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BAD BREATH

Prevalence, periodontal disease, microflora and inflammatory markers

AKADEMISK AVHANDLING

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ABSTRACT

Bad breath usually originates within the oral cavity and is believed to be associated with periodontal disease. The over all aim of the present thesis was to study periodontal conditions in subjects with bad breath as well as the prevalence of bad breath in subjects with periodontal disease. *Paper I* was designed to study the relationships between foetor ex ore, halitophobia, oral hygiene and periodontal disease. The aim of *Paper II* was to study periodontal conditions the presence of certain microorganisms, and inflammatory mediators, in subjects with bad breath. Our hypothesis was that the periodontal condition of patients with bad breath was worse than in subjects without this symptom. In *Paper I*, of 840 men [mean age = 35.7 years (± 2.8 SD)] and 841 woman [mean age = 35.7 years (± 2.9 SD)] who participated in an epidemiological study of periodontal health that was started in 1985, 2.4 % were found to exhibit foetor ex ore and 1 % were diagnosed as having halitophobia. Subjects with both periodontal disease and bad breath demonstrated a higher percentage of teeth with pockets ≥ 5 mm than did individuals without bad breath ($p < 0.001$). Moreover subjects with foetor ex ore showed significantly higher levels of plaque and calculus values than did those without this symptom ($p < 0.01$ and $p < 0.001$ respectively). Of the 28 participants described in *Paper II*, [mean age = 54.4 (± 3.5 SD) years] selected from the same group of patients described in *Paper I*, 8 still had bad breath, 10 suffered from periodontal disease without bad breath and 10 were periodontally healthy non-smokers. Subjects with bad breath had more teeth with pocket depths ≥ 5 mm or ≥ 8 -mm, than did subjects with periodontal disease but without bad breath. In the subjects with bad breath, all of the micro-organisms analysed by the PCR method, were detected and a significantly larger number of subjects with than without bad breath were colonized by *P.g.* ($p < 0.01$).

Conclusions: Paper I: Foetor ex ore is caused by poor oral hygiene, reflected by the amount of calculus and plaque present and visits to the dentist. In subjects with periodontitis, foetor ex ore may be a useful indicator of the severity of the disease. Halitophobia correlated with the presence of a relatively large amount of calculus on the teeth and occurs independently of foetor ex ore. *Paper II:* Subjects with both bad breath and periodontal disease exhibited higher levels of plaque, more gingival inflammation, more severe periodontal disease, and higher levels of PGE₂ and elastase in their gingival crevicular fluid than did periodontal patients without bad breath. More subjects with bad breath harboured *A.a.* and *P.g.* than those without this symptom. In particular *P.g.* was significantly more often detected in the group with bad breath.

General conclusions: The subjects with bad breath and periodontal disease exhibited the same levels of plaque throughout the 18-year study period. Bad breath, which could be a sign of active periodontal disease, does not seem to be influenced by smoking

Key words: Foetor ex ore, bad breath, halitophobia, oral hygiene, periodontal disease, periodontal pathogens and inflammatory markers.

INTRODUCTION

Bad breath

Bad breath usually originates within the oral cavity and is believed to be associated with periodontal disease (Ratkay et al., 1995, Ratcliff and Johnson, 1999). Mouth odour is described with several different names, such as *bad breath*, *foetor ex ore*, *oral malodour* and *halitosis* (Loesche and Kazor, 2002). The belief that one has bad breath, which cannot be detected by others, is called *halitophobia* (Yaegaki and Coil, 2000, Loesche and Kazor, 2002). A classification system for halitosis have been described by Yaegaki and Coil (2000) (see below) (Yaegaki and Coil, 2000). Patients classified as halitophobic need to be counselled with literature support, education and explanation of examination results. They also need assistance from a psychological specialist (Yaegaki and Coil, 1999).

Only a few epidemiological studies have documented the prevalence of bad breath in an entire population. For instance among 1681 young adults (30-40 years of age) in Sweden the prevalence of foetor ex ore was found to be no more than 2.4 % (Söder et al., 2000). The corresponding prevalence's in French and Japanese epidemiological studies were 22 % and 28 % respectively (Loesche and Kazor, 2002). Such pronounced variation probably reflects the use of different methods of examination and analysis of bad breath.

Examination of bad breath

The different methods used to analyze bad breath include *organoleptic measurement* (OR), *gas chromatography* (GC) and *sulphide monitoring*.

Organoleptic measurement is a sensory test scored on the examiner's perception of a subject's oral malodor (Yaegaki and Coil, 2000). The examination of bad breath is carried out by sniffing the patient's breath and scoring the level of oral malodor. Using a scale from 0-5, where 0 represents absence of odour and 5 severe malodor which cannot be tolerated by the examiner (Yaegaki and Coil, 2000). This method can be uncomfortable and embarrassing for the patient.

Gas chromatography (GC) is a method that requires a skilful operator and equipment with a flame photometric detector, which is impractical in the dental office This method is however considered to be the golden standard for measuring oral malodour since it is specific for volatile sulphur compounds (VSC) (Yaegaki and Coil, 2000).

Sulphide monitoring can be achieved by use of the Halimeter (Interscan Co., Chatsworth, CA) a compact sulphide monitor, which exhibits high sensitivity for hydrogen sulphide, but low sensitivity for methyl mercaptan which contributes significantly to bad breath in patients with periodontal disease (Yaegaki and Coil, 2000).

In addition Shimura and co-workers (1997) have described a monitor (New Cosmos Electric Co., Ltd., Osaka, Japan.) using a bag sampling method, which uses a zinc-oxide, thin-film semiconductor sensor (Shimura et al., 1997). Evaluation by this monitor correlates significantly with gas chromatographic readings of exhaled air and with organoleptic scores. These observations suggest that this monitor could be a substitute for the examiner's nose in clinical studies (Shimura et al., 1997).

