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Association between Oral Contraceptive Use and Interleukin-6 Levels and Periodontal Health

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Abstract

Background and aims. Changes in the balance of sexual hormones during pregnancy decrease gingival crevicular fluid levels of interleukin-6 and the resistance of gingival tissue against inflammations. Hormonal contraceptives are agents that are based on the effects of gestational hormones and simulate a state of pregnancy; therefore, they prevent ovulation. This study evaluates the effect of these drugs on periodontal tissues and levels of IL-6 in gingival crevicular fluid.

Materials and methods. Twenty-five patients who had not used oral contraceptives (control) and 35 patients using oral contraceptives (case) were examined clinically and their medical history, dosage and duration of oral contraceptives use were recorded. Periodontal indices such as bleeding on probing, plaque index, probing pocket depth, clinical attachment loss and levels of IL-6 in gingival crevicular fluid were measured. Student's t-test and Mann-Whitney test were used to analyze data.

Results. Mann-Whitney test showed a statistically significant difference in the mean of bleeding on probing in the case and control groups ($P<0.05$). Student's t-test showed a statistically significant difference in the mean of IL-6 levels ($P<0.05$), probing pocket depth ($P<0.05$) and clinical attachment loss between the case and control groups ($P<0.05$) but no statistically significant differences were found between the plaque index of the case and control groups ($P>0.05$).

Conclusion. It seems that use of oral contraceptives may affect the periodontal health status of patients, leading to more gingival inflammation. Therefore, patients must have a strict oral hygiene care.

Key words: **periodontitis, oral contraceptives, interleukin-6, tumor necrosis factor-α.**

Introduction

It has been shown that sexual hormones can affect the cellular proliferation, differentiation and growth in target tissues in the periodontium. These cells include keratinocytes and gingival fibroblasts.[1,2] Pregnancy leads to remarkable alterations in the endocrine system.[3] Progesterone and estrogen levels

increase during pregnancy and this elevation is caused by continues production of these hormones from the corpus luteum.[3] The levels of these hormones increase in the saliva as well.[4] Therefore, more inflammation and gingival bleeding on probing happens in pregnant women in comparison with the general population. Also, susceptibility to infections and periodontal infections increases as a result of immune system's alterations.[5] These effects are due to changes in the dental biofilm, the hormonal levels and the microbial flora.[6]

Hormonal contraceptives are agents that are rely on the effects of gestational hormones and simulate a state of pregnancy; therefore, they prevent ovulation.[7] Reactions to oral contraceptive hormones may be similar to pregnant patients. In general, gingivitis occurs when the hormonal balance is disturbed by some changes in the body system, such as pregnancy, puberty and postmenopause. This fact leads to this hypothesis that pregnancy hormones, specifically estrogen and or progesterone, are the responsible factors.[8]

One of the newest discussions in this field is the influence of estrogen and progesterone on inflammatory mediators that can also be helpful in explaining the increase in inflammation. Interleukin-6 (IL-6) is a pleiotropic cytokine which is produced by many cell types such as human gingival fibroblasts. It is secreted in response to inflammatory challenges such as interleukin-1 (IL-1) and bacterial lipopolysaccharide.[9] Decrease in IL-6 levels during pregnancy due to changes in the balance of sexual hormones, decreases the resistance of gingival tissue against inflammations.[9] IL-6 induces the synthesis of tissue inhibitor metalloproteinase (TIMP) by fibroblasts, decreasing tumor necrosis factor levels.[3] IL-6 is considered a proinflammatory cytokine, but latest research has implied that IL-6 has many anti-inflammatory effects; therefore, it is considered an anti-inflammatory agent.[10] IL-6 decreases IL-1 and tumor necrosis factor levels but induces the release of glucocorticoids and production of natural antagonists against IL-1 and TNF. It also causes the production of acute phase proteins such as fibrinogen and C-reactive protein in the liver, which have anti-inflammatory effects by induction of IL-1ra.[10] In addition, IL-6 may have a protective effect on the destructive patterns of periodontal diseases, decreasing the amount of bone loss.[11]

It has been shown that during gingivitis, the level of IL-6 mRNA increases, especially in fibroblasts.[12] It can be concluded that the decrease in the levels of IL-6 leads to an increase in the inflammatory responses in gingiva and this is exactly what happens under the effect of imbalance in sexual hormones.[3]

Human population control is in the center of administrative programs in many countries and oral contraceptive pills are one of the most commonly used methods in this field. Despite the effects of IL-6 on gingival tissues, there is little research on about this mediator. Therefore, the present study was performed to evaluate the relationship between the periodontal health status and IL-6 levels in gingival crevicular fluid.

