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ORAL HEALTH AND MENOPAUSE

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Academic dissertation

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ABBREVIATIONS

A.a	*Aggregatibacter* (previously *Actinobacillus*) *actinomycetemcomitans*
BMS	Burning mouth syndrome
Ca	Calcium
CEE	Conjugated equine estrogens
CI	Confidence interval
Cl	Chlorine
CPITN	Community Periodontal Index of Treatment Needs
CV	Coefficient of variation
CVD	Cardiovascular disease
DM	Sensation of dry mouth
DMFT	Decayed, missing, filled teeth (index)
DT	Decayed teeth
E1	Estrone
E2	Estradiol (17β-estradiol)
E3	Estriol
ER	Estrogen receptor
ET	Estrogen-only treatment
EPT	Estrogen-progestagen treatment
FSH	Follicle-stimulating hormone
FT	Filled teeth
GCF	Gingival crevicular fluid
HERS	The Heart and Estrogen/progestin Replacement Study
HT	Hormone therapy
HRT	Hormone replacement therapy
IDF	International Dental Federation
IgA/G/M	Immunoglobulin A/G/M
K	Potassium
LNG	Levonorgestrel
LH	Luteinizing hormone
Mg	Magnesium
MMP	Matrix metalloproteinase
MPA	Medroxyprogesterone acetate

MWS	Million Women Study
Na	Sodium
NETA	Norethisterone acetate
NH_3	Ammonia
Ns.	Not significant
OR	Odds ratio
P	Probability value
PCR	Polymerase chain reaction
P.g.	*Porphyromonas gingivalis*
P.i.	*Prevotella intermedia*
PM	Sensation of painful mouth
P.n.	*Prevotella nigrescens*
PT	Progestagen-only treatment
PTG	Panoramic tomography of the jaws
SD	Standard deviation
SE	Standard error
SERM	Selective estrogen-receptor modulators
RR	Risk ratio
T.f.	*Tannerella forsythia* (previously *Bacteroides forsythus*)
WHI	Women´s Health Initiative Study
WHO	World Health Organization

DEFINITIONS

Below are listed some brief definitions for terms that are used in this thesis.

Sex steroid hormones

Female sex hormones are estrogen and progesterone. They are responsible for physiological changes in the different stages of a woman's life, and are feminizing in their action. The ovaries secrete estrogens, small amounts of androgens and progesterone. During pregnancy progesterone is responsible for preparing the uterus for the pregnancy (Ganong 1989).

Male sex hormones, generically known as androgens (testosterone, androstenedione, dehydroepiandrosterone (DHEA)), are responsible for masculinization. The testes secrete large amount of androgens, mainly testosterone, but they also secrete small amounts of estrogens. Androgens are also secreted from the adrenal cortex in both sexes (Ganong 1989).

Estrogens

> ***Steroidal estrogens*** are a group of steroid compounds. The main naturally occurring estrogens in women are estrone (E1; after menopause, estrone levels increase, possibly due to increased metabolism of androstenedione to estrone), estradiol (E2; the primary estrogen from menarche to menopause) and estriol (E3; produced during pregnancy by the placenta).
>
> ***Non-steroidal estrogens*** are compounds which possess estrogenic activity, eg. phytoestrogens and xenoestrogens (Kuhl 2005).

Progestagens (progestogens, gestagens) are a group of naturally occurring (eg. progesterone) as well as synthetic (progestins) hormones having a pregnane skeleton. Progestagens are precursors of other steroids (Kuhl 2005).

Dental caries

Dental caries is a disease process involving progressive, focal demineralization of dental hard tissues by organic acids derived from bacterial fermentation of dietary carbohydrates, especially refined sugars (Featherstone 2000).

Periodontal diseases

Plaque-induced periodontal diseases are divided into two general categories based on whether attachment loss has occurred or not (American Academy of Periodontology 2003).

Gingivitis:

Periodontal disease is categorized as gingivitis based on the presence of gingival inflammation without a loss of connective tissue attachment and where the junctional epithelium remains at its original level and attached to the tooth (Armitage 1995; Beck & Arbes Jr 2006).

Periodontitis:

Periodontal disease is categorized as periodontitis in situations where there is gingival inflammation at sites where there has been pathological detachment of collagen fibers from cementum and the junctional epithelium has migrated apically (Armitage 1995). In addition to these inflammatory events, which are associated with connective tissue attachment loss, the resorption of coronal portions of tooth-supporting alveolar bone occurs (Armitage 1995; American Academy of Periodontology 2003).