Classification of bad breath

Yaegaki and Coil (2000) have described a classification system for halitosis; ***I Genuine halitosis, II Pseudo halitosis*** and ***III Halitophobia***

I Genuine halitosis involves obvious malodour, with intensity beyond social acceptable level, and is subdivided into two categories, *Physiological halitosis* and *Pathologic halitosis*.

Physiological halitosis the malodour arises through putrefactive processes within the oral cavity. Neither specific disease nor pathologic condition that could cause halitosis is found. Origin is mainly the dorsoposterior region of the tongue. Temporary halitosis due to dietary factors (e.g., garlic) should be excluded.

Pathologic halitosis is divided into two categories, i.e., *oral halitosis* and *extra oral halitosis*.

Oral halitosis is caused by disease, pathologic condition or malfunction of oral tissues. Halitosis derived from tongue coating, modified by pathological conditions (e.g., periodontal disease, xerostomia) is included in this subdivision.

Extra oral halitosis the malodour originates from nasal, paranasal and/or laryngeal regions, from the pulmonary- or upper digestive tract or from disorders anywhere else in the body. This odour is blood borne and exhaled via the lungs. Diabetes mellitus, hepatic cirrhosis, uraemia and internal bleeding are examples of conditions that can give rise to extra oral halitosis.

II Pseudo halitosis does not involve an obvious malodour that can be perceived by others, even though the patient stubbornly complains of his/her bad breath. This condition can be improved by counselling (using appropriate literature support, education and explanation of examination results) and simple oral hygiene measures.

III Halitophobia Even after treatment for genuine or pseudo-halitosis, the patient with this condition persists in believing that he/she has halitosis. No physical or social evidence exists to suggest that halitosis is present (Yaegaki and Coil, 2000). Halitophobic patients require professional psychological help (Yaegaki and Coil, 2000).

Dental plaque / biofilm

Dental plaque is a complex and dynamic microbial ecosystem and understanding the formation and development of this biofilm is a key to understanding of the development of periodontal disease (Filoche et al., 2004). The base of the film comprises of non-aggregated cells on which bacteria adhere to each other, thereby rapidly colonising the tooth surface (Filoche et al., 2004). Early colonizers adhere to the supra-gingival area and are predominantly beneficial, Gram - positive microorganisms such as *Actinomyces species* (Li et al., 2004). There does not seem to be an abrupt change in plaque composition at the gingival margin (Ximenez-Fyvie et al., 2000). Thus the bacterial species that colonise above and below the gingival margin are not strikingly different and putative periodontal pathogens can be found in both supra- and sub-gingival plaque samples (Ximenez-Fyvie et al., 2000). However, species such as *Treponema denticola* (*T.d.*), *Tannerella forsythensis* (*T.f.*), *Prevotella intermedia* (*P.i.*) and *Porphyromonas gingivalis* (*P.g.*) have been detected in higher numbers in sub-gingival plaque samples from periodontally diseased subjects than in healthy subjects (Ximenez-Fyvie et al., 2000, Torresyap et al., 2003). *P.g.* is one of the principal bacteria suggested to play a major role in the pathogenesis of periodontal disease and it also exerts systemic effects (Meurman et al., 1997; Morita and Wang, 2001a; 2001b, Nakano et al., 2002). *P.g.* also produces significant amounts of methyl mercaptane (Persson et al., 1990, Torresyap et al., 2003).

Formation of volatile sulphur compounds (VSC)

VSCs includes gases such as hydrogen sulphide (H₂S), methyl mercaptan (CH₃SH) and dimethylsulfide that arise from bacterial metabolism of amino acids, and are primarily responsible for oral malodour (Morita and Wang, 2001a, 2001b, Torresyap et al., 2003).

The periodontal pathogens *T.d.*, *P.g.*, *P.i.* and *T.f.*, are all capable of producing hydrogen sulphide (Torresyap et al., 2003). *T. d.*, *P. g.* and *P.i.* also produces significant amounts of methyl mercaptan (Persson et al., 1990, Torresyap et al., 2003). Even low concentrations of

many of these compounds are highly toxic to tissues, VSCs are potentially capable of altering the permeability of the gingival tissues, inducing inflammatory responses and modulating the functions of gingival fibroblasts (Ratkay et al., 1995, Ratcliff and Johnson, 1999).

Consequently, VSCs may play an important role in the pathogenesis of inflammatory conditions such as gingivitis and periodontitis (Ratkay et al., 1995,Ratcliff and Johnson, 1999, Torresyap et al., 2003).

Methyl mercaptan has been shown to act synergistically with lipopolysaccharide (LPS) and Interleukin-1 β (IL-1 β) to increase secretion of prostaglandin E₂ (PGE₂) and collagenase, important mediators of inflammation and tissue destruction (Ratkay et al., 1995).

Gingivitis

Numerous studies over the years have confirmed the role of microorganisms in the initiation and development of periodontal disease (Löe et al., 1965, Socransky and Haffajee, 1994).

Dental plaque that colonise the sub- and supra-gingival environment is agreed to be the main etiological factor in the development of periodontal disease (Ximenez-Fyvie et al., 2000, Torresyap et al., 2003).

Gingivitis is an immune response to antigens in bacterial plaque and is characterized by alterations in the connective tissue (Offenbacher, 1996) and to alterations in fibroblast functions accompanied by tissue destruction (Ratkay et al., 1995). One of the earliest events in disease development is enhanced permeability of the epithelium in the gingival sulcus (Offenbacher, 1996). VSCs are potentially capable of causing such an alteration and thereby inducing an inflammatory response (Ratkay et al., 1995, Ratcliff and Johnson, 1999). Thus Persson (1990) demonstrated that hydrogen sulphide (H₂S) facilitates the penetration of lipopolysaccharide (LPS) into tissues, thereby eliciting inflammation (Persson (1990).

