The Influence of Anxiety on Gingival Inflammation, Attachment Level and Inflammatory Markers in GCF from Subjects with Periodontal Disease

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Purpose: The aim of this study was to determine if self-reported anxiety had an association with gingival inflammation and attachment level (AL), and with the amounts of prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β) and elastase in gingival crevicular fluid (GCF).
Material and methods: The participants were 51 subjects with adult chronic periodontis (26 men and 25 women), with a mean age of 53.5 (±2.9 SD) years (48-57 years). The subjects were clinically examined and answered questions regarding anxiety in everyday life, as well as smoking habits.
GCF was collected with an intracrevicular washing technique from four sites in each subject. Analysis of variance (ANOVA) and non-parametric Mann Whitney U-test were used as the statistical methods.
Results: Anxious smokers had an average GI of 2.1 (±0.7 SD) compared to the non-anxious smokers 1.3 (±0.9 SD), $p<0.05$. Attachment loss was significantly more pronounced in anxious smokers than in non-anxious smokers, $p<0.05$. No association between self-report anxiety and inflammatory markers in GCF was found.
Conclusion: The present study showed no differences between anxious and non-anxious subjects with periodontitis in relation to the range of biochemical inflammatory markers. Anxious smokers had significantly more gingival inflammation and attachment loss than non-anxious smokers.

Key words: anxiety, gingival inflammation, periodontitis, smoking, inflammatory markers

INTRODUCTION

During the past decade psychological stress has been associated with an increased risk for periodontal disease (Kurer et al, 1995; Linden et al, 1996; Croucher et al, 1997; Deinzer et al, 1998; Genco et al, 1999; Hugosson et al, 2002; Pistorius et al, 2002; Vettore et al, 2003). While the mechanism for a possible association is poorly understood and has not yet been securely established, several mechanisms of action have been suggested. One possibility is an over-production of pro-inflammatory mediators in the local inflammatory lesions (Genco et al, 1998). Pro-inflammatory mediators such as prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β) and tumor necrosis factor (TNFα) have been associated with an increased risk of periodontal disease (Hart and Kornman, 1997). PGE_2, has been implicated both in bone loss and in loss of attachment (Offenbacher

et al, 1986). A series of studies have demonstrated a higher level of IL-1β in gingival crevicular fluid (GCF) in subjects with experimental gingivitis (Kinane et al, 1992) and in periodontally diseased sites as compared to healthy sites (Preiss and Meyle 1994; Figueredo et al, 1999).

The activation of these pro-inflammatory mediators may also affect and increase neutrophil activity, and thus lead to an increased release of elastase, which can be followed by destruction of connective tissue matrix (Champagne et al, 2003). Increased levels of elastase in GCF from gingivitis and periodontitis sites have been reported (Palcanis et al, 1992; Gustafsson et al, 1994; Söder, 1999; Gonzales et al, 2001). The correlations between stress, smoking and levels of IL-1β, IL-4, IL-6 and IL-8 in GCF have also been investigated with varying results. Academic stress increased the level of IL-1β in GCF both under perfect oral hygiene conditions and during plaque accumulation (Deinzer et al, 1999; Waschul et al, 2003). In a study by Giannopoulou et al (2003a) it was found that pocket depth was significantly related to the amounts of all four cytokines, but stress was mostly associated with increased levels of IL-1β, IL-6 and IL-8 levels. On the other hand, Mengel et al (2002) reported no correlation between psychosocial stress and levels of IL-1β and IL-6, in serum. Although associations have been established between levels of these inflammatory markers and presence of periodontal disease, the relationship between these markers and stress/anxiety is, however, still unclear.

In a recent study (Solis et al, 2004) no association between anxiety, stress, depression and periodontitis was found. We have recently shown in an epidemiological study that anxious smokers had significantly more sites with pockets ≥ 5mm, compared to non-anxious smokers (Johannsen et al, 2005). Anxiety, in the present study was assessed by one single question, thereby adjusting to a clinical situation where specialized questionnaires are too time consuming.

The aim of the present study was to determine if self-reported anxiety had an association with gingival inflammation and attachment level (AL) and with the amounts of prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β) and elastase in gingival crevicular fluid (GCF).

