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ANXIETY, EXHAUSTION AND DEPRESSION IN RELATION TO PERIODONTAL DISEASES

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ABSTRACT

Periodontal diseases (gingivitis and periodontitis), are inflammatory processes of the gingiva and supporting structures of the teeth induced by a microbial biofilm. In periodontitis this inflammatory process causes tissue degradation and eventually tooths loss. Initiation and progression of periodontitis are due to a combination of genetic factors and environmental factors *e.g*, neglect of oral hygiene, stress/anxiety/depression, smoking and systemic diseases.

The present thesis includes four studies which all aim at increased understanding of the influence of anxiety and depression on periodontal diseases.

The first specific aim of these studies was to investigate the influence of anxiety on gingival inflammation and periodontal disease in non-smokers and smokers (*Study* I). Secondly we wanted to determine if self-reported anxiety had an association with gingival inflammation and attachment level (AL), as well as with the levels of prostaglandin E_2 (PGE_2), interleukin 1β (IL-1 β) and elastase in gingival crevicular fluid (GCF) in subjects with periodontitis (*Study* II). The third aim was to investigate the importance of stress for the development of periodontitis by comparing oral health status, pro-inflammatory markers, and cortisol in GCF and saliva in patients with stress-related mental depression and in non-depressed controls (*Study* III). The final aim was to investigate periodontal status in relation to inflammatory markers and cortisol in GCF and saliva in women on longterm sick-leave for job-stress related depression compared to non-depressed women (*Study* IV).

The findings of all of these studies revealed that the gingival inflammation was significantly elevated in anxious subjects (*Studies* I, II) and in the depressed patients (*Studies* III, IV).

Anxious smokers with periodontitis had significantly more sites with pockets ≥ 5 mm than non-anxious smokers with periodontitis (*Study* I). Furthermore, anxious subjects with aggressive periodontitis showed more sites with probing depth ≥ 5 mm compared to non-anxious subjects in the same group.

Anxious smokers with periodontitis exhibited a significantly higher degree of loss of attachment than the non-anxious smokers with periodontitis (*Study* II). The levels of PGE_2, IL-1β and elastase in the GCF of these two groups did not differ significantly.

The depressed women in *Study* III had significantly more amount of dental plaque and the depressed women in *Study* IV had significantly more deep pockets compared to the non-depressed women. The levels of interleukin-6 (IL-6) in GCF were significantly higher in the patients in both studies compared to controls, while the levels of IL-1β did not differ between the groups. In *Study* III, the cortisol level in GCF was increased, whereas the patients in *Study* IV surprisingly showed lower cortisol values in GCF than the controls. The level of cortisol in saliva was similar in both groups. Neither did the levels of matrix metalloproteinases-8, -9 (MMP-8, MMP- 9) differ from the control group (*Study* IV), however, the control group reported unexpectedly higher levels of MMP-9 than the depressed patients in *Study* III.

In conclusion, the present thesis findings altogether strengthen the hypothesis that anxiety and depression play an important role in the progression of periodontal diseases. We suggest that this influence is due not only due to behavioural changes, but also to a direct influence on the immune system.

Keywords: anxiety, stress, depression, gingival inflammation, periodontal diseases, smoking, inflammatory markers, cortisol, HPA axis

LIST OF PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text with their roman numbers (I-IV).

I. Johannsen A, Åsberg M, Söder P-Ö, Söder B. Anxiety, gingival inflammation and periodontal disease in non-smokers and smokers – An epidemiological study. J Clin Periodontol. 2005 May;32(5):488-91.

II. Johannsen A & Söder B. The influence of anxiety on gingival inflammation, attachment level and inflammatory markers in GCF from subjects with periodontal disease. Perio 2005;2:91-97.

III. Johannsen A, Rylander G, Söder B, Åsberg M. Dental plaque, gingival inflammation and elevated levels of IL-6 and cortisol in gingival crevicular fluid from women with stress-related depression and exhaustion – J Periodontol in revision

IV. Johannsen A, Rydmark I, Söder B, Åsberg M. Gingival inflammation, increased periodontal pocket depth and elevated IL-6 in gingival crevicular fluid in depressed women on longterm sick-leave – in manuscript

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BOP	Bleeding on probing
CAL	Clinical attachment level
CNS	Central nervous system
CEJ	Cemento-enamel junction
CRH	Corticotropin-releasing hormone
DSM	Diagnostic and Statistical Manual of Mental Disorders
ELISA	Enzyme linked immunosorbant assay
GI	Gingival index
GCF	Gingival crevicular fluid
HPA	Hypothalamus-pituitary-adrenal
ICD	International Classification of Diseases
IL-1β	Interleukin-1β
IL-6	Interleukin-6
LPS	Lipopolysaccarides
LTSL	Longterm sick-leave
MMP-8	Matrix metalloproteinases-8
MMP-9	Matrix metalloproteinases-9
PGE_2	Prostaglandin E_2
PMN	Polymorphonuclear
PPD	Probing pockets depth
SCID	Structured Clinical Interview
SSRI	Selective serotonin uptake inhibitors
TNFα	Tumor necrosis factor α

INTRODUCTION

The goal of periodontal diagnosis, treatment planning and therapy is to reduce the risk for future progression of periodontitis (Page & Beck 1997). Many clinicians, dental hygienists and dentists, regard psychological and psychosocial factors to be of great importance for oral hygiene behaviour and thus for progression of periodontal diseases (Sheiham & Nicolau, 2005).

Several factors have today been identified as possible risk factors for periodontal disease, *e.g.* dental plaque, genetic predisposition, systemic disease, smoking, and stress/ anxiety/ depression (LeResche & Dworkin 2002). The influence of stress and depression has been discussed, but the role that stress plays in the individual's susceptibility to periodontal diseases is not yet established.

This thesis focuses on the relationship between anxiety / depression and periodontal disease in order to investigate if anxious subjects and depressed patients are more prone to develop gingivitis and/or periodontitis compared to controls.

DEFINITIONS - PSYCHOLOGICAL ASPECTS

Anxiety

Anxiety may be defined as apprehension, fear or physical tension elicited by an anticipated danger or misfortune, whether external or internal. Individuals vary in their proneness to react with anxiety to real or imagined threats, and this anxiety proneness is partly constitutional and partially a personality *trait* (SBU, 2005). An anxiety prone individual is thus more likely to react with *state* of anxiety in a wide range of circumstances. Anxiety can also be conceptualized as a symptom which occurs in many mental disorders, including depression and psychosis (SBU, 2005).

There are two major systems for diagnosing mental disorders, the International Classification of Diseases (ICD) issued by the World Health Organization (WHO, 1992) and the Diagnostic and Statistical Manual of Mental Disorders (DSM), issued by the American Psychiatric Association (APA, 2000). Both systems contain a class of anxiety disorders *i.e.,* psychiatric conditions where anxiety is the most prominent symptom. In *Studies* I and II in this thesis, however, the focus is on lower degrees of anxiety, such as anxiety proneness in every day life.

Depression

Depression is defined as pervasive, protracted periods of despondency, feelings of meaninglessness and a sense of hopelessness (SBU, 2004). A diagnosis of depression currently requires that the patient's professional or personal life has been affected. Depression is among the leading causes of ill health, loss of productivity and disability worldwide (SBU, 2004).

Diagnostic criteria for depression are, according to the ICD-10 and the DSM-IV, depressed mood, weight loss or weight gain, insomnia or hypersomnia, fatigue or loss of energy, feelings of insufficiency or guilt, thoughts about death and suicide, among other symptoms (APA, 2000). In Europe, North America and Australia, 5-25 percent of women and 3-10 percent of men suffer at least one depressive episode during their lives (SBU, 2004). In Sweden, the total percentage of mental ill-health has almost doubled from the mid-nineties until 2002, with depression, anxiety and persistent fatigue as dominating symptoms (Socialstyrelsen, 2003).

Exhaustion disorder

Recently, there has been a dramatic increase in long term sick-leave due to mental disorder in Sweden. Although most of these patients have symptoms of depression at one time or another, the background is often a state of profound exhaustion, brought about by stress, most often work related. Exhaustion disorder (utmattningssyndrom) is the officially preferred term (Socialstyrelsen 2003).

Diagnostic criteria have been formulated and accepted by the Swedish Board of Health and Welfare (Socialstyrelsen 2003). The criteria are: physiological or mental symptoms of exhaustion for at least two weeks, an essential lack of psychological energy, and symptoms such as difficulties to concentrate, decreased ability to cope with stress, irritability or emotional lability, sleep disturbances, aches and pains, dizziness, palpitations. The symptoms must be present every day during a two-week period. The symptoms must cause significant clinical suffering, with impaired working capacity, and the symptoms must not be related to other psychiatric diagnoses, substance abuse or medical conditions (Socialstyrelsen, 2003).

Stress

Stress is a slightly ambiguous concept, but in the research context it usually refers to the physiological and psychological reactions that mobilize an organism's defence against external or internal threats to integrity (stressors).

The stress reaction includes activation of the hypothalamus-pituitary-adrenal cortex (HPA) axis with release of corticotropin-releasing hormone (CRH) from the hypothalamus, and of glucocorticoids, including cortisol, from the adrenal cortex (Chrousos 1995). Glucocorticoids may suppress the immunological activation of leukocytes, and inhibit the production of cytokines such as IL-1 β, IL-6 and other mediators of inflammation (Chrousos 1995).

PERIODONTAL DISEASES

Prevalence

Gingivitis affects 20-90 percent of all adults in the world, depending on how gingivitis is defined, and the prevalence varies by age, gender and race (Brown & Löe 1993, Albandar & Rams 2002, Pihlström et al. 2005). Gingivitis, the mildest form of periodontal disease, is caused by accumulation of dental plaque (biofilm) on teeth at a location adjacent to the gingiva. However, gingivitis does not affect the underlying supporting structures of the teeth, and it is reversible.

Periodontitis is a chronic inflammatory disease characterized by destruction of gingival connective tissue, periodontal ligaments, and alveolar bone (Hugoson et al. 1998). The prevalence of periodontitis is estimated to 13-35 percent of the adult population in industrialized countries and approximately 5-8 percent suffer from severe/aggressive periodontitis depending on the definition and severity (Papapanou 1996, Hugosson et al. 1998, Albandar et al. 1999, Sheiham & Netuveli, 2002, Hugosson et al. 2005).

Etiology

Gingivitis

Bacterial biofilms are primary etiological factors for the initiation of gingival inflammation and subsequent destruction of periodontal tissues (Haffajee & Socransky 1994). Both Löe et al. (1965) and Theilade et al. (1966) demonstrated that dental plaque causes gingivitis, and withdrawal of toothbrushing for 28 days resulted in accumulation of plaque on the teeth and development of gingivitis in all periodontally healthy subjects studied, within 10-21 days. Furthermore, removal of the bacterial plaque and a re-establishment of normal oral hygiene procedures resulted in a healthy gingiva after approximately one week. It is also apparent that not all cases of gingivitis progress to periodontitis (Brown & Löe 1993, Prayito et al. 1993). In addition to dental

plaque, hormonal fluctuations, drugs, systemic diseases and malnutrition, can also contribute to the development of gingivitis (Mariotti 1999, Tarakis & Trombelli 2004).