Gingival inflammation is a reversible condition when treated with oral hygiene measures (Löe et al., 1965). The classification of gingivitis is however an important first step in defining a disease which may lead to a more serious periodontal disease (Mariotti, 1999).

Periodontitis

Periodontitis is an inflammation in the tooth-supporting tissue leading to loss of periodontal ligament and alveolar bone. It is generally agreed that human periodontitis is an infectious disease initiated by bacteria that colonise the sub- and supra-gingival environments

(Offenbacher, 1996, Ximenez-Fyvie, 2000) with several other modifying factors being involved in the progression of the disease. A susceptible host is required in addition to the disease related bacteria (Edwardsson, et al.,1999, Kharia et al., 2000) and smoking is probably one of the major aggravating risk factors for development of severe periodontal disease (Grossi et al., 1995, Söder et al.,1995, Genco, 1996).

Periodontal pathogens can be detected in both supra and sub-gingival plaque samples from periodontally healthy subjects. (Ximenez-Fyvie, 2000, Torresyap et al., 2003) Although, bacteria such as *T.d.*, *P.g.*, *P.i.* and *T.f.*, have been detected in higher levels in sub-gingival plaque samples in periodontally diseased subjects than in healthy subjects (Ximenez-Fyvie, 2000, Torresyap et al., 2003). The periodontal pathogens, *T.d.*, *P.g.*, *P.i.*, and *T.f.* are also capable of producing hydrogen sulphide and methyl mercaptan (Torresyap et al., 2003). These VSCs enhances antigen permeation through the epithelium, which results in connective tissue destruction and inflammatory reactions (Ratkay et al., 1995). Data suggest that the VSC levels correlate with the depth of the periodontal pockets (Morita and Wang 2001 a).

Gingival crevicular fluid (GCF)

GCF contains a filtrate of blood and exudates of the inflamed periodontal tissue (Cimasoni, 1983) and efforts to develop diagnostic tests based on host-derived factors have focused on analysis of the components present in this fluid (Page, 1992). The composition of GCF is the result of the interplay between the bacterial biofilm on the tooth surface and the cells of the periodontal tissue (Champagne et al., 2003). Since host response is a critical determinant in the pathogenesis of periodontal disease, the measure of inflammatory mediator levels in the GCF is being used to evaluate risk to develop periodontal disease or disease activity at a site level (Jin et al., 2000, Champagne et al., 2003).

Inflammatory mediators in GCF

Prostaglandin E₂ (PGE₂)

Prostaglandin E₂, one of the most potent biochemical mediators of inflammation, plays a central role in the pathogenesis of periodontal disease (Offenbacher et al., 1986). The presence and levels of this molecule reflect inflammatory and tissue-destructive responses elicited by host-bacterial interactions (Offenbacher et al., 1993b). Thus, the level of PGE₂ can

serve as an indicator of the degree of ongoing disease activity (Offenbacher et al., 1993a, Söder et al., 1999).

Interleukin-1 β (IL-1 β)

Cytokines are small soluble proteins, produced by cells which are undergoing alterations in their behaviour (Okada and Murakami, 1998). The family of cytokines includes *interleukins*, *lymphokines* and *monokines*, as well as growth factors and interferons. Certain cytokines, such as *interleukin-1 β* (IL-1 β), are produced in response to bacterial lipopolysaccharide (LPS), the stimulus for cytokine production that is most commonly studied *in vitro*. LPS is a highly potent toxin to which virtually all types of cells respond (Gemmel et al., 1997).

Matrix metalloproteinase-9 (MMP-9)

At least 6 MMPs designated -1, -2, -3, -8, -9 and -13 have been shown to be present in gingival crevicular fluid (Ingman et al., 1996, Golub et al., 1997, Apajalahti et al., 2003, Kinane et al., 2003). MMPs are involved in degradation of connective tissue (Fosang et al., 1993, Knäuper et al., 1993) and also participate in tissue remodelling associated with diseases of the skin (Birkrdal-Hansen, 1993). MMP-9 may be involved in the destruction of periodontal tissues during periodontal disease (Sorsa et al., 1988, Ingman et al., 1996).

Granulocyte elastase

Elastase a neutral serine protease produced by and stored in granulocytes (Giannopoulo et al., 1992), is capable of degrading a wide variety of connective tissue proteins and thereby altering the permeability of this tissue. Clearly, this protease plays a significant role in the destruction of connective tissue associated with inflammatory processes (Cimasoni et al., 1983, Söder et al., 2002, Figueredo et al., 2004). Thus, the level of neutrophil elastase activity in GCF provides a useful marker of the activity of intra crevicular polymorphonuclear leukocytes (PMN) (Gustafsson, 1996, Söder et al., 2002, Figueredo et al., 2004).

Polymerase chain –reaction (PCR)

PCR is an analyse method that can be used to detect bacteria in sub- gingival plaque samples at concentrations as low as 5 to 50 cells *per* sample. *Actinobacillus actinomycetemcomitans* (*A.a.*) and *Porphyromonas gingivalis* (*P.g.*) have been suggested to be useful and specific risk-indicators in the diagnosis and treatment of human periodontal disease (Genco, 1996, Torresyap et al., 2003). The PCR method can be used to detect both of these bacteria in sub-gingival plaque samples in a single reaction in less than four hours with high sensitivity (Wahlfors et al., 1995, Morillo et al., 2004).

AIMS

The overall aim of the present thesis was to study periodontal conditions in subjects with bad breath as well as the prevalence of bad breath in subjects with periodontal disease.

Paper I

The aim was to study the relation between foetor ex ore, halitophobia, oral hygiene and periodontal disease.