Materials and Methods

This descriptive analytical research was performed on 60 female patients aged 17–40 years, who were using oral contraceptives and were seeking dental care at Azad Dental School of Khorasgan (Isfahan). Pregnant patients, patients with any systemic diseases, patients under any medication, patients undergoing any periodontal treatment, patients receiving any wide-spectrum antibiotics, smokers and patients drinking alcohol were excluded from the study.

Twenty-five patients who did not use oral contraceptives were assigned to the control group and 35 patients taking oral contraceptives were assigned to the case group. The patients were informed about the study design, and their medical history, dosage and duration of oral contraceptives they were taking were recorded. Periodontal indices such as bleeding on probing, plaque index, probing pocket depth and clinical attachment loss were recorded for each patient as well.

Patients were asked to rinse their mouths. After isolation, sampling of gingival cervical fluid was performed at three sites (two anterior regions and one posterior region) by means of three #25 paper points. Each paper point was placed in the gingival sulcus and left there for three minutes. Then it was removed and inserted into a test tube filled with normal saline and intermediate fluid and transferred to the laboratory. The samples were kept at 2−6ºC temperature in a specific box filled with dry ice.

The samples were placed on a shaker for 15 minutes and then centrifuged for 10 minutes at 3500 rpm. A diagnostic kit for IL-6 was used (BD OptEIA, BD Biosciences, San Diego, US).

All the reagents, working standards and samples were prepared according to manufacturer guidelines.

Reagent preparation

All the reagents were brought to room temperature before use. To prepare standards, one vial of lyophilized standard was reconstituted with required vol-

ume of standard/sample diluent to prepare a 1000-pg/mL stock standard. The standard was allowed to equilibrate for 15 minutes before making dilutions and mixed with gentle vortex. 300 µL of standard/sample diluent was added to 6 tubes and labeled as 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL and 15.6 pg/mL. Serial dilutions were performed by adding 300 µL of each standard to the next tube and vortexing between each transfer. The undiluted standard served as the high standard (1000 pg/mL). The standard/sample diluent served as zero standard (0 pg/mL).

Sixty test wells were placed in a well holder and coded with a number. 100 µL of ELISA Diluent were added to each well. 100 µL of each standard sample were added to appropriate wells. The plate was gently shaken for five seconds to mix. The wells were covered with plate sealer and incubated for two hours at room temperature.

Working detector was prepared within 15 minutes prior to use and an equal volume of detection antibody was added to a clean tube by means of a pipette. Then an equal quantity of enzyme concentrate was add and mixed.

Contents of each well were aspirated. The wells were washed by filling with at least 300 µL/well of wash buffer, followed by aspirating. This washing method was repeated four times. After the last wash, the plate was blotted on an absorbent paper to remove any residual buffer. Complete removal of liquid is required for proper performance.

200 µL of prepared working detector was added to each well. The wells were covered with plate sealer and incubated for one hour at room temperature. The wells were washed seven times as described above. In this final wash step, the wells were soaked in wash buffer for 30 seconds for each wash because thorough washing at this step is very important. 200 µL of TMB One-Step Substrate Reagent was added to each well and the plate was incubated without plate sealer for 30 minutes at room temperature in darkness. Finally, 50 µL of Stop Solution was added to each well.

Absorbance was read at 450 nm within 30 minutes of stopping the reaction by means of a Microplate Reader STAT FAX 2100 (Awareness Technology, Inc. New York, USA).

Mann-Whitney test was used to compare the means of BOP and student's t-test was used to compare the mean of PD, PI, CAL in the case and control groups.

Results

The means of IL-6 levels, bleeding on probing, probing pocket depths, plaque indexes and clinical attachment losses in the case and control groups are presented in Table 1.

Mann-Whitney test showed statistically significant differences in the means of bleeding on probing between the case and control groups ($P<0.05$).

Student's t-test showed statistically significant differences in the means of IL-6 levels (($P<0.05$), probing pocket depths ($P<0.05$) and clinical attachment losses between the case and control groups ($P<0.05$) but no statistically significant differences were found between the plaque index of the case and control groups ($P>0.05$) (Figures 1 and 2).