Periodontitis

In contrast to our relative understanding of the etiology of gingivitis, the etiology of periodontitis is more complex, involving both genetic and environmental factors. The risk factors for onset and progression of this inflammatory process involve cigarette smoking, extensive stress, inadequate coping behaviour and low socio-economic status, as well as systemic disease (Page & Beck 1997, Kornman & di Giovine 1998, Norderyd et al. 1999, Hanson & Persson 2003, Shapira et al. 2005, Klinge & Nordlund 2005, Sheiham & Nicolau 2005, Kinane et al. 2006).

Pathogenesis

Gingivitis

Gingivitis induced by dental plaque involves inflammation of the gingiva caused by bacteria present at the gingival margin (Albandar & Tinoco 2002, Sheiham & Netuveli 2002). In the early stages the supragingival bacterial plaque contains primarily gram-positive aerobic species, *e.g, Streptococcus* and *Actinomyces*. Subsequently this composition changes to a more anaerobic, gram-negative flora which also increases in time (Socransky & Haffajee 2005). The host mounts an inflammatory response to this microbial challenge and the flow of gingival crevicular fluid is increased**.** Associated clinical changes involve increased edema, reddening in colour and an increased tendency to bleeding in response to mechanical probing. Moreover, alterations in the permeability of blood vessel walls occur, resulting in an enhanced flow of sulcular fluid (Mariotti 1999).

The development and progression of plaque-induced gingival inflammation can be influenced substantially by systemic factors, both inherited and related to the environment (Mariotti 1999, Tarakis & Trombelli 2004). For instance, the gingiva is a target for steroid hormones, which can exacerbate gingivitis during periods of hormonal fluctuation, *e.g.*, puberty, the different stages of the menstrual cycle, pregnancy and menopause (Mariotti 1994, Bimstein & Mattson 1999, Sorry 2000, Tilakaratne 2000). However, the use of hormonal oral contraceptives has not increased the prevalence of gingivitis (Taichman & Eklund, 2005) and supplementation of post-menopausal women with estrogens has actually been associated with reduced gingival inflammation (Norderyd et al. 1993, Reinhardt et al. 1999).

Periodontitis

Periodontitis, a chronic inflammatory response to the subgingival bacteria, produces irreversible destruction of periodontal tissue and tooth loss. This condition is diagnosed clinically by loss of attachment between the tooth and the supporting tissues, thus creating a pocket between the root of the tooth and the supporting tissues, and/or by radiography showing bone loss (Page 1998). The main causative factor is the formation of a microbial biofilm at the gingivocervical margin, which evokes an inflammatory response in the gingival tissue, which progresses deeper into the periodontal tissue (Axelsson et al. 2002).

Oral bacteria gather and coaggregate in colonies on the tooth surface, first supragingivally and thereafter subgingivally. With time, Gram-negative anaerobic microorgansims *e.g., Treponema denticola, Porphyromonas gingivalis, Fusobacterium nucleatum,* and *Tannerella forsythensis (former Bacteroides forsythus.)* become more prevalent in the subgingival plaque, thereby enhancing its pathogenicity (Darveau et al. 1997, Ximenez-Fyvie et al. 2000). The "red complex" that appears later in connection with the development of the biofilm consists of species that are considered to be periodontal pathogens, namely, *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythensis,* which occur more commonly in deeper pockets (Socransky & Haffajee 2005).

The ecological plaque theory proposes that the increasing quantity of plaque provides an appropriate environment for colonization by and growth of more pathogenic bacteria (Marsh 1994). In this manner, the dynamic relationship whereby the inflammatory response results in environmental changes subgingivally, produces a shift in the balance of the resident microflora which changes the gingivally healthy situation to gingivitis. Bacteria then move further through the junctional epithelium and into underlying tissues, thereby predisposing to periodontal disease (Marsh 1994). Dental plaques/biofilms consist of a community or ecosystem of diverse microorganisms growing on the surface of the teeth in an extracellular matrix containing polymers of both host and bacterial origin (Filoche et al. 2004, Marsh 2005, Socransky & Haffajee 2005).

Host response

The periodontal pathogens present in the subgingival biofilm are capable of releasing a number of products including lipopolysaccarides (LPS) and proteases, metabolic and toxic products, which can invade the host defence mechanisms and

change or inhibit the immune/inflammatory response (Teng 2003, Madianos et al. 2005). The inflammatory responses activate immune cells such as neutrophils and monocytes/macrophages, which are primary protective components of the first-line defence (Teng 2003). The LPS of gram-negative bacteria are known for their ability to stimulate macrophages/monocytes to produce pro-inflammatory cytokines, *e.g*, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), prostaglandin E_2 (PGE_2) and interleukin-6 (IL-6) (Page 1991, Madianos et al. 2005).

These cytokines and inflammatory mediators can stimulate alveolar bone resorption and thereby be deleterious to the host (Teng 2003, Madianos et al. 2005). These factors are responsible for the development of local inflammatory processes, including increased vascular permeability followed by the migration of polymorphonuclear (PMN) leucocytes, monocytes and lymphocytes through the capillary walls, an increase in the flow rate of gingival crevicular fluid, and loss of connective tissue and swelling (Shapira et al. 2005). In addition, neutrophils, which constitute the first level of defence against bacteria and their virulence factors (Kantarci et al. 2003**),** may be activated to release certain mediators, such as oxygen radicals and proteolytic enzymes, which can directly induce tissue damage.

The mechanisms underlying the progression of gingivitis into periodontitis have not yet been established. Possibly, the bacteria themselves and/or their products (*e.g.*, LPS) acquire the ability to penetrate deeper into the connective tissue, and/or perturbation of the host defences allows deeper penetration (Graves & Cochran 2003).

RISK FACTORS

The term "risk factor" is used to refer to an aspect of lifestyle, an environmental situation, or an inborn or inherited characteristic, that is associated with a given disease. Risk factors may be part of the causal chain in a disease and /or may predispose the host to develop the disease. An individual exposed to one or more risk factors demonstrates an elevated probability of developing the disease and/or a more severe form of the disease.

Ageing

Increasing age has been associated with the prevalence, extent and severity of periodontitis and certain studies indicate that ageing is a risk factor for loss of alveolar bone and loss of attachment (Papapanou et al. 1989). This relationship is thought to

reflect the accumulation of periodontal breakdown with time, rather than other factors related to the ageing process itself (Albandar 2002, Stanford & Rees 2003).

Genetics

It has become evident that there is a genetic component to many diseases, including periodontitis (Kinane & Hart 2003) and genes appear to play a role in both the predisposition to and progression of periodontal disease (Hodge & Michalowicz 2001, Kinane & Hart 2003, Persson 2003). Such a genetic predisposition is suggested by the familiar aggregation of aggressive periodontitis (Kinane & Hart 2003), although it must be borne in mind that families usually share a common environment as well. Studies on twins have also found a genetic predisposition for periodontal disease (Michalowicz et al. 2000, Boomsma et al. 2002). Furthermore, a genetic polymorphism in the Interleukin-1 gene cluster appears to influence susceptibility to periodontal disease (Kornman et al. 1997). Accordingly, certain individuals with a "high-susceptibility profile" for periodontitis may carry a number of genetic polymorphisms that are unfavourable in the pathogenesis of periodontitis (Kinane & Hart 2003).

Smoking

Cigarette smoking, one of the major risk factors for a number of diseases in humans (Ezzati et al. 2002), is also strongly associated with both the prevalence and severity of periodontitis (Norderyd & Hugosson 1998, Norderyd et al. 1999, Albandar et al. 2002, Jansson & Lavstedt 2002, Bergström 2003). Numerous studies have revealed an increased level of periodontal disease in terms of deeper periodontal pockets, greater loss of attachment, and more loss of alveolar bone (Preber & Bergström 1986, Grossi et al. 1994, Grossi et al. 1995, Elter et al. 1999, Machtei et al. 1999) in subjects who currently smoke than in those who are former-smokers or non-smokers. Moreover, smoking is associated with poor oral hygiene and impaired healing following non-surgical periodontal treatment (Söder et al. 1999, Papantonpoulos 1999, Amarasena et al. 2002, Kerdvongbundit & Wikesjö 2002).

Smokers with experimental gingivitis (Giannopoulou et al. 2003) and periodontitis (Bergström & Boström 2001) have been reported to display a lower degree of gingival inflammation than non-smokers. According to these investigators, the smokers had a higher proportion of small and lower proportion of large gingival blood vessels than did non-smokers, suggesting that the symptoms of gingival inflammation were suppressed

in smokers. However, in a more recent study no significant differences in experimental gingivitis were between detected in smokers and non-smokers (Salvi et al. 2005).

Systemic diseases

Systemic factors that have been linked to disease activity enhance the susceptibility to periodontal disease progression. Several investigations have documented the presence of a more severe periodontal condition in diabetic than in non-diabetic adults (Verma & Bhat 2004). Patients with well-controlled diabetes do not appear to be at higher risk for periodontal disease than people without diabetes, but patients with poorly controlled diabetes are at higher risk for periodontitis and progressive bone loss (Tervoner & Oliver 1993, Taylor 2001, Soskolne & Klinger 2001). In addition, cardiovascular diseases have also been shown to be associated with periodontal disease (Mattilla et al 2002, Buhlin et al. 2003, D'Aiuto et al. 2004, Söder et al. 2005). Certain investigations indicate that osteoporosis enhances an individuals susceptibility to periodontal breakdown (Payne et al. 1999, Yoshihara et al. 2004) and women with osteoporosis are at elevated risk for periodontal loss of attachment. This risk could be attenuated by oestrogen replacement therapy (Ronderos et al. 2000).

Psychosocial stress

Psychosocial stress can have a negative impact on the immune system, and has therefore been studied as a risk factor for periodontal diseases during the last decade. Some studies have reported that stress might initiate and /or contribute to periodontal destruction (Genco et al. 1999, Elter et al. 2002, Winner et al. 2005). There are also studies who reported no association between stress and periodontitis (Solis et al. 2004). Psychological stress has been associated with increased levels of pro-inflammatory mediators such as IL-1β and IL-6 in GCF and serum in periodontitis patients (Giannopoulou et al. 2003, Kamma et al. 2004), while other authors found no such association (Mengel et al. 2002).

Psychiatric disorder

Studies of patients with stress related psychiatric conditions have also yielded contradictory results. Thus, stress or increased workload often leads to increased cortisol levels in saliva, particularly in the morning (Melamed et al. 1999, De Vente et al. 2003, Grossi et al. 2005) whereas suppressed levels of cortisol have been seen in some psychiatric disorders (Peeters et al. 2003, Rohleder et al. 2004). Patients with

major depression and patients with posttraumatic stress disorder have also been found to have increased levels of IL-1β and IL-6 in serum (Maes et al. 1997, 1999, Owen et al. 2001, Kiecolt-Glaser et al. 2003).

BIOCHEMICAL MARKERS IN SALIVA AND GINGIVAL CREVICULAR FLUID

Saliva

Saliva is produced by three major glands, the parotid, submandibular, and subgingival as well as by numerous minor glands (Mandel 1987). In addition to secretions from these glands, saliva contains *e.g.,* gingival crevicular fluid, desquamated epithelial cells, microrganisms, leukocytes, food residue and blood (Sahingur & Cohen 2004). It has been suggested that increased levels of certain host-derived salivary enzymes such as collagenase, elastase, and gelatinase may be correlated with periodontitis (Ingman et al. 1993, Niemonen et al. 1993) and periodontal therapy was found to reduce the levels of active collagenase and elastase.

