# Effect of Contraception on the Expression of Cytokeratin 1 in Epithelial Cells of the Palatal Mucosa and Salivary Estrogen

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**Abstract**

Sex hormones may affect keratinization of the epithelial cells. Cytokeratin 1 (CK1) is expressed in keratinized cells of oral mucosa. The aim is to analyze the effect of contraception on the CK1 expression of epithelial cells in the palatal mucosa and salivary estrogen. Forty females were divided equally into pill, injection, intrauterine device, and non-contraception (control) groups. Saliva samples were collected by unstimulated method. Salivary estrogen was measured by using an ELISA kit. Epithelial cells of the palatal mucosa were swabbed using cytobrush then treated by immunohistochemistry staining. The primary antibody of CK1 was used to analyze the expression of the protein. The results of ANOVA revealed the significant effect of contraception on salivary estrogen and CK1 expression ($p < 0.05$). The Games–Howell post hoc test indicated a significant difference between salivary estrogen level and CK1 expression among the control, pill, and injection groups ($p < 0.05$); however, no significant difference between the control and IUD groups ($p > 0.05$) was found. Pearson correlation analysis indicated a strong correlation between salivary estrogen and CK1 expression ($r = 0.880$). Hormonal contraception could increase the keratinization of epithelial cells by increasing CK1 expression and correlate with increasing salivary estrogen.

**Experimental article(J Int Dent Med Res 2021; 14(2): 629-632)**

**Keywords:** Contraception, epithelial cell, palatal mucosa, salivaray estrogen, cytokeratin 1.

**Received date:** 17 Fabruary 2021 **Accept date:** 20 April 2021

## Introduction

Contraception is often used by women to prevent pregnancy and includes hormonal (e.g., pills, injection) and non-hormonal (e.g., intrauterine device [IUD]) methods. Keratinization of the oral mucosa may be influenced by various hormones, including sex hormones. Changes in hormone level may affect the oral mucosa. The female sex hormone may affect oral ulceration, mood variation and recurrent herpetic lesion, gingival bleeding,[1]or periodontitis.[2] Cytokeratins (CKs) make up the basic protein structure of epithelial cells. CK expression is abundant in the oral epithelia or salivary glands during odontogenesis. CK is also a biomarker for diagnostic pathology.[3,4,5] The pattern of CK expression is related to the maturation of the mucosa.[6] Keratinized epithelial cells express CK1 and CK10 in the suprabasal layer and CK5 and CK14 in the basal layer.[7,8] Several studies have shown that hormonal contraception could increase saliva volumes, pH,[9] and α-amylase levels.[10] CK1 is highly expressed in keratinized oral epithelia, including the palatal mucosa (8) and expression of CK19 is increase in azo-exposed.[11] Previous study also indicated there was positive correlation between salivary estrogen and epithelial cell-expressed cytokeratin 5, and depend on age.[12] The aim of this study is to analyze the effect of contraception on the CK1 expression of epithelial cells in the palatal mucosa.

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## Materials and methods

The present study adopted a cross-sectional design, and the study protocol was approved by the ethics committee of the Faculty of Dentistry, Universitas Gadjah Mada (Ethical Clearance No. 001502/KKEP/FKG-UGM/EC/2018). The study participants included 40 females who were divided into four groups (n

= 10), namely, the pill group, the injection group, the intrauterine device (IUD) group, and the non-contraceptive (control) group. The inclusion criteria were as follows: (1) contraceptive use for at least 3 months, (2) age range of 20–35 years, (3) of generally good health and with acceptable Simplified Oral Hygiene Index scores, and (4) did not wear dentures or orthodontic appliances. Participants were recruited from Kampung KB at Banyurojo Village, sub-district of Mertoyudan, Magelang District, Jawa Tengah Province, Indonesia. Contraceptive participants either took pills (Mycrogynon, Bayer, Indonesia) every day or used IUDs (Andalan, Indonesia) or accepted injections (Depo-Provera, Mayo Clinic) every 3 months. Informed consent was obtained from each participant in accordance with the Helsinki II declaration.

Saliva samples were collected by using the unstimulated method at 15:00–17:00. Salivary estrogen was measured by using ELISA kits (DRG® Estradiol ELISA, DRG International Inc., USA). Absorbance was read at 450 nm.