Discussion

Individuals taking oral contraceptives have conditions similar to pregnant women due to high levels of estrogen and progesterone.[3] Therefore, their clinical features are similar to pregnant women but these changes happen in an extended duration in oral contraceptive users and as a result they show different degrees of gingivitis. It should be noted that the incidence and severity of gingivitis may decrease with good oral hygiene maintenance in these individuals.[3,13]

Lindhe and Björn[14] found out that regular use of oral contraceptive pills for 12 months increases the exudation of gingival pockets in anterior regions. In another study by Kaufman and Gan[15] it was observed that weakly consumption of progestonic and strongly estrogenic contraceptives resulted in hyperplastic gingivitis and a pregnancy tumor in one patient. Jensen et al[16] found a 16-fold increase in the population of *Bacteroides* species in oral contraceptive consumers and Klinger et al[17] found a 4.8% increase in *P. intermedia* population in woman using oral contraceptive pill but could detect no increase in the population of *P. gingivalis* and *A. actinomycetemcomitans*. Current oral contraceptive pills contain low doses of estrogen (0.05 mg/day) and 1.5 mg/day of progestin, and this formulation is different from the early pills that contained higher concentrations of sex steroid hormones (20−25 µg estrogen and 0.15-4

Table 1. The mean of IL-6 levels in GCF and clinical parameters in the test and control groups

	Level of IL-6 in GCF (ng/dL) Mean (SD)	Bleeding on probing Mean	Probing pocket depth(mm) Mean (SD)	Plaque index (%) Mean (SD)	Clinical attachment loss(mm) Mean (SD)
Case group	63.82 (12.14)	41.11	3.78 (1.66)	32.40 (12.29)	1.87 (1.62)
Control group	135.04 (42.14)	15.64	0.78 (1.07)	29.24 (10.61)	0.24 (0.56)

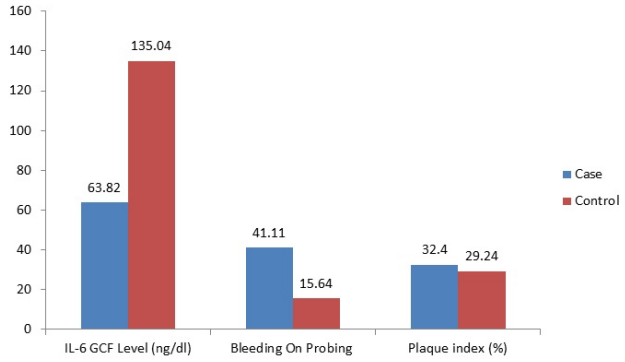

Figure 1. The Mean of IL-6 levels in GCF, bleeding on probing and plaque index in the test and control groups.

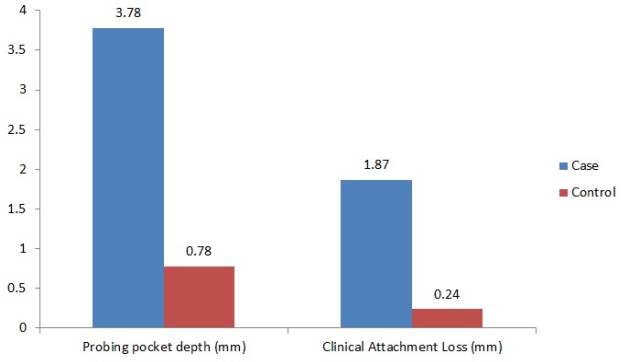

Figure 2. The Mean of probing pocket depths and clinical attachment loss in the test and control groups.

mg progesterone); therefore, it is assumed that at low plaque levels and good oral hygiene maintenance during oral contraceptive consumption, their effects on the periodontium may be minimized.[3,7,18,19]

In the present study the mean of IL-6 levels in the gingival crevicular fluid, decreased significantly in individuals taking oral contraceptives, followed by a significantly higher amount of bleeding on probing, clinical attachment loss and probing pocket depths in comparison with the control group, similar to some other studies performed earlier.[20,21,22]

On the other hand, Taichman et al[23] could not find any relations between the use of oral contraceptives and an increase in the incidence of gingivitis and periodontitis in their subjects. Also in another study, Haerian-Ardakani et al[24] found more gingivitis and bleeding on probing in individuals using oral contraceptives but could not find any significant differences in the probing pocket depth and clinical attachment loss amounts. The reason for the discrepancy in the results of the present study and the two mentioned above may be attributed to the dosage of oral contraceptives used or the cooperation of patients and the levels of plaque indices, which has not been mentioned in those studies. The designs of these studies may differ as well.