Indices

Different indices have been developed to be used for quantifying the amount and severity of different oral diseases or conditions in individuals or populations. Some indices are mainly used in clinical practice, while other indices are used in epidemiological surveys (Lindhe et al. 2006).

Dental indices

DMFT–index

The dental caries index, DMF-index, describes the prevalence of dental caries in an individual and is obtained by calculating the number of decayed (D), missing (M) and filled (F) teeth. Originally, the DMF-index was developed to describe the dental status and need for treatment of elementary school children, but the World Health Organization (WHO) has standardized the use of the DMF-index for oral health surveys for adults and elderly people as well (Larmas 2010).

Periodontal indices

CPITN-index

International Dental Federation (IDF) and WHO jointly developed the Community Periodontal Index of Treatment Needs (CPITN) index system (WHO 1978). The CPITN index places each subject in a category representing the most severe finding in the mouth. CPITN codes 1 and 2 indicate gingivitis and scores 3 and 4 (periodontal pockets ≥4mm) indicate periodontitis. The need for periodontal treatment is classified in one of four treatment needs categories based on the highest CPITN code. Those with codes 1 to 2 require no treatment or only require improvement in their personal oral hygiene, whereas those with codes 3 to 4 require scaling and root planning along with instructions for improving oral hygiene (Ainamo et al. 1982).

ABSTRACT

Unpleasant symptoms connected to menopause are treated with hormone therapy (HT). HT is widely used to relieve those symptoms in order to increase the well-being of the women treated. HT is also used to prevent age-related diseases such as cardiovascular diseases and osteoporosis. The benefits as well as side-effects of HT are well documented. Oral discomfort is found in many menopausal women in addition to general climacteric complaints. The principal peri- and postmenopausal oral symptoms are dry mouth, sensation of painful mouth (PM) due to various causes and less frequently burning mouth syndrome (BMS). BMS is also known as glossodynia, since the tongue is most frequently affected. BMS is characterized by burning oral mucosal pain without any visible signs of mucosal pathology. The pain also does not follow the anatomy of peripheral nerves.

Profile studies on HT users have indicated that these women are more health-conscious than non-users. Therefore, the hypothesis of the present study was based on the idea that women who had chosen to take HT in the first place were those who also had better oral health and health habits in general.

A questionnaire study of 3173 women of menopausal age (50-58 years old) was done to investigate the prevalence of self-assessed sensations of PM and dry mouth (DM) or xerostomia. Xerostomia is the medical term for the subjective complaint of DM. Special attention was paid to the association between HT and oral symptoms. Of those women participating in the questionnaire study, a random sample of 400 (200 using, 200 not using HT) was then examined clinically in a two-year follow-up study. Dental and periodontal status was recorded according to WHO methods using DMFT and CPITN indices, and unstimulated and stimulated saliva flows were measured. Salivary total protein, albumin and immunoglobulin concentrations as well as selected periodontal micro-organisms were analysed. Panoramic tomography of the jaws was taken at baseline and at the follow-up. The patients also filled in a structured questionnaire on their systemic health, medication and health habits. The results were analysed statistically between and within the groups.

According to our questionnaire study there was no significant difference in the occurrence of self-assessed PM or DM between the HT users and non-users. Climacteric symptoms were reported by 24% (n=761) of the total sample. According to logistic regression analyses, climacteric complaints significantly correlated with the occurrence of PM (p=0.000) and DM (p=0.000) irrespective of the

use of HT, indicating that PM and DM are associated with climacteric symptoms in general. In the clinical study the DMFT index was 20.1 ± 4.3 in the HT users at baseline. There was no difference in DMFT index values at follow up, and there was also no difference between HT users and non-users in this index. The number of filled teeth (FT) showed a significant ($p<0.05$) increase in the HT group at follow-up, however. This result was cautiously interpreted as indicating that women using HT were more active for seeking dental treatment than non HT users. Periodontitis (at least one sector having a score of 3 in the CPITN index) was diagnosed in 79% of HT users at baseline and in 71% at the follow-up. The values for non-HT users were 80% vs. 76%, respectively (Ns.). Severe periodontits (CPITN score of 4) is diagnosed when there are at least one gingival pocket ≥ 6mm deep. The mean numbers of ≥ 6 mm deep periodontal pockets were 0.9 ± 1.7 at baseline vs. 1.1 ± 2.1 two years later in the HT group, and 1.0 ± 1.7 vs. 1.2 ± 1.9, respectively, in the non-HT group. In a large Finnish national health survey, the prevalence of peridontitis of women of this age group was lower, but the prevalence of severe periodontitis seemed to be higher than in our study.