Gingival crevicular fluid (GCF)

Gingival crevicular fluid (GCF), a filtrate of blood and inflammatory exudates in periodontal tissue (Goodson 2003), contains substances derived from the serum, leukocytes, and structural cells of the periodontium as well as oral bacteria. The host-derived substances present in this fluid include antibodies, cytokines, enzymes and products formed in connection with tissue degradation (Uitto 2003). GCF can be employed as site-specific diagnostic indicator of periodontal condition and disease processes (Champagne et al. 2003, Griffiths 2003).

Interleukin-1β (IL-1β)

IL-1β, a pleiotropic cytokine, is one of the most potent and multifunctional pro-inflammatory regulators of inflammatory reactions (Dinarello 2000). This mediator is produced primarily by monocytes/macrophages, natural killer cells and B cells but also by other tissue cells such as fibroblasts, keratinocytes and endothelial cells. IL-1β can induce the release of other cytokines, prostaglandins, LPS and matrix metalloproteinases (MMPs) (Dinarello 1997).

In subjects suffering from severe or chronic periodontitis, it has been proposed that enhanced production of cytokine IL-1β induces bone resorption (Figueredo et al. 1999, Graves & Cochran 2003, Holmlund et al. 2004). Furthermore, IL-1β is also produced

by cells of the central nervous system (CNS), *e.g.*, microglia cells, astrocytes and endothelial cells (Besedovsky & del Rey 1996). Interestingly, the levels of IL-1β in the GCF of subjects with periodontitis (Giannopoulou et al. 2003, Kamma et al. 2004) and in serum of patients with major depression (Owen et al. 2001) are elevated in connection with psychological stress.

Interleukin-6 (IL-6)

IL-6, another pleiotropic cytokine, is secreted by several different types of cells, including monocytes/macrophages, T-cells, fibroblasts and endothelial cells (Van Snick 1990). Release of this cytokine from activated monocytes/macrophages is triggered by LPS, IL-1β and TNFα (Kishimoto 1989). IL-6 is involved in the regulation of inflammatory processes in tissues and has been shown to promote bone resorption (Takashiba et al. 2003) as well as induce the initiation and progression of periodontal disease (Teng 2003). Production of this cytokine can be stimulated directly by depression and other negative emotional and stressful experiences (Maes et al. 1999, Kiecolt-Glaser et al. 2003, Motivala et al. 2005).

Prostaglandin E_2 (PGE_2)

Prostaglandin E_2, (PGE_2), one of the most potent biochemical mediators of inflammation, has also been implicated in the progression of periodontal disease (Offenbacher et al. 1986, 1993, Söder 1999, Champange et al. 2003).

Granulocytes elastase

Elastase, a neutral serine protease stored in the azurophilic granules of granulocytes, can degrade most of connective tissue components, including collagen, laminin, fibronectin, proteoglycan and elastine (Hassel 1993). In connection with inflammatory processes in periodontal tissue, elastase plays a role in the destruction of connective tissue (Armitage et al. 1994, Söder et al. 2002, Figueredo et al. 2005).

Matrix metalloproteinases -8 and -9

Matrix metalloproteinases (MMP´s), a group of zinc- and calcium-dependent enzymes, are involved in degrading collagen, both in connection with normal tissue remodelling and during pathogenic processes such as periodontal disease (Birkedahl-Hansen 1993, Mäntylä et al. 2003, Sorsa et al. 2004). The expression and activity of MMPs have been associated with various inflammatory conditions that may lead to

tissue destruction, including periodontal disease (Kinane et al. 2003, Smith et al. 2004, Pozo et al. 2005).

MMP-1 and MMP-8 are interstitial collagenases that have the capacity to degrade the triple helical structures of the native type I collagen found in the periodontal ligament. The degradation of these collagen fibres can be considered as rate limiting for disease progression in periodontitis. Several investigations have shown increased levels of MMP-8 in sites with deep pockets and attachment loss (Ingman et al. 1996, Golub et al. 1997, Söder et al. 2002, Uitto et al. 2003, Sorsa et al. 2004), and the levels were reduced following non-surgical treatment of subjects with periodontitis (Kinane et al. 2003, Figueredo et al. 2004).

Within the MMP family, the gelatinases form a subgroup of enzymes that includes MMP-9, which can degrade the collagen located in basement membranes, and elastin. Enhanced levels of MMP-9 have been observed in the GCF of subjects with periodontitis (Ingman et al. 1996, Smith et al. 2004).

Cortisol

Cortisol, one of the most important glucocorticoids, is a hormone produced by the adrenal cortex and regulated by the hypothalamic-pituitary-adrenal (HPA) axis (Chrousos & Gold 1992). Glucocorticoids including cortisol are the primary mediators of responses to stress participating in many of the interactions between HPA axis and immunologically mediated inflammation, thereby inhibiting the accumulation and functions of lymphocytes, monocytes/macrophages, eosinophils and neutrophils at sites of inflammation (Chrousos 1995). Cortisol is present in serum and saliva (Aardal & Holm 1995, Genco et al. 1998, De Vente et al. 2003) and also in GCF (Axtelius et al. 1998).

AIMS OF THE THESIS

GENERAL AIM

This thesis focuses on the relationship between anxiety/ depression and periodontal diseases. Periodontal state was compared between subjects with and without self-reported anxiety, and between patients on longterm sick-leave for a stress related affective disorder and population controls.

Specific aims

The specific aims were the following:

- to investigate the influence of self-reported low grade of anxiety on gingival inflammation and periodontal disease in smokers and non-smokers (*Study* I).

- to determine if self-reported low grade of anxiety had an association with gingival inflammation and attachment level (AL), and with the levels of prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β) and elastase in gingival crevicular fluid (GCF) in subjects with chronic periodontitis (*Study* II).

- to investigate the importance of stress-related depression for the development of periodontitis by comparing oral health status, pro-inflammatory markers, and cortisol in GCF and saliva in patients (women) with stress-related mental depression and in non-depressed women (*Study* III).

- to investigate periodontal status in relation to inflammatory markers and cortisol in GCF, and saliva in a more homogenous group of women on longterm sick-leave for job-stress related depression in comparison to non-depressed women (*Study* IV).

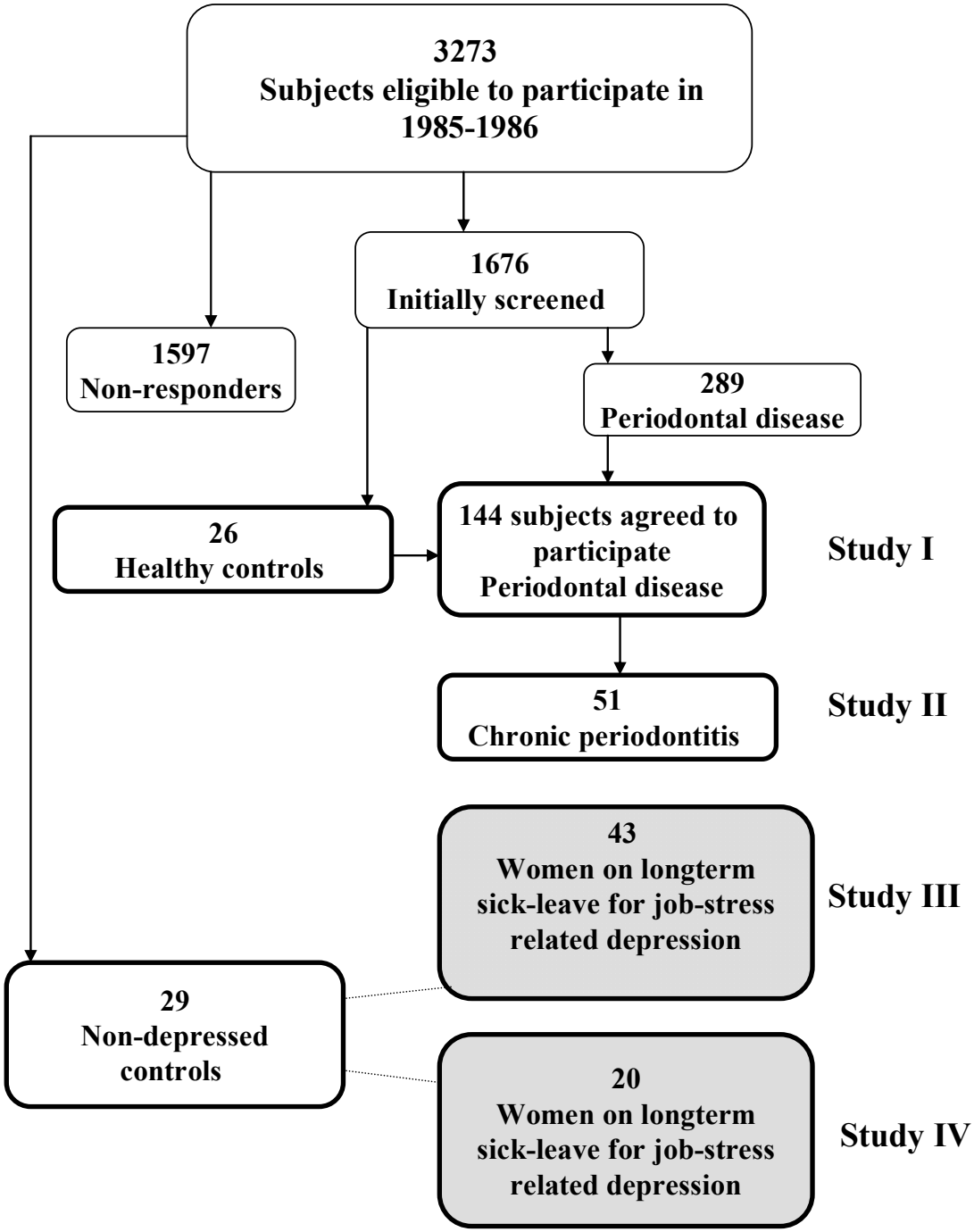

Fig 1. Selection of the subjects involved in *Studies* I-IV

MATERIAL AND METHODS

SUBJECTS

The individuals in *Studies* I, II and control group in *Studies* III and IV were recruited from the same epidemiological sample in the Stockholm region (Söder et al. 1994). A sample of 3273 people was selected from the registry file of all inhabitants of the Stockholm area, born on the 20th day of any month from 1945 to 1954. From this group 1681 individuals (840 males and 841 females), were examined clinically and 289 individuals were diagnosed with periodontal disease. 144 aged 31-40 years agreed to participate in the clinical study (Fig 1). All of them were offered further treatment.

Study population

The participants in *Study* I involved 144 subjects with periodontal disease (65 women and 79 men), with a mean age of 36.2 ± SD 2.8 years, who had participated in an epidemiological investigation on periodontal health (Fig 1). Of these, 122 subjects had been diagnosed as suffering from chronic periodontitis (CP-group) and the other 22 subjects were diagnosed as aggressive periodontitis (AP-group). The healthy control group was randomly selected from the individuals who initially agreed to participate in the epidemiological study and consisted of 16 women and 10 men with a mean age 35.4 ± SD 3.5 years who had no signs of periodontal disease.

