The Evaluation of Clinical Parameters and Gingival Crevicular Fluid Prostaglandin E_2 Level in Postmenopausal Females: A Pilot Study

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The purpose of this study was to evaluate the effects of postmenopausal alterations on clinical measurements and to assess the relationship between prostaglandine E_2 (PGE_2) level and clinical parameters after the menopause. Thirty-five postmenopausal women and 35 women who menstruated were selected. Plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) were measured. Gingival crevicular fluid PGE_2 level was determined using the enzyme immunoassay technique. The statistical tests used were Student t-test and Pearson Correlation Analysis. In the test group, the levels of PGE_2 and the mean values of GI, PPD and CAL were found to be significantly higher than in the controls, and the correlation between PGE_2 level and PPD was found to be significant ($p<0.05$). It can be speculated that clinical parameters may be affected depending on the negative effects of the menopause, and PGE_2 level may increase directly with increasing probing depth in this selected group of postmenopausal females.

Key words: menopause, GCF PGE_2 level, clinical parameters

INTRODUCTION

The relationship between changes in postmenopausal women and periodontal condition has been investigated by various studies (Groen et al, 1968; Tezal et al, 2000; Amar and Chung, 1994; Payne et al, 1997; Reinhardt et al, 1994). It has been demonstrated that women with functional disorders of the ovaries experienced increased severity of periodontal disorders together with reduced mineral density of the mandible, compared with healthy women with normally functioning ovaries (Moschil et al, 1991). Also, it was speculated in a study that the incidence of periodontitis was correlated with signs of generalised osteoporosis (Groen et al, 1968).

It was mentioned in a study that bone loss is a common feature of both osteoporosis and periodontal disease, and both diseases begin to demonstrate their effects mainly after the age of 35 (Tezal et al, 2000). Osteoporosis in postmenopausal women is not considered to be an aetiological factor in periodontal disease, but it is thought to affect the severity of pre-existing periodontitis (Amar and Chung, 1994). A study has reported a three-fold increase in the frequency of sites losing ≥ 0.4mm of interproximal alveolar bone height in estrogen-deficient postmenopausal women following active treatment of moderate/severe periodontitis, compared to estrogen-sufficent patients over a one-year period. However, the clinical changes had not been analysed (Payne et al, 1997). Also, oestrogen deficiency is thought to play a role in the progression of periodontal disease by affecting oral bone resorption, attachment and tooth loss during menopause, since it may lead to a reduc-

tion in the synthesis and maintenance of collagen (Payne et al, 1997; Reinhardt et al, 1994). In a prospective study it was shown that oestrogen supplementation reduced the signs of gingival inflammation and the frequency of clinical attachment loss in postmenopausal women (Reinhardt et al, 1994). However, little information exists regarding the influence of postmenopausal alterations on clinical features of periodontal disease compared with pre-menopausal conditions.

Prostaglandine E_2 (PGE_2), a major arachidonic acid metabolite, has been implicated as a key pro-inflammatory mediator in periodontal disease (Lamster and Grbic, 1995; Offenbacher et al, 1993; Lamster, 1997; Heasman et al, 1998). It is released locally and has many pro-inflammatory effects on periodontal tissues, including vasodilatation, enhancement of vascular permeability at sites of inflammation, release of collagenase by inflammatory cells, activation of osteoclasts and mediation of bone resorption (Lamster and Grbic, 1995; Offenbacher et al, 1984; Offenbacher et al, 1986; Genco, 1992; Page, 1991). Gingival crevicular fluid (GCF) levels of cytokines such as IL-1, and IL-6 had been investigated in postmenopausal women previously (Pacifici et al, 1990; Jilka et al, 1992; Reinhardt et al, 1994). However, we were unable to find a study that had focused on GCF PGE_2 levels in postmenopausal females.

The purpose of the present study was to evaluate the effects of postmenopausal alterations on clinical measurements, to evaluate the influence of PGE_2 levels on gingival tissues and to assess its relationship to clinical parameters in a group of Turkish women during the menopause.

STUDY DESIGN AND RESULTS

Study Population

Thirty-five postmenopausal females within at least four years of the menopause and a control group of 35 women who were experiencing a normal menstrual cycle were selected for the study. Detailed medical and dental histories of the patients revealed that they were systemically healthy, non-smokers and they had neither used any antibiotics/anti-inflammatory drugs, nor received any kind of periodontal treatment within the past three months. All subjects had attended the Menopause Clinic in the Department of Reproductive Medicine at the University of Istanbul, Faculty of Medicine, and a written informed consent was obtained prior to participation. Also, none of the women were receiving hormone replacement therapy.

Clinical Measurements

The clinical measurements and the gingival crevicular fluid collection were made with a Williams probe (Hu-Friedy, Chicago IL, USA) by the same examiner. Full mouth plaque index (PI) (Silness and Löe, 1964), Gingival Index (GI) (Löe and Silness, 1963) scores, probing pocket depths (PPD) and clinical attachment level (CAL) were recorded at six sites (mesio-facial, disto-facial, mid-facial, mesio-lingual, disto-lingual, mid-lingual) per tooth.

Gingival Crevicular Fluid Sample Collection

Sites selected for GCF sampling were isolated using cotton rolls. A gentle stream of air was directed parallel to the root surface for five to 10 seconds to dry the area. GCF samples were collected from two deepest interproximal pockets in the anterior region of each patient using standardised paper strips (Periopaper, Proflow, Amityville, NY, USA). Mesio-buccal or disto-buccal aspects of anterior teeth were selected in order to facilitate isolation from saliva. The selected area was dried for 10 seconds, then the filter paper strip was inserted into the base of the pocket until a slight resistance was felt and left in place for 30 seconds. Samples containing blood were discarded. The amount of GCF on the strips was measured by weighing the accumulated fluid. Then the paper strips were placed individually into uniquely labelled 0.5-ml microcentrifuge sterile tubes and stored under -30°C to prevent degradation of PGE_2 until the assay procedures.