Different reasons have been proposed to explain the relation between the increase in the levels of sexual hormones and increase in the response of gingival tissues to local stimulating factors, including changes in microvasculature, an increase in gingival permeability, an increase in prostaglandin synthesis, especially prostaglandin E1 and E2, a decrease in the chemotaxis of neutrophils, immunosuppression of cell-mediated immune system, a decrease in the phagocytosis ability and the responses of T-cells and finally an imbalance in the fibrinolytic system mediated by progesterone.[25,26,27,28] It has been shown that progesterone stimulates the production of inflammatory mediators and prostaglandin E2 and enhances the accumulation of polymorphonuclear leukocytes in the gingival sulcus.[29] On the other hand, low levels of estradiol reduce the chemotaxis of polymorphonuclear leukocytes while progesterone enhances this ability.[30]

As it was mentioned earlier, an increase in the levels of sexual hormones induces a decrease in the levels of IL-6 and leads to the deficiency in the defense system of gingiva prior to inflammatory processes.[9]

Longitudinal studies have shown that users of oral contraceptive drugs show more clinical attachment loss and probing pocket depth in comparison to individuals that do not use these drugs but have the same oral hygiene level and plaque index.[21] It must also be pointed out that sex steroid hormones may modulate the production of cytokines[31] and progesterone can downregulate the production of IL-6 by human gingival fibroblasts to 50%.[9,32] This fact makes IL-6 a suitable inflammatory mediator for diagnosis and prognosis processes in the future.

Conclusion

The present study showed that IL-6 gingival crevicular fluid level decreases significantly in women consuming oral contraceptives and bleeding on probing, probing pocket depth and clinical attachment loss increase significantly with the same oral hygiene status.

It seems that use of oral contraceptives may affect the periodontal health status of patients and lead to more gingival inflammation. Therefore, patients must be informed to have a strict oral hygiene care program to decrease the levels of inflammation when they are using oral contraceptive pills.