Paper II

The aim was to study periodontal conditions, the presence of certain micro-organisms and inflammatory mediators, in subjects with bad breath.

Our hypothesis was that the periodontal condition of patients with bad breath was worse than in subjects without this symptom.

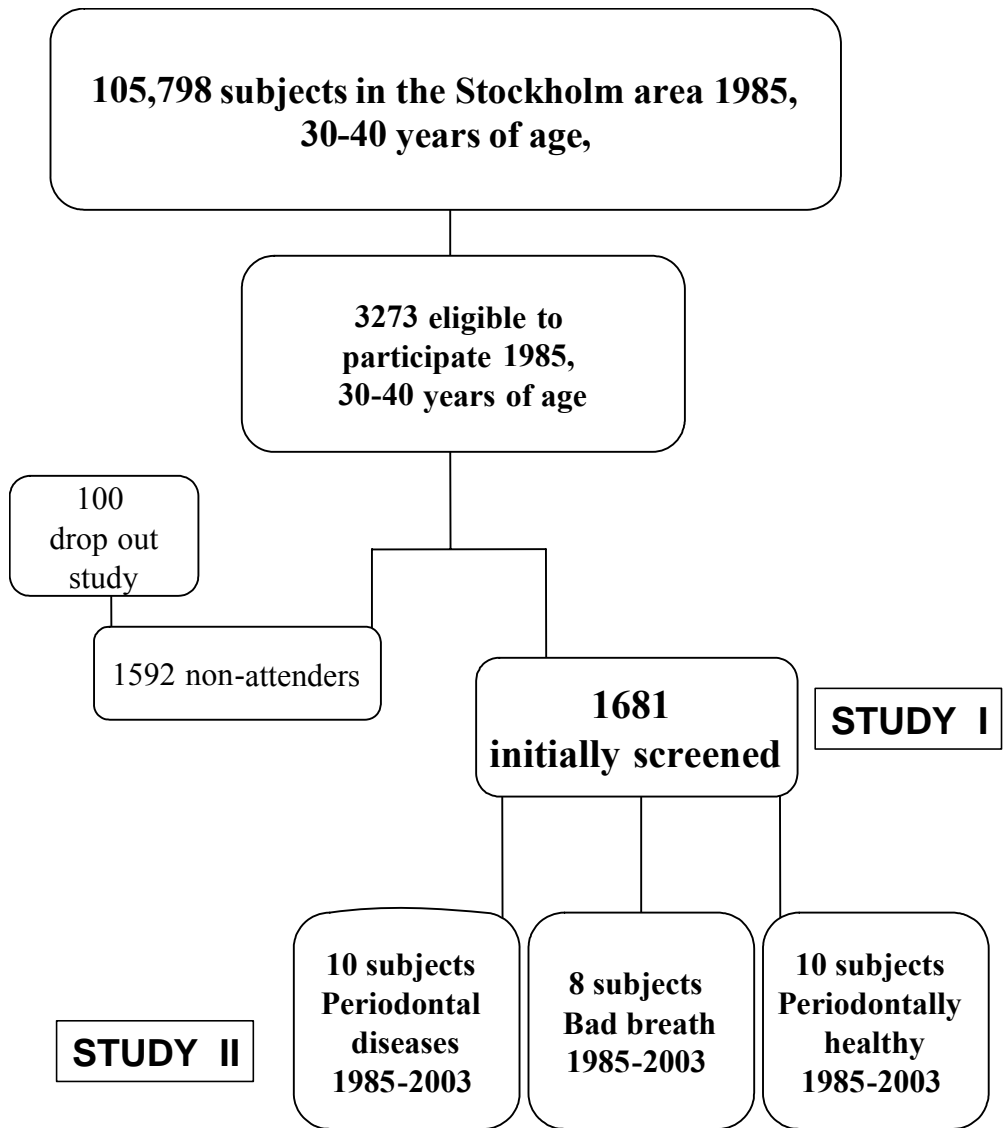


Fig. 1. Selection of the subjects to be included in Studies I and II

MATERIALS AND METHODS

Paper I, involved 840 men (mean age =35.7 (\pm 2.8 SD) years) and 841 woman (mean age =35.7 (\pm 2.9 SD) years) participating in an epidemiological study of periodontal health that was started in 1985 (Söder P-Ö et al., 1994, Söder B et al., 1995) (Fig.1). All of these subjects were examined by six experienced clinicians working at community dental centres in the Stockholm area. A pre-study calibration was performed including six secessions in which the same parameters were recorded. The entire oral cavity was examined for pathological changes.

The participants in *Paper II* were 28 subjects (mean age = 54.4 (\pm 3.5 SD) years), 8 (5 women, 3 men) had bad breath; 10 (7 women, 3 men) (5 smokers, 5 non-smokers), suffered from periodontal disease without bad breath and 10 (4 women, 6 men) were periodontally healthy non-smokers. These subjects were selected from the same group of patients who had taken part in the epidemiological study of periodontal health that started in 1985 and continued up to 2003. The 8 patients with bad breath were those who still exhibited this symptom 2003 despite receiving intensive dental therapy including scaling and rootplaning during the 18-year follow-up period (Fig .1).

The clinical examination in *Paper I* consisted of determining the number of remaining teeth (excluding third molars) and the oral hygiene status, using the simplified oral hygiene index (OHI-S) (Greene and Vermillion, 1964), including the debris (DI-S) and calculus indices (CI-S). After probing, a modified gingival index (GI-M) was used. Following this examination, the dentists recorded separately the presence or absence of foetor ex ore, defined as a strong evil-smelling odor emanating from the mouth of the patient which had an effect on the examiner and made the oral examination extremely unpleasant for the examiner.

