The Role of Estrogen Receptor Beta on Severity of Recurrent Aphthous Stomatitis (RAS)

Sri Utami Sunardi[1], Tri Budi Wahyuni Rahardjo[2,3], Ali Baziad[4], Elza Ibrahim Auerkari[1,2]*

1. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
2. Center for Ageing Studies, Universitas Indonesia, Depok, Indonesia.
3. Department of Prosthodontics, Faculty of Dentistry, Unversitas Indonesia, Jakarta, Indonesia.
4. Department of Obstetrics & Gynaecology, Faculty of Medicine, Unversitas Indonesia, Jakarta, Indonesia.

Abstract

The aim of this study was to examine the expression of the estrogen receptor Beta (ER β) in female oral mucosa and to equate Oral Hygiene Index status (OHI-S) with the severity of minor recurrent aphthous stomatitis (RAS). The subjects (26 women, 17–40 years old, with minor RAS but no hormonal therapy or hysterectomy) were investigated to see the whether the severity correlated with the onset pattern, recurrence, level of pain, position, number of lesions, and OHI-S. The expression of ER β in the mouth was detected by immuno cytochemistry. Logistic regression was used to analyze the significance of factors related to the severity of minor RAS. The results showed a significant relation between ER β and the severity of minor RAS controlled by OHI-S ($p<0.05$), with the OR for ER β = 8.67. In conclusion, expression of ER β in oral mucosa may be related to the severity of minor RAS, reflecting the role of oral hygiene. Hence, maintaining oral hygiene is important for preventing the worsening of RAS.

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Introduction

Recurrent aphthous stomatitis (RAS) or ulcer is a chronic inflammatory disease that attacks the mouth mucosa and is marked by the recurrence of ulcers several times, along with pain, but without any signs of other diseases.[1,2] The RAS clinical picture is divided into three types: minor, major, and herpetiform. The minor type, or Mikulicz's aphthae, is the most common RAS type, accounting for 80% or more of the cases. It occurs frequently in the second decade of life, with 1 to 5 ulcers of diameter 4–5 millimeters. Shallow ulcers take the form of a circle or an oval and affect the non-keratin mucosa, especially the labial and buccal mucosa, and the lateral and dorsal parts of tongue; these ulcers are grey or yellowish in color at the bottom and red at the periphery. They take from 7 to 10 days to heal and do not leave scars. The interval of recurrence is from 1 to 4 months, although this can vary widely.[1-4]

At present, the etiology of RAS remains unknown and is considered idiopathic. One of the triggering factors assumed to contribute to RAS pathogenesis is an endocrinal or hormonal imbalance.

Estrogen stimulation regulates cell growth, proliferation, differentiation, and buccal mucosa cornification. The effects of estrogen are mediated by the estrogen receptor (ER). Two estrogen receptor subtypes have been identified: ER α and ER β. Estrogen receptors are included in the family of transcription factors activated by ligand binding to estrogen response elements of DNA.[5,6] ER β is an intracellular protein that defines the role of estrogen in the oral mucosa.

The oral hygiene status (OHI-S) related to the condition of oral micro-organisms, can be expected to influence the severity of RAS. Clinical experience shows that RAS patients normally have poor oral hygiene, probably due to pain that discourages commitment to proper oral maintenance.

Välimaa (2004) found that ER β expression is high in the epithelial mucosa of

Corresponding author:
Elza Ibrahim Auerkari
Department of Oral Biology
Faculty of Dentistry, Universitas Indonesia
E-mail: ei_auerkari@yahoo.com

the buccal, gingival, and salivary glands, whereas ER α was not expressed. This explains that the influence of estrogen hormonal changes in the mouth is based on ER β[7], but Leimola et.al. did not find any cells with positive ER immunehisto chemical expression in the buccal mucosa, however they found the existence of ER mRNA and immune reactive ER protein that shows estrogens have a biological role in oral mucosa and salivary glands.[8]

In pathologic conditions, ER expression occurs in pyogenic granulomas that occur during pregnancy[9] and in cases of peripheral giant cell granulomas in the mouth.[10] Another research report indicated high positive expression of ER in female gingiva with periodontitis ($p<0.01$), which suggested a direct effect of estrogen on the gingiva, operating through the ER.[11]

Several studies have reported various microorganisms as predisposing factors for RAS. Oral hygiene (OH) relates to the condition of micro organisms in the mouth, so OH affects the eventual seriousness of RAS. Among the range of microorganisms that play significant roles in the pathogenesis of RAS is *Streptococcus sanguis.* A cross reaction that occurs between microorganisms and the oral mucosa is known as the mechanism of immunopathology.[3]

Estrogen influences inflammation and tumor regulation, and RAS is an inflammatory disease that is likely to be affected by estrogen. The OH shows the hygiene status of mouth health as it relates to the condition of microorganisms in the mouth, so OH influences the severity of RAS.

The aim of the present study was to compare the expression of ER β in female oral mucosa and OHI-S with the severity of minor RAS.

Materials and Methods

The study was approved by the Academic Ethical and Research Commission of Faculty of Dentistry UI and informed consent was obtained from all subjects.

The subjects were 26 women (17–40 years old, with minor RAS but no hormonal therapy or hysterectomy) investigated to see whether the severity correlates with the onset pattern, recurrence, level of pain, position, number of lesions, and OHI-S.

The expression of ER β in the mouth was detected by immunocytochemistry, based on previous cytology with Papanicolaou to determine cell distribution and firmness. Mucosa samples were taken from the RAS lesions by cytobrush swabbing and placed on poly-L-Lysine–coated slides.

The slides were immediately transferred to ethanol for further Papanicolaou staining. This was followed by immunocytochemistry analysis using ER β monoclonal antibody from Novo (Streptavidin-Biotin).