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References

1. Mariotti A. Sex steroid hormones and cell dynamics in the periodontium. *Crit Rev Oral Biol Med* 1994; 5: 27-53. doi:10.1111/j.1600-0757.2011.00424.x
2. Mealey L, Moritz J. Hormonal influences on periodontium. *Periodontol 2000* 2003; 32: 59-81. doi:10.1046/j.0906-6713.2002.03206.x
3. Markou E, Eleana B, Lazaros T, Antonios K. The influence of sex steroid hormones on gingiva of women.*Open Dent J* 2009;3:114-9. doi:10.2174/1874210600903010114
4. Yokoyama M, Hinode D, Masuda K, Yoshioka M, Grenier D. Effect of female sex hormones on Campylobacter rectus and human gingival fibroblasts. *Oral MicrobiolImmunol* 2005; 20: 239- 43. doi:10.1111/j.1399-302x.2005.00222.x
5. Brabin J. Epidemiology of infection in pregnancy. *Rev Infect Dis* 1985; 7: 579-603. doi:10.1093/clinids/7.5.579
6. Al Habashneh R, Guthmiller M, Levy S, et al. Factors related to utilization of dental services during pregnancy. *J Clin Periodontol* 2005; 32: 815-21. doi:10.1111/j.1600-051x.2005.00739.x
7. Güncü GN, Tözüm TF, Cağlayan F. Effects of endogenous sex hormones on the periodontium--review of literature. *Aust Dent J* 2005;50:138-45. doi:10.1111/j.1834-7819.2005.tb00352.x
8. Silness J, Loe H. Periodontal disease in pregnancey. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121-35. doi:10.3109/00016356408993968
9. Lapp A, Thomas E, Lewis B. Modulation by progesterone of interleukin- 6 production by gingival fibroblasts. *J Periodontol* 1995; 66: 279-84. doi:10.1902/jop.1995.66.4.279
10. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol Today* 1997;18:428-32. doi:10.1016/S0167-5699(97)01103-1
11. Irwin CR, Myrillas TT. The role of IL-6 in the pathogenesis of periodontal disease.*Oral Dis* 1998;4:43-7. doi:10.1111/j.1601-0825.1998.tb00255.x
12. Takahashi K, Takashiba S, Nagai A, Takigawa M, Myoukai F, Kurihara H, et al. Assessment of interleukin-6 in the pathogenesis of periodontal disease. *J Periodontol* 1994;65:147-53. doi:10.1902/jop.1994.65.2.147
13. Richards D, Rutherford RB. The effects of interleukin 1 on collagenolytic activity and prostaglandin-E secretion by human periodontal-ligament and gingival fibroblast.*Arch Oral Biol* 1988;33:237-43. doi:10.1016/0003-9969(88)90184-7
14. Lindhe J, Bjorn L. Influence of hormonal contraceptives on the gingiva of women. *J Period Res* 1967; 2: 1-6. doi:10.1111/j.1600-0765.1967.tb01989.x
15. Kaufman A, Gan R. An oral contraceptive as an etiologic factor in producing hyperplastic gingivitis and a neoplasm of the pregnancy tumor type.*Oral Surgery Oral Med Oral Pathol* 1969; 28: 666-70. doi:10.1016/0030-4220(69)90411-3
16. Jensen J, Liljemark W, Bloomquisti C. The effect of female sex hormones on subgingival plaque.*J Periodontol* 1981; 52: 599-602. doi:10.1902/jop.1981.52.10.599
17. Klinger G, Eick S, Klinger G, Pfister W, Gräser T, Moore C, et al. Influence of hormonal contraceptives on microbial flora of gingival sulcus. *Contraception* 1998; 57: 381-4. doi:10.1016/S0010-7824(98)00044-4
18. Soory M, Ahmad S. 5 alpha reductase activity in human gingiva and gingival fibroblasts in response to bacterial culture supernatants, using [14C]4-androstenedione as substrate. *Arch Oral Biol* 1997; 42: 255-62. doi:10.1016/s0003-9969%2897%2900028-9
19. Tilakaratne A, Soory M, Ranasinghe AW, Corea SMX, Ekanayake SL, De Silva M. Effects of hormonal contraceptives on the periodontium, in a population of rural Sri-Lankan women. *J ClinPeriodontol* 2000;27:753-7. doi:10.1034/j.1600-051x.2000.027010753.x
20. Mullally BH, Coulter WA, Hutchinson JD, Clarke HA. Current oral contraceptive status and periodontitis in young adults.*J Periodontol* 2007 ;78:1031-6. doi:10.1902/jop.2007.060163
21. Seck-Diallo A, Cissé ML, Benoist HM, Diouf A, Ahnoux-Kouadio A, Diallo T, et al. Periodontal status in a sample of Senegalese women using hormonal contraception. *Odontostomatol Trop* 2008;31:36-42.
22. Geivelis M, Turner DW, Pederson ED, Lamberts BL. Measurements of interleukin-6 in gingival crevicular fluid from adults with destructive periodontal disease. *J Periodontol* 1993;64:980-3. doi:10.1902/jop.1993.64.10.980
23. Taichman LS, Eklund SA. Oral contraceptives and periodontal diseases: rethinking the association based upon analysis of National Health and Nutrition Examination Survey data. *J Periodontol* 2005;76:1374-85. doi:10.1902/jop.2005.76.8.1374
24. Haerian-Ardakani A, Moeintaghavi A, Talebi-Ardakani MR, Sohrabi K, Bahmani S, Dargahi M. The association between current low-dose oral contraceptive pills and periodontal health: a matched-case-control study. *J Contemp Dent Pract* 2010;11:33-40.
25. Kalkwarf KL. Effect of oral contraceptive therapy on gingival inflammation in humans.*J Periodontol* 1978;49:560-3. doi:10.1902/jop.1978.49.11.560
26. Raber-Durlacher JE, Leene W, Palmer-Bouva CC, Raber J, Abraham-Inpijn L. Experimental gingivitis during pregnancy and post-partum: immunohistochemical aspects. *J Periodontol* 1993;64:211-8. doi:10.1902/jop.1993.64.3.211
27. Lopatin DE, Kornman KS, Loesche WJ. Modulation of immunoreactivity to periodontal disease-associated microorganisms during pregnancy. *Infect Immun* 1980;28:713-8.
28. Kinnby B, Matsson L, Astedt B. Aggravation of gingival inflammatory symptoms during pregnancy associated with the concentration of plasminogen activator inhibitor type 2 (PAI-2) in gingival fluid. *J Periodontal Res* 1996;31:271-7. doi:10.1111/j.1600-0765.1996.tb00493.x
29. Ferris M. Alteration in female sex hormones: their effect on oral tissues and dental treatment. *Compend Contin Educ Dent* 1993; 14: 1558-70. doi:10.1902/jop.1992.63.1.28
30. Miyagi M, Aoyama H, Morishita M, Iwamoto Y. Effects of sex hormones on chemotaxis of human peripheral polymorphonuclear leukocytes and monocytes. *J Periodontol* 1992; 63: 28-32. doi:10.1902/jop.1992.63.1.28
31. Tabibzadeh S, Santhanam U, Sehgal B, May T. Cytokine-induced production of IFN-beta 2/IL-6 by freshly explanted human endometrial stromal cells. Modulation by estradiol 17-beta.*J Immunol* 1989; 142: 3134-9. doi:10.1111/j.1749-6632.1989.tb24059.x
32. Gordon M, Leboff S, Glowacki J. Adrenal and gonodal steroids inhibit Il-6 secretion by human marrow cells. *Cytokine* 2001; 16: 178-86.