In *Paper II* the clinical examinations, following the sampling, included measurement of dental plaque (PLI; Silness and Løe, 1964), calculus (CI-S, Greene and Vermillion, 1964) and determination of the degree of gingival inflammation using a non-invasive modification of the gingival index (GI; Løe and Silness, 1963). Pocket depth and loss of attachment were assessed. Periodontal disease was defined as the presence of three teeth with probing depths of \geq 5 mm and radiographic evidence of alveolar bone loss. Furthermore bleeding on probing (BOP) was registered and expressed as the percentage of bleeding sites per patient. Bad breath was evaluated using organoleptic measurement. The deepest site in each quadrant was selected for sampling of gingival crevicular fluid (GCF) and subsequently examined for elastase activity, levels of prostaglandin E₂ (PGE₂), matrix metalloproteinase-9 (MMP-9) and

interleukin 1-beta (IL-1 β). GCF was collected by using an intracrevicular washing technique (Jin et al., 1995a, 1995b) (Fig.2).

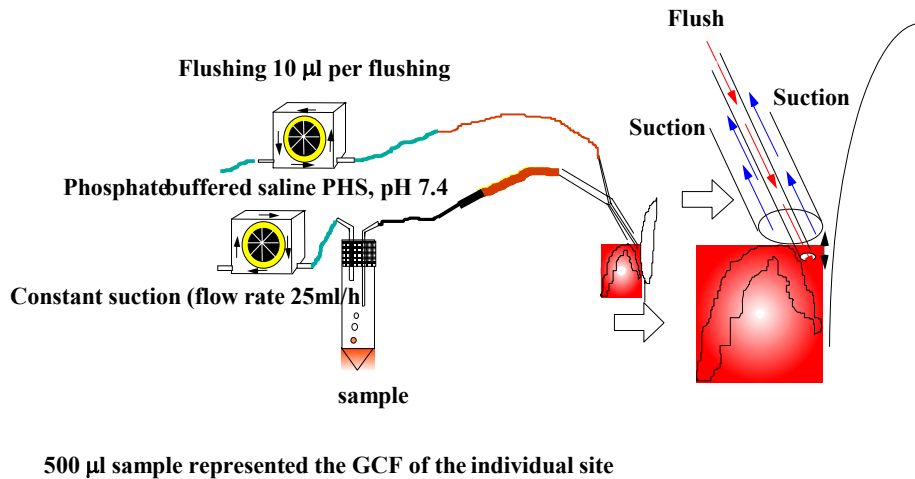


Fig.2. Schematic representation of the intracrevicular washing technique

Statistical analyses

In *Paper I* statistical analysis of the number or percentage of remaining teeth with PD \geq 5 mm, each tooth was regarded as a separate unit. Analyses of variance (ANOVA) Student's unpaired *t*-test, logistic regression, multiple linear regression and the chi-square test were employed and a P-value of < 0.05 considered significant.

The programs SAS/Stat¹ and StatView version 5.0 for Machintosh (¹ SAS[®] Institute Inc. SAS Circle, Cary, NC, USA) were utilised for these analyses.

In *Paper II* the clinical parameters for each individual patient were expressed as averages of the values for the four sites examined. The means and standard deviations for these parameters were calculated for descriptive statistics. The paired Student's *t*-test, Mann-Whitney test and chi-square test was employed, with differences between data sets exhibiting a P-value of <0.05 being regarded as statistically significant. These analyses were performed with the SPSS [®] software package, version 13 (SPSS Inc. Chicago, IL, USA)

RESULTS

Paper I

Of the 1681 subjects investigated, 2.4 % (41 subjects, 25 males and 16 females) exhibited foetor ex ore, (Tables 1A and 1B, in *Paper I*), while 1 % were diagnosed as having halitophobia (Table 5 in, *Paper I*). In subjects with foetor ex ore 51.2 % were smokers in contrast to subjects without foetor ex ore where 36.4 % were smokers. Among all of these subjects, 289 (17.2 %) had periodontal disease, with a mean of 18.5 % (± 1.1 SE) of their teeth with pocket depths ≥ 5 mm. The, 7.3 % of these individuals with periodontal disease who also had foetor ex ore demonstrated significantly ($p < 0.001$) higher percentages of teeth with pocket depths ≥ 5 mm (Tables 1A, 1B, in *Paper I*). The correlation between the presence of foetor ex ore and periodontal disease was statistically significant (chi-square 34.2, $p < 0.001$).

Table 1 A. Comparison of mean values with 95% confidence intervals(CI) of the difference for plaque(DI-S), calculus index(CI-S) and modified gingival index (GI-M), number and percent of remaining teeth with at least one site with pocket depth(PD) \Rightarrow 5 mm, and number of missing teeth between males with (n=24) or without foetor ex ore (n=816)

Index	Malodor Mean (95% CI)	No malodor Mean (95% CI)	p
DI-S	1.31 (1.01-1.61) ^a	0.77 (0.74-0.81) ^b	< 0.001
CI-S	1.28 (0.94-1.62) ^a	0.51 (0.47-0.55) ^b	< 0.001
GI-M	2.00 (1.81-2.19)	1.35 (1.31-1.39)	< 0.001
No. of Teeth with PD \Rightarrow 5 mm	3.76 (1.61-5.91)	0.94 (0.73-1.15)	< 0.001
% of Teeth with PD \Rightarrow 5 mm	13.43 (6.07-21.91)	3.37 (2.62-4.12)	< 0.001
No. Missing Teeth	3.44 (1.64-5.53)	1.22 (1.05-1.40)	< 0.001

^a one value is missing

^b three values are missing

Table 1 B. Comparison of mean values with 95% confidence intervals (CI) of the difference for plaque (DI-S), calculus index (CI-S) and modified gingival index (GI-M), number and percent of remaining teeth with at least one site with pocket depth (PD) \Rightarrow 5 mm, and number of missing teeth between females with (n =16) and without oral malodor (n = 825)

