DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
182

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182

ENE-RENATE PÄHKLA

Factors related to the efficiency of treatment of advanced periodontitis

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CONTENTS

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Paper I: Study design, clinical evaluation and treatment of patients, collecting a specimen, data analysis.

Paper II: Study design, clinical evaluation and treatment of patients, collecting a specimen, data analysis, writing the paper.

Paper III: Study design, clinical evaluation and treatment of patients, collecting a specimen, data analysis, writing the paper.

Paper IV. Study design, clinical evaluation of patients and controls, data analysis, writing the paper.

Paper V. Study design, data analysis, writing the paper.

ABBREVIATIONS

AAP	American Academy of Periodontology
ANOVA	Analysis of Variance
AP	Adult Periodontitis
AP-PCR	Arbitrary Primed Polymerase Chain Reaction
ATC	Anatomical Therapeutic Chemical Classification
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CEJ	Cementoenamel Junction
CFU	Colony Forming Unit
CP	Chronic Periodontitis
CPI	Community Periodontal Index
CPTIN	Community Periodontal Index of Treatment Needs
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetate
GCF	Gingival Cervicular Fluid
HPLC	High Pressure Liquid Chromatography
ID	Unique Identifier
PGE	Prostaglandin E
MGI	Modified Gingival Index
MIC	Minimal Inhibitory Concentration
MUG	4-Methylumbelliferyl-β-D-Galactopyranoside
OR	Odds Ratio
PAL	Periodontal Attachment Level
PCR	Polymerase Chain Reaction
PI	Plaque Index
PPD	Periodontal Probing Depth
PD	Probing depth
PRFs	Prognostic risk factors
QC	Quality Control
RAL	Relative Attachment Level
RIs	Risk Indicators
RFs	Risk Factors
RMs	Risk Markers
RPs	Risk Predictors
SD	Standard Deviation
SRP	Scaling and Root Planing
SUP	Suppuration Index
TSBV	Tryptone Soya Vancomycin Bacitracin Agar
UV	Ultraviolet Light
VMGA	Viability Medium, Göteborg, Anaerobically Prepared and Sterilized
VPI	Visible plaque index
WHO	World Health Organization

1. INTRODUCTION

Periodontal tissues consist of gingiva, root cementum, periodontal ligament and alveolar bone. Inflammatory periodontal diseases, gingivitis and periodontitis, affect this tooth attachment apparatus. Gingivitis is defined as inflammation of the marginal gingiva, whereas periodontitis is characterized by a chronic inflammatory process, accompanied by degradation of the periodontal tissues. Loss of connective tissue attachment and alveolar bone resorption are consequences of the inflammatory process initiated by bacteria and of the ensuing complex defence mechanisms of the host (Page, 1986; Socransky and Haffajee, 1992). Formation of periodontal pockets, a clinical feature of periodontitis, results from the apical migration of epithelium along root surfaces previously occupied by connective tissue fibres (Heitz-Mayfield *et al*., 2003).

Destructive periodontal disease is the result of complex interactions between subgingival microflora and nonbacterial factors, specifically host and environmental factors (Axelsson, 2002). Periodontal disease is an infectious disease caused by the presence of bacterial plaque (Timmermann *et al*., 2001). Susceptibility to bacterial infection, caused by environmental factors, increases the risk of progressive periodontitis in a compromised host (Heitz-Mayfield, 2005). These microorganisms colonize the gingival region of the tooth surfaces, supragingivally as well as subgingivally**,** forming dentogingival plaque, also-called biofilm. In diseased pockets, microorganisms also grow subgingivally, without attaching to the tooth surfaces, and may invade the periodontal tissues (Allenspach-Petrzilka and Guggenheim, 2005).

Nearly 700 species of microorganisms have been isolated from periodontal pockets; it is likely that only a small percentage of these are aetiological agents (Haffajee and Socransky, 2005; Aas *et al*., 2005).

At the American Workshop on Periodontology (American Academy of Periodontology, 1996), there was consensus that *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,* and *Tannerella forsythensis* in particular should be considered as causative periopathogens.

If *A. actinomycetemcomitans* and *P. gingivalis* are true exogenous pathogens, avoidance of exposure to these organisms is relevant to the prevention of periodontal disease: their mere presence would be an indication for intervention (Slots, 2003). The prevention of opportunistic infections, however, implies the continuous control of ecological conditions regulating growth of the resident flora (Kõll-Klais *et. al.*, 2005). From this point of view, qualitative and quantitative tests would be required for the detection of putative pathogens.

The bacterial culture test is regarded as the "gold standard" microbiological assay against which other tests are compared and validated. Bacterial culture testing enables the recovery of the widest range of bacterial species and is the method of choice for determining antibiotic susceptibility and resistance – information of great importance in planning treatment of periodontal infections.

The ecological plaque hypothesis is based on the theory that the unique local microenvironment influences the composition of the oral microflora. According to this theory, periodontitis might be prevented not only by inhibiting the putative pathogens but also by interfering with the factors responsible for the transition of the plaque microflora from commensal to pathogenic relationship to the host (Marsh, 1991).

Reducing probing depths, surgical or nonsurgical, has long been successful in the treatment of periodontal disease, achieving an immediate, dramatic ecological change that favours a facultative anaerobic gingival microflora, and depriving the subgingival anaerobic microflora of its anaerobic environment at the base of deep pockets (Suvan, 2005).

The microbial aetiology of inflammatory periodontal disease provides the rationale for the use of antimicrobial medication in periodontal therapy. Antibiotics may be prescribed for periodontal patients who do not respond to conventional mechanical therapy, for patients with acute periodontal infections associated with systemic manifestations, as prophylaxis in medically compromised patients, and as an adjunct to surgical or nonsurgical periodontal therapy (Slots, 2003; Pihlstrom *et al*., 2005). This concept is based on the premise that specific microorganisms cause destructive periodontal disease and that the antibiotic agent *in vivo* can exceed concentrations necessary to kill or inhibit the pathogen(s).

Unnecessary antibiotic administration is contrary to sound clinical practice and may cause overgrowth of intrinsically resistant pathogens (Rams *et. al*., 1990; Olsvik *et al*., 1995) or may unnecessarily increase *in vivo* resistance to antibiotics that are valuable in potentially fatal medical infections (Slots and Pallasch, 1996; Roberts, 2002).

Data from numerous cross-sectional and longitudinal studies indicate that tobacco use, particularly smoking, is a powerful external (environmental) risk indicator for tooth loss and periodontal diseases (Dietrich *et al*., 2007, Axelsson, 2002). Among older adults, smokers are significantly more susceptible to the loss of periodontal attachment than are non-smokers (Beck *et al*., 1995; Machtei *et al*., 1997; Axelsson, 2002).

The first part of this study assessed the presence of periodontal pathogens in comparison with the total level of microorganisms after nonsurgical periodontal therapy. The clinical purpose was to assess the need for application of antimicrobial therapy for patients not responding to nonsurgical therapy.

The second part of the study compared the concentrations of metronidazole in plasma, saliva and gingival crevice fluid in patients with periodontitis after multiple administrations. The benefit of this information is that the general pharmacokinetic data for metronidazole, which have been established in numerous trials, can also be applied in the treatment of periodontal disease and in the design of respective treatment regimens.

The third part of the study compared the longitudinal effect of the combination of nonsurgical periodontal therapy with systemic antibiotic treatment in smoking and non-smoking patients.

The fourth part of the study evaluated the risk for periodontal diseases in children of periodontally diseased and healthy mothers. This data is important for the selection of high-risk children. Treatment of periodontitis in its early stage or proper prevention of the disease may help reduce the incidence of periodontitis in the future.

Finally, the fifth part of the study determined the involvement of the dental profession in the diagnosis and treatment of periodontitis. The research analysed the prescribed systemic medicine spectrum to study the suitability of systemic antibiotics that were used for treatment of periodontitis.

All studies for this dissertation were carried out in the Department of Stomatology, University of Tartu; Department of Clinical Microbiology, United Laboratories of Tartu University Clinics; Institute of Technology, University of Tartu; Department of Pharmacology, Department of Public Health, University of Tartu, Institute of Computer Science, University of Tartu and in the Polyclinic of the Tartu University Dental Clinic.

2. REVIEW OF THE LITERATURE

2.1. Classification of periodontal diseases

Plaque-induced periodontal diseases are mixed infections associated with relatively specific groups of indigenous oral bacteria (Moore and Moore, 1994; Haffajee and Socransky, 2005; Zambon, 1996). Susceptibility to these diseases is highly variable and depends on host responses to periodontal pathogens (Ishikawa *et al*., 1997; Kornman, 1997; Marsh and Martin, 1999). Although bacteria cause inflammatory periodontal diseases, the progression and clinical characteristics of these diseases are influenced by both acquired and genetic factors that can modify susceptibility to infection (Ronderos and Ryder, 2004; Axelsson, 2002; Heitz-Mayfield, 2005).

Periodontal diseases range in severity from early inflammation of the gingival margin to advanced loss of periodontal support and tooth loss. Traditionally, periodontal diseases have been classified as gingivitis or periodontitis. Gingivitis is caused by the oral microflora that colonize the tooth surfaces, forming plaque along the gingival margin. With control of gingival plaque, it is a reversible condition (Armitage, 2004).

In the absence of control of gingival plaque, the inflammation of the gingiva may progress to periodontitis, or irreversible loss of periodontal support (destruction of periodontal ligament as well as alveolar bone). Although untreated, infectious, inflamed gingival sites do not always progress to loss of periodontal support, periodontitis is always preceded by gingivitis.

The diagnosis and classification of these diseases is still based almost entirely on traditional clinical assessments (Armitage, 1996). Clinical features may include combinations of the following signs and symptoms: oedema, erythema, gingival bleeding upon probing, suppuration, attachment loss, tooth mobility and tooth loss.

The criteria used for diagnosis of periodontal diseases are: 1) presence or absence of clinical signs of inflammation (e.g., bleeding upon probing); 2) probing depths; 3) extent and pattern of loss of clinical attachment and bone; 4) patient's medical and dental histories; and 5) presence or absence of miscellaneous signs and symptoms, including pain, ulceration, and amount of observable plaque and calculus (Lang, 1996; Greenstein, 1997).

At the 1999 International Workshop for Classification of Periodontal Diseases and Conditions, a reclassification of the different forms of plaque-induced periodontal diseases was developed (Armitage, 1999). This revised classification includes seven general types of plaque-induced periodontal diseases: 1) gingivitis, 2) chronic periodontitis, 3) aggressive periodontitis, 4) periodontitis as a manifestation of systemic diseases, 5) necrotizing periodontal diseases, 6) abscesses of the periodontium, and 7) periodontitis associated with endodontic lesions (Armitage, 1999).

The major changes from the previous classification system are: 1) the term "chronic periodontitis" has replaced "adult periodontitis" (AP), and 2) the term "aggressive periodontitis" (AgP) has replaced "early-onset periodontitis" (AAP, 1999). Epidemiological data and clinical experience suggest that the form of periodontitis commonly found in adults can also be seen in adolescents. Thus the term "adult periodontitis" seems to be inaccurate in spite of the fact that periodontitis is more prevalent in adults and the elderly than in adolescents and young adults.

Chronic periodontitis is initiated and sustained by gingival biofilms at any age, but internal (genetics) and external (e.g., smoking) modifying factors play an integral role in its pathogenesis (Heitz-Mayfield 2005). Chronic periodontitis can be further characterized by extent and severity. Lindhe and Newman (1975) classified AP as *periodontitis levis*, *gravis* or *complicata*, according to the extent and pattern of alveolar bone loss, infrabony pockets, and furcation lesions. Additionally, diagnosis was given separately for each tooth in the dentition. Extent can be classified as localized if 30% or fewer of the sites are affected, and generalized if more than 30% of the sites are affected (Armitage, 1999). According to probing (clinical) attachment loss and pocket depth measurements, the severity of AP has been descriptively classified into mild or slight=1 to 2 mm clinical attachment loss (CAL), moderate=3 to 4 mm CAL, and severe=5 mm or more CAL (Armitage, 1999). The criterion for distinguishing advanced CP from less severe forms of the disease has been defined as attachment loss of greater than one third of the supporting periodontal tissues (AAP, 1999). Periodontal probing depths of >6 mm with attachment loss of >5 mm and radiographic evidence of bone loss are the clinical features for advanced destruction (AAP, 1999). However, the extent of tissue destruction is not further categorized, but may include localized lesions involving one area of a single tooth or generalized destruction affecting several teeth of the entire dentition. Overall, due to differences in the clinical definition of periodontitis it is difficult to compare data from different epidemiological and clinical studies, which reveals a need for precise criteria to determine the distinct categories of CP.

Based on the current classification system, the diagnosis should fall within one of the main classification categories or its subdivisions (Table 1).

Table 1. The periodontal disease classification system of the American Academy of Periodontology (Armitage, 1999; Wiebe and Putnins, 2000)

I	Gingival Diseases A. Dental plaque-induced gingival diseases B. Non-plaque-induced gingival lesions
II	Chronic Periodontitis A. Localized B. Generalized
III	Aggressive Periodontitis A. Localized B. Generalized
IV	Periodontitis as a Manifestation of Systemic Diseases A. Associated with haematological disorders B. Associated with genetic disorders C. Not otherwise specified
V	Necrotizing Periodontal Diseases A. Necrotizing ulcerative gingivitis B. Necrotizing ulcerative periodontitis
VI	Abscesses of the Periodontium A. Gingival abscess B. Periodontal abscess C. Pericoronal abscess
VII	Periodontitis Associated With Endodontic Lesions A. Combined periodontic-endodontic lesions
VIII	Developmental or Acquired Deformities and Conditions A. Localized tooth-related factors that modify or predispose B. Mucogingival deformities and conditions around teeth C. Mucogingival deformities and conditions on edentulous ridges D. Occlusal trauma

2.2. Prevalence of periodontitis

Periodontitis, viewed for years as primarily an outcome of infection, is now seen as resulting from a complex interplay between bacterial infection and host response, often modified by behavioural factors (Hujoel *et al*., 2003). The host response is now seen as a key factor in the clinical expression of periodontitis (Page *et al*., 1997). Only 20% of periodontal diseases are now attributed to bacterial variance, whereas some 50% of periodontal diseases are attributed to genetic variance and more than 20% to tobacco use, although the role of tobacco has also been estimated as higher (Hujoel *et al*., 2003; Darveau *et al*., 1997; Heitz-Mayfield, 2005).

Chronic periodontitis occurs mostly in adults, but it can be seen in younger people as well. Current epidemiological evidence indicates that severe periodontitis occurs in a few teeth in a relatively small proportion of people in any given age cohort, and the proportion affected increases with age (Brown *et al*., 1996). On the other hand, mild gingival inflammation is common (Russak *et al*., 1984) and many adults have mild-to-moderate loss of periodontal attachment at some tooth sites. In Europe, the proportion of 35- to 44-year-old adults with shallow periodontal pockets (3.5–5.5 mm) ranges from 13% to 57%, and the mean proportion of adults with deep periodontal pockets (>5.5 mm) is 14% (Sheiham and Netuveli, 2002).

In the United States, periodontitis is common, with mild-to-moderate forms affecting 30% to 50% of adults and the severe generalized form affecting 5% to 15% of all adults (Brown *et al*., 1996). Periodontitis is even more prevalent in developing countries and is considerably variable globally, although the prevalence of the severe generalized disease appears to be similar in most populations (Pihlstrom *at al*., 2005).

Epidemiological data available at the World Health Organisation (WHO) are confirmed by studies which show that the prevalence and severity of periodontal disease tend to be higher in older age groups than in younger age groups (WHO, 2003).

Therefore, certain indicator age groups are identified by WHO for inter-country comparisons and to assess the impact of oral health systems on periodontal health (WHO, 1997). The essential age groups comprise 15 to 19 years, 35 to 44 years, and 65 to 74 years. The Community Periodontal Index (CPI) was introduced by WHO to provide profiles of periodontal health status in countries and to enable countries to plan intervention programmes for effective control of periodontal disease.