MATERIALS AND METHODS

Subjects

The participants were 51 subjects with adult chronic periodontitis (26 men and 25 women), with a mean age of 53.5 (±2.9 SD) years (range 48–57 years). The subjects were randomly selected from a group of patients with periodontitis identified in Söder et al (1994). The criterion of periodontal disease was based on probing depth and the subjects had at least four interproximal sites with ≥ 5mm in at least two different teeth. The subjects were in good general health as assessed by a health questionnaire. None had received antibiotics during the previous six months. Subjects with self-reported psychiatric disorder or use of psychotropic medications were not included.

Ethics

This study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden. The subjects gave their informed consent to participate in the study.

Questionnaire

Before the clinical examination all subjects filled in a 78-items questionnaire. This concerned their last visit to the dentist, self-reported evaluation of periodontal health, smoking habits, marital status and use of dental home care devices. The questionnaire contained one single question concerning anxiety -namely: "Do you feel anxious in your every day life?" - with the response alternatives (1) no, never, (2) yes, sometimes and (3) yes, often. None of the subjects reported that they were often anxious. The two anxiety response categories were therefore collapsed into one "anxious" category, while all those who reported no anxiety were classified as "non-anxious". The questionnaire also included a question concerning depression – namely: "Do you feel depressed?" – with the response alternative (1) no, (2) yes. Two subjects (women) reported that they were depressed and were excluded from the study.

Subjects were classified as smokers or non-smokers. Smoking was quantified by the number of cigarettes smoked per day.

Clinical Examination

The clinical examination included presence of dental plaque on lingual and buccal surfaces, gin-

gival index (GI) (Löe, 1967) and the number of remaining teeth, excluding third molars. Probing depth (PD) and clinical attachment level (CAL) were measured to the nearest mm, using a standard probe (Hu-Friedy, USA) graded at 2mm intervals and with a tip diameter of 0.5mm. All the teeth were probed at six sites for each tooth - mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual and mid-lingual. AL was measured with a probe from the cemento-enamel junction (CEJ). Bleeding on probing (BOP) was assessed by probing intracrevicularly, using a probe with a tip diameter of 1 mm (Hu-Friedy, USA). Bleeding within 60 seconds was recorded as "bleeding on probing". The occurrence was expressed as a percentage of bleeding teeth per patient. One examiner performed all measurements.

Gingival Crevicular Fluid Sampling

In order to avoid a selective retention of GCF in paper strips the samples were collected with an intracrevicular washing technique (Salonen et al, 1991), modified with a quantitatively controlled delivery system (Compu-Pet $^{800.}$, Alphamedics, NJ, USA) and a peristaltic pump for aspiration (Pharmacia, Uppsala, Sweden) (Jin et al, 1995). The sites to be sampled (second premolar in each quadrant) were isolated with cotton rolls, gently air-dried and supragingival plaque carefully removed. The ejection needle of the instrument was gently inserted into the crevice to a level 1mm below the gingival margin. The gingival pocket was then flushed with an aliquot of 15µl of phosphate buffered saline (PBS, pH 7.4) and simultaneously drained through the collection needle into Eppendorf tubes by constant suction (flow rate 25ml/h). The gingival washings were diluted up to a final volume of 500µl. The samples were immediately centrifuged (8000 g) for 5 min at 4°C, and then the supernatants were frozen at –70°C pending analysis. The results of the GCF analyses are presented as amounts per site.

Assay of PGE2

Gingival crevicular fluid supernatants (30 µl) were assayed for PGE2 levels by radioimmunoassay (^{125}I RIA Kit, E.I. Du Pont de Nemours & Co., Inc., NEN® Research Products, Boston, MA, USA), according to the manufacturer's instructions. The levels of PGE2 were determined as total amount per site (pg/site).

Assay of IL-1β

IL-1β was measured with ELISA Quantikine HS Immunoassay Kits (R & D Systems Europe Ltd, Oxon, UK) according to the manufacturer's instructions manual. The levels of IL-1β were determined as total amount per site (pg/site).

Assay of Neutrophil Elastase Activity

Neutrophil elastase activity was measured with a low molecular weight chromogenic substrate specific for neutrophil elastase, L-pyroglutamyl-L-prolyl-L-valine-p-nitroanilide (SA-2484, Haemochrone Diagnostica AB, Mölndahl, Sweden) (Kramps et al. 1983). The activity was expressed as absorbance/site.