Index	Malodor	No malodor	P-Value
	Mean (95% CI)	Mean (95% CI)	
DI-S	1.21 (0.85-1.57)	0.61 (0.59-0.64) ^a	< 0.001
CI-S	1.24 (0.64-1.84)	0.37 (0.33-0.40) ^a	< 0.001
GI-M	1.84 (1.55-2.13)	1.17 (1.14-1.21)	< 0.001
No. of Teeth with PD \Rightarrow 5 mm	5.69 (2.14-9.23)	0.65 (0.49-0.80)	< 0.001
% of Teeth with PD \Rightarrow 5 mm	20.31 (7.66-32.97)	2.31 (1.76-2.85)	< 0.001
No. Missing Teeth	1.25 (0.21-2.29)	1.22 (1.07-1.36)	NS ^b

^a two values are missing

^b Not significant

One percent (10 males and 7 females) of the 1681 subjects complained of bad breath. In 16 of these cases bad breath could not be confirmed and was therefore diagnosed as halitophobia (Table 4, in *Paper I*).

Table 4. Comparison of mean values with 95% confidence intervals(CI) of the difference for plaque(DI-S), calculus index(CI-S) and modified gingival index (GI-M), number and percent of remaining teeth with at least one site with pocket depth(PD) \Rightarrow 5 mm, and number of missing teeth between subjects with (n = 16) and without "imaginated" halitosis (n = 1664)

Index	suspected halitosis Mean (95% CI)	no halitophobia Mean (95% CI)	p
DI-S	0.87 (0.58-1.17)	0.71 (0.68-0.73) ^a	NS ^b
CI-S	0.85 (0.51-1.20)	0.45 (0.43-0.48) ^a	0.01
GI-M	1.35 (1.17-1.53)	1.28 (1.25-1.30)	NS
No. of Teeth with PD \Rightarrow 5 mm	0.31 (-0.23-0.85)	0.88 (0.74-1.02)	NS
% of Teeth with PD \Rightarrow 5 mm	1.12(-0.82-3.05)	3.14 (2.66-3.63)	NS
No. Missing Teeth	1.00 (0.20-1.80)	1.26 (1.14-1.37)	NS

^a six values is missing

^b Not significant

On the basis of the entire population sample, the multiple regression analysis with foetor ex ore as the dependent variable showed that calculus (CI-S) ($p < 0.001$), plaque (DI-S) ($p < 0.01$) and dental visits less than once every 3 years ($p < 0.01$) had a significant independent effect on foetor ex ore (Table 5, in *Paper I*).

Table 5. Multiple linear regression analysis with oral malodor as dependent variable based on the entire population sample. n = 1668; 13 values are missing; F = 14.91, P < 0.001

	Regression Coefficient	Standard Error	Standard coefficient	t-value	P-value
Gender(Male=1; Female=0)	-0,004	0,007	-0,012	-0,5	NS ^a
Smoking(Yes=1; No=0)	0,005	0,008	0,016	0,68	NS
Dental Visits(1-3 yrs= 1; <3 yrs = 0)	-0,055	0,017	-0,079	-3,28	< 0.01
DI-S(0.0-3.0)	-0,025	0,009	-0,080	-2,68	< 0.01
CI-S(0.0-3.0)	-0,027	0,008	-0,101	-3,39	< 0.001
GI-M (0.0 - 3.0)	-0,007	0,009	-0,023	-0,71	NS
No. of Teeth with PD \Rightarrow 5 mm	-0,154	0,100	-2,875	-1,54	NS
% of Teeth with PD \Rightarrow 5 mm	0,041	0,028	2,726	1,46	NS
No. Missing Teeth	-0,002	0,002	0,029	-1,19	NS
Suspected halitosis (Yes=1; No=0)	0,016	0,036	0,011	0,452	NS

^a Not significant

Paper II

In subjects with bad breath, the clinical parameters recorded in 2003 did not differ significantly from those recorded in 1985. Subjects with bad breath had more severe periodontal disease than subjects with periodontal disease but without bad breath both in 1985 and in 2003. BOP was 2003 for subjects with periodontal disease and bad breath in mean, [53.2 (\pm 32.7 SD)], for subjects with periodontal disease but without this symptom [28.6 (\pm 24.8 SD)] and for controls [14.3 (\pm 11.9 SD)] % bleeding sites. Significant difference was found between subjects with bad breath and controls ($p < 0.01$).

In 2003, GI in subjects with bad breath was also higher [1.8(\pm 1.0 SD)] than in subjects with periodontitis but without bad breath [0.9(\pm 0.4 SD)] or in controls [0.1(\pm 0.1 SD)]. As was also the case in 1985, the difference between subjects with bad breath and controls with respect to this parameter was statistically significant ($p < 0.001$). These clinical values are documented in the table below.

Table Clinical oral and radiographic data in patients with bad breath, periodontal diseases and controls 1985 and 2003