The CPI databank was recently updated, and the population data are available in the WHO Global Oral Health Data Bank. The CPI data are expressed in mean percentages of persons with certain CPI scores and the mean number of sextants with CPI scores, and the data are presented for the three age groups of adults in relation to the WHO region. The most severe score or sign of periodontal disease (CPI score 4) varies worldwide from 10% to 15% in adult

populations; however, the most prevalent score in all regions is CPI score 2 (gingival bleeding and calculus), which primarily reflects poor oral hygiene.

Determining the prevalence of periodontitis in the population of the United States is complicated by the various case definitions used (AAP, 2005). If periodontitis is defined as the identification of at least one site with CAL of ≥2 mm, around 80% of all adults are affected, and around 90% of those aged 55 to 64 (U.S. Public Health Service, 1972; NHANES III). When the case definition is at least one site with CAL of ≥ 4 mm, the prevalence in those aged 55 to 64 drops to around 50%; when it is CAL of ≥6 mm, prevalence is less than 20% (NHANES III). Using pockets of ≥4 mm as a case definition, 30% of adults had met that criterion on at least three to four teeth (Oliver *et al*., 1998).

Today, however, it is well documented that only 5% to 15% of any population suffers from severe generalized periodontitis, even though moderate disease affects a majority of adults (Oliver *et. al*., 1998). This clustering of serious disease in a subset of the population has been recorded among well-treated patients (Kornman, 2001; Tonetti, 1998) as well as in epidemiological studies of populations which do not receive modern dental care (Baelum, 1988; Baelum, 1987). Epidemiologically, the majority of almost any adult population has chronic periodontitis to some degree, but mild attachment loss, as measured by CAL of 2 mm or so, is compatible with good health and function for many years.

The basic clinical measures for periodontitis, apart from gingival bleeding and radiographic assessment of bone loss, are clinical attachment loss (CAL) and probing depth (PD). The standard protocol used today for measuring CAL and PD with a manual probe was first described more than 65 years ago and has not changed much since. According to the position of the American Academy of Periodontology (AAP, 2005) various scaled indexes have been used in the past, but these were "composite" indexes which scored gingivitis and periodontitis on the same scale. Composite indexes (i.e., CPI) are now considered invalid and have thus been discarded. Although CAL, a measure of accumulated past disease at a site rather than current activity, remains a diagnostic "gold standard" for periodontitis, the absence of consensus on how best to incorporate CAL and PD into a case definition of periodontitis continues to hamper clinical and epidemiological research. Consequently, the major dilemma in the epidemiology of periodontal diseases is that there are still no international standards or recommendations.

2.3. Aetiology and pathogenesis

Periodontitis, viewed for years as primarily the outcome from infection, is now seen as resulting from a complex interplay between bacterial infection and host response, often modified by behavioural factors (Page *et. al*., 1997), Figure 1.

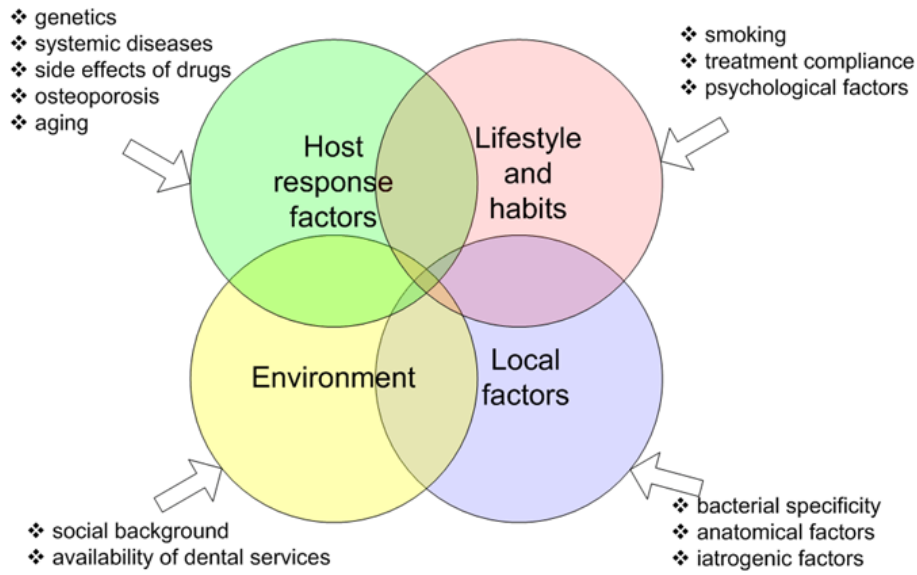

Figure 1. Aetiology of periodontitis

2.3.1. Biofilm and bacterial complexes

Although more than 700 species of microorganisms have been isolated from periodontal pockets (Haffajee and Socransky, 2005; Paster *et al*., 2006), it is likely that only a small percentage of these are aetiological agents (Moore and Moore, 1994). The subgingival microflora in a healthy oral cavity consists of facultative anaerobic gram-positive species, but in gingivitis the proportion of gram-negative bacteria increases (van Palenstein Heldreman, 1981). It is now accepted that three species – *P. gingivalis, A. actinomycetemcomitans* and *T. Forsythensis* – are true periodontal pathogens and the primary aetiological agents in periodontitis (Proceedings of the 1996 World Workshop in Periodontics). Additional putative pathogens include *Prevotella intermedia, Prevotella nigrescens, Campylobacter rectus, Fusobacterium nucleatum, Micromonas micros,* and spirochetes (Socransky and Haffajee, 1992; Proceedings of the 1996 World Workshop in Periodontics).

Consequently, periodontal disease appears to be a mixed infection. It is possible that only one or two species are the principal pathogens, the rest being involved in contributing to further injury once the lesion is initiated.

Intrafamilial transmission is possible and seems to be associated with individual susceptibility factors. When these bacterial forms are present, all the infected members of a given family carry the same clonal type. Usually, this same clonal type occurs in given pockets of family members (Socransky and Haffajee, 1994; Socransky and Haffajee, 2005; Dogan *et. al.,* 2008;, 2008, Zambon *et.al.,* 1996). Also parents and siblings of an individual with *A. Actinomycetemcomitans* may have an increased susceptibility to periodontitis (Dogan *et. al.,* 2008, Zambon *et.al.,* 1996).

Two fundamental features of bacterial plaque explain the difficulty in controlling its growth and eliminating it. First, subgingival plaque is organised as a biofilm, and second, the bacterial species it harbours interact to form bacterial complexes.

In the biofilm, the microorganisms live in well organised symbiosis; they are supplied with nutrients via microchannels through the plaque matrix and are inaccessible to phagocytozing leucocytes (polymorphonuclear leukocytes and macrophages), chemical plaque control agents, and antibiotics.

The different bacterial species interact by facilitating or inhibiting the progression of periodontal disease and form bacterial complexes. Some are exogenous, while others are normally present in dental plaque (Socransky *et al*., 1998). Socransky *et. al*. proposed a classification system that attributes four bacterial complexes to distinct colour categories, and it has since become a reference.

Red complex: *P. gingivalis, Treponema denticola, T. forsythensis.*

These exogenous species are found in great numbers in subgingival plaque, deep pockets, and advanced lesions. They invade periodontal tissues and the cementum and produce proteolytic enzymes.

Orange complex: *F. nucleatum, P. intermedia, P. nigrescens, M. micros, C. rectus.*

P. intermedia is systematically associated with *F. nucleatum* in deep pockets. Both of these endogenous pathogenic, anaerobic microorganisms are responsible for opportunistic commensal infections. *M. micros* and *C. rectus* are frequently observed in sites related to mobile teeth. *P. intermedia*, *C. rectus*, and *M. micros* are frequently found in the saliva of patients with advanced periodontitis.

Yellow and green complex: *Eikenella corrodens, Streptococcus sanguis.*

These pathogens are seldom found associated with those belonging to the orange and red complexes. There is probably an antagonistic relation between these subgroups: coexistence of species from the red or orange groups with species from this group is difficult or impossible. The so-called beneficial species are found in this group.

An increase in the percentage of certain suspected periopathogens in cultivable bacterial flora has been shown to correlate with a higher risk of disease progression (Socransky and Haffajee, 2002). On the other hand, the absence of periodontal pathogens is an indicator of periodontal stability (Rooney *et. al.*, 2002). Suspected periodontal pathogens have been detected not only in the subgingival area, but also in oral mucosal surfaces, as in the dorsum of the tongue, the vestibulum and the tonsils as well as in saliva.

A. actinomycetemcomitans is an exogenous pathogenic microorganism, which means that it is not considered part of the commensal flora and not classified within any complex.

Knowledge of the differences between health and disease should help the therapist to define microbial endpoints in the treatment of periodontal infections (Socransky *et. al*., 2002).

2.3.2. Intrafamilial transmission of the periodontal pathogens

It is well established that severe periodontitis clusters in families (Van der Velden *et. al*., 1993; Van der Velden *et. al*., 1996).

Many similar clones of periodontal pathogens are in found within families suggesting that the aggregation of periodontitis in families can be due to intrafamilial transmission of periodontal pathogens (Petit *et. al*., 1994; Könönen *et. al*., 2000). Children obviously acquire oral bacteria via the saliva of their frequent close contacts, the mother being the most important one (Li and Caufield, 1995).

Some studies have shown that if children harbour *A. Actinomycetem-comitans*, usually one or two parents harbour the same strain. However, identical genotypes in family members are not 100% proof of transmission, as there are not an infinite number of genotypes and finding identical genotypes may have occurred by chance (Asikainen *et. al*., 1996). The frequency of vertical transmission of *A. actinomycetemcomitans* is between 30% and 60% based on detection of identical genotypes in children and parents.

The research indicates that some periodontopathic bacteria, such as *P. gingivalis*, are not so easily transmitted from parents to children. On the other hand, periodontopathic bacteria such as *T. denticola* and *C. rectus* seem to transmit among family members more often (Musilova *et. al*., 2008).

Data indicate that different species within the *P. intermedia* group have different colonization patterns in childhood and the periodontal status reflects, qualitatively, their presence in maternal saliva. Intrafamilial transmission of *P. nigrescens* and *Prevotella pallens* can occur in early childhood (Könönen *et. al.*, 2000).

Children and young adults with chronic periodontal disease were previously studied along with patients having localized aggressive periodontitis and generalized aggressive periodontitis. In most studies, intrafamilial spread of periodontal diseases was subjected to investigation of aggressive periodontitis

and to single specific pathogens, but there are no data available about the spread of sulcular microflora in the case of chronic periodontitis.

In addition, there are no data about the relationship between mothers with chronic periodontitis and their children's periodontal status although chronic periodontitis is one of the most common forms of periodontal disease.

2.3.3. Tobacco smoking

Tobacco smoking appears to be one of the most significant risk factors in the development and progression of periodontal disease (Isamail *et al*., 1990; Beck et al., 1995; Horning *et al*., 1992; Hyman *et al*., 2003; Heitz-Mayfield, 2005).

In studies in the United States and other countries, individuals who smoke (cigarettes and pipes) have six to seven times more alveolar bone loss than non-smokers (Grossi *et al*., 1995; Tomar *et al*., 2000). Patients with periodontitis are three to five times more likely to be smokers than those without attachment loss (Grossi *et al*., 1995; Bergström and Boström, 1987; Bergström, 2004)**.**

Smoking has been identified as one of the major predictive variables for response to periodontal therapy. Studies about the effectiveness of nonsurgical therapy have shown less probing depth reduction (Grossi *et al*., 1997; Renvert *et al*., 1998; Van der Velden *et al*., 2003) and less attachment gain (Ah *et al*,. 1994; Haffajee *et al*., 1997) in smokers compared with non-smokers. Among the patients who have been surgically treated for periodontitis and then longitudinally followed, smokers exhibited less reduction in probing depths (Ah *et al*., 1994; Kaldahl *et al*., 1996), less gain in clinical attachment levels (Ah *et al*., 1994; Kaldahl *et al*., 1996), and less gain in bone height than non-smokers (Boström *et al*., 1998a,b).

Several studies have demonstrated that the severity of periodontal disease appears to be related to the duration of tobacco use, smoking status, and the amount of daily tobacco intake (Grossi *et al*., 1994; Grossi *et al*., 1995; Krall *et al*., 1997).

Studies on the periodontal microflora of cigarette smokers showed no difference between smokers and non-smokers (Stoltenberg *et al*., 1993; Preber *et al*., 1992), although recent studies involving large sample sizes suggest that certain periopathogens are more prevalent among smokers (Grossi *et al*., 1997; Grossi *et al*., 1996; Van der Velden *et al*., 2003).

Clinical studies have consistently shown that smokers respond less favourably to scaling and root planing, and that tobacco users have a poorer response to surgical pocket therapy. Hence, few studies have examined the effect of the combination of nonsurgical therapy with systemic antibiotic therapy on the treatment response of smoking and non-smoking patients (Labriola *et al*., 2005; Jin *et al*., 2000; Gamal, 2002). More information is needed about the longitudinal effect of the combination of nonsurgical periodontal therapy with systemic antibiotic in smoking and non-smoking patients.

2.3.4. Pathogenesis

The pathogenesis of human periodontal disease was described by Page and Schroeder (1981) and is currently accepted (Proceedings of the 1996 World Workshop in Periodontics; American Academy of Periodontology, 1999). The pathogenesis of periodontal lesion occurs in initial, early, established, and advanced stages, of which the first three are characterized as gingivitis and the last as periodontitis. In initial and early stages, accumulation of dental plaque in the gingival sulcus enhances leukocyte and neutrophil migration to the junctional epithelium and underlying connective tissue, results in collagen loss and fibroblast alteration in the marginal gingiva, and induces the proliferation of the basal cells of the junctional epithelium (Bosshardt and Lang, 2005). Later, in the established lesion, plasma cells predominate, the connective tissue loss continues, and the junctional epithelium migrates apically, starting the conversion to pocket epithelium (Zappa, 1995). Destruction of alveolar bone and connective tissue in the gingiva and periodontal ligament, periodontal pocket formation, and several inflammatory reactions take place in the advanced periodontal lesion (Davenport *et. al*., 1982).

Kornman and colleagues (1997) felt that although bacteria were essential for the development of periodontal disease, bacteria did not directly destroy the bone or connective tissue. Indirectly, they activated an inflammatory process in the periodontal tissue (Kornman *et al*., 1997, Kornman, 2008); for example, the release of lysosymal enzymes during phagocytosis, or the production of inflammatory mediators and cytokines that can stimulate soft tissue and bone resorption (Leibur *et al*., 1999; Jin *et al*., 1999; Sorsa *et al*., 2006). There is consensus that the bacteria initiate a challenge, which is then modified by a combination of genetic and acquired (e.g., smoking, diabetes) risk factors that amplify the response (AAP, 1996; Nieri *at al*., 2002; Van Dyke and Serhan, 2003; Kornman, 2008). See Figure 2.

It is clear that the initiation and propagation of most forms of gingivitis are dependent upon the presence and persistence of bacterial plaque. Although a high proportion of sites that experience periodontal attachment loss display signs of gingival inflammation, there is little evidence demonstrating that gingivitis lesions will always progress to become destructive periodontitis lesions (Lang *et al*., 2009). It has been shown that several factors are required for periodontal disease activation: host susceptibility, the presence of pathogenic agents, the presence of pathogens that produce virulence factors in amounts exceeding a threshold tolerable to the host, and the absence of beneficial bacterial species that create a favourable environment (Kornman, 2008). However, at this stage, the pathological processes that trigger the initiation of attachment loss have not been identified.