Epithelial cells of the palatal mucosa were swabbed by using a cytobrush moistened with 0.09% NaCl and then smeared on poly L-lysine glass slides. The slides were fixed with a solution of freshly prepared methanol–acetate (3:1). Immunostaining was performed by using a mouse ABC staining system (ImmunoCruz™ sc-2017, Santa Cruz Biotechnology Inc., USA) according to the manufacturer's instructions. The slides were washed with PBS three times for 5 minutes each time and then incubated with 1% $H_2O_2$ diluted with methanol for 5 minutes to quench endogenous peroxidase activity. Thereafter, the slides were washed three times with PBS for 5 minutes each time and incubated with 1.5% normal blocking serum with PBS for 1 hour. Samples were washed three times with PBS for 5 minutes each time and incubated overnight with the primary antibody of CK1 (NB100-2756, Novus Biological USA) at 4 °C. The primary antibody was diluted to 1:500 with PBS. Samples were washed three times with PBS for 5 minutes each time.

Next, slides were incubated with a biotinylated secondary antibody for 30 minutes at room temperature and then washed three times with PBS for 5 minutes each time. The slides were incubated with AB enzyme reagent containing avidin and biotinylated HRP reagent for 30 minutes at room temperature and then washed three times with PBS for 5 minutes each time.

The samples were incubated in peroxidase substrate for 30 seconds to 10 minutes or until the desired stain intensity developed. At least 100 epithelial cells of the palatal mucosa were collected for each sample. Positive results were confirmed by a brown stain in the nucleus and cytoplasm of the cells. Observation of positive cells was conducted by using a light microscope with 400× magnification, and cells positive for CK1 were counted on a computer monitor with 100× magnification.

Data were processed by using IBM SPSS Statistics version 22 for Windows (IBM SPSS Inc., USA), and the significance level was set to $p < 0.05$. Normality and homogeneity of variance were respectively calculated by using the Shapiro–Wilk and Levene tests. Data were analyzed by using ANOVA, the Games–Howell post hoc test, and Pearson correlation analysis.

## Results

CK1 expression manifests as a brown stain in the nucleus and cytoplasm of epithelial cells of the palatal mucosa, as shown in Figure 1. Salivary estrogen levels and number of cells with positive expression of CK1 per 100 cells are displayed in Table 1.

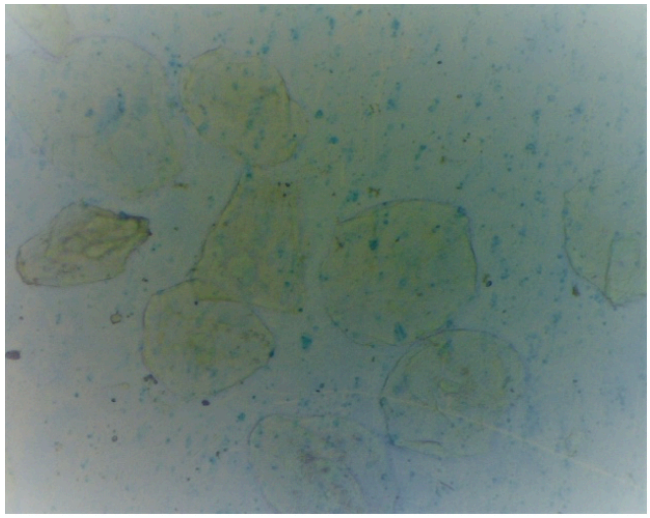

**Figure 1.** Positive expression of cytokeratin 1 is manifested as a brown stain in the nucleus and cytoplasm of epithelial cells of the palatal mucosa.

Data were normally distributed according to the Shapiro–Wilk test (CK1 expression: $p = 0.245$; salivary estrogen: $p = 0.557$). The

homogeneity of the data was assessed by using Levene's test, and CK1 expression showed p = 0.264 while salivary estrogen showed p = 0.000. These results reveal that the data of salivary estrogen are not homogeneous. ANOVA revealed a significant difference ($p < 0.05$) the effect contraceptive on the epithelial cell, which means contraceptive use may have an effect on salivary estrogen levels and CK1 expression. The results of the Games–Howell post hoc test revealed a significant difference between salivary estrogen and CK1 expression among the control, pill, and injection groups ($p < 0.05$); however, no significant difference between the control and IUD groups ($p > 0.05$) was found. Pearson correlation analysis showed a strong correlation between salivary estrogen and CK1 expression (r = 0.880)