Subject with bad breath (n=8)	1985	2003	p
Plaque Index (PLI) (mean \pm SD)	1.0 \pm 0.7	1.0 \pm 1.0*	NS
Gingival Index (GI) (mean \pm SD)	2.3 \pm 0.3***	1.8 \pm 1.0***	NS
Calculus Index (CaI) (mean \pm SD)	1.2 \pm 1.2	0.6 \pm 1.1	NS
No of teeth with PD \geq 5 mm (mean \pm SD)	9.7 \pm 5.1**	9.0 \pm 8.4**	NS
Number of missing teeth (mean \pm SD)	1.3 \pm 1.4	2.9 \pm 2.6*	NS
Bone % on radiographs (mean \pm SD)	89.6 \pm 3.4	82.4 \pm 7.4**	<0.05
Subject with periodontal diseases (n=10)			
Plaque Index (PLI) (mean \pm SD)	1.1 \pm 0.3**	0.4 \pm 0.3*	=0.001
Gingival Index (GI) (mean \pm SD)	2.0 \pm 0.6**	0.9 \pm 0.4*	<0.01
Calculus Index (CaI) (mean \pm SD)	1.0 \pm 0.7*	0.2 \pm 0.2	<0.01
No of teeth with PD \geq 5 mm (mean \pm SD)	8.2 \pm 2.0**	7.6 \pm 6.7**	NS
Number of missing teeth (mean \pm SD)	1.5 \pm 0.5	4.1 \pm 3.1**	<0.01
Bone % on radiographs (mean \pm SD)	87.7 \pm 9.3	82.2 \pm 10.3*	<0.001
Controls (n=10)			
Plaque Index (PLI) (mean \pm SD)	0.6 \pm 0.4	0.2 \pm 0.2	=0.001
Gingival Index (GI) (mean \pm SD)	1.2 \pm 0.4	0.1 \pm 0.1	<0.001
Calculus Index (CaI) (mean \pm SD)	0.3 \pm 0.7	0.1 \pm 0.0	NS
No of teeth with PD \geq 5 mm (mean \pm SD)	0	0	NS
Number of missing teeth (mean \pm SD)	0.2 \pm 0.6	0.6 \pm 0.8	NS
Bone % on radiographs (mean \pm SD)		93.0 \pm 4.2	

PD = Pocket Depth. Significant compared to the same parameter in controls, 1985 and 2003,

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Subjects with bad breath had more pockets ≥ 8 mm than subjects with periodontal disease but without bad breath, as well as more teeth with pockets ≥ 5 mm, shown in Fig.3.

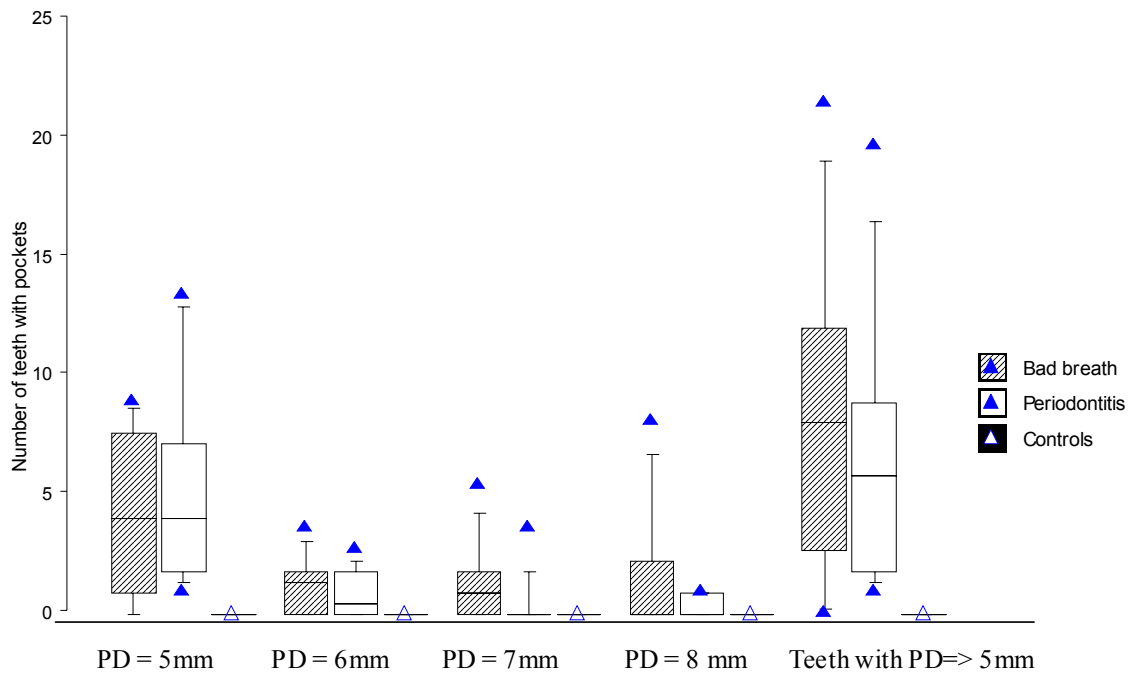


Fig.3. Distribution of teeth with pocket dept (PD) 5 - > 8 mm and total number of teeth with PD=> 5 mm in subjects with bad breath, subjects with periodontal disease without bad breath and controls.

Among the subjects with bad breath, all the micro-organisms analyzed were detected (Fig4). Significantly more subjects with than without bad breath had *P.g.* (Chi-square 6.67, $p < 0.01$). In addition, more subjects with bad breath had *A.a.* and *P.g.* than those with periodontal diseases but no bad breath symptom.

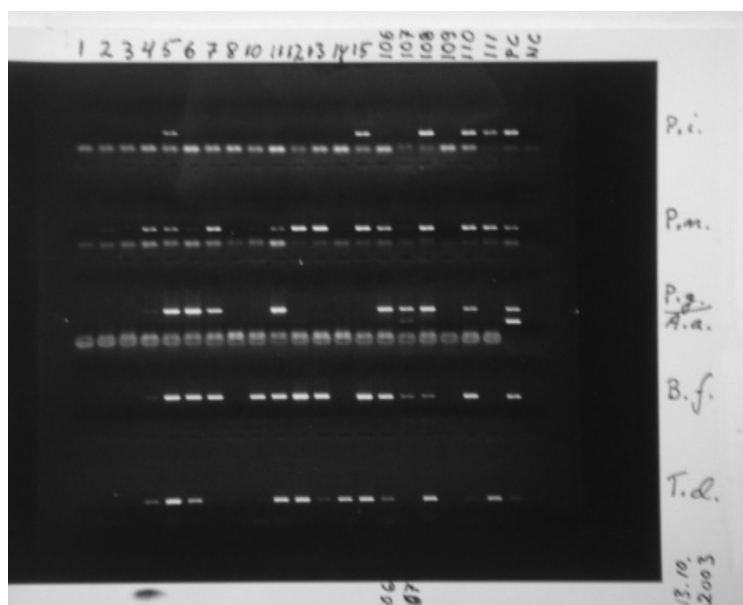


Fig.4. PCR analyses of subgingival plaque samples from subjects with and without bad breath