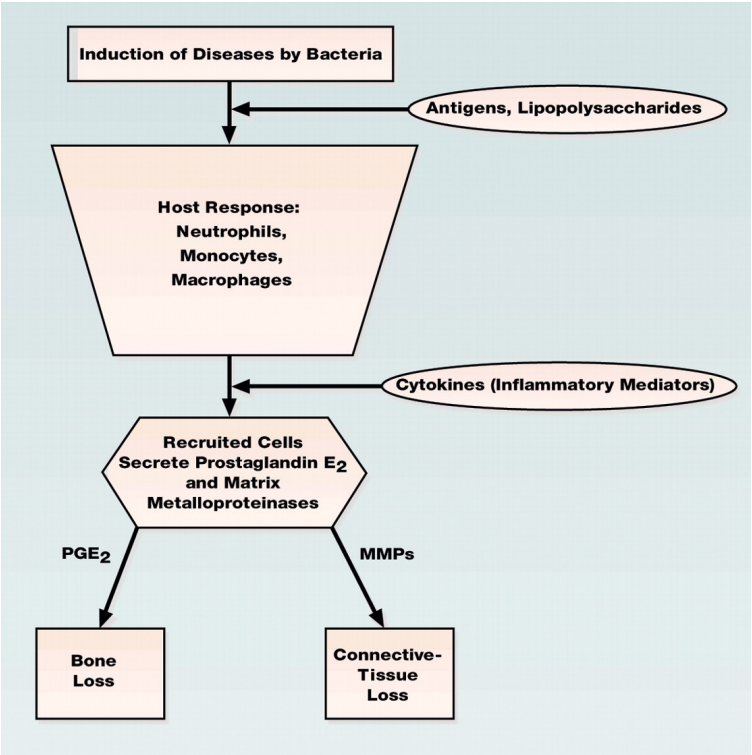

Figure 2. Model of disease pathogenesis. Adapted with permission of Quintessence Publishing Co. Inc. from Greenstein and Lamster 2000

2.4. Diagnostics in periodontitis

2.4.1. Clinical examination

Periodontal diagnosis relies on the information collected during the clinical periodontal examination of a patient. The information routinely collected during a periodontal examination includes demographic data (age, gender), medical history, history of previous and current periodontal problems, periodontal probe measurements (probing depths, CAL, etc.), radiographic findings, and miscellaneous clinical features or observations (e.g., gingival inflammation, plaque/calculus, mobility, occlusal problems) (AAP, 2003).

All traditional methods for determining the progression of periodontitis assess the degree of damage that has occurred in periodontal tissues over a given period of time. Measurements of probing depth, attachment level and tooth mobility provide historical information of past periodontal activity. They help in appreciating severity but do not necessarily reflect the degree of pathological activity. Pathological activity is characterized by objective signs,

such as the intensity of inflammation and presence of suppuration, and subjective signs, such as discomfort, tenderness, pain and halitosis. The most significant sign is bleeding on probing.

In clinical practice, conventional periodontal probes are widely used to obtain two important measurements: probing depth (PD) and clinical attachment loss (CAL). PD is defined as the distance from the gingival margin to the base of the probe-able crevice. PD measurements are clinically important since they provide a useful overall assessment of the depth of periodontal pockets which are the principal habitats of periodontal pathogens. In addition, PD measurements can be rapidly recorded and give a good assessment of the distribution of periodontal problems for a given patient (AAP, 2003).

Clinical attachment is the distance from the cemento-enamel junction (CEJ) to the base of the probable pocket. In certain situations, a landmark other then CEJ is used as a reference point from which attachment loss measurements are made (e.g., the cervicular margin of a restoration, the edge of the stent, or the occlusal surface of a tooth). Measurements made from these landmarks are referred to as relative attachment level.

CAL assessments are more difficult to accurately measure, but they give a better overall estimate of the amount of damage to the periodontium than do PD measurements. In prospective studies, CAL measurements are the most valid method of assessing treatment outcomes (Armitage, 1996). Multiple studies indicate that, in the hands of experienced practitioners, CAL measurements taken with conventional periodontal probes at different visits are repeatable to within ±1 mm more than 90% of the time (Armitage, 1996). The standard deviation of repeated CAL measurements of the same site by an experienced examiner with a manual probe is around 0.8 mm (Haffajee and Socransky, 1986). Accordingly, the change in attachment level in a clinical study needs to be at least 2 mm (i.e., two to three times the standard deviation) before the investigators can be confident that they are seeing real change rather than measurement error (Haffajee *et. al*., 1985; Lindhe *et. al.*, 1983). CAL progression of at least 3 mm over a given time period has been the criterion for change in other studies (Haffajee, 1991; Brown *et. al.*, 1994).

Probing depths of ≥ 5mm in deep residual pockets after initial periodontal therapy signify risk of disease progression, whereas moderate 4–5 mm deep periodontal pockets are poor indicators of future disease progression.

Consequently, it seems that the extent of inflammation prior to periodontitis is the best indicator of future disease progression and that it is not possible to predict future disease progression by using clinical means alone (Griffiths *et. al*., 1988).

There is general agreement that bleeding on probing is a reliable sign of gingival inflammation. Studies have shown that a 30% probability for future attachment loss may be predicted for sites that repeatedly exhibit bleeding on probing (Claffey *et. al*., 1990; Vanooteghem *et. al.*, 1987). Because the absence of bleeding on probing indicates periodontal stability, with negative predictive

value of 98% to 99% (Lang *et. al*., 1990), this is the most reliable clinical variable for monitoring a patient's progress in daily practice.

The presence and/or the amount of supragingival plaque is usually measured by one of the established plaque indices. The plaque index is based on estimated measurements of plaque by examination of the whole parts of the dentition. Each of the four gingival areas of the tooth is given a score from 0 to 3; this is the Visible Plaque Index (VPI) for the area. By adding the area scores for each tooth and dividing by the number of teeth examined, the plaque index for the individual is obtained. A major criticism of the Silness and Löe plaque index is the subjectivity involved in estimating plaque, which becomes apparent when several examiners are participating in a study. It is therefore recommended that single examiners be trained and used with each group of patients throughout a clinical trial (Axelsson, 2002). The Plaque Control Record is a procedure for evaluating the efficacy of oral hygiene programmes in daily practice. The disclosed plaque accumulation on all teeth is scored, on a dichotomous basis. Four or six surfaces per tooth are recorded. The number of positively scored units is divided by the total number of tooth surfaces evaluated, and the results are multiplied by 100 to express the index as a percentage. With this method, the topographic distribution of plaque through the dentition can be readily assessed.

2.4.2. Radiographic diagnosis

Radiographs provide information on the height and configuration of the interproximal alveolar bone (Tugnait *et. al*., 2000). The presence and severity of furcation involvements can also be determined from radiographs (Jeffcoat *et. al*., 1995). The extent of bone loss can be measured by using a grid or root-length ruler, and the result is generally expressed as a percentage of the root length. Panorama radiographs and periapical radiographs are comparable methods for detecting alveolar bone lesions (Hausmann, 2000; Persson *et. al*., 2003). Recently developed image-processing techniques enhance the detection of small osseous changes over short periods of time. These include digital subtraction radiography and computer-assisted densiometric image analysis (Hausmann, 2000; Park *et. al*., 2007).

2.4.3. Microbiological diagnosis

Clinicians faced with a multitude of clinical forms of periodontal diseases in their patients can count on laboratory examinations to help them in the therapeutic process (Pihlstrom *et. al.*, 2005). These biological examinations can offer help in five areas of application: support in diagnosis and prognosis, verification of the efficiency of the treatment, indication of the correct antibiotic therapy, and choice of the most appropriate medicine (Sixou, 2003; Loomer, 2004; Chapple, 2008).

Microbiological tests have been proposed for planning the treatment of new patients, selecting the appropriate recall interval, and monitoring periodontal therapy (van Winkelhoff and Winkel, 2009). By including examination of the subgingival microflora in treatment planning, it is possible to limit the risk of adverse sequels of infection by periodontal pathogens and to enhance a favourable clinical outcome (Mombelli *et. al*., 2003; Mombelli, 2005). Matchei *et. al*. (1997) showed that the odds ratio for further attachment loss in sites infected by *T. forsythensis* and *P. gingivalis* were 7.5 and 6.0 respectively.

Despite their diverse oral microbiological profiles, the majority of patients with chronic periodontitis respond well to mechanical periodontal treatment. For these patients, a sustainable reduction in the total level of periodontopathic bacteria in their pockets is usually adequate to stop the progression of the disease (Loesche *et. al*., 2002; Pihlstrom, 2005). However, there are some patients who do not respond well to traditional periodontal therapy. They continue to lose clinical attachment and alveolar bone (Pihlstrom, 2005).

Determining the composition of the subgingival biofilm and administration of antimicrobial agent may provide an additional therapeutic benefit for the patient (Listgarten and Loomer, 2003; Mombelli, 2005). Because these patients may have had previous empirical antibiotic therapy, resulting in antimicrobial resistance and the emergence of novel periodontal pathogens such as enteric species or yeasts, bacterial culture and antibiotic sensitivity tests are the assays of choice.

The efficiency of periodontal treatment can be judged by the disappearance of clinical symptoms and of the major pathogens involved in this pathology (Teles *et. al*., 2006). If, following immediate mechanical treatment, inflammatory phenomena persist in certain sites and specific periopathogens present, the treatment should be re-initiated to ameliorate clinical signs and destroy the pathogens involved (van Winkelhoff *et. al.,* 2009).

The nature of isolated microorganisms can help therapists to prescribe antibiotic therapy. Eradication of *A. actinomycetemcomitans* may require systemic antibiotic therapy (Saxen *et. al*., 1990; Takashi *et. al.*, 2007). In contrast, in adult periodontitis with subgingival microflora of moderate virulence, antibiotic therapy is not necessary (Slots and Jorgensen, 2000).

There is general agreement that microbiological tests for periodontal pathogens are not indicated in all patients, but it is mandatory for certain

indications: patients with aggressive periodontitis (Schenkein, 1994), patients with refractory disease (van Dyke, 1994; Teles *et. al*., 2006; Pihlstrom, 2005); patients about to undergo extensive prosthetic, implant or regenerative therapy (Slots and Rams, 1990; Teles *et. al*., 2006; Pihlstrom *et. al*., 2005) and patients with cardiovascular disease (Pallasch and Slots, 1996; Dajani *et. al*., 1997; Teles *et. al*., 2006; Pihlstrom *et. al*., 2005).

2.5. Treatment of chronic periodontitis

The goals of periodontal therapy are to preserve the natural dentition, to maintain and improve periodontal health, comfort, aesthetics and function. Currently accepted clinical signs of a healthy periodontium include: the absence of inflammatory signs of disease such as redness, swelling, suppuration and bleeding on probing; the maintenance of functional periodontal attachment level; and minimal or no recession in the absence of interproximal bone loss (AAP, 2001).

2.5.1. Nonsurgical periodontal therapy

Nonsurgical periodontal therapy is directed towards removal of the microbial biofilm from the root surfaces of periodontally diseased teeth. The aim of this treatment is to eliminate both living bacteria in the microbial biofilm and calcified microorganisms – that is, dental calculus – from the root surface and from the subgingival area without surgical reflection of the soft tissues surrounding the teeth (Cobb, 2002; Adriaens and Adriaens, 2004). As a consequence, the host tissues can better cope with remaining microorganisms, thereby reducing the inflammatory changes of the soft tissues and producing a varying degree of closure of the subgingival pocket (Cobb, 2002; Adriaens and Adriaens, 2004). The host should therefore be able to better control the microbial recolonization of the dentogingival area by personal oral hygiene measures (Loesche *et. al*., 2002; Adriaens and Adriaens, 2004).

Nonsurgical mechanical therapy includes different treatment modalities: depuration using hand instruments, sonic or ultrasonic instruments, motor driven instruments, and any combination of these instruments (Cobb, 2002; Adriaens and Adriaens, 2004).

Nonsurgical periodontal therapy induces beneficial changes to the periodontal tissues by the reduction of gingival inflammation and probing pocket depth, and a gain in clinical attachment level (Heitz-Mayfield *et. al.*, 2003). A number of studies indicate that none of the instrumentation techniques is totally effective in eliminating all bacteria and calculus from the subgingival surface of the tooth (Umeda *et. al*., 2004; Doungudomadcha *et. al*., 2001; Loesche *et. al*., 2002).

Nonsurgical treatment leads to a relative increase in the total numbers of beneficial bacteria and a reduction of bacterial load and the amount of pathogenic species present in all sites, except in furcations where the bacterial load decrease is less significant (Ishikawa and Baehni, 2004).

P. gingivalis is eliminated from most sites whereas *A. Actinomycetem-comitans* tends to persist in different sites (Renvert *et. al*., 1990, Doungu-domdacha *et.al.,* 2001). Failure to eliminate *A. actinomycetemcomitans* after mechanical instrumentation may relate to the ability of the organism to invade periodontal tissues (Johnson *ed. al.,* 2008). In deep pocket sites (probing depths exceeding 6 mm), only limited changes occur in the subgingival microbiota.

Controlled clinical trials show that manual and ultrasonic debridement can be used to treat most patients with mild-to-moderate chronic periodontitis (Greenstein, 2000; Aslund, 2008).

The percentage of the treated root surface with residual plaque or calculus is directly related to the probing pocket depth present at the time of instrumentation (van der Weijden and Timmerman, 2002; Adriaens and Adriaens, 2004). The treatment of single-rooted teeth is better than that of multirooted teeth. Problematic areas (e.g., furcations and osseous defects) may not respond optimally to nonsurgical therapy, because at these sites limited access impedes removal of bacterial deposits (Ehnevid, 2001). The effect of the treatment also depends on environmental factors such as the quality of oral hygiene (Pastagia, 2006), and the smoking status of the patient (Labriola *et. al*., 2005).

There were few data about the microbiological profile of chronic periodontitis in the Estonian population. Patients with periodontitis from Southern Estonia have been investigated microbiologically after mechanical debridement. This information helps clinicians to choose appropriate treatment modalities and to provide evidence-based periodontal practice.

2.5.2. Surgical periodontal therapy

A surgical approach to the treatment of periodontitis is utilized in an attempt to: 1) provide better access for removal of aetiological factors, 2) reduce probing depth, and 3) regenerate or reconstruct lost periodontal tissues (AAP, 1996).

Periodontal surgery is used when it is not possible to accomplish scaling and root planing without exposing the root surface. In addition, to gain access to the subgingival surfaces, the goal of periodontal surgery is to create an anatomically favourable gingival contour that facilitates the patients' home care. For this purpose, pocket elimination techniques (e.g., gingivectomy, apically positioned flap) and pocket reduction techniques (e.g., modified Widman flap) have been used (Wang and Greenwell, 2001). Both nonsurgical and surgical therapies have been shown to result in similar mean improvements of clinical scores, which, in general, suggests stability in attachment levels following therapy (Heitz-Mayfield *et. al*., 2003). However, the data for the

possible adjunctive effect of surgical procedures on patients/sites unresponsive to initial therapy are scarce (Claffey, 2004).

From a meta-analysis applied to study differences in the treatment effect between nonsurgical and surgical therapy, surgical therapy is the treatment of choice in probing pocket depth reduction for moderate and deep pockets (Wang and Greenwell, 2001). In increasing attachment level gain, nonsurgical therapy is of greater benefit for shallow (1–3 mm) and moderate (4–6 mm) pockets, and open flap debridement for deep pockets (≥6 mm) (Heitz-Mayfield, 2005). However, in many cases, the area to be treated includes a combination of shallow, moderate, and deep pockets.

According to studies performed by Levy *et. al*. (1999), surgery induces a significant decrease in bacterial counts from the orange complex (Levy *et. al*., 2002). *P. gingivalis* populations are completely eliminated from surgically treated sites. After bone surgery, *A. actinomycetemcomitans* and *P. gingivalis* are no longer detected. When bone surgery is not performed, there is an increase in *A. actinomycetemcomitans* numbers, although the *P. gingivalis* counts remain stable (Tuan *et. al*., 2000).

The choice of therapy may depend not only on the outcome measure of probing depth reduction and clinical attachment level gain, but also on the influence of other variables, including the evaluation of adverse effects and patient-centred outcomes.