In *Study* II, 51 adult subjects (26 men and 25 women), with a mean age of 53.5 ± SD 2.9 years, and with chronic periodontitis were included (Fig 1). They were randomly selected from the group of 144 patients diagnosed as suffering from periodontitis described above (Söder et al. 1994).

The participants comprised of 24 non-anxious subjects (7 smokers and 17 non-smokers) and 27 anxious subjects (14 smokers and 13 non-smokers). The smokers had smoked for an average of 30 years, and approximately 11-15 cigarettes/day. There where also 23 former smokers, who had not smoked for 5 years who were classified as non-smokers.

The patients in *Study* III were recruited from an ongoing study of stress-related exhaustion and depression and drawn from lists maintained by a Swedish insurance company (Alecta). These patients had received a diagnosis of affective disorder and been on long term sick-leave for longer than 3 months. 89 women were asked to participate and 44 agreed.

43 patients were included in the study with a mean age of 42.0 ± SD 9.3 years. 40 of these patients had a diagnosis of current major depression, or a major depression in partial remission. Three patients had a maladaptive stress reaction, one of them with depressive symptoms. 28 of these patients were using antidepressants (serotonin uptake inhibitors), and a subgroup analysis was carried out both for these patients and for the 15 patients who did not take antidepressants. None of the patients was pregnant, receiving hormonal treatment, or had taken antibiotics during the previous three months. One patient was excluded because she used an oral contraceptive.

In *Study* IV the patients were recruited from an ongoing study of women on longterm sick-leave (LTSL) for job stress related depression and all of these patients were insured by another Swedish company (AFA). All patients lived in the Stockholm area and worked in the public health care or social service sector. These women had been on full-time sick-leave for 3-8 months, because of a psychiatric disorder. 31 were asked to participate and 22 agreed to participate. 20 patients were finally included in the study with a mean age 48.5 ± SD 6.9.

18 of these patients had a diagnosis of current major depression, or a major depression in partial remission, and two patients had a maladaptive stress reaction with depressed mood. Nine patients were using antidepressants (serotonin uptake inhibitors), and a subgroup analysis of these patients and of the 11 patients not taking antidepressants, was also performed. None of the patients were pregnant, took oral contraceptives, or had taken antibiotics during the previous three months. Two patients were receiving estrogen replacement therapy and were excluded.

The control group in *Studies* III and IV (Fig 1) consisted of 45 women who were recruited randomly from the initial file of 3273 subjects in Stockholm investigated previously Fig 1). These women were contacted by telephone. 8 women could not be reached because they had no listed telephone number or did not answer repeated calls, and 4 women declined to participate in the study. Three other women had moved away from Stockholm and could not participate. 30 women wished to participate and 29 of these subjects with a mean age of 54.5 ± SD 2.9 years were included in the study. The criteria for exclusion were the presence of self-reported psychiatric disorder, use of psychotropic medications, pregnancy or use of oral contraceptive pills or estrogens. One subject didn't meet up to these criteria and was excluded due to use of estrogens.

CLINICAL EXAMINATION

In *Study* I the presence of supragingival plaque on teeth was evaluated in accordance with the criteria of Silness & Löe (1964). In *Studies* II-IV the procedure were somewhat simplified; the presence (1) or absence (0) of dental plaque on the lingual and buccal surfaces of each tooth was determined.

Gingival inflammation around the teeth was evaluated in *Study* I with the gingival index by Löe & Silness (1963). This index is based on clinical characteristics of different grades of inflammation which is determined by pressure of the gingiva. In *Studies* II and IV the gingival index of Löe (1967) was used, which is determined by probing of the gingiva.

In addition, gingival bleeding on probing (BOP) using a probe with a tip diameter of 0.5 mm (Hu-Friedy, USA) was recorded at six sites for each tooth (excluding the third molar) in *Studies* II-IV, and expressed as the percentage of bleeding sites per patient.

Probing pocket depth (PPD) (*Studies* I-IV) and clinical attachment level (CAL) (*Studies* II-IV), were measured to the nearest mm and was recorded at six sites (mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual, mid-lingual) around each tooth using a standard probe (Hu-Friedy, USA) graded at 2 mm intervals and with a tip diameter of 0.5 mm. The CAL was measured from the cemento-enamel junction (CEJ).

In *Study* I, periodontal disease was defined as the presence of at least one site with probing depth ≥ 5 mm and the subjects were divided into three groups, aggressive periodontitis group (AgP) with ≥ 20 sites with pockets ≥ 5 mm, chronic periodontitis group (CP) with < 20 sites with pockets ≥ 5 mm and the control group with no pockets ≥ 5 mm. In *Study* II, the criterion of periodontal disease was based on probing depth and at least four interproximal sites with ≥ 5 mm from at least two different teeth.

QUESTIONNAIRES AND PSYCHIATRIC EXAMINATION

All subjects involved in *Study* I and II answered a questionnaire evaluating periodontal health, which also contained one 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. The two anxiety response categories were collapsed into one “anxious” category, while all those who reported no anxiety were classified as “non-anxious”. Subjects who reported a psychiatric disorder, or the use of psychotropic medications were excluded.

The patients in *Studies* III and IV were seen for a thorough psychiatric examination, including a diagnostic interview by the Structured Clinical Interview (SCID) (First et al. 1997a, 1997b), which yields a diagnosis in according to the Diagnostic and Statistical Manual for Mental Disorders, Forth Edition (DSM-IV). Axis I and II (psychiatric syndrome and personality disorder, respectively) and Axis IV (type and degree of stressfactors) were used for the present study.

In *Study* IV, the stress factors were required to be work related and of longer duration than 6 months. The absence of any psychiatric disorders in the controls in *Studies* III and IV was established by self-report (health declaration and brief interview by the current investigator).

All subjects were classified as smokers or non-smokers (*Study* I-IV) and former smokers were also registered in *Study* III and IV. Smoking was quantified by number of cigarettes smoked per day.

COLLECTION OF SALIVA

Saliva was collected in *Studies* III and IV, prior to the clinical examination and in all cases between 8.30 am and 9.00 am. The patient was instructed not to eat, drink or smoke later than 60 minutes before collection of the sample, in order to avoid both increases in cortisol concentration and contamination of the oral cavity. In addition, brushing the teeth was not allowed for one hour preceding this collection to minimize the risk of contamination by blood.

The patients were instructed to spit all saliva produced during a 5 min period into a test tube. These samples were immediately centrifuged at 8000 g for 15 minutes at 4^{o}C and the supernatants thus obtained were frozen at -70^{o}C until analysis. The levels of cortisol in these saliva samples were determined as described above.

SAMPLING OF GINGIVAL CREVICULAR FLUID

In *Studies* II-IV, gingival crevicular fluid was collected with an intracrevicular washing technique (Salonen et al, 1991), modified by the addition of a quantitatively controlled delivery system (Compu-Pet $^{800.}$, Alphamedics, NJ, USA) and a peristaltic pump for aspiration (Pharmacia, Uppsala, Sweden) (Jin et al. 1995). In brief, the sites to be sampled were isolated with cotton rolls, and air dried gently following a careful supragingival plaque removal. The ejection needle of the instrument was then inserted gently into the crevice to a level 1 mm below the gingival margin. The gingival pocket was subsequently flushed with 15 µl phosphate buffered saline (PBS, pH 7.4), with

simultaneous drainage by constant suction through the collection needle into Eppendorf tubes at a flow rate of 25 ml/h. The total amount of gingival washing collected in this manner was 500 µl from each pocket and thereafter immediately centrifuged (8000 g) for 15 min at 4º C, following which the supernatants were frozen at -70º C until analysis. Since this washing technique does not allow determination of the volume of the samples or the calculation of the concentrations our results are presented as amounts per site.

These samples of gingival crevicular fluid were analyzed for elastase activity (*Study* II), prostaglandin E_2 (PGE_2) (*Study* II), interleukin-1β (IL-1β) (*Studies* II-IV), interleukin-6 (IL-6) (*Studies* III-IV), matrixmetalloproteinase-8 (MMP-8) (*Study* IV), and matrixmetalloproteinase -9 (MMP-9) (*Studies* III-IV), and cortisol in GCF (*Studies* III-IV).

ASSAYS

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 (A-2484, AB Kabi Diagnostica, Stockholm, Sweden) (Kramps et al. 1983) The elastase activity measured with this substrate derived from free elastase and from complexed elastase with α-2-macroglobulin (Travis & Salvesen 1983). The activity was expressed as absorbance/site.

PGE_2

The samples of gingival crevicular fluid were assayed for PGE_2 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 PGE_2 were determined as total amounts per site (pg/site).

IL-1β and IL-6

IL-1β and IL-6 were 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 and IL-6 were determined as total amounts per site (pg/site).

MMP-8 and MMP-9

In gingival crevicular fluid the levels of MMP-8 and MMP-9 (both free and complexed) were measured with the ELISA kit (R & D Systems Europe Ltd, Oxon, UK), according to the manufacturer's instructions manual. The levels of MMP-8 and MMP-9 were determined as total amounts per site (ng/site).

Cortisol in GCF and saliva

Cortisol levels were measured with a sensitive radioimmunoassay procedure (RIA Kit Orion Diagnostica AB, Espoo, Finland), according to the manufacturer's instructions. The levels of cortisol were determined as total amounts per site (nmol/l).

STATISTICAL ANALYSIS

In *Studies* I and II, 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. Moreover, in *Study* I Fisher's exact probability test was employed to determine the significance of differences between the non-anxious and anxious smokers in the AP-group. When determining the significance of difference in pocket depth between the sites with ≥ 5 mm in smokers and non-smokers, the Mann-Whitney U-test was used *(Study I)*. Furthermore, in this same study analysis of covariance (ANCOVA) was utilized to control for smoking when comparing gingival inflammation in the different groups, as well as to evaluate the potential correlation between anxiety and gingival inflammation.

In *Study* II, the differences in the clinical data and levels of inflammatory markers for anxious and non-anxious subjects, both for all participants combined and for smokers and non-smokers separately, were analyzed statistically employing the Mann-Whitney U-test.

In *Studies* III and IV, when comparing clinical observations and levels of inflammatory markers, and cortisol (mean and standard deviations) for depressed patients and non-depressed subjects, an analysis of covariance (ANCOVA) was utilized to remove the influence of age and smoking. To correct the P-values for multiple comparisons Bonferroni's method was applied.