GENERAL DISCUSSION

It is always easy to recognise bad breath, but objective assessment of this condition has not provided a reliable estimate of the true prevalence. In several epidemiological studies documenting this prevalence in an entire population the values have ranged from 2.4 % to 28 % (Loesch et al., 1996, Söder et al., 2000), a variation which probably reflects the different methods of examination and analysis of bad breath employed. The low prevalence of 2.4 % reported in *Paper I* might be explained by the relatively young age of the participants together with the fact that they were well treated by their dentists.

In *Paper I* 17 of the subjects involved in the study complained of having bad breath. In 16 of these cases the dentist could not confirm this symptom and these individuals were therefore diagnosed, in this study, as suffering from halitophobia. In other studies this same condition has been referred to as delusional halitosis, olfactory reference syndrome, halitophobia or suspected halitosis (Pryse-Philips, 1971, Yasuno et al., 1989, Rosenberg, 1996, Yaegaki and Coil, 1999).

Different causes for this condition have been suggested. Iwakura et al. (1994) believe that imaginary halitosis may reflect the attitudes of other people (Iwakura et al., 1994) while Iwu and Akpata (1990) pointed out that suspected halitosis demonstrates features identical with those of a psychiatric disorder (Iwu and Akpata, 1990). Patients classified as halitophobic can clearly benefit from professional psychological assistance (Yaegaki and Coil, 1999).

The study reported in *Paper I* revealed no relationship between halitophobia and periodontal disease (chi-square 1.002; $p=0.96$). Bad breath and halitophobia also occurred independently, but halitophobia was correlated with a relatively large amount of calculus present on the teeth. This might explain, at least in part, why these patients believed they have bad breath.

Paper I also documents a significant correlation between bad breath and poor oral hygiene, as reflected in the amount of dental plaque and calculus.

Paper II shows that subjects with bad breath did not improve their plaque or gingival indices between 1985 and 2003, in contrast to periodontitis patients without bad breath and healthy controls, whose PLI and GI improved significantly (Table, in *Paper II*). This is in accordance with studies by Morita and Wang (2001), who found that inflamed pockets exhibit significantly higher total sulphide levels than non-inflamed pockets (Morita and Wang, 2001a, 2001b, Torresyap et al., 2003). In *Paper II* subjects with bad breath had more pockets of ≥ 8 mm depth than subjects with periodontal disease but without bad breath, and more teeth with pocket depths ≥ 5 mm (Fig.3). This is in accordance with Morita and Wang (2001) who

showed that the VSC levels are directly correlated to with the depth of the periodontal pockets (Morita and Wang 2001a).

The development of periodontal disease is an interaction between bacterial challenge and host response (Jin *et al.*, 2000; Ximenez-Fyvie *et al.*, 2000; Söder *et al.*, 2002) but putative periodontal pathogens such as *T.d.*, *P.g.* and *T.f.* can also produce copious amounts of VSCs, which are responsible for mouth odor (Persson *et al.*, 1990; Figueredo *et al.*, 2002).

These VSCs can probably enhance permeation of antigens through the epithelium, leading to inflammatory reactions and connective tissue destruction and (Ratkay *et al.*, 1995).

In *Paper II* more subjects with than without bad breath harboured *P. g.* This is interesting since *P.g.* is one of the principal bacteria suggested to play a major role in the pathogenesis of periodontal disease and it also exerts systemic effects (Figueredo *et al.*, 2002, Meurman, 2004). Further more *P. g.* is one of the bacteria that produce significant amounts of methyl mercaptan (Ratkay *et al.*, 1995). Methyl mercaptan acts synergistically with bacterial lipopolysaccharide (LPS) and Interleukin-1b (IL-1 β) to stimulate secretion of prostaglandin E₂ (PGE₂) and collagenase, which are important mediators of inflammation and tissue destruction (Ratkay *et al.*, 1995; Söder, 1999). The subjects In *Paper II* with bad breath demonstrated higher levels of PGE₂ and elastase in their GCF.

The higher levels of PGE₂ and elastase may reflect the more severe periodontal disease in subjects with bad breath than subjects with periodontal disease but without the symptom.

Thus our present findings are in agreement with those of Ratkay (1995) and may indicate that bad breath is a sign of active periodontal disease (Ratkay *et al.*, 1995).

CONCLUSIONS

Paper I

Foetor ex ore is caused by poor oral hygiene, reflected by the amount of calculus, plaque and visits to the dentist. In subjects with periodontitis, foetor ex ore may be a useful indicator of the severity of the disease. Halitophobia correlated with the presence of a relatively large amount of calculus on the teeth and occurs independently of foetor ex ore.

Paper II

Subjects with both bad breath and periodontal disease exhibited higher levels of plaque, more gingival inflammation, more severe periodontal disease, and higher levels of PGE₂ and elastase in their gingival crevicular fluid than did periodontal patients without bad breath. More subjects with bad breath harboured *A.a.* and *P.g.* than those without this symptom. In particular *P.g.* was significantly more often detected in the group with bad breath.

GENERAL CONCLUSION

The subjects with bad breath and periodontal disease exhibited the same levels of plaque throughout the 18-year study period. Bad breath could be a sign of active periodontal disease.

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