2.5.3. Systemic antimicrobial therapy

The biological rationale for using antibiotics in the treatment of periodontal diseases is that bacteria are the major aetiological factor. Antibiotics are used systemically in the treatment of periodontitis as an adjunct to initial periodontal treatment to prevent the need for surgery only after traditional periodontal therapy has failed to achieve an adequate response (Van Winkelhoff and Winkel, 2009; Valenza *et. al.,* 2009, Ehmke *et al.,* 2005). Conditions that may call for systemic antimicrobial periodontal therapy are: continuing periodontal attachment loss despite diligent conventional mechanical treatment, periodontitis that is refractory to conventional mechanical and surgical periodontal therapy, aggressive types of periodontitis, medical conditions that predispose patients to periodontitis, and acute periodontal infection (Heitz-Mayfield, 2009; Slots, 2004; van Winkelhoff and Winkel, 2009).

Rational use of systemic antibiotics in periodontics requires adequate clinical diagnosis of the disease, thorough mechanical debridement, microbiological analysis of the subgingival plaque, and susceptibility testing. The drug must attain effective concentrations in gingival cervicular fluid (GCF) for an adequate length of time. The efficacy of periodontal antibiotic therapy is determined by the antimicrobial spectrum and the pharmacokinetic characteristics of the drug, and by the local environmental factors. A major concern

associated with the use of antibiotics is the potential for development of resistant bacterial strains (Kullik *et. al*., 2008). There are only a few controlled, double-blind studies on the efficacy of systemic antimicrobial agents in the treatment of periodontitis, and the evidence to support the validity of these agents is based on a series of case reports rather than placebo-controlled clinical trials (Ellen and McCulloch, 1996). From a meta-analysis applied to study differences in the treatment effect of systemically administered antibiotics as compared to controls on clinical measures of attachment level, the use of systemically administered adjunctive antibiotics with and without scaling and root planing and/or surgery appeared to provide a greater clinical improvement in CAL than therapies not employing these agents (Heitz-Mayfield, 2009). Due to the lack of sufficient sample sizes for many of the antibiotics tested, it is difficult to provide guidance as to the more effective antibiotics (Haffajee *et. al*., 2003).

Systemic drug therapy offers several benefits compared with local drug delivery. Systemic drugs can be delivered via serum to the base of the pocket and can affect tissue-invasive organisms (e.g., *A. actinomycetemcomitans*). They also can affect reservoirs of bacterial reinfection – the saliva, tonsils and mucosa. Furthermore, systemic drugs are often less costly and require less time to treat patients compared to locally delivered drugs (vanWinkelhoff *et. al*., 1988; Müller *et. al*., 1995; Asikainen and Chen, 1999).

The disadvantages of systemic antibiotic therapy as compared to locally applied antimicrobial agents include inability of systemic drugs to achieve high gingival crevice fluid concentrations (Goodson, 1994), an increased risk of adverse drug reactions (Walker, 1996), increased selection of multiple antibiotic resistant microorganisms (Walker, 1996; van Winkelhoff and Winkel, 2009; Kullik *et. al*., 2008), and uncertain patient compliance (Loesche, 1993). The precondition of efficient antibiotic therapy is ascertaining the microbe(s) causing the disease, though the broad spectrum of periodontal pathogens is a problem (Paster *at al*., 2001).

Metronidazole is an antibacterial compound widely used in the treatment of some types of periodontal disease. The pharmacokinetics of metronidazole in plasma has been well-described but few data exist about the penetration of the drug to the gingival crevice fluid.

Knowledge of several factors gives an overview about dental practice: the involvement of dental professionals engaged in diagnosing and treating periodontitis, the frequency with which antibiotics are prescribed, the frequency with which microbial analyses are taken before the cure with antibiotics, and the spectrum of drugs used. This information will help to provide further evidence-based guidelines for periodontal treatment in Southern Estonia.

The following table (Table 2) gives an overview of the preparations and doses used around the world, based on the data of the American Academy of Periodontology.

Table 2. International therapy schemes of periodontitis (American Academy of Periodontology, 2004)

Antibiotic/ Combination	**Dose (mg)**	**Administering (times a day)**	**Length of therapy in days**
Metronidazole	500	3	8
Clindamycin	300	3	8
Doxycycline/ Minocycline	100–200	4	8
Azitromycine	500	4	4–7
Metronidazole + amoxicillin	á 250	2	8

Periodontal treatment based on the mechanical approach has been used in the treatment of advanced periodontitis for many decades. However, in some cases, a combined treatment is needed that includes systemic antibiotic therapy. Although abundant data are available about pharmacology – side-effects, microbiological and clinical effectiveness of systemic antimicrobial treatment of periodontitis – the applicability of that information for treatment planning and for predicting the treatment outcome is still an open question in clinical practice.

It is not clear how patients should be selected for additional antimicrobial therapy, how patient-related and local health care factors may influence the treatment outcome, and how the recognition of patients susceptible to periodontal breakdown before manifestation of the disease can be simplified in clinical practice.

3. AIMS OF THE STUDY

The overall aim of this study was to evaluate problems related to the treatment of advanced periodontitis.

The specific objectives of the present investigation were

1. To assess the success rate of periodontal debridement, according to the presence of periodontal pathogens in comparison with the total level of microorganisms after nonsurgical periodontal therapy, and the need for application of antimicrobial therapy for patients not responding to nonsurgical therapy.
2. To test the concentration of metronidazole in plasma, saliva and gingival crevice fluid in patients with periodontitis after multiple administration of the drug, in order to provide clinicians with assurance in choosing the proper route of administration for the antimicrobial agent.
3. To compare the longitudinal effect of the combination of nonsurgical periodontal therapy with systemic antibiotic treatment in smoking and non-smoking patients.
4. To evaluate the risk of periodontal diseases in children of periodontally diseased and healthy mothers.
5. To determine the involvement of the dental profession in the diagnosis and treatment of periodontitis, including the selection of antibiotics for antimicrobial therapy.

4. MATERIAL AND METHODS

4.1. Study subjects and objects

Table 3. Study subjects, objects and performed investigations

Study	Subjects/Objects	Type of investigation
Investigation of subgingival microflora after nonsurgical periodontal therapy **(Paper I)**	140 patients with chronic periodontitis	Clinical and microbiological investigation
Comparison of the effect of the combination of nonsurgical periodontal therapy / systemic antibiotic treatment in smoking and non-smoking patients **(Paper III)**	28 adult patients with periodontitis (14 smokers and 14 non-smokers)	Clinical and microbiological investigation Comparison of the effect of the combination of nonsurgical periodontal therapy / systemic antibiotic treatment
Evaluation of the risk of periodontal diseases in children of periodontally diseased and healthy mothers **(Paper IV)**	20 mothers with periodontitis 13 periodontally healthy mothers 34 children of mothers with periodontitis 15 children of periodontally healthy mothers	Clinical and microbiological investigation Comparison of the clinical parameters of children of healthy and diseased mothers
		Comparison of the distribution of pathogens among different study groups
		Identification of intrafamilial spread of pathogens
Comparison of the concentration of metronidazole in plasma, saliva and periodontal pockets **(Paper II)**	11 adult patients with periodontitis	Clinical investigation Measuring the concentration levels of metronidazole (saliva, crevicular fluid, plasma) using the high-performance liquid chromatographic method
Investigation of the clinical and microbiological diagnosis and antibiotic treatment of chronic periodontitis in Southern Estonia **(Paper V)**	2102 prescriptions 409 ordered microbiological analyses	Screening the involvement of dental professionals Screening the testing of microbial analysis before the cure with antibiotics Screening the frequency of prescribing antibiotics and the spectrum of drugs used

4.1.1. Subjects

An overview of the material and methods used is presented in Table 3.

Paper I. The study material was collected from 140 adult patients with chronic generalized severe periodontitis referred to the Policlinic of the Tartu University Dental Clinic. The patients were systemically healthy and had not received antibiotics within the three months prior to entering the investigation.

Paper II. Twenty eight patients with generalized severe chronic periodontitis that did not respond well to previous mechanical periodontal treatment were recruited consecutively from new referrals to the Clinic of Stomatology of the Tartu University Clinics. The patients were healthy and had no systemic conditions known to affect periodontal tissues, nor had they had antibiotic therapy during the preceding six months. Cigarette consumption was determined on the basis of verbal questioning (10–20 cigarettes per day for ≥5 years). Of the patients, aged between 25 and 65, 14 smokers (S) and 14 non-smokers (NS) were consecutively selected.

Paper III. Eighty two patients were recruited consecutively from new referrals to the Policlinic of the Tartu University Dental Clinic. The following four study groups were included in this study: the first group included 20 female patients with untreated generalized severe chronic periodontitis (median age 35, ranges 31–44 years) and the second group was composed of their children (21 female and 13 male; median age 12, range 5–17 years). The third group included 13 periodontally healthy mothers (median age 36, range 29–43 years) and the fourth group was comprised of their children (9 female and 4 male; median age 12, range 10–16 years). All patients had no history of systemic disease or antibiotic therapy within the six months prior to sampling. The main inclusion criteria of the study were as follows: healthy and periodontally diseased mothers with their children (fully erupted first incisors and first molars) with first permanent teeth, up to 18 years old.

Paper IV. Eleven patients (six male, five female) with severe generalized chronic periodontitis were selected for the study. The mean age of patients was 46.3 ± 12.8 (range 24–60) years. Patients were in good general health and did not take any other medication.

4.1.2. Objects

Paper V. The study analysed 2102 prescriptions registered in pharmacies and sent to the Health Insurance Fund in the time period 01.01.2001–31.12.2006 on which the health service providers' area of workplace was Tartu County and the code of diagnosis was "K05.3" (chronic periodontitis). The following data was collected from the prescriptions: ID (the unique identifier of the patient), the name of the active ingredient of the drug, the code of the ATC (*Anatomical Therapeutic Chemical classification*) of the drug, the date of issue of the

prescription, the data of the health service provider (ID, the data of health institutions and doctors are substitution codes). The 409 samples of periodontal pockets sent to the United Laboratories of Tartu University Hospital for microbiological analysis during the same time period were analysed.

4.1.3. Ethical considerations

Participation in the studies was voluntary. Informed consent was obtained from study subjects, in accordance with the procedures of the Ethics Review Committee on Human Research of the University of Tartu.

4.2. Clinical examination

The baseline examination included the registration of dental plaque, gingival inflammation and the presence of suppuration on probing at four sites (distal, mesial, lingual and buccal), and periodontal probing depth and periodontal attachment level at six sites (distal, mid and mesial aspects for both buccal and lingual sites) for each tooth, excluding third molars.

The plaque status of an individual was given as the plaque index (PI) and as the frequency of plaque positive surfaces expressed as a total number of surfaces. Gingival inflammation was given as the modified gingival index (MGI: 0, healthy gingiva with no bleeding on probing; 1, bleeding on probing; 2, immediate and overt bleeding on probing; 3 spontaneous bleeding) and as the frequency of bleeding sites on probing, expressed as a percentage of all sites. Suppuration on probing was recorded as present or absent and results were given as the frequency of pus positive sites expressed as a percentage of all sites. Periodontal probing depth (PPD) was measured to the nearest millimetre from the gingival margin to the bottom of the gingival sulcus/pocket and attachment level (CAL) from the cementoenamel junction to the bottom of the periodontal pocket with a WHO periodontal probe. The mean across all sites formed the PPD and attachment level (CAL) of the patient. Alveolar bone loss was measured from panoramic tomography.

Severe cases of generalized chronic periodontitis were diagnosed based on gingival inflammation; periodontal breakdown with pocket depth greater than 6 mm in all sextants; minimum radiographic marginal alveolar bone loss >1/3 of the root length in at least two quadrants and a CPITN (Community Periodontal Index of Treatment Needs) score of 4 in at least three sextants. All the patients had at least 22 natural teeth.

Gingivitis was defined as gingival redness, swelling, loss of contour, marginal bleeding and pseudopockets in the absence of bone loss (Clerehugh *et al*., 2001).

Healthy individuals were defined as having no radiographic or clinical evidence: absence of deepened pockets and ≤20% bleeding on probing, the criterion that predicts periodontal inflammation.

All patients were recruited consecutively from new referrals to the Policlinic of the Tartu University Dental Clinic. The same examiner performed all clinical measurements.

4.3. Clinical procedures

Nonsurgical periodontal treatment (scaling and root planing under local anaesthesia at between four and six appointments over two to three weeks) was performed and, three weeks after completion of the treatment, periodontal status was clinically re-evaluated. Samples from periodontal pockets were obtained from patients with clinical signs of inflammation **(Paper I)**.

Clinical parameters were recorded at the baseline, two to three weeks after the first mechanical treatment and 14 months after combined treatment during a regular check-up visit. Following initial examination, each patient subsequently underwent quadrant scaling and root planing under local anaesthesia over a four-week period at up to six appointments. Two to three weeks after the last nonsurgical treatment visit, patients were reviewed, and initial healing was evaluated. As the patients did not respond to the conventional periodontal therapy, showing an inadequate resolution of inflammation, microbiological analyses were taken and a combination of systemic amoxicillin 500 mg × 3 and metronidazole 250mg × 2 for seven days was prescribed (Berglundh *et al*., 1988; Slots, 2002), **(Paper III**).

Nonsurgical periodontal treatment (scaling and root planing under local anaesthesia at four to six appointments during two to three weeks) was performed. Mechanical debridement and root planing under local anaesthesia was finished at least three weeks before the present trial. Metronidazole (500 mg tablet, Nycomed SEFA, Põlva, Estonia) was administered orally two or three times daily for at least two days before sample collection **(Paper II).**

4.4. Microbiological investigation of the subgingival microflora

4.4.1. Collection and transport of specimens

In patients with periodontitis, the six deepest inflamed periodontal pockets – three in the upper jaw and three in the lower jaw – were selected for sampling. In healthy persons and persons with gingivitis, six sulci (first molars and first incisors in opposite jaws) were selected. The pooled samples were obtained with a sterile Gracey 11/12M and 13/14 M curette. The plaque was transferred to the 2 mL VMGA III medium vials and the samples were processed in the laboratory within 4 hours (Dahlen and Möller, 1992).

4.4.2. Preparation and cultivation of specimens

The samples were homogenized with a Vortex mixer. The bacterial suspension was then serially diluted in five-fold steps in the Brucella broth (Oxoid, Basingstoke, Hampshire, UK). 100 µl aliquots from the dilutions were inoculated onto two agars: 1) Brucella agar (Oxoid) enriched with 5% horse blood and 1% menadione, and 2) tryptic soy-serum-bacitracin-vancomycin (TSBV) agar (Oxoid) for anaerobic and facultative anaerobic bacteria.

The Brucella agar plates were incubated in an anaerobic chamber (Sheldon Manufacturing Inc., Cornelius, Oregon, USA; gas mixture: 5% CO2, 5% H2, 90% N2) and the TSBV plates under microaerobic (Oxoid, CampyPak) conditions.

4.4.3. Identification and counting of microorganisms

After incubation at 35°C for five to seven days, the isolates were identified according to colonial and cellular morphology; the potency disk pattern (Vancomycin, Kanamycin, Colistin, Brilliant Green, and Oxgall); catalase, oxidase and spot indole reactions; long-wave UV light fluorescence; and MUG assay (Slots, 1986, Jousimies-Somer *et al*., 2002). All anaerobic micro-organisms were tested for absence of growth under microaerobic conditions.

The total level of microbial load of specimens collected from gingival pockets was calculated as the logarithm value of colony-forming unit per millilitre (log10CFU/ml), **Papers I, III**.

Microbiologically similar interfamilial species of pathogens were compared by arbitrary-PCR (Arbitrary Primed Polymerase Chain Reaction), **Paper IV**.

4.4.4. Genotyping of pathogens

Three- to four-day-old cultures on Wilkins-Chalgren Blood Agar (Oxoid) were suspended in 500 ml of lysis buffer (50 mM Tris-hydrochloride [pH 8.0], 5 mM EDTA, 50 mM sodium chloride, 1 mg of pronaseB (Roche) per ml, 1% sodium dodecyl sulfate), and the mixture was incubated for 60 min at 56°C. After centrifugation (12 000 g) for 10 min, DNA was extracted twice with 70% phenol-water-chloroform, adjusted to pH 7.6 with 1 M Tris-hydrochloride (pH 8.3), and precipitated with a 0.8 volume of isopropyl alcohol and 0.1 volume 3M potassium acetate. A DNA pellet was rinsed with ice-cold 70% ethanol and resuspended in distilled water.