Table 1. Subjects and methods of the four *Studies* if the thesis

Study	Population	Methods	Data analysis	Results
I	22 subjects with aggressive periodontitis, 122 with chronic periodontitis, 26 healthy controls, 30-40 yrs of age	Clinical examination: PLI, GI, PPD, Periodontal disease was defined: at least one site with PPD => 5 mm. Question: "Do you feel anxious in your every day life?"	ANOVA, ANCOVA Fisher's exact probability test Mann-Whitney U-test	Self-reported anxiety was associated with an adverse effect on the gingiva.
II	51 subjects with chronic periodontitis, 26 men and 25 women, mean age of 53.5 yrs	Clinical examination; PLI, GI, BOP, PPD,CAL Periodontal disease was defined: at least four interproximal sites with PPD => 5 mm on at least two different teeth. Assays: PGE_2, Il-1β and elastase. Question: "Do you feel anxious in your every day life?"	ANOVA, Mann-Whitney U-test	Anxious smokers had significantly more gingivitis and clinical loss of attachment than did non-anxious smokers. There were no significant differences in any of the inflammatory markers.
III	43 - women on long-term sick-leave for job-stress related depression, mean age 42.0 yrs 29 non-depressed controls, mean age 54.5 yrs	Clinical examination: PLI, GI, BOP, CAL, PPD, number of teeth. Assays: IL-1β, IL-6, MMP-9 and cortisol in GCF, cortisol in saliva. DSM-IV.	ANCOVA, (adjusting for age and smoking), Multiple comparison Bonferroni's method was used to corret P-values	Depressed patients had significantly more PLI, GI and significantly higher levels of IL-6 and cortisol in GCF than controls. Controls had significantly higher levels of MMP-9 than the patient group.
IV	20 - women on long-term sick-leave for job-stress related depression, mean age 48.5 yrs 29 non-depressed controls, mean age 54.5 yrs	Clinical examination PLI, GI, BOP, PPD, CAL, number of teeth. Assays: IL-1β, IL-6, MMP-8, MMP-9 and cortisol in GCF, cortisol in saliva. DSM-IV	ANCOVA, (adjusting for age and smoking), Multiple comparison Bonferroni's method was used to corret P-values	Depressed patients had significantly more GI, PPD and significantly higher levels of IL-6 in GCF than controls. The levels of cortisol in GCF was lower in the patients than controls.

RESULTS

Study I

Anxious subjects exhibited a significantly higher degree of gingival inflammation than non-anxious subjects ($p<0.01$) (Table 2), when controlling for smoking. Furthermore, the average GI for anxious non-smokers in the CP-group was 2.1 ± SD 0.3 versus 1.8 ± SD 0.4 for the non-anxious non-smokers, ($p<0.05$). Moreover, anxious smokers (n = 38) with periodontitis had significantly more sites with pockets ≥ 5 mm compared to non-anxious smokers (n = 42), ($p<0.05$).

Study II

The anxious smokers displayed a significantly higher degree of gingival inflammation compared to non-anxious smokers, ($p<0.01$) (Table 2) and moreover, loss of attachment was significantly more pronounced in anxious smokers than in non-anxious smokers, 4.2 ± SD 1.3, versus 3.2 ± SD 0.5, respectively; $p<0.05$. The levels of prostaglandin E_2, interleukin 1β and elastase in the GCF of these groups did not differ significantly.

Study III and Study IV

The patients on longterm sick-leave for job-stress related depression (*Studies* III and IV) demonstrated significantly higher degree of gingival inflammation ($p<0.001$) compared to the non-depressed controls (Table 2), after adjusting for age and smoking. Moreover, the patients in *Study* III had significantly more plaque ($p<0.003$) and patients in *Study* IV had a significantly larger number of deep pockets ($p<0.003$). There were no differences between the patients and control group with respect to the number of current smokers or the number of cigarettes smoked per day in *Studies* III and IV, although in Study IV there were more former smokers in the control group.

The patients in both studies demonstrated significantly higher levels of IL-6 in GCF compared to the controls, after adjusting for age and smoking ($p<0.003$ and $p<0.000$, respectively) (Table 3). In Study III the patients exhibited a 10-fold higher mean level of cortisol in GCF than did the control subjects, whereas in Study IV the patients had lower cortisol values in GCF than in controls. The level of cortisol in saliva did not differ between the groups. Furthermore, in *Study* III and *Study* IV the levels of IL-1β in GCF and in *Study* IV the levels of MMP-8 were similar in both groups, although the control group was characterized by significantly higher levels of MMP- 9 in *Study* IV, ($p<0.004$).

When the 28 patients and 9 patients in *Study* III and IV, respectively, who were taking antidepressant medication, were analyzed separately, none of their clinical parameters or biochemical markers differed significantly from those of patients not on such medication.

Table 2.
Levels of Gingival inflammation in *Studies* I-IV
n = number of subjects

Study	Gingival Index	Statistical analysis	Values means ± SD	P- value
I	Löe & Silness (1963)	ANCOVA (controlling for smoking)	Anxious (n=76) 2.03 ± 0.48 Non-anxious (n=92) 1.87 ± 0.54	< 0.01
II	Löe (1967)	Mann-Whitney U-test	Anxious smoker (n=14) 2.1 ± 0.7 Non-anxious smoker (n=7) 1.3 ± 0.9	<0.05
III	Löe (1967)	ANCOVA (controlling for age and smoking)	Depressed patients (n=43) 1.53 ± 0.26 Controls (n=29) 0.89 ± 0.35	< 0.000*
IV	Löe (1967)	ANCOVA (controlling for age and smoking)	Depressed patients (n=20) 1.59 ± 0.34 Controls (n=29) 0.89 ± 0.35	< 0.000*

* Multiple comparisons according to Bonferroni's method

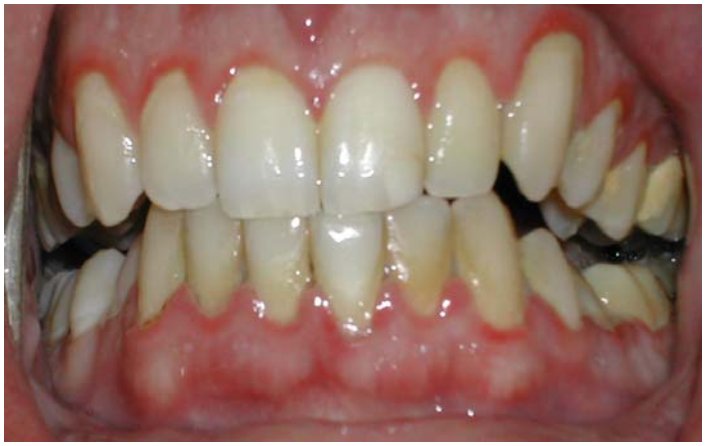

Fig 2. Gingival inflammation

Table 3.

Interleukin-1β, interleukin-6, matrixmetalloproteinas -8 -9, cortisol in GCF and cortisol in saliva in patients and controls, after controlling for age and smoking, by analysis of covariance (ANCOVA), in *Studies* III and IV.

n = number of subjects

		Patients *Study* III (n=43)		Controls *Study* III (n= 29)				Patients *Study* IV (n=20)		Controls *Study* IV (n= 29)			
Parameter	unit	Mean	± SD†	Mean	± SD	F_{DF}§	P	Mean	± SD	Mean	± SD	F_{DF}§	P
IL-1β	pg/site	35.42	(28.53)	31.66	(20.61)	$0.37_{1,69}$	0.546	32.07	(14.92)	31.66	(20.61)	$0.82_{1,45}$	0.370
IL-6	pg/site	**2.03**	(1.62)	**0.79**	(1.83)	$9.48_{1,72}$	**0.003***	**3.84**	(1.58)	**0.79**	(1.83)	$22.91_{1,45}$	**0.000***
MMP-9	ng/site	**19.40**	(12.11)	**30.56**	(18.49)	$9.05_{1,68}$	**0.004***	28.26	(20.14)	30.56	(18.49)	$0.15_{1,45}$	0.704
Cortisol in GCF‡	nmol/l	**3.46**	(3.25)	**0.30**	(0.25)	$26.89_{1,72}$	**0.000***	0.17	(0.33)	0.30	(0.25)	$0.62_{1,45}$	0.436
Cortisol in saliva	nmol/l	9.92	(5.40)	10.50	(5.07)	$0.20_{1,72}$	0.653	12.20	(4.79)	10.50	(5.07)	$0.01_{1,45}$	0.907
MMP-8								12.49	(9.90)	11.98	(5.56)	$0.10_{1,45}$	0.920

* Multiple comparisons according to Bonferroni's method

† Standard deviation of the mean

‡ Gingival crevicular fluid (GCF)

§ DF (degree of fredom)

GENERAL DISCUSSION

In this study of periodontal state and biochemical markers in relation to self-reported anxiety, and to stress related affective disorder severe enough to lead to longtem sick-leave, the following findings were made. In subjects with signs of periodontal disease, self-reported anxiety was associated with worse gingival inflammation, and smoking was associated with deeper pockets and more loss of attachment in the anxious than in the non-anxious group, in spite of the absence of a difference in biochemical markers between the two groups. Patients on longterm sick-leave for stress-related depression and exhaustion disorder, diagnosed by a psychiatrist using diagnostic criteria, had more dental plaque and gingival inflammation, and more deep pockets than healthy controls.

Both groups of depressed patients had significantly higher levels of IL-6 in GCF compared to the non-depressed controls. The levels of cortisol in GCF were higher in the patients than in control group in *Study* III, in contrast to *Study* IV, where the levels were lower in the depressed patients. Moreover, in *Study* III, we observed, unexpectedly, that the levels of MMP-9 in GCF of the depressed patients were lower than in the controls, while in *Study* IV, no such differences were found. Furthermore, the levels of IL-1β and MMP-8 in GCF of the depressed patients were similar to the control group. None of the depressed patient groups had a cortisol concentration in saliva than differed from the controls.

The increased level of gingival inflammation in these subjects can be explained by both an indirect and a direct influence, in which the indirect influence would involve behavioural changes, *e.g,* neglect of oral hygiene, lack of regular dental check-ups, and changes in dietary, that could influence periodontal health. The direct influence might involve modulation of the HPA axis, leading to an endocrine imbalance and consequently lowered host resistance, as suggested by Genco et al. (1998).

Furthermore, anxious subjects (*Studies* I and II) had more deep pockets and more loss of attachment than controls, in agreement with other investigations (Genco et al. 1999, Winner et al. 2005). Winner et al. (2005) reported that chronic periodontitis patients with a defensive/ suppressive coping style (possible related to anxiety) exhibited significantly more loss of attachment and much less improvement after two years of non-surgical periodontal therapy than did patients with active coping strategies. Moreover, an investigation by Genco et al. (1999) showed that clinical attachment loss and smoking was significantly associated with financial strain / distress (likely related to anxiety) and periodontitis.

In *Studies* I and II, we used only one question regarding anxiety, in addition to the standard health questionnaire including questions concerning general diseases, which was filled out by the subjects. These subjects considered themselves healthy, and none suffered from any psychiatric disorder, *i.e.,* the degree of anxiety was probably comparatively low. The results from these studies suggest that the answer to a single simple question concerning the experience of anxiety was able to discriminate people at risk for periodontal disease. This has an obvious clinical relevance.

The present studies (I and II) support the assumption that anxious subjects smoked more, as has also been shown previously (Monteiro da Silva et al. 1998, Genco et al. 1999), and confirmed in a longitudinal study (Breslau et al. 1998).

In order to examine the association between anxiety and periodontal disease in greater detail we also measured the levels of various biochemical markers (*Study* II). Although the levels of these markers would be expected to be elevated in anxious subjects, we found similar values in both groups. The reason for this could be that the degree of psychological disorder in our subjects was low.