No difference was found between the groups in the prevalence of the periodontal bacteria studied. Salivary analyses showed that albumin, IgG and IgM concentrations decreased in the HT group during the 2-year follow up ($p<0.05$), possibly indicating an improvement in epithelial integrity. No other differences were seen in any other salivary parameters between or within the groups.

In conclusion, the present findings showed that 50 to 58 year old women living in Helsinki have fairly good oral and dental health. According to a questionnaire the occurrence of PM and DM seemed to be associated with climacteric symptoms in general, and the use of HT did not affect the oral symptoms studied. This result did not support our original study hypothesis. More data and a longer follow-up period are needed to determine the long-term effects of HT on oral and dental health, however.

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals. This thesis contains also some additional unpublished data.

I Tarkkila L, Linna M, Tiitinen A, Lindqvist C, Meurman JH. Oral symptoms at menopause-the role of hormone replacement therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:276-280.

II Tarkkila L, Furuholm J, Tiitinen A, Meurman JH. Oral health in perimenopausal and early postmenopausal women from baseline to 2 years of follow-up with reference to hormone replacement therapy. Clin Oral Invest 2008;12:271-277.

III Tarkkila L, Kari K, Furuholm J, Tiitinen A, Meurman JH. Periodontal disease-associated micro-organisms in peri-menopausal and post-menopausal women using or not using hormone replacement therapy. A two-year follow-up study. BMC Oral Health 2010;10:10.

IV Tarkkila L, Furuholm J, Tiitinen A, Meurman JH. Saliva in perimenopausal and early postmenopausal women. A two-year follow-up study. Submitted.

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2. INTRODUCTION

In a woman`s life at middle age, during the climacteric process, circulating sex hormone levels change and this understandably results in some clinical effects, i.e. climacteric symptoms, which also affect the quality of life (Speroff & Fritz 2005; Jalava-Broman et al. 2008; Kase 2009). Therefore in the climacterium and post-climacterium, hormone therapy (HT) which consists of administration of estrogens, progestins, and estrogen-progestin combinations, is used to alleviate climacteric complaints and also to prevent age-related diseases (te Velde & Van Leusden 1994; Stefanick 2005; Nelson 2008).

Oral health means much more than healthy teeth, and the relationship between oral and general health has been shown (WHO 2010). The majority of pain and discomfort in the oral cavity is due to diseases of the mouth. However, there are situations where oral symptoms are a consequence of systemic diseases or systemic alterations in physiological conditions.

Oral discomfort is found in many menopausal women. The principal peri- and postmenopausal oral symptoms are dry mouth, sensation of painful mouth (PM) of several causes, and less frequently burning mouth syndrome (BMS).

PM has been associated with reduced salivary flow rate (Lamey & Lamb 1988) and the presence of removable dentures (Main & Basker 1983). Mandibular dysfunction (Lamey & Lamb 1988) and also diffuse gingival atrophy or oral ulcerations can be present with oral dryness, causing PM (Forabosco et al. 1992). Other possible causative factors of PM are oral candidosis (Samaranayake et al. 1989; Wardrop et al. 1989), pernicious anemia (Hjørtin-Hansen & Bertram 1986), and some nutritional deficiencies (Lamey et al. 1986).

BMS is defined as a chronic condition characterized by a burning sensation of the oral mucosa, with or without dysgeusia (the distortion of the sense of taste) and xerostomia, in the setting of no identifiable clinical lesions, laboratory abnormalities, or causative systemic disease (Torgerson 2010; Zakrewska et al. 2005). This chronic pain is described by patients as a burning or stinging sensation of the tongue, lips or other oral mucosal surfaces (Zakrewska 1995; Zakrewska et al. 2005; Hagqvist et al. 2009; Ni Riordain et al. 2010). The intensity of the pain is moderate to severe and it generally occurs bilaterally (Woda & Pionchon 1999). The etiology of the BMS is, however, not known, although lately neuronal mechanisms have been suggested to be involved in its

occurrence (Jääskeläinen et al. 1997; Forssell et al. 2002). Nevertheless comprehensive data on BMS prevalence are lacking.