For genotyping, AP-PCR (arbitrary primed polymerase chain reaction) was carried out with the primer set of ERIC1R (5‘-ATGTAAGCT CCTGGGG-ATTCAC-3‘) and ERIC2 (5‘-AAGTAAGTGACTGGGGTGAGCG-3‘). DNA amplification was performed for 35 cycles, with each cycle comprising 45 sec at 95°C, 1 min at 45°C, and 1 min at 65°C, with a single final extension step of 5 min at 72°C. DNA amplification was done in a DNA thermal cycler Master-cycler Personal (Eppendorf). In brief, DNA amplification was performed in a 50 µl reaction mixture consisting of 7.5 mM Tris-hydrochloride (pH 8.8), 20 mM ammonium sulfate, 0.1% (v/v) Tween 20, 4 mM magnesium chloride, deoxynucleoside triphosphate at a concentration of 400 mM, and 2.5 U of Taq DNA polymerase (Solis Biodyne). The reaction products were subjected to agarose gel electrophoresis with a 1.5% agarose (SeaKem) gel and were visualized under UV transillumination following ethidium bromide staining.

4.5. High pressure liquid chromatography

4.5.1. Collection of samples

Samples for drug determination were collected 2 hours after the last dose. Venous blood was collected by direct venepuncture into tubes containing Li-heparin anticoagulant, and was immediately centrifuged and plasma separated. Crevice fluid was collected from the eight deepest pockets (clinical pocket depth ≥6 mm) with micropipettes and pooled. Automatic 50 µl pipettes (Lenpipet, St. Petersburg, Russia) with adapted and curved tips were used. Crevice fluid collected from each patient was pooled into a weighed and labelled plastic microtube per patient. The amount of fluid in the tubes was calculated by weighing the tubes after sample collection. Before the gingival fluid was sampled, the teeth surfaces were isolated with cotton rolls and saliva injector and air-dried to prevent contamination of the sampling area with saliva.

Until analysis, the samples of collected biological material were stored at –20°C in PCR-clean microtubes (Eppendorf, Hamburg, Germany) without any conservant added.

4.5.2. Laboratory assay method

Metronidazole concentrations in plasma, saliva and gingival fluid samples were analysed by a HPLC (high-performance liquid chromatography) method. The same procedure was used for preparation of plasma, saliva and gingival fluid samples, for calibration curve construction and for quality control (QC) samples.

The samples were prepared for analysis by extraction with acetonitrile, and were thawed at room temperature. Fifty microliters of plasma or saliva was transferred to the 1.5 ml vial; gingival fluid samples were prepared in the sample collection vial. The exact amount of gingival fluid was estimated by weighing the vial before and after sample collection. 50 µl of 50 µg/ml tinidazole solution was added to this sample and vortex-mixed for 10 sec. Then 600 µl of acetonitrile was added and the sample was vortex-mixed for 1 min. The resulting sample was centrifuged at 6000 × g for 15 min at 10°C. Vials were kept frozen overnight at –20°C. 500 µl of organic layer was removed from frozen samples with a pipette and transferred to the 1.5 ml vial. The solvent was evaporated to dryness under a stream of air. The sample was reconstituted in 120 µl of mobile phase, vortex-mixed for 0.5 min and centrifuged at 8000 × g for 10 min at 10°C. Ninety microliters of this solution was transferred to the autosampler vial. The chromatographic system consisted of Lichrosorb RP-18 pre-column (Merck, Darmstadt, Germany), Lichrosorb RP-18, 5 µ, 250 ×3.2mM column (Merck), and an ultraviolet detector measuring at 318 nm. Mobile phase consisted of acetonitrile–0.01M phosphate solution (NaH2PO4), 15:85 (v:v), flow rate was 0.7ml/min., column temperature 22–25°C (room temperature). The analytical method was validated before the determination of study samples with regard to the following parameters: specificity, limit of detection and quantification, linearity, precision and accuracy, inter-day variability, intra-day variability, stability in the freezer and stability in the autosampler. The minimum quantifiable concentration (lowest concentration in the calibration curve) was 0.1 µg/ml. The method was linear over concentration range 0.1–50 µg/ml. No interfering peaks were registered at the retention times of the drug and internal standard. A measure of goodness of fit of linear regression, r2, was above 0.9995 in all calibrations showing good linearity. Mean intra-day precision (coefficient of variation in the determination results of QC samples) of the determinations was 5.51–9.19% and mean intra-day accuracy (bias % of QC samples) was from 1.20 to 4.53% in all concentrations. Mean inter-day precision of the determinations was 8.03–8.16% and mean inter-day accuracy was from –3.66 to 0.33% in all concentrations.

4.6. The involvement of dental professionals in the diagnosis and treatment of periodontitis

Doctors and health institutions that have issued relevant prescriptions were counted annually. The spectrum of antibiotics prescribed annually for the treatment of periodontitis and the groups of drugs were counted in each year.

The yearly number of prescriptions issued for diagnosis K05.3 was measured by counting prescriptions year on year and comparing this number to the number of periodontal pocket samples accepted by the laboratory of microbiology.

4.7. Statistical analysis

Statistical analysis (Descriptive statistics and Spearman's correlation) was carried out with the SigmaStat 2.0 (Jandel Scientific, San Rafael, CA, USA) program and Excel (Microsoft Corporation, Redmond, WA, USA).

The following tests were employed: repeated measures of analysis of variance (ANOVA) were used for analysing the data; Bonferroni's multiple comparison test was used to compare the plasma concentrations with concentrations in saliva and crevicular fluid; data were analysed with GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA, USA).

The baseline clinical data between smokers and non-smokers was compared using the Mann-Whitney test. The Signed Rank test was used to compare the changes in clinical parameters after systemic antibiotic therapy. The characteristics of healthy and diseased mothers and their children were compared with the Mann-Whitney U test and Fisher's test. Percentages were compared with the proportion test. The R version 2.4 (The R Foundation for Statistical Computing) was used for statistical analysis.

The differences in p-values less than 0.05 were considered statistically significant.

5. RESULTS

5.1. The microbiological status of patients with periodontitis in Southern Estonia after nonsurgical periodontal therapy (Paper I)

After instrumentation, no periodontal pathogens were isolated in 46 (33%) patients, whereas 94 patients (67%) were infected with one to five different periodontal pathogens. 53 patients harboured one, 27 harboured two, 12 harboured three and two patients harboured five pathogens. The pathogens isolated from the periodontal pockets of Estonian patients with chronic generalized severe periodontitis (n=140) are depicted in Figure 3.

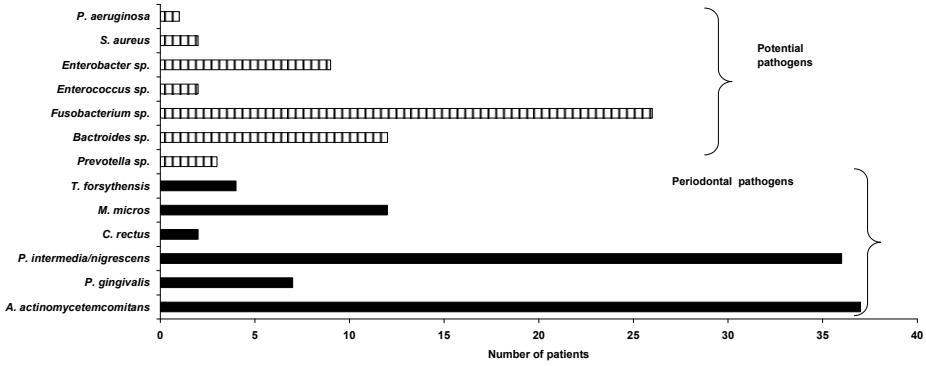

Figure 3. Specific and potential periodontal pathogens in Estonian patients with periodontitis (n=140) after nonsurgical periodontal therapy

Among the periodontal pathogens, *Prevotella intermedia/nigrescens* (37 patients) and *A. actinomycetemcomitans* (36 patients) were predominant, *M. micros* was detected in 12, *P. gingivalis* in 7, *T. forsythensis* in 4, and *C. rectus* in two patients. Proportional recovery of P. *intermedia/nigrescens* varied from 2.3 to 63% (median 16.9), of *A. actinomycetemcomitans* from 2.4 to 100% (median 23), of M. micros from 3 to 44% (median 21.8), of *P. gingivalis* from 9 to 65% (median 29), of *T. forsythensis* from 6.3 to 20% (median 9.6), and of *C. rectus* from 4.5 to 33% (median 18.8). The total level of microbial load (log10 CFU/ml) of all isolated microbes varied from zero to 8.4 log, whereas sterile samples occurred rarely, four cases per 140. The median of colonization after treatment was 5.5 log (according to 3x105CFU/ml), indicating the substantial effect of instrumentation in viable counts of microbes.

5.2. Metronidazole concentrations in plasma, saliva and periodontal pockets in patients with periodontitis (Paper II)

All included patients completed the study. All patients declared that they took the drug as prescribed. No adverse events were registered during the study. All planned samples were collected from the patients; the volume of gingival crevice fluid obtained per patient ranged from 8 to 35 µl. Metronidazole was quantified in all measured samples. Concentrations ranged from 4.8 to 30.6 mg/l. Individual measured concentrations with mean values and standard deviations are presented in Table 4. The highest concentrations were measured in saliva, the lowest in crevicular fluid. However, the difference between plasma and crevicular fluid or between plasma and saliva did not reach statistical significance.

Table 4. Number of tablets taken by patients per day and individual and mean ± SD metronidazole concentrations (mg/l) in plasma, saliva and gingival crevice fluid

Patient no.	No. of 500mg tablets per day	Plasma	Saliva	Crevice fluid	Crevice fluid/ Plasma (%)
1	2	10.02	7.58	6.46	64.5
2	3	26.19	30.61	27.75	106.0
3	2	13.68	16.82	12.42	90.8
4	2	14.45	14.70	10.52	72.8
5	3	7.55	7.21	7.18	95.1
6	2	6.33	9.35	4.81	76.0
7	3	13.84	16.59	12.03	86.9
8	2	8.59	8.30	6.87	80.0
9	3	25.82	23.09	27.41	106.2
10	2	12.05	11.93	9.53	79.1
11	3	19.11	20.53	16.47	86.2
Mean		14.33	15.15	12.86	85.78
SD		6.80	7.40	7.99	13.17

SD–standard deviation

5.3. The efficacy of nonsurgical and systemic antibiotic treatment on smoking and non-smoking periodontitis patients (Paper III)

5.3.1. The effect of systemic antibiotic therapy in combination with nonsurgical treatment

Systemic antibiotic therapy in combination with nonsurgical treatment was effective in all cases. There were significant improvements in most clinical parameters (Table 5). The level of post-treatment oral hygiene also improved significantly.

Table 5. Changes in clinical parameters in response to combined treatment

Clinical parameters	Total		Smoking patients		Non-smoking patients	
	Before-treatment median (ranges)	14 mos. follow-up median (ranges)	Before-treatment median (ranges)	14 mos. follow-up median (ranges)	Before-treatment median (ranges)	14 mos. follow-up median (ranges)
Visible plaque index (%)	56.8 (24.1–84.6)[1]	33.3 (5.0–69.4)[1]	51.9 (24.1–82.3)[5]	33.6 (13.4–69.4)[5]	62.5 (34.8–84.6)[8]	33.3 (5.0–65.7)[8]
Bleeding on probing (%)	46.9 (16.9–81.0)[2]	27.7 (9.2–46.2)[2]	44.8 (21.9–81.0)[6]	32.2 (15.8–46.2)[6, 13]	51.5 (16.9–76.3)[9]	22.1(9.2–38.2)[9, 13]
Suppuration index (%)	2 (0–12)[3]	0 (0–5)[3]	2.5 (0–8)	2 (0–5) [14]	2 (0–12)[10]	0 (0–2)[10, 14]
Probing pocket depth (mm)	4.0 (3.1–6.3)[4]	3.6 (2.4–4.7)[4]	4.2 (3.1–6.3)[7]	3.8 (3.1–4.2)[7]	3.9 (3.3–5.5)[11]	3.4 (2.4–4.7)[11]
Relative attachment level (mm)	4.2 (3.2–6.3)	4.1 (3.0–6.1)	4.2 (3.3–6.3)	4.4 (3.5–5.1)	4.1 (3.4–6.1)	4.0(3.0–6.0)
Modified gingival index (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–2)	3 (2–3)[12]	2 (0–3)[12]

[1 … 14] $P<0.05$

5.3.2. Comparison of clinical parameters between smokers and non-smokers

Table 5 demonstrates the effect of smoking on the post-treatment improvement of clinical parameters. Despite the general improvement of clinical parameters, there were no significant post-treatment changes in suppuration (SUP), relative attachment level (RAL) and MGI in smokers.

Although at baseline the clinical parameters of smokers and non-smokers were similar ($p>0.05$), it was estimated that there would be differences in treatment responses. The reduction in bleeding on probing (BOP) and suppuration at 14 months was significantly lower in the smokers than in the non-smokers. The smokers' group showed continuing attachment loss and less reduction in BOP values compared to non-smokers. Post-treatment clinical parameters (except suppuration index) in non-smokers improved significantly ($p<0.05$). The poorer response to therapy may not be due to oral hygiene levels, because there were no significant differences in VPI values between smokers and non-smokers.

5.3.3. Microbiological results

After instrumentation, no periodontal pathogens were isolated in 11 patients (39%), while 17 patients (61%) were infected with one to three different pathogens. Among the pathogens, *P. intermedia/nigrescens* (10 patients) and *A. actinomycetemcomitans* (8 patients) were predominant. The total level of microbial load (log10 CFU/ ml) and the spectrum of pathogens in smoking and non-smoking patients remained similar.

5.4. Periodontal disease in mothers indicates risk in their children (Paper IV)

5.4.1. Clinical results

When comparing children of healthy mothers and mothers with periodontitis, we found that children of diseased mothers had more frequent periodontal diseases, especially gingivitis (Table 6).

Table 6. Periodontal status of children of diseased and healthy mothers

Periodontal status	Children of diseased mothers (n=34)	Children of healthy mothers (n=13)	p value
	% (n)	% (n)	
Healthy	29% (10)	85% (11)	<0.001
Gingivitis	56% (19)	15% (2)	0.02
Periodontitis	15% (5)	None	0.313

Furthermore, clinical parameters of gingival inflammation (BOP, MGI) were more expressed in this group of children, and oral hygiene (VPI, VPI%) was worse (Table 7). We found that such oral hygiene parameters as VPI and VPI% differed significantly when the diseased and healthy mothers were compared ($p<0.001$).

Table 7. Comparison of clinical parameters of children of healthy and diseased mothers

Clinical parameters		Groups		p value
		CHM	CMP	
BOP%	Median	23.6	35.9	<0.001
	Ranges	10.8–38.3	12.1–67.5	<0.001
MGI	Median	0	2	NM
	Ranges	0	2–3	NM
VPI	Median	0.9	1.3	0.006
	Ranges	0.3–1.8	0.9–2.3	0.006
VPI%	Median	28.1	45.3	<0.0001
	Ranges	15.8–42.5	24.4–73.5	<0.001

CHM–children of healthy mothers; CMP–children of mothers with periodontitis; BOP–bleeding sites on probing; MGI–modified gingival index; VPI–Visible Plaque Index

5.4.2. Microbiological results

The most commonly isolated oral pathogens in our study were *P. intermedia/ nigrescens* (21 isolates) and *A. actinomycetemcomitans* (16 isolates). Also, coexistence of two pathogens occurred in six events. The children of healthy mothers harboured pathogens less frequently than the children belonging to the diseased mothers study group (Table 8).