All patients in *Studies* III and IV were women and all were suffering from depression and were on long-term sick-leave (LTSL). It is well known that depression is much more common in women than in men (Riise & Lund 2001, SBU 2004, Jurado et al. 2005). This was also born out in *Study* III, where the number of men that could be recruited for the study was so small that analysis would have been meaningless. In *Study* IV, female sex was an inclusion criterion. The depressed women in *Study* IV all were workers in the human services, an occupation that previous research has shown to be stressful due to the difficulty of balancing between concern and detachment (Iacovides et al. 2003). The cause of the illness in *Study* IV was in all cases related to the subjects work. This group can thus be seen as representative of longterm stress, leading to exhaustion and subsequent depression.

The depressed patients (*Studies* III and IV) had more dental plaque, higher degree of gingival inflammation and more deep pockets than the non-depressed controls. They may also be at increased risk for a poorer response to periodontal treatment for their oral problems, as suggested by Elter et al (2002), who found that treatment of periodontitis patients suffering from clinical depression (defined according to DSM-IV), was less effective than treatment of those without depression.

In order to elucidate the association between depression and periodontal disease in greater detail, we expanded the number of biochemical markers analyzed to include IL-1β, IL-6, and MMP-8 (only *Study* IV), MMP-9 and cortisol (in both GCF and saliva).

The level of IL-6 in GCF was significantly higher in the patients in both studies, suggesting that depression may stimulate the production of IL-6, which has in fact been confirmed in other studies on patients with different psychiatric disorders (Berk et al. 1997, Maes et al. 1997, Dentino et al. 1999, Kiecolt-Glaser et al. 2003). Depressed patients exhibit alterations in both the cellular (Herbert & Cohen 1993, Maes et al. 1994) and humoral immune responses (Maes 1995) that may impair immunological defence mechanisms and promote accumulation of periodontopathogens which exacerbate periodontal diseases.

In none of our clinical groups was the level of IL-1β in GCF increased, while IL-6 was increased in the patient group. Interestingly, Chrousos (1995) in his review of the HPA axis and immune mediated inflammation states that systemic IL-6 concentrations increase during stress unrelated to inflammation, and that IL-6 inhibits the secretion of IL-1. There mechanisms may possibly contribute to the explanation of our findings.

The amount of cortisol in the GCF was higher in the patients with stress-related depression and exhaustion compared to the control group in *Study* III, in contrast to *Study* IV, where GCF cortisol was lower in the depressed patients than in the control group. Today, no clear explanation for these results can be given. The two patient groups are similar in many ways and both are on LTSL. However, one difference between the two groups is that the patients in *Study* IV were more homogenous with regard to eliciting cause (job-stress) and to profession. Interestingly, the patients in *Study* IV also participated in a challenge study of HPA axis function, where the reaction of both cortisol and adrenocorticotropin hormone (ACTH) to corticotrophin-releasing hormone (CRH) -dexamethasone challenge was attenuated (Rydmark et al. submitted). This was in contrast to expectation and was interpreted as evidence of an exhaustion state after prolonged stress, rather than classical depression disorder (which would have been expected to yield an exaggerated, rather than attenuated reaction to the hormonal challenge).

Evidence of dysregulation of the HPA axis is found in several psychiatric illnesses, but the nature of the disturbance reported varies between studies. This may be due to differences between psychiatric disorders (*e.g.,* high activation in classical depression (Kunugi et al. 2004), low in atypical depression (Gold & Chrousos 2002) and in post traumatic stress disorder (Kasckow et al. 2001)), between different stages in the illness, and between different times of the day. It has also been proposed that prolonged strain on the HPA axis may blunt the regulation of this system in response to stress (McEwen,

1998). Discrepancies can also depend on many other factors, including genetic factors, and gender, or early experiences of stress, as suggested by Heim et al. (2000).

To our knowledge there is only one previous study which has investigated cortisol in GCF (Axtelius et al. 1998), and the function - if any - of the cortisol present in the gingival crevicular fluid is far from clear and should be elucidated.

Axtelius et al. (1998) concluded that cortisol concentrations varied from different teeth, and that concentrations were higher in subjects with poor oral health. It is difficult to compare this study to ours because of the different GCF sampling method. We used an intracrevicular washing technique to obtain GCF which results in more dilute samples, while Axtelius et al. (1998) utilized absorption on a filter paper.

In addition, the sampling time at which the samples were taken might also influence the results obtained. In our studies the samples of GCF and saliva were all taken at the same time in the morning. A limitation is that the analysis is based on one single saliva sample

Our studies revealed no differences between any of the patient groups and the controls with respect to salivary concentrations of cortisol. This is in contrast to Genco et al. (1998), who demonstrated that subjects with periodontitis and a high level of stress and inadequate coping had elevated levels of cortisol in their saliva. Possibly, our failure to find increases in cortisol in saliva may be related to the long period of time our subjects had been under stress, and the subsequent break-down in social functioning, evidenced by their inability to continue working. This issue might be resolved by longitudinal studies, where stress levels and cortisol in saliva are simultaneously monitored.

It has been proposed that enhanced levels of cortisol in depressed patients may exert a number of negative effects on immunological defences, including attenuation of the functions of T-helper cells, antibody production and activity, and neutrophil function (Chrousos 1995, Maes 1995, Genco et al. 1998), which could increase the risk for periodontal disease.

In the explanation of our findings it should be to taken into consideration that GCF and saliva are derived from different sources. GCF is derived from the periodontal pocket and thus contains molecules that reflect the periodontal disease process. It represents a local site-specific sample, whereas saliva is a more general oral sample, as suggested by Kinane (2000).

Moreover we observed, unexpectedly, that the levels of MMP-9 in GCF of the depressed patients (*Study* III) were actually lower than in the controls, which might be

explained by the fact that glucocorticoids down-regulate the expression of MMPs, as suggested by Bosse et al. (1999). Yang et al. (2002) reported that subjects with higher plasma levels of cortisol also had lower levels of MMP-2, but no association between plasma cortisol and MMP-9 was detected. These authors suggested that the plasma cortisol levels in their patients, who were not clinically depressed, were not high enough to modulate the expression of MMP-9. This might contribute to the explanation of the results of *Study* IV, where the similar levels of MMP-9 in the depressed patients and controls were accompanied by low levels of cortisol in both groups.

Overall, our findings demonstrate that anxiety and depression constitute significant risk factors for enhanced gingival inflammation and for progression of periodontitis. We propose that this elevation in risk is due not only to behavioural changes, but also to a direct influence on the immune system.

CLINICAL IMPLICATIONS AND FURTHER RESEARCH

The findings in this thesis showed a possible link between gingival inflammation and psychosocial factors including anxiety and depression. Patients with such problems may experience difficulties in initiating dental check-ups, and a more active approach from the dental hygienists or dentists may be in order. Maintenance care intervals should be shorter, and the patients should also be informed that their psychological problems may affect their immune status negatively, and that it is important for them to try to keep regular routines for oral hygiene.

The present thesis suggests some lines for further research:

- The interaction of cortisol with bacteria in the gingival crevicular fluid and saliva should be studied.
- Possible gender differences in the relationship between anxiety / depression and periodontal diseases need to be explored.
- It is also of interest to follow these patients after treatment for their depression to investigate if this intervention has an influence on the periodontal status.

CONCLUSIONS

- Self-reported anxiety was associated with adverse affects on the gingiva. Anxiety appeared to be associated with an increased severity of periodontal disease in smokers.

- Anxious smokers exhibited significantly higher levels of gingival inflammation and loss of attachment than non-anxious smokers, but there were no differences between the anxious and non-anxious subjects with periodontitis with respect to a range of biochemical inflammatory markers.

- Women suffering from stress-related depression and exhaustion demonstrated more accumulation of plaque, gingival inflammation and more deep pockets and elevated levels of IL-6 in comparison to non-depressed individuals, suggesting that depression may suppress immunological functions and thereby impair periodontal health.

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REFERENCES

Aardal E. Holm AC. Cortisol in saliva—reference ranges and relation to cortisol in serum. *Eur J Clin Chem Clin Biochem* 1995;**33**:927-932.

Albandar JM. Brunelle JA. Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 1999;**70**:13-29.

Albandar JM. Tinoco EM. Global epidemiology of periodontal diseases in children and young persons. *Periodontol 2000* 2002;**29**:153-176

Albanar JM. Global risk factors and risk indicators for periodontal diseases. *Periodontol 2000* 2002;*29*:177-206.

Albandar JM. Rams TE. Global epidemiology of periodontal diseases: an overview. *Periodontol 2000* 2002;**29**:7-10

Amarasena N. Ekanavaka AN. Herath L. Miyazaki H. Tobacco use and oral hygiene as risk indicators for periodontitis. *Community Dent Oral Epidemiol* 2002;**30**:115-123.

APA. (2000). *Diagnostic and statistical manual of mental disorders. 4th ed*. (DSM-IV) Washington DC: *American Psychiatric Association*

Armitage GC. Jeffcoat MK. Chadwick DE. Taggart EJ Jr. Numabe Y. Landis JR. Weaver SL. Sharp TJ. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. *J Periodontol* 1994;**65**:120-128.

Axelsson P. Albander JM. Rams TE. Prevention and control of periodontal diseases in developing and industrialized nations. *Periodontol 2000* 2002;**29**:235-246.

Axtelius B. Edwardsson S. Theodorsson E. Svensäter G. Attström R. Presence of cortisol in gingival crevicular fluid. A pilot study. *J Clin Periodontol* 1998;**25**:929-932.

Bergström J. Tobacco smoking and risk for periodontal disease. *J Clin Periodontol* 2003;**30**:107-113.

Bergström J. Boström L. Tobacco smoking and periodontal hemorrhagic responsiveness. *J Clin Periodontol* 2001;**28**:680-685.

Besedovsky HO. del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocr Rev* 1996;**17**:64-102.

Berk M. Wadee AA. Kuschke RH. O´Neill-Kerr A. Acute phase proteins in major depression. *J Psychosom Res* 1997;**43**:529-534.

Boomsma D. Busjahn A. Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* 2002; **3**:872-882.

Bimstein E. Matsson L. Growth and development considerations in the diagnosis of gingivitis and periodontitis in children. *Pediatr Dent* 1999;**21**:186-191.

Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodontal Res* 1993;**28**:500-510.

Bosse M. Chakir J. Rouabhia M. Boulet LP. Audette M. Laviolette M. Serum matrix metalloproteinase-9:Tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am J Respir Crit Care Med* 1999;**159**:596-602.

Breslau N. Peterson EL. Schultz LR. Chilcoat HD. Andreski P. Major depression and stages of smoking. A longitudinal investigation. *Arch Gen Psychiatry* 1998;**55**:161-166.

Brown LJ. Löe H. Prevalence, extent, severity and progression of periodontal disease. *Periodontol 2000* 1993;**2**:57-71.

Buhlin K. Gustafsson A. Pockley AG. Frostegård J. Klinge B. Risk factors for cardiovascular disease in patients with periodontitis. *Eur Heart J* 2003;**24**:2099-2107.

Champagne CM. Buchanan W. Reddy MS. Preisser JS. Beck JD. Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol 2000* 2003;**31**:167-180.

Chrousos GP. Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA*1992;**4**:1244-1252.

Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;**18**:1351-1362.

D'Aiuto F. Ready D. Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J Periodontal Res* 2004;**39**:236-241.

Darveau RP. Tanner A. Page RC. The microbial challenge in periodontitis. *Periodontol 2000* 1997;**14**:12-32.