Statistical Analysis

Analysis of variance (ANOVA) and differences between data sets with a probability of less than 0.05 were regarded as significant, and means ±SD were given. The data analysis was performed using the statistical packages of Stat View 5.0.1 (SAS Institute Inc. SAS Campus Drive Cary, NC 27513, USA). The differences between clinical data and inflammatory markers of anxious and non-anxious subjects, among all participants and among smokers and non-smokers, were statistically calculated by using the non-parametric Mann-Whitney U-test, due to the wide variations in standard deviations.

RESULTS

The mean values and standard deviation (SD) of the clinical data in non-anxious-, anxious-, non-smoking and smoking subjects are shown in Table 1. There were 24 non-anxious subjects and 27 anxious subjects, and 21 were smokers. The non-anxious smokers (n=7) had smoked in average 29.9 (±6.9 SD) years and smoked 10.9 (±6.8 SD) cigarettes/day. The anxious smokers (n=14) had smoked an average 29.6 (±12.1 SD) years and smoked 14.7 (±7.1 SD) cigarettes/day. There were 23 former smoker, but since no one of them had smoked for five years, they were classified as non-smokers.

The anxious smokers had an average GI of 2.1 (±0.7 SD) compared to non-anxious smokers' 1.3 (±0.9 SD). The difference was statistically significant, $p<0.05$ (Table 1). Attachment loss was sig-

Table 1 Mean values (± standard deviation) of plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), number of pockets depth ≥ 5 mm and attachment level (AL) in non-anxious and anxious and non-smoking and smoking subjects with periodontitis.
n = number of subjects

	Non-Anxious		Anxious			Non-Anxious Non-Smokers		Anxious Non-Smokers			Non-Anxious Smokers		Anxious Smokers		
Parameters	n=24 Mean ≥ SD		n=27 Mean ± SD		p-value	n=17 Mean ± SD		n=13 Mean ± SD		p-value	n=7 Mean ± SD		n=14 Mean ± SD		p-value
PLI	0.7	(0.6)	1.0	(0.7)	NS	0.8	(0.6)	1.1	(1.6)	NS	0.5	(0.5)	0.9	(0.7)	NS
GI	1.4	(0.8)	1.8	(0.9)	NS	1.5	(0.7)	1.6	(0.9)	NS	1.3	(0.9)	2.1	(0.7)	0.05*
BOP	45.0	(20.4)	44.9	(31.6)	NS	47.6	(22.5)	46.4	(31.9)	NS	38.8	(13.6)	41.8	(32.2	NS
PD	2.9	(0.5)	3.1	(0.8)	NS	2.9	(0.5)	2.8	(0.5)	NS	2.9	(0.4)	3.5	(0.8)	NS
Number of PD ± 5 mm	15.1	(9.6)	18.7	(17.3)	NS	15.1	(10.3)	12.6	(9.6)	NS	15.1	(8.5)	24.4	(20.9)	NS
AL	3.6	(0.8)	3.9	(1.1)	NS	3.8	(0.9)	3.7	(0.8)	NS	3.2	(0.5)	4.2	(1.3)	0.05*

* Significance of differences between non-anxious and anxious smokers, calculated with the non-parametric Mann-Whitney U-test
NS = not significant

nificantly more pronounced in anxious smokers than in non-anxious smokers, $p<0.05$ (Table 1). The amount of dental plaque, probing depths and number of sites with pocket depths ≥ 5mm were higher in anxious smokers compared to non-anxious smokers, but the differences were not significant.

The results of the inflammatory marker measurements in non-anxious-, anxious-, non-smoking and smoking subjects are shown in Table 2. No statistically significant differences were found.

DISCUSSION

The objective of this study was to determine if self-reported anxiety had an association with gingival inflammation and attachment level and on inflammatory markers in GCF. No significant differences were observed in any of the biochemical inflammatory markers, but anxious smokers showed significantly more gingival inflammation and attachment loss than non-anxious smokers. This is in agreement with Mengel et al (2002) who did not find any correlation between between IL-1β and stress, but in disagreement with Giannopoulou et al (2003a) who reported increased levels of IL-1β in GCF in the presence of stress in subjects with various degrees of periodontal disease. One explanation for this discrepancy could be the different instruments for assessing anxiety/stress. In our study we used one single question regarding anxiety, and Mengel et al (2002) used a few questions concerning stress, while Giannopoulou et al (2003a) assessed stress with a standardized questionnaire. It should be noted that in the present study no differentiation between different kinds of anxiety was made. The purpose was to find out if the mere feeling of anxiety in a patient can have an influence on periodontal disease. The question concerning anxiety, in a clinical setting, can thus be successfully used to identify patients with an increased risk of periodontal disease, indicating different needs for periodontal maintenance care.