Table 8. The distribution of pathogens among healthy and diseased mothers and their children

Presence of pathogens	Number of pathogens (%)					
	HM (n=13)	MP (n=20)	p value	CHM (n=13)	CMP (n=34)	p value
A. actinomycetemcomitans	0 (0%)	6 (30%)	0.06	0 (0%)	10 (29%)	0.043
P. intermedia/nigrescens	2 (15%)	9 (45%)	0.132	1 (8%)	9 (26%)	0.244
M. micros	0 (0%)	1 (5%)	1	0 (0%)	2 (6%)	1
Any pathogen	2 (15%)	13 (65%)	0.011	1 (8%)	18 (53%)	0.007
Two or more pathogens	0 (0%)	3 (15%)	0.261	0 (0%)	3 (9%)	0.55

HM–healthy mothers; MP–mothers with periodontitis; CHM–children of healthy mothers; CMP–children of mothers with periodontitis

In nine families with diseased mothers, we found pathogens of the same species in two or more family members. Healthy mothers and their children did not share similar pathogens. Using genotyping, we found that from nine suspected cases, the interfamilial spread of the same genotype was confirmed in six cases (Figure 4). The sharing of *P. intermedia/nigrescens* was more frequent (5 families) than *A. actinomycetemcomitans* (2 families). However, the prevalence of *P. intermedia/nigrescens* in studied families was quite similar to the frequency of *A. actinomycetemcomitans*.

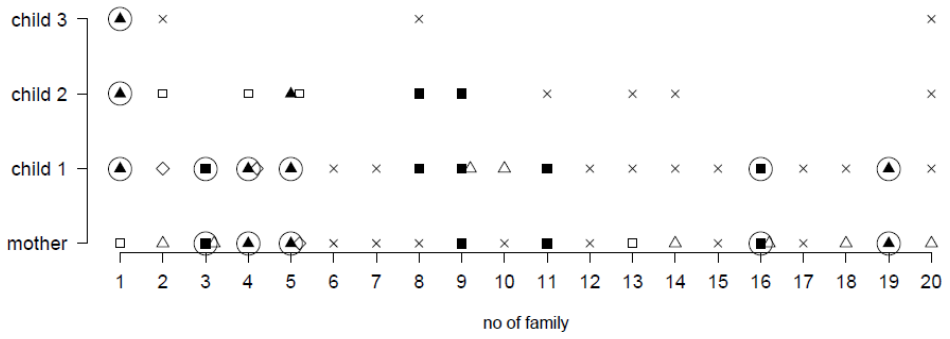

Figure 4. The interfamilial spread of pathogens of mothers with periodontitis and their children.
□ *A. actinomycetemcomitans*; Δ *P. intermedia/nigrescens*, ◊ *M. micros*, × no pathogens, dark dots–similar pathogens among family, O–same clone among family

5.5. Diagnosis and anti-infective therapy of chronic periodontitis in Southern Estonia (Paper V)

5.5.1. Doctors and institutions who have diagnosed periodontitis in the time period 2001–2006

The number of institutions and doctors who have diagnosed periodontitis in Southern Estonia increased from 13 to 27 and 38 to 61 respectively during the research period. Although there were yearly fluctuations, the opportunities of periodontal diagnostics improved significantly (Figure 5).

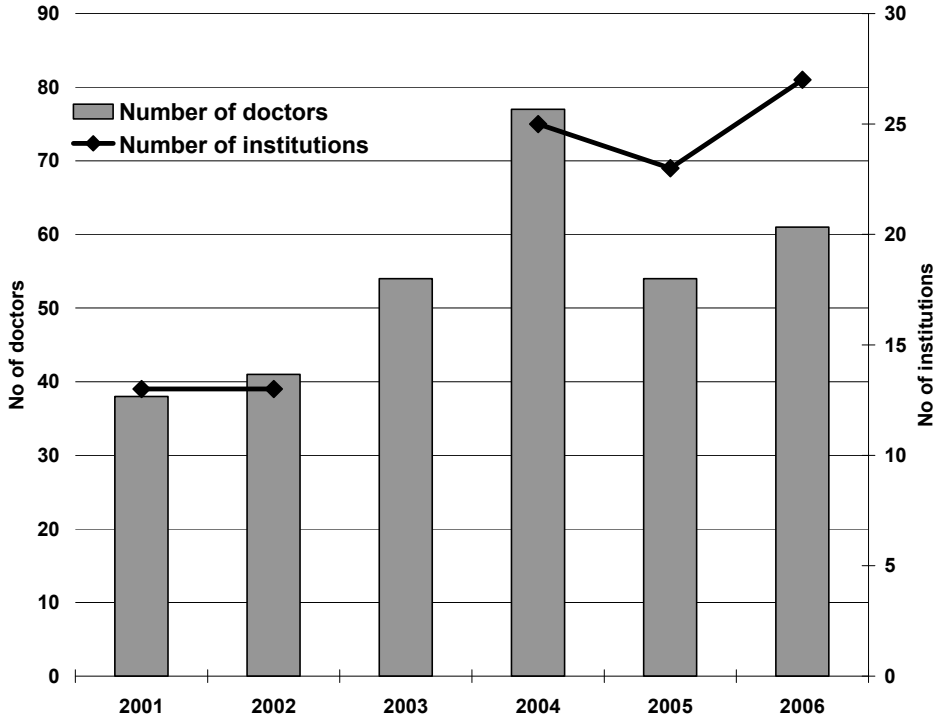

Figure 5. The number of doctors and institutions who diagnosed periodontitis in years 2001–2006

5.5.2. Antibiotics prescribed for diagnosis K05.3 (chronic periodontitis) in the time period 2001–2006

Although the number of antibiotics prescribed increased from year to year, the preferences of antibiotics remained the same in those years. Similarly to drugs used in the therapy of periodontitis globally, the broad-spectrum penicillins (40.7%) and linkosamides (24.8%, mostly clindamycin) were most often prescribed in Tartu County, and other antibiotics were used more rarely (penicillins with ß-lactam inhibitor 10.3%; cefalosporins, tetracyclines, macrolides, fluoroquinolons, narrow-spectrum penicillins 4.7% in total). The use of metronidazole as the first-choice treatment for anaerobic infection grew year after year, (Figure 6).

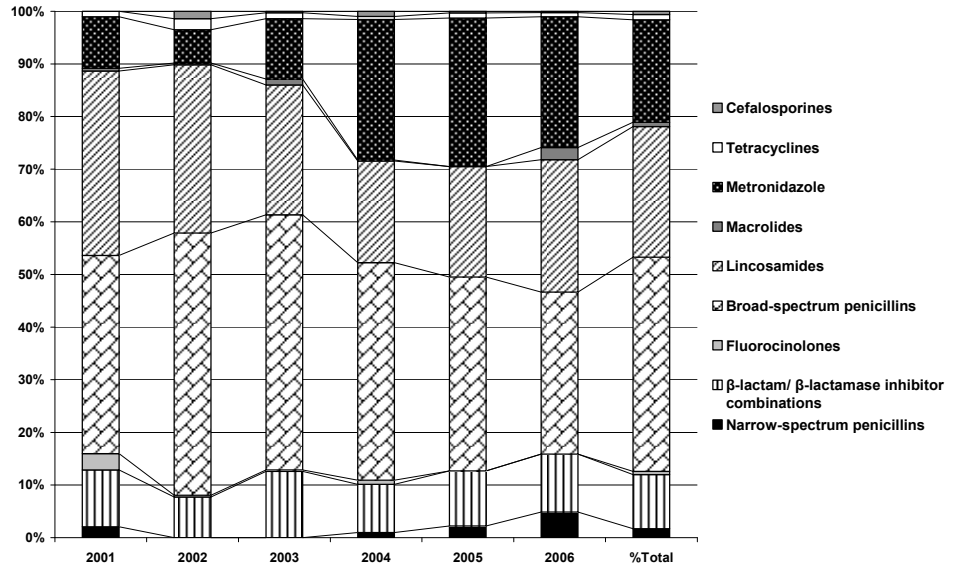

Figure 6. Antibiotics prescribed annually for diagnosis K05.3 (chronic periodontitis) in the time period 2001–2006 in Southern Estonia

5.5.3. The correlation between prescribing antibiotics and microbiological diagnostics of periodontitis

Significantly fewer microbiological samples were taken than prescriptions issued. However, there was a correlation between the two indicators (r=0.88, p=0.02), and taking microbiological samples grew year after year (Figure 7).

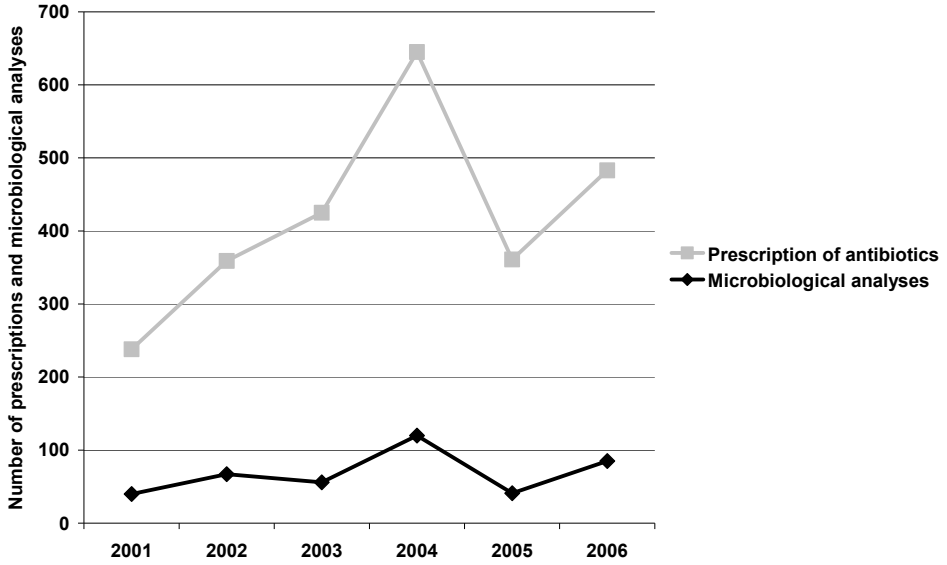

Figure 7. The correlation between taking microbiological samples and issuing prescriptions in years 2001–2006

6. DISCUSSION

Today, there is great public awareness about periodontal diseases and there are more treatment options for patients than ever before. However, although advancements in periodontal therapy continue to develop, periodontal diseases remain the most common cause of adult tooth loss. From a theoretical viewpoint, there have been no paradigm shifts but a gradual accumulation of knowledge about epidemiology, aetiology, pathogenesis and treatment.

Since the ethiopathogenesis of periodontitis is multifactorial and the distribution of the amount of inflammation and tissue destruction is very individual, evidence-based treatment suggestions are very scarce and additional studies are needed in this field. Consequently, we have investigated some aspects in the treatment of advanced periodontitis in order to clarify the need for individualized microbiological testing of subgingival microflora and to provide reassurance in choosing the route of administration for antibacterial drugs. We also developed the easiest and most cost-effective method to identify patients at risk and to evaluate the local periodontal treatment from the viewpoint of good clinical practice.

These data may help to develop Estonian guidelines for the management of patients with periodontal diseases.

6.1. The microbiological status of patients with periodontitis in Southern Estonia after nonsurgical periodontal therapy

In our study, one third of the patients remained pathogen-free after nonsurgical periodontal therapy, in concordance with the results of other authors, where primary therapy reduced the amount of pathogens (Petersilka *et. al.*, 2002; Ximenez-Fyvil *et. al.*, 2000). On the other hand, we found that 67% of the patients treated with primary therapy were equally infected with mostly one pathogen, but also with combinations of different pathogens.

Scaling and root planing alone do not eliminate periodontal pathogens effectively (Lowenguth and Greenstein, 1995). Antibiotics are used systematically in the treatment of periodontitis as an adjunct to initial periodontal treatment to prevent the need for surgery only after traditional periodontal therapy has failed to achieve an adequate response. Microbiological analysis of the subgingival plaque is therefore carried out following initial periodontal treatment to determine whether a need for antimicrobial therapy exists if the clinical response to the primary therapy is unsatisfactory.

Scaling and root planing alone reduce the numbers of microorganisms in the subgingival area, but due to the limitations of cultivation (detection limit 10^3) there may be false negative results. Additionally, the bacteria in periodontal

pockets may not be cultivable; the bacterial cells may already be dead or may be initially viable but unable to survive the accumulated stress of sampling. Hence, a higher microbial load was positively correlated ($p<0.001$) with the number of residual pathogens.

Information gathered from curette samples usually differs from that obtained from paper-point samples because a curette collects plaque from the entire pocket, whereas the plaque adsorbed onto a paper point is derived mostly from the outer layers of the biofilm, which may contain the more pathogenic microflora. We chose the curette for sampling because the paper-point samples would not accurately represent the flora at the base of the pocket where the disease is progressing.

It was anticipated that using a homogenous study population and examining the whole dentition would allow more powerful conclusions to be drawn about the treatment response in patients with well-defined periodontitis than could be achieved by examining selected teeth or a few dental sites in patients exhibiting various periodontal diseases.

Periodontal microbes can be divided into different risk groups according to their association with periodontal disease, where the "red" group includes real pathogens and the "green" group comprises the normal oral microflora (Socransky and Haffajee, 2002).

Along with the well known pathogens of periodontitis, some other potentially pathogenic bacteria (e.g. *Fusobacterium* sp., *Bacteroides* sp., *Prevotella* sp., enterococci, enterobacteria, and others) can play a role in different populations.

The spectrum of most of the important gingival pathogens found in Estonian patients is similar to those reported in the literature (Asikainen and Alasluusua, 1993; Jousimies-Somer *et. al.,* 2002). However, information about pretreatment microbial ecology is needed in order to compare the Estonian data with the findings of other studies.

In the case of periodontitis with developed deep gingival pockets, the increased amount of microflora creates good possibilities for the overgrowth of anaerobes, also indicating the presence of periodontal pathogens. Therefore, due to the occurrence of residual microorganisms after nonsurgical mechanical treatment, information about the pattern of residual pathogens is needed in order to apply appropriate antimicrobial therapy to patients not responding to nonsurgical treatment. The higher level of microbial load in gingival pockets, including both pathogenic and non-pathogenic species, may be one of the determinants for the presence of residual pathogens after nonsurgical mechanical therapy. For individualized treatment with systemic antibiotics, it is important to determine the relative proportions of pathogens rather than their mere presence, because most periodontal pathogens are commensal microorganisms. For empirical treatment, it is advisable have data about the spectra of pathogens in that specific geographical region.

6.2. Metronidazole concentrations in plasma, saliva and periodontal pockets in patients with periodontitis

The findings of this study indicate that metronidazole penetrates well into gingival crevicular fluid and saliva. Therefore, the present study supports the results of some earlier experiments. The main advantages of the present study were the use of patients without any induction of the crevicular fluid flow and the use of validated, specific and sensitive HPLC methodology.

The flow rate of crevicular fluid can differ several times between the normal state and patients with periodontitis (Goodson, 2003).

It is possible that the diffusion of compounds from the plasma to the crevicular fluid depends on the fluid's flow rate. As antibiotics are used in the treatment of periodontitis, penetration into crevicular fluid during the increased secretion induced by the disease state is a key factor of effectiveness.

All patients included in the present study were diagnosed as having severe periodontitis which did not respond to mechanical debridement. This patient population is typical of other populations in which the antibacterial treatment (including metronidazole) is used (Winkel *et. al*., 1997; Slots and Ting, 2002). The assay method used in the present study was developed in the laboratory of the Department of Pharmacology, University of Tartu, to measure metronidazole concentrations in samples of different origin with a volume of 5–50 µl.

As part of a larger project (including non-published sponsored trials), full validation of the method was performed according to good laboratory practice principles. This assay was also used to measure metronidazole concentrations during the microdialysis (Karjagin *et. al*., 2004).