Dentino AN. Pieper CF. Rao MK. Currie MS. Harris T. Blazer DG. Cohen HJ. Association of interleukin-6 and other biologic variables with depression in older people living in the community. *J Am Geriatr Soc*1999;**47**:6-11.

De Vente W. Olff M. Van Amsterdam JG. Kamphuis JH. Emmelkamp PM. Physiological differences between burnout patients and healthy controls: blood pressure, heart rate, and cortisol responses. *Occup Environ Med* 2003;**60** Suppl 1:54-61.

Dinarello CA. Interleukin-1. *Cytokine Growth Factor Rev* 1997;**8**:253-265.

Dinarello CA. Proinflammatory cytokines. *Chest* 2000;**118**:503-508.

Elter JR. Beck JD. Slade GD. Offenbacher S. Etiologic models for incident periodontal attachment loss in older adults. *J Clin Periodontol* 1999;**26**:113-123.

Elter JR. White BA. Gaynes BN. Bader JD. Relationship of clinical depression to periodontal treatment outcome. *J Periodontol* 2002;**73**:441-449.

Ezzati M. Lopez AD. Rodgers A. Vander Hoorn S. Murray CJ. Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. *Lancet* 2002;**2**:1347-1360.

Figueredo CM. Ribeiro MS. Fischer RG. Gustafsson A. Increased interleukin-1beta concentration in gingival crevicular fluid as a characteristic of periodontitis. *J Periodontol* 1999;**70**:1457-1463.

Figueredo CM. Areas A. Miranda LA. Fischer RG. Gustafsson A. The short-term effectiveness of non-surgical treatment in reducing protease activity in gingival crevicular fluid from chronic periodontitis patients. *J Clin Periodontol* 2004;**31**:615-619.

Figueredo CM. Fischer RG. Gustafsson A. Aberrant neutrophil reactions in periodontitis. *J Periodontol* 2005;**76**:951-955.

Filoche SK. Zhu M. Wu CD. In situ biofilm formation by multi-species oral bacteria under flowing and anaerobic conditions. *J Dent Res* 2004;**8**3:802-806.

First MB. Spitzer RL. Gibbon M. Williams JBW. Structured Clinical Interview DSM-IV Axis I Disorders (SCID-I), Clinican Version. 1997a. Washington D.C., *American Psychiatric Press.*

First MB. Gibbon M. Spitzer RL. Williams JBW. Benjamin LS. Structured Clinical Interview DSM-IV Axis II Personality Disorders (SCID-II). 1997b.Washington D.C., *American Psychiatric Press*.

Genco RJ. Ho AW. Kopman J. Grossi SG. Dunford RG. Tedesco LA. Models to evaluate the role of stress in periodontal disease. *Ann Periodontol* 1998;**3**:288-302.

Genco RJ. Ho AW. Grossi SG. Dunford RG. Tedesco LA. Relationship of stress, distress and inadequate coping behaviors to periodontal disease. *J Periodontol* 1999;**70**:711-723.

Giannopoulou C. Kamma JJ. Mombelli A. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. *J Clin Periodontol* 2003;**30**:145-153.

Gold PW. Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry* 2002;**7**:254-275.

Golub LM. Lee HM. Greenwald RA. Ryan ME. Sorsa T. Salo T. Giannobile WV. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 1997;**46**:310-319.

Goodson JM. Gingival crevice fluid flow. *Periodontol 2000* 2003;**31**:43-54.

Graves DT. Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 2003;**74**:391-401.

Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontol 2000* 2003;**31**:32-42.

Grossi G. Perski A. Ekstedt M. Johansson T. Lindström M. Holm K. The morning salivary cortisol response in burnout. *J Psychosom Res* 2005;**59**:103-111.

Grossi SG. Zambon JJ. Ho AW. Koch G. Dunford RG. Machtei EE. Norderyd OM. Genco RJ. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994;**65**:260-267.

Grossi SG. Genco RJ. Machtei EE. Ho AW. Koch G. Dunford R. Zambon JJ. Hausmann E. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* 1995;**66**:23-29.

Haffajee AD. Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994;**5**:78-111.

Hanson WL. Persson GR. Periodontal conditions and service utilization behaviors in a low income adult population. *Oral Health Prev Dent* 2003;**1**:99-109.

Hassel TM. Tissues and cells of the periodontium. *Periodontol 2000* 1993;**3**:9-38.

Heim C. Ehlert U. Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 2000;**25**:1-35.

Herbert TB. Cohen S. Stress and immunity in humans: a meta-analytic review. Psychosom *Med* 1993;**55**:364-379.

Hodge P. Michalowicz B. Genetic predisposition to periodontitis in children and young adults. *Periodontol 2000* 2001;**26**:113-134.

Holmlund A. Hänström L. Lerner UH. Bone resorbing activity and cytokine levels in gingival crevicular fluid before and after treatment of periodontal disease. *J Clin Periodontol* 2004 ;**31**:475-482.

Hugoson A. Norderyd O. Slotte C. Thorstensson H. Distribution of periodontal disease in a Swedish adult population 1973, 1983 and 1993. *J Clin Periodontol* 1998;**25**:542-548.

Hugoson A. Ljungquist B. Breivik T. The relationship of some negative events and psychological factors to periodontal disease in an adult Swedish population 50 to 80 years of age. *J Clin Periodontol* 2002;**29**:247-253.

Hugoson A. Koch G. Gothberg C. Helkimo AN. Lundin SA. Norderyd O. Sjödin B. Sondell K. Oral health of individuals aged 3-80 years in Jönköping, Sweden during 30 years (1973-2003). II. Review of clinical and radiographic findings. *Swed Dent J* 2005;**29**:139-155.

Iacovides A. Fountoulakis KN. Kaprinis S. Kaprinis G. The relationship between job stress, burnout and clinical depression. *J Affect Disord* 2003;**75**:209-221.

Ingman T. Sorsa T. Konttinen YT. Liede K. Saari H. Lindy O. Suomalainen K. Salivary collagenase, elastase- and trypsin-like proteases as biochemical markers of periodontal tissue destruction in adult and localized juvenile periodontitis. *Oral Microbiol Immunol* 1993;**8**:298-305.

Ingman T. Tervahartiala T. Ding Y. Tschesche H. Haerian A. Kinane DF. Konttinen YT. Sorsa T. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* 1996;**23**:1127-1132.

Jansson L. Lavstedt S. Influence of smoking on marginal bone loss and tooth loss--a prospective study over 20 years. *J Clin Periodontol* 2002;**29**:750-756.

Jin LJ. Söder P-Ö. Åsman B. Bergström K. Granulocyte elastase in gingival crevicular fluid: improved monitoring of the site-specific response to treatment in patients with destructive periodontitis. *J Clin Periodontol* 1995;**22**:240-246.

Jurado D. Gurpegui M. Moreno O. Fernandez MC. Luna JD. Galvez R. Association of personality and work conditions with depressive symptoms. *Eur Psychiatry* 2005;**20**:213-222.

Kamma JJ. Giannopoulou C. Vasdekis VGS. Mombelli A. Cytokine profile in gingival crevicular fluid of aggressive poeriodontitis: influence of smoking and stress. *J Clin Periodontol* 2004;**31**:894-902.

Kantarci A. Oyaizu K. Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. *J Periodontol* 2003;**74**:66-75.

Kasckow JW. Baker D. Geracioti TD Jr. Corticotropin-releasing hormone in depression and post-traumatic stress disorder. *Peptides* 2001;**22**:845-851

Kerdvongbundit V. Wikesjö UM. Prevalence and severity of periodontal disease at mandibular molar teeth in smokers with regular oral hygiene habits. *J Periodontol* 2002;**73**:735-740.

Kiecolt-Glaser JK. Preacher KJ. MacCallum RC. Atkinson C. Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci* 2003;**22**;9090-9095.

Kinane DF. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. *Periodontol 2000* 2000;**24**:215-225.

Kinane DF. Hart TC. Genes and gene polymorphisms associated with periodontal disease. *Crit Rev Oral Biol Med* 2003;**14**:430-449.

Kinane DF. Darby IB. Said S. Luoto H. Sorsa T. Tikanoja S. Mantyla P. Changes in gingival crevicular fluid matrix metalloproteinase-8 levels during periodontal treatment and maintenance. *J Periodontol Res* 2003;**38**:400-404.

Kinane DF. Peterson M. Stathopoulou PG. Environmental and other modifying factors of the periodontal diseases. *Periodontol 2000* 2006;**40**:107-119.

Kishimoto T. The biology of interleukin-6. *Blood* 1989;**74**:1-10.

Klinge B. Nordlund A. A socio-economic perspective on periodontal diseases: a systematic review. *J Clin Periodontol* 2005;**32** Suppl 6:314-325.

Kornman KS. Crane A. Wang HY. di Giovine FS. Newman MG. Pirk FW. Wilson TG Jr. Higginbottom FL. Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;**24**:72-77.

Kornman KS. di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann Periodontol* 1998;**3**:327-338.

Kramps JA. van Twisk C. van der Linden AC. L-Pyroglutamyl-L-prolyl-L-valine-p-nitroanilide, a highly specific substrate for granulocyte elastase. *Scand J Clin Lab Invest* 1983;**43**:427-432.

Kunugi H. Urushibara T. Nanko S. Combined DEX/CRH test among Japanese patients with major depression. *J Psychiatr Res* 2004;**38**:123-128.

LeResche L. Dworkin SF. The role of stress in inflammatory disease, including periodontal disease: review of concepts and current findings. *Periodontol 2000* 2002;**30**:91-103.

Löe H. Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand* 1963**;21**:533-551.

Löe H. Theilade E. Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965;**36**:177-187.

Löe H. The gingival index, the plaque index and the retention index system. *J Periodontol* 1967;**38**:610-616.

Machtei EE. Hausmann E. Dunford R. Grossi S. Ho A. Davis G. Chandler J. Zambon J.Genco RJ. Longitudinal study of predictive factors for periodontal disease and tooth loss. *J Clin Periodontol* 1999;**26**:374-380.

Madianos PN. Bobetsis YA. Kinane DF. Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. *J Clin Periodontol* 2005;**32** Suppl 6:57-71.

Maes M. Meltzer HY. Stevens W. Calabrese J. Cosyns P. Natural killer cell activity in major depression: relation to circulating natural killer cells, cellular indices of the immune response, and depressive phenomenology. *Prog Neuropsychopharmacol Biol Psychiatry* 1994;**18**:717-730.

Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry* 1995;**19**:11-38.

Maes M. Bosmans E. De Jongh R. Kenis G. Vandoolaeghe E. Neels H. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 1997;**9**:853-858.

Maes M. Lin AH. Delmeire L. Van Gastel A. Kenis G. De Jongh R. Bosmans E. Elevated serum interleukin-6 (IL-6) and IL-6 receptor concentrations in posttraumatic stress disorder following accidental man-made traumatic events. *Biol Psychiatry* 1999;**1**:833-839.

Mandel ID. The functions of saliva. *J Dent Res* 1987;**66**:623-627.

Mantilla CB. Horlocker TT. Schroeder DR. Berry DJ. Brown DL. Frequency of myocardial infarction, pulmonary embolism, deep venous thrombosis, and death following primary hip or knee arthroplasty. *Anesthesiology* 2002;**96**:1140-1146.