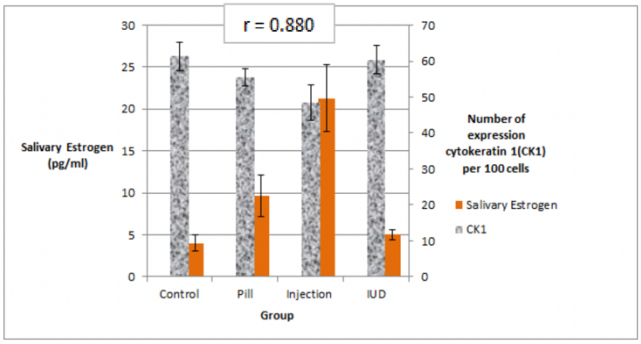


**Table 1.** Salivary estrogen level (pg/ml) and number of cells with positive cytokeratin 1 (CK 1) expression per 100 cells. The highest level of salivary estrogen and the lowest number of cells positive for CK1 are observed in the injection group.

## Discussion

CK1 is expressed in the nucleus and cytoplasm (Fig. 1). All groups showed CK1 expression (Table 1), and the highest level of expression was observed in the control group, followed by the IUD, pill, and injection groups. According to Rao et al.,[13] CKs make up the basic protein structure of epithelial cells. Among its many functions. CK1 protects epithelial cells from stress and apoptosis.

Levels of salivary estrogen were highest in the injection group, followed by the pill, IUD, and control groups, thereby indicating that hormonal contraceptives increase salivary estrogen. This study supported to previous result that hormonal contraception could increase expression CK5 and have correlation with epithelial maturation.[14] Hormonal contraceptives contain estrogen components. Pills, for example, contain ethinyl estradiol, while injectables contain estradiol cypionate. IUDs contain a component of progesterone called progestin. A strong correlation between salivary estrogen and CK1 expression was observed in the injection group. This result supports the findings of a previous study, which showed that the oral mucosa is sensitive to the effect of sex hormones, particularly estrogen. Keratinization of the oral mucosa normally occurs under the influence of hormones.[1] Changes in the pattern of differentiation may be used as an early indicator of premalignancy so that prevention and treatment can be implemented earlier. The effect of hormonal contraceptive use on periodontal disease remains debatable. The results of a previous study indicated that the use of contraception affects gingivitis and loss of gingival attachment.[15] Although the results of other studies in postmenopausal female subjects showed no correlation between estrogen levels and periodontal status.[16]

The results of this study supported previous research that Estrogen Receptor β (ER β) as an intracellular protein could show the role of estrogen in the oral mucosa. The presence of ER β expressed in the epithelial oral mucosa is thought to strengthen the possible role of estrogen in the keratinization process of the oral mucosa. In the case of Recurrent Aphtous Stomatitis (RAS), the expression of ER β were little or negative in the epithelial cells of the oral mucosa. It indicated that the complex between the estrogen receptor hormone and DNA was not formed, causing interference with the signal for mRNA transcription activation as well as disruption of translation by ribosomes including those transferred to the growth response, proliferation, differentiation, and development. In these circumstances the oral mucosa was more susceptible to developing RAS.[17]

When gingivitis occurs, keratinization, which could be characterized by an increase in CK1 expression, increases. Increased CK1 expression was correlated with higher salivary estrogen in the injection group (Fig. 2). Estrogen may influence the process of keratinization by increasing CK1 expression. According to a previous study,[18] estrogen plays important roles in regulating tissue homeostasis and the proliferation and differentiation of cells in female

reproductive organs through estrogen receptor 1 (ESR1). Epithelial ESR1 could integrate estrogen and growth factor signaling to mediate the regulation of cell proliferation during squamous differentiation. This mechanism may also occur in the palatal mucosa, which has a stratified keratinized squamous type similar to that of the vaginal epithelium. The results of this study reveal that hormonal contraception could affect the keratinization of the palatal mucosa, which may play an important role in the mechanism of periodontal disease (e.g., gingivitis), by increasing CK1 expression. This study helps determine the mechanisms behind changes in the differentiation and maturation of palatal epithelial cells after hormonal contraceptive use.

## Conclusion

A strong correlation between salivary estrogen and CK1 expression was found. The findings indicate that hormonal contraception could increase epithelial cell keratinization by increasing CK1 expression.

## Acknowledgments

This study was supported by a grant from the Faculty of Dentistry, Universitas Gadjah Mada (Contract Research No: 4471/UN1/KG/Set.KG/LT/2018).

## Declaration of Interest

The authors have no conflict of interest to declare.

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