One aim of the study was to compare the metronidazole penetration into gingival crevicular fluid with drug penetration into muscular tissue. Comparisons with the same patients were not made during this study but with results that were obtained at the same laboratory using the same assay method, therefore the results were comparable.

The microdialysis study revealed that equilibrium between metronidazole concentrations in muscle and plasma was reached 2–3 hours after drug administration and concentrations in muscle accounted for about 85% of total plasma concentrations. In the present study, the mean concentration in gingival crevice fluid compared with plasma was 86%. According to the literature, the protein binding of metronidazole is 10–15% (Lamp *et. al.*, 1999; Tracy and Webster, 1996). Therefore, our study indicated that metronidazole concentrations in gingival crevicular fluid are well correlated with protein-unbound metronidazole concentrations in plasma.

One limitation of the present study was the single time point for sample collection after drug administration. Collection of crevice fluid can cause gingival tissue irritation or even damage. This probably also influences the crevicular fluid flow rate (Lamster *et. al*., 1985; Goodson, 2003). Therefore, the

single sample collection point was chosen at the time when equilibrium between plasma and tissue was reached.

The present study was performed under steady state conditions, which also supports the equilibrium between all distribution compartments. The elimination half-life of metronidazole is about 8 hours, and 48 hours was considered sufficient for reaching the steady state. As evident from the results table, the percentage of metronidazole concentration in crevice fluid compared with plasma is not dependent on the plasma drug concentrations.

Significant inter-individual differences in metronidazole concentrations were observed. This can be explained by the different treatment regimens used and different distribution volumes for the drug which depend on body weight and height (Lau *et. al*., 1992; Lamp *et. al*., 1999). There are no questions about the general effectiveness of metronidazole in the treatment of periodontitis, but problems can be encountered in the treatment of infections caused by strains with increased resistance (Roberts, 2002).

In this case, high concentrations of the antimicrobial agent at the infection site are of critical importance. The present experiment confirmed that metronidazole penetrates well into crevicular fluid. High concentrations were also measured in saliva; the mean concentration was very similar to those found in plasma. Although saliva does not have access to the inside of the gingival pocket, a high antibiotic concentration in saliva eradicates microbes outside the gingival structure and prevents the spread of infection to non-affected gingival areas.

In summary, the present study revealed that metronidazole penetrates well into crevicular fluid and saliva. Therefore, the general pharmacokinetic data of metronidazole, which has been established in numerous trials, can also be applied in the treatment of periodontal disease and in the design of respective treatment regimens.

6.3. The efficacy of nonsurgical and systemic antibiotic treatment on smoking and non-smoking periodontitis patients

This study was designed to investigate routine periodontal therapy in advanced cases as close as possible to clinical practice for better comprehension of a clinical management strategy.

We found that smoking habits affected the results of combined treatment. Few data are available about the validation of the effect of the combination of nonsurgical therapy with systemic antibiotics on treatment response in smokers and non-smokers. According to Palmer *et al*., smokers had a poorer treatment response to scaling and root planing, regardless of the application of adjunctive metronidazole (Palmer *et. al.*, 1999). The differences in bleeding on probing

and the suppuration index were assessed for smoking and non-smoking patients, while using metronidazole, and, in the smoking group, only the reduction in probing depth was less (Palmer *et. al.*, 1999).

We found that the combination of nonsurgical treatment and systemic antibiotic treatment with two antimicrobial compounds was effective in treating generalized severe chronic periodontitis. There was a significant improvement in some clinical parameters after the treatment: a reduction in the visible plaque index, bleeding on probing and probing pocket depths. Additionally, from the literature, it seems possible that some patients with severe generalized periodontitis may benefit from systemic antimicrobial therapy in the initial stage of active treatment (Nieminen *et. al*., 1996). We chose a time for administration of systemic antibiotics two to three weeks after the completion of nonsurgical treatment because this gave us the opportunity to evaluate the primary healing reaction in order to ensure that these patients needed additional anti-inflammatory treatment. Also, from the literature, it is well known that antimicrobial treatment is much more effective after the disruption of the biofilm (Socransky *et. al*., 2002).

Moreover, this time period supports the host defence mechanisms in overcoming the infection by killing subgingival pathogens that remain after conventional mechanical treatment. The selection of potent antibiotics presupposes adequate microbiological analysis and susceptibility testing where indicated (Berglundh *et. al*., 1988; Slots, 2002).

In the present study, the predominant pathogens before the administration of systemic antibiotics were *P. intermedia/nigrescens*, *A. actinomycetemcomitans*, *Enterobacter* spp. and *T. forsythensis*. Therefore the decision was made to use a combination of metronidazole and amoxicillin as an adjunct to mechanical periodontal debridement (Berglundh *et. al*., 1988; Slots, 2002; Preber *et. al*., 1990).

The improvement of clinical parameters indicates the effectiveness of the chosen treatment method. Nevertheless, precise investigation of both patient groups showed a better improvement of clinical parameters among non-smoking patients, which in turn had an effect on the combined treatment modality. Therefore, essential risk factors should be considered in estimating the effect of the treatment.

The deposition of plaque is associated with environmental, behavioural, and healthcare variables. According to Skaleric *et. al*., poorer health conditions were associated with males, plus lower levels of education and lower frequency of tooth brushing (Skaleric *et. al*., 2000). All of our patients received an oral hygiene training programme consisting of oral hygiene instruction, regular plaque control and a motivation session during every scheduled treatment visit. Therefore the poorer response to therapy may not be due to oral hygiene because there were no significant differences between visible plaque index values between smokers and non-smokers at the baseline.

The current report is in agreement with similar findings from the literature (Ismail *et. al*., 1983; Tonetti *et. al.*, 1995). There are also reports in which alveolar bone destruction has been found in patients with an excellent level of oral hygiene (Bergström and Boström, 1987). We found that smoking mostly influenced parameters associated with disease activity, such as suppuration and bleeding. The poorer response of smokers is probably a result of the general effect of smoking, which compromises the response to periodontal treatment. In smokers, the host's immune response is adversely affected by impaired production of immunoglobulins, which makes smokers more susceptible to infections and re-infections (Lamster, 1992; Graswinckel *et. al*., 2004). *In vitro* exposure to nicotine suppresses the ability of macrophages to kill oral pathogens (Pabst *et. al*., 1995), and leads to lowered elastase and neutrophil levels in the oral cavity (Pauletto *et. al*., 2000). Cigarette smoking also compromises periodontal ligament cell adhesion to root-planed surfaces, which might affect periodontal regeneration following therapy (Gamal *et. al*., 2002).

These findings may explain the disadvantages of smoking, especially in the case of clinical markers closely related to inflammation. Although smoking affects treatment results, the qualitative and quantitative extent of the effect remains unclear.

However, it was expected that the use of a homogenous study population and examination of the whole dentition would allow more powerful conclusions to be reached about the treatment response in patients with well-defined periodontitis than would have been achieved by examining selected teeth or only a few dental sites in patients exhibiting various periodontal diseases. The combination of nonsurgical and systemic antibiotic treatment was effective in the treatment of generalized severe chronic periodontitis. However, smoking habits adversely affected the results of combined treatment, especially bleeding, on the index of probing and suppuration.

6.4. Periodontal disease in mothers indicates risk in their children

We found a significant association between severe periodontitis in mothers and the presence of periodontal disease in their children. Although other studies have shown severe forms of periodontitis clusters in families (Armitage, 1999; Oliver *et. al*., 1998), no correlation between mothers and children carrying periodontal diseases has been demonstrated. In the present study, we were looking for risk markers of periodontal disease. Our suggestion is that maternal severe periodontitis is a factor associated with the probability of their children having the disease in the future. In most cases, children of diseased mothers had gingivitis but not periodontitis. However, gingivitis has been shown as an important factor predicting further development of periodontitis (Lindhe *et. al*., 1975; Page and Schroeder, 1981; Schätzle, 2004).

We found worse oral hygiene in diseased mothers (as compared with healthy ones) and their children (as compared to children of healthy mothers). It is possible that mothers' oral hygiene habits can influence the habits of their children and thus predispose them to the development of periodontitis.

However, the role of VPI and VPI% as indicators of oral hygiene and risk factors of periodontitis are not fully understood yet. According to Van der Velden (Van der Velden, 2006), the amount of supragingival plaque does not adequately reflect personal oral hygiene habits but people with abundant inflammation develop massive plaque. In contrast, Axelsson and (Albandar *et. al*., 1995; Axelsson *et. al*., 1974) established the importance of dental plaque as the primary aetiological factor in the development of gingival inflammation and chronic periodontitis. Based on this finding, Albandar *et. al*. (1995) concluded that secondary prevention of periodontitis in children is of prime importance and may be achieved through early detection of high-risk patients. The weakness of VPI indices is the fact that some patients may brush their teeth directly before the dental visit. Measurement of supragingival plaque might provide data about local factors present in the subject's mouth and enable their habits and attitudes to be evaluated. However, based on present knowledge, VPI indices alone are insufficient to predict periodontitis.

Our study revealed sharing of periodontal pathogens of the same species and, in some cases, also the sharing of similar genotypes, among families with diseased mothers. Detailed knowledge regarding the acquisition and transmission of infectious agents facilitates a more comprehensive approach towards prevention. One difficulty in investigating the influence of parental sulcular microflora on the child's periodontal health is that periodontitis is not a single bacterial infection; it displays heterogeneity even in the same mouth. In addition, oral and family environmental factors, and immunological and inflammatory host profiles may modulate the colonization and establishment of periodontal pathogens in early childhood.

Children and young adults with chronic periodontal disease have been previously studied along with patients having localized aggressive periodontitis and generalized aggressive periodontitis. In most studies, interfamilial spread of periodontal diseases was subjected to the investigation of aggressive periodontitis and to single specific pathogens, but there are no data available about the spread of sulcular microflora in the case of chronic periodontitis.

Some studies have shown that if children harbour *A. actinomycetem-comitans*, then usually one or two parents harbour the same strain. However, identical genotypes in family members are not 100% proof of transmission, as there is not an infinite number of genotypes and finding identical genotypes may have occurred by chance (Asikainen *et. al*., 1996). The frequency of vertical transmission of *A. actinomycetemcomitans* is between 30% and 60% based on detection of identical genotypes in children and parents.

According to our data, in diseased families 45% of cultivated pathogens were of the same genotype for mothers and their children. In healthy families,

we did not find even the same species of pathogens. Our study provides evidence that such maternal indicators as periodontitis, hygiene habits, and periodontal microflora are risk factors for periodontal diseases in children. Also, the maternal disease might be predictive for periodontitis in children and adolescents. The identification of high-risk children and their early treatment may help to reduce the development of periodontal disease in the future**.**

6.5. Diagnosis and anti-infective therapy of chronic periodontitis in Southern Estonia

We found that prescribing antibiotics and taking microbiological samples were positively correlated. This is a positive indication of the awareness of Estonian dental professionals and compliance with good clinical practice.

The number of institutions and doctors that have diagnosed periodontitis has increased during the period from 2001 to 2006. Yet our research could not assess how many and what types of periodontitis were diagnosed in this period. One of the reasons is that the Health Insurance Fund does not reimburse adult dental therapy and the data about diagnosed or treated diseases remain in the health institutions. As antibiotics belong to subsidized drugs, we got data only about prescriptions issued to the people diagnosed with periodontitis who are insured by the Health Insurance Fund.

Another reason could be that the only publicly usable distribution of diagnoses of periodontitis is the WHO classification recognized by the Ministry of Social Affairs in 1996. At the same time, rapid development in the knowledge of periodontitis and the systematization of data took place around the world. In 1999, as a result of this process, the materials from an international workshop for the classification of periodontal diseases and conditions (1999 International Workshop for a Classification of Periodontal Diseases and Conditions) were published. Consequently, the classification of diseases that is used in Estonia at present does not allow for the procurement or processing of adequate and scientifically substantial data in this field. This problem needs a quick solution from the perspective of fostering stomatology.

When prescribing systemic antibiotics, the dentist needs to know the pathogens of the patient's periodontal pockets and their sensitivity to antibiotics, in order to avoid using inefficient antibiotics. Taking inappropriate drugs contributes to the overgrowth of pathogens in the oral cavity and deteriorates the prognosis for periodontitis (Helovuo and Paunio, 1989).

Because of data protection requirements, it was not possible to observe the prescribing of antibiotics individually and therefore it was not possible to assess the share of combined therapy. At the same time, the combinations of metronidazole with amoxicillin-clavulanic acid and amoxicillin have been successful in the case of *A. actinomycetemcomitans* infection (Tinoco *et al*., 1998).

Microbiological analysis is routinely made after mechanical therapy to estimate the need for further therapy, including systemic antibiotic therapy. The clinical and microbiological assessment of the results of therapy should take place one to three months after taking antibiotics to check the elimination and suppression of pathogens and ascertain the possible generators of superinfection like enterobacteria, pseudomonades and fungi (U.S. Public Health Service, 1987).

Our results showed that fewer microbiological samples were taken than prescriptions issued. The combinations of antibiotics prescribed to different patients should be taken into consideration where, in response to one sample, several antibiotics were used.

Table 2 presents an overview of the preparations and doses used around the world, based on the data of American Academy of Periodontology 2004.

There are no definite positions on therapy and the only recommended treatment guidelines concern the dental care of children (Saag and Russak, 2005). At the same time the preparations used in other parts of the world are not always in compliance with Estonian antibiotics policy.

Our research showed that 19.5% of preparations prescribed in Tartu County were made up of metronidazole. As shown by our investigation, metronidazole achieves sufficient effective concentration in the fluid of gingival sulcus and can therefore stop the progression of periodontitis in the case of *P. gingivalis or P. intermedia/nigrescens* infection (Haffajee *et al*., 2003). Systemic metronidazole therapy combined with mechanical therapy may contribute to the regeneration of tooth attachment tissue (Loesche *et al*., 1992).

40.7% of prescriptions issued were of broad-spectrum penicillins, amoxillin and ampicillin. In periodontitis therapy, amoxillin is preferred because, in systemic administration, the concentration of the preparation is higher in serum than in ampicillin and its effect on the pathogens of periodontitis is beneficial (Goodson, 2003). In addition, penicillins including amoxicillins are sensitive to ß-lactamase which is produced by many anaerobes. Studies have shown that in the samples of periodontal pockets of more than 60% of adults suffering from periodontitis, ß-lactamase is active (Helovuo and Paunio, 1989). Therefore ß-lactamase sensitive penicillins, amoxicillin as a single preparation among them, are not advised for the treatment of people suffering from periodontitis because in some cases this therapy scheme may even aggravate the destruction of periodontal tissues. The generally accepted strategy is to use amoxicillin with ß-lactamase inhibitor (clavulanic acid) because microbial stems producing ß-lactamase are sensitive to this preparation (Helovuo and Paunio, 1989).

We found that 24.8% of prescriptions issued were made up by clindamycin. According to literature data, clindamycin has been successfully used in refractory periodontitis therapy. Compared to other preparations, clindamycin achieves the best effective antimicrobial concentration (Goodson, 2003). However, limited use of this preparation is recommended because of life-endangering side-effects such as the overgrowth of *Clostridium difficile* and the

development of pseudomembranous colitis. The reason why Estonian doctors prefer clindamycin may be the vigorous sales campaign in 2000–2002. The preparation was actively introduced at fairs of dental care, and sales representatives promoted it among dental professionals giving them pre-filled sample prescriptions. From conversations with colleagues it emerged that this was a relatively successful argument in the choice of the preparation.

It is interesting to note that tetracycline had a very small share among the prescribed preparations although tetracycline is popular around the world especially in the treatment of *A. actinomycetemcomitans* infections. Moreover, tetracycline has a positive effect on suppression of gingival collagenases.

We therefore found that the number of doctors diagnosing and treating periodontitis is increasing in Tartu County and that one of the therapy methods chosen is systemic antibiotic therapy. According to literature data, most of the antibiotics prescribed have an effect on anaerobic microflora. However, it is known that unsubstantiated drug prescribing substantially influences the health of patients and increases the formation of drug resistance. Though prescribing systemic antibiotics is positively correlated with determining the microflora of periodontal pockets, the increase of microbiological analyses could be beneficial.