PGE_2 Assay Procedures

The laboratory procedures in order to evaluate levels of PGE_2 in GCF were performed as described previously (Preshaw et al, 1998; Yalcin et al, 2002). Briefly; immediately prior to the assay, the paper strips were removed from storage and a 50 µL of corresponding assay buffer (tris buffered saline), which was provided within each kit, was pipetted onto each tube. The tubes were left at room temperature for 30 minutes and vortexed every five minutes. The aliquots of the extracted samples were used in the assay procedures, as described within

Table 1 The means and standard deviations (SD) of age and clinical parameters and the statistical significance of mean differences between groups

	Group	Means ± SD	Mean Difference (2-tailed Sig.)
AGE	Test	49.69 ± 4.22	p > 0.05
	Control	48.63 ± 2.91	
PI	Test	1.65 ± 0.55	p > 0.05
	Control	1.73 ± 0.48	
GI	Test	1.56 ± 0.72	p > 0.05
	Control	1.05 ± 0.66	
PPD	Test	2.48 ± 1.05	p > 0.05
	Control	2.00 ± 0.75	
CAL	Test	2.71 ± 1.15	p > 0.05
	Control	2.12 ± 0.73	
PGE_2 (ng/mg)	Test	52.36 ± 11.31	p > 0.05
	Control	33.73 ± 7.14	

n = 35 in the test and control groups.

the kit instructions. Finally, enhancement solution was added to stop the reaction, and the optical density of the color produced was immediately measured using a microplate reader at 405. The level of PGE_2 in GCF samples was determined using optical density values and expressed as ng/ml. Mouth median GCF PGE_2 levels were calculated as the summary statistic for the individual patient.

STATISTICAL ANALYSIS

In the present study, the patient was used as the unit of observation. The differences between two groups regarding plaque index, gingival index, probing pocket depths and GCF PGE_2 levels were evaluated using Student t-test. The effect of PGE_2 levels on clinical parameters was evaluated using Pearson Correlation Analysis. Statistical analysis of the study was carried out using a software program (SPSS for Windows, Release 10.0, 1999, SPSS Inc., USA) and statistical significance was established at $P<0.05$.

The mean age and clinical data including means of plaque index, gingival index, probing pocket depth scores and PGE_2 levels of the test and control groups are shown in Table 1. The mean age of the test and control groups did not differ from each other ($p>0.05$) (Table 1).

There was no statistical significance between the groups regarding plaque index ($p>0.05$) (Table 1). In the test group, the mean values of GI, PPD and CAL were found to be significantly higher than the controls ($p<0.05$) (Table 1). The levels of PGE_2 in GCF were significantly higher in the test group compared with the controls ($p<0.05$).

The effect of GCF PGE_2 level on clinical parameters was evaluated using Correlation Analysis, and only the correlation between GCF PGE_2 level and PPD in the test group was found to be statistically significant ($P<0.05$) (Table 2).

Table 2 The correlation between PGE2 levels and clinical parameters in the test and control groups.

	Control Group				Test Group			
r	PI	GI	PPD	CAL	PI	GI	PPD	CAL
PGE_2	0.22	0.34	0.47	0.44	0.06	0.32	0.57*	0.29

** Statistical significance at P < 0.05.*

DISCUSSION

In the light of previous studies, oestrogen deficiency is considered to be involved in the progression of periodontal disease during postmenopausal period (Payne et al, 1997; Reinhardt et al, 1994). However, information about the effects of postmenopausal alterations on clinical signs of periodontal disease compared with the premenopausal condition is limited. The present study was designed to evaluate the influence of postmenopausal alterations on the periodontal condition, and mean values of GI, PPD and CAL were found to be significantly higher in postmenopausal females. Since the differences between the mean age of the test and control groups did not represent statistical significance, all subjects were systemically healthy and non-smokers and none of them were receiving oestrogen supplementation, the test and control groups can be considered to be matched. For this reason, this finding may be due to the possible negative effects of the menopause. Although the clinical changes had not been analysed, another study has reported a three-fold increase in the frequency of sites losing alveolar bone height following active periodontal treatment in oestrogen-deficient postmenopausal women over a one-year period (Payne et al, 1997). Reinhardt et al (1994) further analysed the influence of oestrogen on clinical periodontitis in postmenopausal women and reported a reduction in the signs of gingival inflammation and the frequency of clinical attachment loss caused by oestrogen supplementation. In order to achieve the purpose of our study, the postmenopausal women in the test group were not receiving hormone replacement therapy and women having a menstrual cycle served as the control group. Therefore, we did not have the chance to compare clinical data of the two studies.

The level of PGE_2 in GCF were significantly higher in the postmenopausal female group compared with the controls. Since the mean values of GI, PPD and CAL were significantly higher in the test group and the role of PGE_2 in gingival inflammation has been well established, this finding may not be surprising. Also, in the test group the elevation in GCF levels of PGE_2 accompanied an increase in probing depths. Because of these findings we suggest that levels of PGE_2 in GCF might be used as a marker of gingival inflammation in order to determine the effects of periodontal therapy in the menopause. However, we were unable to find a study on GCF PGE_2 levels in postmenopausal females that could provide us with more information.

The data from this pilot study lead us to speculate that the corresponding impact of hormonal changes on periodontal status during the menopause, makes proper periodontal evaluation a prerequisite. The possible effects of the menopause must be taken into consideration during evaluation of periodontal findings in women. Also, the cooperation between menopause clinics and periodontology departments may be helpful in the prevention of severe periodontal conditions in postmenopausal women, considering cost-benefit accounts.

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