It is well known that the levels of the biochemical inflammatory markers in GCF are increased in subjects with gingivitis or periodontal destruction. The amounts of PGE_2 and elastase have not earlier, to our knowledge, been analysed in relation to stress. In our study no significant associations between inflammatory markers and anxiety were

Table 2 Mean (± standard deviation) amounts of prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β) and mean activity of elastase in non-anxious and anxious and non-smoking and smoking subject with periodontitis.
n = number of subjects

		Non-Anxious		Anxious			Non-Anxious Non-Smokers		Anxious Non-Smokers			Non-Anxious Smokers		Anxious Smokers		
	Unit	n=24 Mean ± SD		n=27 Mean ± SD		p-value	n=17 Mean ± SD		n=13 Mean ± SD		p-value	n=7 Mean ± SD		n=14 Mean ± SD		p-value
PGE_2	pg/site	9.9	(20.3)	5.4	(10.9)	NS	13.0	(23.5)	7.5	(2.1)	NS	2.5	(3.4)	3.4	(9.6)	NS
IL-1β	pg/site	13.0	(12.9)	11.0	(9.9)	NS	12.7	(14.8)	9.0	(8.3)	NS	13.5	(7.3)	12.4	(11.3)	NS
Elastase	abs/site	0.041	(0.06)	0.059	(0.1)	NS	0.042	(0.07)	0.083	(0.15)	NS	0.037	(0.02)	0.037	(0.02)	NS

NS = not significant

found. The lack of correlation is due to the fact that the clinical findings are based on all sites while the GCF samples only were taken from four fixed sites (mesial surface of each second premolar). It should be borne in mind that the subjects in our study were assessed by self-reported anxiety and maybe there is a need for individuals with a higher degree of psychological disturbance with a diagnostic criterion to confirm a relationship. However, looking at the clinical data, it can be seen that the anxious smokers showed significantly higher gingival inflammation and attachment loss than non-anxious smokers, and there was also a tendency towards more sites with probing with ≥ 5mm in those subjects.

Smoking is a major risk factor that contributes to the pathogenesis of periodontitis. In the present study, the amounts of IL-1β do not differ between smoking and non-smoking subjects, and this is in agreement with other studies (Giannopoulou et al, 2003b; Rivera-Hidalgo, 2003; Kamma et al, 2004), which reported no association between IL-1β and smoking status.

The anxious smokers in the present study showed significantly more gingival inflammation, compared to non-anxious smokers. This is in line with an earlier study from the group (Johannsen et al, 2005) as well as Kurer et al (1995), where an association was demonstrated between anxiety and gingival inflammation. In the present study, attachment loss was also significantly more pronounced in anxious smokers than in non-anxious smokers, and this is similar to Genco et al (1999) who reported that increased attachment loss was significantly associated with smoking and also with depression. However, in our study the influence of smoking on attachment level could be difficult to interpret, since almost all subjects had been smokers. These findings suggest that risk factors such as anxiety and smoking may increase the possibility of destruction of the tissue, which may subsequently reduce the resistance to plaque and gingival inflammation, thus leading to periodontal disease. Anxiety may also induce behavioural changes, such as poor oral hygiene and more smoking, which can influence periodontal disease directly. Further studies are required to determine whether the possible association between anxiety and an increased risk for periodontitis is due to neglect of oral hygiene or to differences in the inflammatory response pattern.

In conclusion, the present study showed no differences between anxious and non-anxious subjects with periodontitis in relation to range of biochemical inflammatory markers. Anxious smokers had significantly more gingival inflammation and attachment loss than non-anxious smokers.

Acknowledgement

We thank Prof. M Åsberg, Department of Clinical Neuroscience, Karolinska Institutet, Huddinge, Sweden, for her kind assistance and generous support. This study was supported by the Swedish Patent Revenue Research Fund (2003) and the Karolinska Institute.

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