Mariotti A. Sex steroid hormones and cell dynamics in the periodontium. *Crit Rev Oral Biol Me.* 1994;**5**:27-53.

Mariotti A. Dental plaque-induced gingival diseases. *Ann Periodontol* 1999;**4**:7-19.

Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res* 1994;**8**:263-271.

Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. *J Clin Periodontol* 2005;**32** Suppl 6:7-15.

McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998:**338**;171-179.

Melamed S. Ugarten U. Shirom A. Kahana L. Lerman Y. Froom P. Chronic burnout, somatic arousal and elevated salivary cortisol levels. *J Psychosom Res* 1999;**46**:591-598.

Mengel R. Bacher M. Flores-De-Jacoby L. Interactions between stress, interleukin-1beta, interleukin-6 and cortisol in periodontally diseased patients. *J Clin Periodontol* 2002;**29**:1012-1022.

Motivala SJ. Sarfatti A. Olmos L. Irwin MR. Inflammatory markers and sleep disturbance in major depression. *Psychosom Med* 2005;**67**:187-194.

Michalowicz BS. Diehl SR. Gunsolley JC. Sparks BS. Brooks CN. Koertge TE. Califano JV. Burmeister JA. Schenkein HA. Evidence of a substantial genetic basis for risk of adult periodontitis. *J Periodontol* 2000 ;**71**:1699-1707.

Monteiro da Silva AM. Newman HN. Oakley DA. O'Leary R. Psychosocial factors, dental plaque levels and smoking in periodontitis patients *J Clin Periodontol* 1998;**25**:517-523.

Mäntylä P. Stenman M. Kinane DF. Tikanoja S. Luoto H. Salo T. Sorsa T. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J Periodontal Res* 2003;**38**:436-439.

Nieminen A. Nordlund L. Uitto VJ. The effect of treatment on the activity of salivary proteases and glycosidases in adults with advanced periodontitis. *J Periodontol* 1993;**6**4:297-301.

Norderyd OM. Grossi SG. Machtei EE. Zambon JJ. Hausmann E. Dunford RG. Genco RJ. Periodontal status of women taking postmenopausal estrogen supplementation. *J Periodontol* 1993;**64**:957-962.

Norderyd O. Hugoson A. Risk of severe periodontal disease in a Swedish adult population. A cross-sectional study. *J Clin Periodontol* 1998;**25**:1022-1028.

Norderyd O. Hugoson A. Grusovin G. Risk of severe periodontal disease in a Swedish adult population. A longitudinal study. *J Clin Periodontol* 1999;**26**:608-615.

Offenbacher S. Odle BM. Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *J Periodontal Res* 1986;**21**:101-112.

Offenbacher S. Heasman PA. Collins JG. Modulation of host PGE2 secretion as a determinant of periodontal disease expression. *J Periodontol* 1993;**64**(5 Suppl):432-444.

Owen BM. Eccleston D. Ferrier IN. Young AH. Raised levels of plasma interleukin-1beta in major and postviral depression. *Acta Psychiatr Scand* 2001;**103**:226-228.

Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. *J Periodontal Res* 1991;**26**:230-242.

Page RC. Beck JD. Risk assessment for periodontal diseases. *Int Dent J* 1997;**47**:61-87.

Page RC. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. *Ann Periodontol* 1998;**3**:108-120.

Papapanou PN. Periodontal diseases: epidemiology. *Ann Periodontol* 1996;**1**:1-36.

Papapanou PN. Wennström JL. Gröndahl K. A 10-year retrospective study of periodontal disease progression. *J Clin Periodontol* 1989;**16**:403-411.

Papantonopoulos GH. Smoking influences decision making in periodontal therapy: a retrospective clinical study. *J Periodontol* 1999;**70**:1166-1173.

Payne JB. Reinhardt RA. Nummikoski PV. Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. *Osteoporos Int* 1999;**10**:34-40.

Persson R. Periodontal Risk Management (PRM). 2003 *Oral Health Prev Dent* 2003;**1**: Suppl 1:361-381.

Peeters F. Nicholson NA. Berkhof J. Cortisol responses to daily events in major depressive disorder. *Psychosom Med* 2003;**65**:836-841.

Pihlström BL. Michalowicz BS. Johnson NW. Periodontal diseases. *Lancet* 2005 **19;**366:1809-1820.

Pozo P. Valenzuela MA. Melej C. Zaldivar M. Puente J. Martinez B. Gamonal J. Longitudinal analysis of metalloproteinases, tissue inhibitors of metalloproteinases and clinical parameters in gingival crevicular fluid from periodontitis-affected patients. *J Periodontol Res* 2005;**40**:199-207.

Prayitno SW. Addy M. Wade WG. Does gingivitis lead to periodontitis in young adults? *Lancet* 1993;**342**:471-472.

Preber H. Bergström J. The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *J Clin Periodontol* 1986;**13**:319-323.

Reinhart RA. Payne JB. Maze CA. Patil KD. Gallagher SJ. Mattson JS. Influence of estrogen and osteopenia/osteoporosis on clinical periodontitis in postmenopausal women. *J Periodontol* 1999;**70**:823-828.

Riise T. Lund A. Prognostic factors in major depression: a long-term follow-up study of 323 patients. J Affect Disord 2001;**65**:297-306.

Rohleder N. Joksimovic L. Wolf JM. Kirschbaum C. Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biol Psychiatry* 2004;**1**:745-751.

Ronderos M. Jacobs DR. Himes JH. Pihlström BL. Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III. *J Clin Periodontol* 2000;**27**:778-786.

Sahingur SE. Cohen RE. Analysis of host responses and risk for disease progression. *Periodontol 2000* 2004;**34**:57-83.

Salonen JI. Paunio KU. An intracrevicular washing method for collection of crevicular contents. *Scand J Dent Res* 1991;**99**:406-412.

Salvi GE. Ramseier CA. Kandylaki M. Sigrist L. Awedowa E. Land NP. Experimental gingivitis in cigarette smokers: a clinical and microbiological study. *J Clin Periodontol* 2005;**32**:441-447.

SBU 2004. Behandling av depression - en systematisk litteraturöversikt. Statens beredning för medicinsk utvärdering.

SBU 2005. Behandling av ångestsjukdomar - en systematisk litteraturöversikt. Statens beredning för medicinsk utvärdering.

Shapira L. Wilensky A. Kinane DF Effect of genetic variability on the inflammatory response to periodontal infection. *J Clin Periodontol* 2005;**32** Suppl 6:72-86.

Sheiham A. Nicolau B. Evaluation of social and psychological factors in periodontal disease. Periodontol *2000* 2005;**39**:118-131.

Sheiham A. Netuveli GS. Periodontal diseases in Europe. *Periodontol 2000* 2002;**29**:104-121.

Silness J. Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22**:121-135.

Smith PC. Munoz VC. Collados L. Ovarzun AD. In situ detection of matrix metalloproteinase-9 (MMP-9) in gingival epithelium in human periodontal disease. *J Periodontal Res* 2004;**39**:87-92.

Socialstyrelsen. (2003). Utmattningssyndrom. Stressrelaterad psykisk ohälsa (No. 2003-123-18). Stockholm: Socialstyrelsen.

Socransky SS. Haffajee AD. Periodontal microbial ecology. *Periodontol 2000* 2005;**38**:135-187.

Solis AC. Lotufo RF. Pannuti CM. Brunheiro EC. Marques AH. Lotufo-Neto F. Association of periodontal disease to anxiety and depression symptoms, and psychosocial stress factors. *J Clin Periodontol* 2004;**31**:633-638.

Soory M. Hormonal factors in periodontal disease. *Dent Update* 2000;**27**:380-383.

Sorsa T. Tjäderhane L. Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* 2004;**10**:311-318.

Soskolne WA. Klinger A. The relationship between periodontal diseases and diabetes: an overview. *Ann Periodontol* 2001;**6**:91-98.

Stanford TW. Rees TD. Acquired immune suppression and other risk factors/indicators for periodontal disease progression. *Periodontol 2000* 2003;**32**:118-135.

Söder PÖ. Jin LJ. Söder B. Wikner S. Periodontal status in an urban adult population in Sweden. *Community Dent Oral Epidemiol* 1994;**22**:106-111.

Söder B. Neutrophil elastase activity, levels of prostaglandin E2, and matrix metalloproteinase-8 in refractory periodontitis sites in smokers and non-smokers. *Acta Odontol Scand* 1999;**57**:77-82.

Söder B. Nedlich U. Jin LJ. Longitudinal effect of non-surgical treatment and systemic metronidazole for 1 week in smokers and non-smokers with refractory periodontitis: a 5-year study. *J Periodontol* 1999;**70**:761-771.

Söder B. Jin LJ. Wickholm S.Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. *J Clin Periodontol* 2002;**29**:384-391.

Söder P. Söder B. Nowak J. Jogestrand T. Early carotid atherosclerosis in subjects with periodontal diseases. *Stroke* 2005;**36**:1195-1200.

Takashiba S. Naruishi K. Murayama Y. Perspective of cytokine regulation for periodontal treatment: fibroblast biology. *J Periodontol* 2003;**74**:103-110.

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**:1374-1385.

Tatakis DN. Trombelli L. Modulation of clinical expression of plaque-induced gingivitis. I. Background review and rationale. *J Clin Periodontol* 2004;**31**:229-238.

Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. *Ann Periodontol* 2001;**6**:99-112.

Teng YT. The role of acquired immunity and periodontal disease progression. *Crit Rev Oral Biol Med* 2003;**14**:237-252.

Tervonen T. Oliver RC. Long-term control of diabetes mellitus and periodontitis. *J Clin Periodontol* 1993;**20**:431-435.

Theilade E. Wright WH. Jensen SB. Löe H. Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *J Periodontal Res* 1966;**1**:1-13.

Tilakaratne A. Sorry M. Ranasinghe AW. Corea SM. Ekanavake SL. de Silva M. Periodontal disease status during pregnancy and 3 months post-partum, in a rural population of Sri-Lankan women. *J Clin Periodontol* 2000;**27**:787-792.

Travis J. Salvesen GS. Human plasma proteinase inhibitors. *Annu Rev Biochem* 1983;**52**:655-709.

Yoshihara A. Seida Y. Hanada N. Miyazaki H. A longitudinal study of the relationship between periodontal disease and bone mineral density in community-dwelling older adults. *J Clin Periodontol* 2004;**31**:680-684

Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990;**8**:253-278.

Verma S. Bhat KM. Diabetes mellitus--a modifier of periodontal disease expression. *J Int Acad Periodontol* 2004;**6**:13-20.

WHO. 1992. International statistical classification of diseases and related health problems (Vol. Tenth revision). Geneva: World Health Organization.

Wimmer G. Kohldorfer G. Mischak I. Lorenzoni M. Kallus KW. Coping with stress: its influence on periodontal therapy. *J Periodontol* 2005;**76**:90-98.

Uitto VJ. Gingival crevice fluid--an introduction. *Periodontol 2000* 2003;**31**:9-11.

Ximenez-Fyvie LA. Haffajee AD. Socransky SS. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *J Clin Periodontol* 2000;**27**:648-657.

Yang EV. Bane CM. MacCallum RC. Kiecolt-Glaser JK. Malarkey WB. Glaser R. Stress-related modulation of matrix metalloproteinase expression. *J Neuroimmunol* 2002;**133**:144-150.