CONCLUSIONS

1. Microbial testing may provide guidance in choosing a specific antibiotic agent mainly in periodontal cases that do not respond to initial therapy. Successful diagnosis and therapy of periodontitis should be based on individual microbiological examinations.

2. The present study revealed that metronidazole penetrates well into gingival crevice fluid and saliva. Metronidazole concentrations in crevice fluid are about equal to the protein-unbound drug concentrations in plasma. Metronidazole distribution to crevice fluid conforms to the distribution of the compound into soft tissues with good circulation (e.g., muscular tissue). Therefore, the general pharmacokinetic data of metronidazole, which have been established in numerous trials, can be also applied in the treatment of periodontal disease.

3. The combination of nonsurgical and systemic antibiotic treatment is effective in the treatment of generalized severe chronic periodontitis. However, smoking habits adversely affect the results of combined treatment. We found continuous attachment loss in smoking patients, and less improvement on bleeding on probing and suppuration indices.

4. Maternal indicators such as periodontitis, hygiene habits, and periodontal microflora are risk factors for periodontal diseases in children. We can conclude that maternal disease might be predictive for periodontitis in children and adolescents.

5. The number of doctors diagnosing and treating periodontitis is increasing in Tartu County. In Tartu County, prescribing systemic antibiotics is positively correlated with determining the microflora of periodontal pockets. This is a positive indication of the awareness of Estonian dental professionals and compliance with good clinical practice.

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SUMMARY IN ESTONIAN

Parodontiidi ravi efektiivsust mõjutavad tegurid

Sissejuhatus

Parodondiit on krooniline põletikuline haigus, mille tagajärjel hävivad parodondi koed (gingiva, juuretsement, parodontaalligament ja alveolaarluu). Tähtsaimaks igemepõletiku tekkepõhjuseks peetakse peamiselt hambakatus paiknevaid mikroorganisme. Mikroobid on võimelised kahjustama hammast ümbritsevaid kudesid otseselt või kaudselt, vallandades immuunsüsteemi vastuse ja põhjustades parodondi põletikku. Selle protsessi käigus vabanevad mitmesugused põletikumediaatorid, mis võivad põhjustada nii pehmete kudede kui ka luukoe hävimise ja igemetaskute moodustumise. Parodontiidi tekkemehhanism on multifaktorilise etioloogiaga: haiguse tekkimiseks on oluline roll subgingivaalsel mikroflooral, lokaalstel faktoritel suuõõnes, organismi vastupanuvõimel ja keskkonnateguritel. Üldine seisukoht on, et parodontiit on mikrobiaalse biokile poolt põhjustatud krooniline infektsioosne haigus.

Suuõõs on koloniseeritud paljude erinevate mikroorganismidega. Kroonilise parodontiidi tekitajad on peamiselt erinevad gram-negatiivsed mikroorganismid (*A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. denticola* ja *T. forsythensis*).

Seetõttu on nende mikroobide esinemine aktiivse ravi näidustuseks. Haiguse profülaktikas on omakorda oluline ökoloogilise keskonna pidev kontrollimine, mis reguleerib residuaalset mikrofloorat. Kliiniliselt saab igemetasku mikrofloorat uurida erinevate kvalitatiivsete ja kvantitatiivsete mikrobioloogiliste testide abil. Haiguse profülaktika seisneb seega potentsiaalsete patogeenide mahasurumises ja nende faktorite elimineerimises, mis soodustavad hambakatu mikrofloora muutumist potentsiaalselt patogeenseks.

Igemetaskute sügavuse vähendamist kirurgilisel või mittekirurgilisel teel peetakse parodontiidi eduka ravi aluseks. Ravi tagajärjel toimuvad kohesed olulised ökoloogilised muutused, mis igemetaskutes suruvad alla subgingivaalse anaeroobse mikrofloora ja loovad eelise soodsamale igememikrofloorale.

Antibakteriaalsete ravimite kasutamine põhineb parodontiidi mikrobioloogilisel etioloogial. Süsteemseid antibiootikume ordineeritakse patsientidele, kelle haigus ei allu esmasele mehaanilisele ravile. See seisukoht tugineb eeldusel, et parodontiiti põhjustavad spetsiifilised mikroobid ja et manustatava ravimi kontsentratsioon *in vivo* ületab ravimi mikroobe inhibeerivat kontsentratsiooni.

Mitmed teised faktorid võivad samuti mõjutada parodontiidi levimust, tekkimise aega ja progresseerumist. Multivariatiivsete analüüside tulemused näitavad, et haiguse levimus on oluliselt suurem suitsetajatel.

Haiguse ravi on seda efektiivsem, mida varajasemas staadiumis haigus an avastatud.

Parodontiidi ravi on kompleksne ja seotud oluliste kulude ja kannatustega, seetõttu on oluline parema haiguse profülaktika saavutamiseks tuvastada juba varakult riskipatsiendid.

Töö eesmärk

Uuringu eesmärk oli saada uusi teadmisi parodontiidi ravi efektiivsuse kohta. Selleks pöörasime tähelepanu mittekirurgilise ravi edukuse mikrobiaalsele hindamisele, metronidasooli farmakokineetikale parodondi kudedes, suitsetamise mõjule parodontiidi ravis, riski patsientide välja selekteerimisele ja kohalikule parodontiidi ravi praksisele.

Kitsamad ülesanded olid järgmised:

1. Hinnata mehaanilise ravi edukust (patogeenide elimineerimine), patsientide igemetasku patogeenide spektrit ja igemetasku kogu mikrofloora kasvutiheduse seost seal leiduvate erinevate patogeenide arvuga. Selgitada antimikrobiaalse ravi vajadust patsientidel, kellel põletik ei allunud mittekirurgilisele ravile.
2. Määrata süsteemselt manustatud metronidasooli kontsentratsiooni vereplasmas, süljes ja igemevao vedelikus.
3. Võrrelda mittekirurgilise ja süsteemse antibiootilise parodontiidi ravi kombinatsiooni pikaajalist raviefekti suitsetajatel ja mittesuitsetajatel.
4. Hinnata parodontiidi tekkimise riski parodontiidhaigete ja tervete emade lastel.
5. Hinnata parodontiidi diagnostika ja raviga tegeleva arstkonna haaratust ning ordineeritud antibiootikumide valikut ning sobivust parodontiidi raviks.

Materjalid ja meetodid

Mehaanilise ravi edukuse uuringus osales 140 täiskasvanut generaliseerunud kaugelearenenud kroonilise parodontiidiga patsienti.

Igemetaskute mikrofloora määrati rutiinse laboratoorse analüüsina. Igemetasku kaabe võeti 6 sügavamast parodontaaltaskust VMGA transportsöötmesse, millest tehti järjestikused lahjendused ja külvati Brucella ja TSBV agarile ning inkubeeriti vastavalt anaeroobses ja mikroaeroobses keskkonnas. Patogeenid samastati pesade morfoloogia, Grami järgi värvumise, katalaasi, indooli ja oksüdaasi testide, autofluorestsentsi ja diagnostiliste diskidega.

Mikroobide kasvutihedust väljendati kümnendlogaritmina PMÜ (pesa moodustav ühik)/ml-s. Patogeenide arvu ja mikrofloora kolonisatsioonitiheduse vahelise korrelatsiooni hindamiseks kasutati Spearmani testi.

Igemetasku patogeene ei sedastatud 33%-l eelnevalt ravitud patsiendil, samas isoleeriti ülejäänud 67%-l patsientidest 1–5 erinevat patogeeni.

Ravitud patsientide igemetasku erinevate mikroobide kolonisatsioonitihedus varieerus 0-st kuni 8,4 log PMÜ/ml (mediaan 5,5 PMÜ/ml) ja oli positiivselt seotud isoleeritud patogeenide arvuga ($p<0,001$), olles patogeenide esinemise võimalikuks indikaatoriks. Igemetasku tõestatud patogeenidest domineerisid *P. intermedia*/*nigrescens*'i grupp ja *A. actinomycetemcomitans* vastavalt 37-l ja 36 patsiendil, *M. micros* esines 12-l, *P. gingivalis* 7-l, *T. forsythensis* neljal ja *C. rectus* kahel patsiendil. Lisaks üldtunnustatud parodontiidi tekitajatele leidsime patsientide igemetaskutest ka muid potentsiaalseid patogeene. Isoleeritud patogeenide arv ühel patsiendil seostus otseselt igemetasku mikrofloora rohkusega.

Metronidasooli farmakokineetika uuringus osales 11 generaliseerunud kaugelearenenud parodontiidiga patsienti (6M ja 5N), vanuses 24–60 aastat (keskmine 46,3±12,8) Metronidasooli annustati 500 mg kaupa kaks või kolm korda päevas, vähemalt kaks päeva enne proovi võtmist. Proov võeti 2h peal viimast doosi. Metronidasooli kontsentratsiooni määrati kõrgsurve vedelik-kromatograafia meetodil.

Keskmised ravimikontsentratsioonid plasmas, süljes ja igemevao vedelikus olid vastavalt 14,33; 15,15 ja 12,86 µg/ml. Erinevused plasma ja igemevao vedeliku või plasma ja sülje vahel ei olnud statistiliselt olulised.

Süsteemse antibiootilise ravikombinatsiooni pikaajalist efekti uuringus suitsetajatel ja mittesuitsetajatel osales 28 generaliseerunud kaugelearenenud kroonilise parodontiidi haiget, kellele esmane mittekirurgiline ravi ei toiminud. Patsiendid olid vanuses 25–65, 14 suitsetajat ja 14 mittesuitsetajat.

Patsientidel määrati kogu hammaskonna igemetaskute sügavus, suhteline kinnituskudede tase, veritsuse-, mädavoolu ja nähtava hambakatu indeksid.

Haigetele manustati vastavalt kliinilisele pildile ja mikrobioloogilisele näidustusele süsteemselt amoksitsilliini 500 mg×3 ja metronidasooli 200mg×2 7 päeva jooksul.

Raske generaliseerunud parodontiidi korral osutus efektiivseks mikrobioloogilisel analüüsil põhinev süsteemne antibiootikumravi. Mittesuitsetajate kliinilised parameetrid (v.a. supuratsiooniindeks) paranesid kombineeritud ravi järgselt. Suitsetajate parameetrid ei olnud vaatamata ravi üldisele edukusele ühegi näitaja osas oluliselt muutunud, lisaks jätkus suitsetajatel kinnituskudede häving ja igemete veritsemine.

Parodontiidi tekkimise riski uuringus parodontiidhaigete ja tervete emade lastel osales 82 patsienti (kokku 4 uuritavate gruppi: (I) 20 generaliseerunud kaugelearenenud kroonilise parodontiidihaiget ema; (II) 34 haigete emade last; (III) 13 tervet ema ja (IV) 13 tervete emade last).

Kliiniliselt määrati kõigil patsientidel modifitseeritud igemeindeks, nähtava hambakatu- ja veritsuse indeksid. Kõigilt uuritavatelt võeti igemetasku mikrofloora proov. Mikroobid samastati biokeemilise ja morfoloogilise profiili järgi. Perekonnas esinenud samaliigilisi patogeene võrreldi arbitrary-PCR meetodil.

Haigete emade lastel esines sagedamini parodondi haigusi, eriti gingiviiti, neil olid halvemad hügieeninäitajad ja kõrgemad põletikunäitajad. Haigete ja tervete emade VPI ja VPI% näitajad erinesid statistiliselt oluliselt.

Kõige sagedamini isoleeritud igemetasku patogeenid olid *P. intermedia /nigrescens* ja *A. actinomycetemcomitans*. Tervete emade lastel esines patogeene harvemini kui haigete emade lastel.

Perekonniti esines sagedamini *P. intermedia/nigrescens* (5 perekonda), seejärel *A. actinomycetemcomitans* (2 perekonda).

Kroonilise parodontiidi diagnostika ja antibiootikumravi uuringus Lõuna-Eestis ajavahemikul 2001–2006 uuriti Eesti Haigekassa andmetel väljakirjutatud 2102 retsepti diagnoosiga K053 (krooniline parodontiit) ja 409 SA TÜK Ühendlaborist tellitud igemetasku mikrofloora uuringu põhjal Tartu maakonnas parodontiidi diagnostika ja raviga tegeleva arstkonna haaratust. Samuti ordineeritavate ravimite valikut ning kasutatavate süsteemsete antibiootikumide sobivust parodontiidi ravis ja igemetasku mikrofloora analüüside tellimist SA TÜK ühendlaborist.

Väljakirjutatud antibiootikumide spekter oli järgmine: laiatoimespektriga penitsilliinid 40,7%, metronidasool 19,5%, linkosamiidid 24,8% ja β-laktaamid/β-laktamaasi inhibiitorid kombinatsioonis 10,3%. Muude antibiootikumide kasutamine oli suhteliselt harv (4,7%). Väljastatud antibiootiliste preparaatide arv oli positiivses korrelatsioonis teostatud mikrobioloogiste analüüside arvuga ($p=0,002$, $r=0,88$).

Tulemused ja järeldused

1. Meie uuring näitas, et patsientidele, kelle haigus ei allu rutiinsele mehaanilisele ravile, on näidustatud residuaalse mikrofloora testimine. Saadud tulemused kinnitasid, et individuaalne potentsiaalsete parodontiidi patogeenide määramine on adekvaatse diagnostika ja sobiva antibiootilise ravi määramise edukuse aluseks.
2. Meie uuring kinnitas, et metronidasool tungib hästi igemevao vedelikku ja sülge. Metronidasooli kontsentratsioon igemevao vedelikus oli võrdeline proteiiniga seondumata ravimi kontsentratsiooniga plasmas. Metronidasooli jaotumine igemevao vedelikus on sarnane ravimi jaotumisega teistes hea verevarustusega kudedes nagu näiteks lihaskude. Seetõttu saab parodontiidi ravis rakendada kõiki metronidasooli kohta käivaid farmakokineetilisi teadmisi. See teadmine aitab kaasa sobiva antimikrobiaalse ravi manustamise viisi valikule.

3. Raske generaliseerunud parodontiidi korral osutus mittekirurgiline ravi kombineeritud mikrobioloogilisel analüüsil põhineva süsteemse antibiootikumiraviga üldiselt efektiivseks, kuid suitsetamine halvendas oluliselt parodontiidi ravitulemusi. Suitsetajatel jätkus kinnituskudede häving. Samuti oli igemete veritsemine sondeerimisel ja mädavoolu esinemine igemetaskutest suitsetajatel ravijärgselt suurem.
4. Haigete vanemate lapsed on enam eksponeeritud parodondi haigustele, neil leidub ka rohkem parodontiidi patogeene ning nende suuhügieen on halvem ja põletiku näitajad kõrgemad. Seega võib ema haigus omada prognostilist väärtust laste ja noorukite parodondi seisundile.
   Kuna varakult alanud haigus seostub halvema prognoosiga, on spetsiifilise profülaktika vajadus parodontiidihaigete perekonnaliikmetel kõrge. Kliinilise töö ja rahvatervishoiu seisukohast võib antud uuring olla suhteliselt lihtsa ja oluliselt odava haiguse riskiga patsientide selekteerimise meetodi väljatöötamise aluseks.
5. Leidsime, et parodontiiti diagnoosivate arstide arv Tartu maakonnas kasvab. Süsteemsete antibiootikumide väljakirjutamine oli positiivses korrelatsioonis igemetasku mikrofoora määramisega. Enamik väljakirjutatud antibiootikume toimis sarnaselt kirjanduse andmetele anaeroobsesse mikrofloorasse.

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Teadustöö

Peamised uurimisvaldkonnad:

- prodontiit, igemetasku mikrofloora, diagnostika
- parodontiidi kombineeritud ravi efektiivsus
- metronidasool, igemetasku vedelik, farmakokineetika
- parodontiidi risk haigete emade lastel
- parodontiidi diagnostika, antibiootiline ravi

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