localization in oral PV. Gornstein et al. stated that ER is localized in

periodontal tissues, similar to other hormone receptors.<sup>(15)</sup>

Since clinical observations have shown gingival enlargement during pregnancy, and gingival atrophy and desquamation during menopause, some investigators have proposed the gingiva as another “target organ” for estrogen.<sup>(10)</sup>

In the current study, ER expression intensity in cutaneous and oral PV equaled 33% and 17%, respectively, and a significant correlation was observed between ER expression intensity and different types of PV ( $P=0.003$ ). Valimaa et al. hypothesized that the incongruity between ER expression and estrogen severity in various studies, can be due to different subtypes of ERs in oral tissues,<sup>(8)</sup> as ER $\alpha$  was not found in the buccal mucosa, gingiva or salivary glands, but ER $\beta$  was widely expressed in oral tissues such as keratinocytes and acinar and ductal cells of salivary glands. Consequently, it can be concluded that estrogen affects oral tissues through ER $\beta$ , and therefore hormonal changes can influence oral mucosa and salivary gland secretions.

To date, no similar study has been performed on this topic. The present study was performed to evaluate that whether estrogen could affect the oral mucosa, especially the gingiva in immunologic diseases such as PV. Since Yih et al,<sup>(5)</sup> evaluated many cases of chronic desquamative gingivitis (CDG), which could be a manifestation of immune-mediated diseases such as PV, lichen planus, etc., It has been suggested that CDG may be hormone (estrogen)-mediated. Several studies have reported cases of gingival enlargement concurrent with the onset of puberty and also during pregnancy and menopause. These findings have led some researchers to believe that the gingiva is a target tissue for estrogen. Although, their findings show no correlation between immunological and idiopathic diseases and ER expression in desquamative gingivitis.<sup>(5)</sup>

Finally, further studies focusing on different types of ER and definite sites of ER expression are recommended. It is suggested

that future studies examine both ER $\alpha$  and ER $\beta$ , using larger sample sizes.

### Conclusion:

According to the results, higher positive expression of ER was observed in cutaneous lesions of PV (89.4%) in comparison with the oral lesions (35%). Furthermore, there were significant differences in ER expression intensity between cutaneous and mucosal PV.

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### Conflicts of Interest:

The authors of this manuscript certify that they have no conflicts of interest regarding this research.

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