The cell assumed to have ER β protein was treated with the primary ER β mouse monoclonal antibody, followed by incubation with the secondary biotinylated rabbit antimouse (BRAM) antibody and addition of the diamino benzidine (DAB) chromogen to generate a brown color and counterstaining with hematoxylin. ER β is an intracellular protein that resides on the cell nucleus. Microscopic examination revealed a brown precipitate.

The staining intensity of ER β was estimated on a semi quantitative scale, and scored as follows: 0 or negative: no reactivity; 1+: weak intensity; 2+: medium intensity; 3+: strong intensity.[9,12-14]

Results

Under the microscope, at a magnification of 100×, expression of ER β appeared as shown in Figures 1–3. The multivariate analysis results shown in Table 1 indicate a significant relation between ER β and the severity of minor RAS controlled by the OHI-S variable ($p=0.045$) and an Odds Ratio for ER β = 8.667, which means the estrogen receptor β has an 8.67 times greater chance to identify minor RAS if there is an OHI-S.

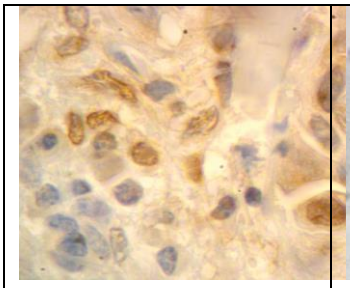

Figure 1: Positive control ER β (100 x): Brown precipitation in nucleus showing protein ERβ with strong intensity.

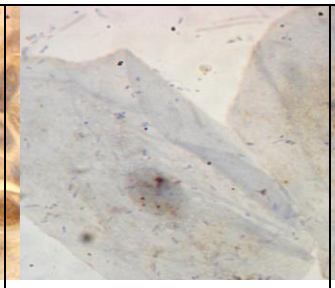

Figure 2: Minor RAS, ERβ + weak (100x): Brown precipitation in nucleus with weak intensity compared to the positive control, showing protein ERβ with weak intensity

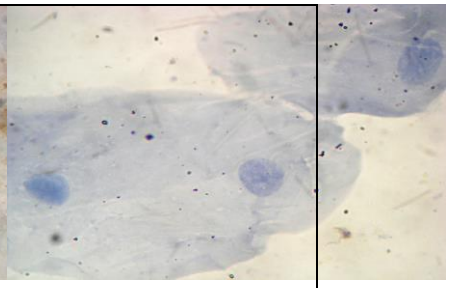

Figure 3: Negative control. Blue nucleus shows no reactivity, or no expression of ERβ

Figure 1-3

Table 1. Multivariate Analysis of the Logistic Regression Between ER β and OHIS

Variable	B	SE	Wald	Sig	OR	95% CI
ESTRO-B	2.159	1.077	4.019	**0.045***	8.667	1.049-71.569
OHI-S			2.491	0.288		
OHIS (1)	2.159	1.382	2.441	0.118	8.667	0.577-130.111
OHIS (2)	8.668	36.663	0.056	0.813	5814.169	0.000-9E+034

Notes: -2 Log Likelihood = 26.901; G = 7.745; p = 0.052

ESTRO-B: estrogen receptor beta
OHIS (1): oral hygiene medium to good
OHIS (2): oral hygiene bad to good
*p: significance < 0.05 (**0.045***)*
OR: odds Ratio
n = 26

Discussion

ER β is an intracellular protein that defines the role of estrogen in the oral mucosa. The present research findings indicate that estrogen activity, shown through ER β, is very weak. Of the 26 cases of minor RAS, 6 of them showed positive weak expression. Estrogen, through ER β, is assumed to have an indirect influence on the lesions of minor RAS and, generally, to show a negative expression of ER β. One endocrinal irritation is the disturbance of the target cell response towards the related hormone.[13] The lack of expression of ER β in a target cell or tissue would indicate that no complex formed between the estrogen-receptor hormone and DNA, which would disrupt the signaling for the activation of mRNA transcription. Consequently, a disturbance happens in the translation by the ribosome that transfers to the responses of growth, proliferation, differentiation, and development. As a result, the oral mucosa become more vulnerable and RAS readily occurs. However, Valimaa found that ER β, but not ER α, was detected in all layers of the gingival and buccal mucosal epithelium and salivary glands. Identification of ER in the oral epithelium suggests that estrogen has an important role in the maturation of the oral epithelium. Estrogen may therefore act via ER β in oral tissues and

this could explain the effect of hormonal changes on the oral mucosa.[7]

At this point, no reports have documented the patterns of expression of ER α and β, or Progesterone receptors (PrR) on RAS lesions.

In this study, most respondents were female students of the Faculty of Dentistry UI who were already aware and had knowledge of the importance of maintaining the hygiene status of the mouth. Therefore, their OHI-S statuses were generally good. Clinical experience indicates that people with bad OHI-S tend to have severe RAS.

The OHI-S shows the hygiene status of mouth health as it relates to the microorganisms in the mouth, so OHI-S influences the severity of RAS. Several studies have reported on the roles of various microorganisms as predisposing factors for RAS. One of these that plays an important role in RAS pathogenesis is *Streptococcus sanguis.* RAS cases reportedly show a cross reaction between microorganisms and the oral mucosa, which is called the mechanism of immunopathology.[3,11] Clinical experience shows that RAS patients normally have bad oral hygiene, since the pain of RAS can reduce the diligence of the patients in maintaining their oral hygiene.

Conclusion

Expression of ER β in oral mucosa is related to the severity of minor RAS and reflects the role of oral hygiene. Maintaining oral hygiene is therefore essential for preventing the worsening of RAS.

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