Salivary gland hypofunction has been divided into three different entities: xerostomia (a subjective sensation of dry mouth), hyposalivation (decresed saliva flow rate) and altered saliva composition (determined by laboratory testing) (Nederfors 2000). However, there is no global consensus concerning the terminology associated with dry mouth and therefore there can also be some confusion in clinical practice as well as in interpretation of different published data eg. xerostomia and hyposalivation are quite commonly used as synonyms. Women seem to suffer from xerostomia or hyposalivation more often than men (Sreebny & Valdini 1988; Nederfors et al. 1997). A recent study shows that patients with xerostomia display varying degrees of discomfort related to the quality of life depending on the etiology of xerostomia (Cho et al. 2010). The prevalence of xerostomia in the population varies with wide range (Nederfors 2000). Sex-related variations in salivary flow rates are well documented. In a Finnish study a higher flow rate was found in men than in women and a higher flow rate in women of premenopausal than of postmenopausal age (Parvinen 1984). A study from the USA did not find any differences in flow rates between premenopausal and menopausal women (Ship et al. 1991b). The effect of hormonal changes related to menopause on salivary secretion or composition is not well established. A reduced salivary flow rate has been suggested to cause oral symptoms during menopause. Altered saliva composition is not analysed routinely in clinical practise, and there are no prevalence data available to show altered saliva compositions in menopause. However, the importance of changes in salivary protein composition should not be underestimated (Nederfors 2000).

Dental caries and periodontal diseases have historically been considered the most important global oral health burdens (WHO 2010). Both dental caries and periodontal diseases are the result of the initiation and progression of multiple interrelated processes in which oral bacteria are one prominent factor (Thylstrup & Fejerskov 1994; Kinane et al. 2006). Host response and also oral hygiene habits are involved in the progression of both diseases. Hormonal changes as seen during and after menopause have been associated with osteoporosis but there is a lack of studies linking menopause or an estrogen-deficient state to a higher susceptibility to periodontal disease (Kinane et al. 2006). Also there are hardly any data on dental health at menopause (Meurman et al. 2009).

Before the HERS and WHI randomized trials demonstrated that the use of HT in healthy and asymptomatic postmenopausal women did not have overall health benefits that had been assumed

earlier, it seemed that women using HT tended to have healthier lifestyles than non-users (Hemminki et al. 1993; Matthews et al. 1996). Therefore we hypothesized that HT users would also be more interested in taking care of their oral health. The present series of studies (**I, II III and IV**) were conducted in order to evaluate the oral health of perimenopausal and early postmenopausal women with respect to the use of HT.

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3. REVIEW OF THE LITERATURE

3.1 Oral health

According to the policy of the WHO Oral Health Programme, oral health is integral and essential to general health. A major theme of the CAPP-report (http://www.whocollab.od.mah.se/index.html) is that oral health means much more than healthy teeth. WHO defines oral health as follows: "Oral health implies being free of chronic oro-facial pain, oral and pharyngeal (throat) cancer, oral tissue lesions, birth defects such as cleft lip and palate, and other diseases and disorders that affect the oral, dental and craniofacial tissues, collectively known as the craniofacial complex" (Petersen 2003; WHO 2010). We often take the function of these oral tissues for granted, although dysfunction of any of these tissues destabilizes our well being. The major functions of these tissues are to allow humans to speak, smile, smell, taste, touch, chew, swallow and cry out in pain. Humans also use oral tissues to convey their feelings and emotions through facial expressions. In addition, oral tissues provide protection against microbial infections and environmental insults (WHO 2010).

Dental caries and periodontal diseases are considered the most important global oral health threats, and losing the teeth is still seen by many people as a natural consequence of ageing (Petersen 2003). However, according to WHO reports there has been a positive trend, as tooth loss among adults has decreased in recent years. The distribution and severity of oral diseases and unfortunately the access to oral health services vary markedly in different parts of the world and even within the same country and region (WHO 2002b; WHO 2010).

3.2 Oral mucosa

The mouth or oral cavity is situated at the beginning of the gastrointestinal tract and it is a complex organ with various soft and hard tissue anatomical structures. The condition of the oral cavity can be seen as a reflection of the general health of the individual. Changes due to diseases, such as diabetes or vitamin deficiency, or the local effects of long-term tobacco or alcohol use, are seen as alterations in the oral mucosa (Squier & Kremer 2001).

The major role of the oral mucosa is to protect the underlying structures from mechanical damage and from the entry of some microorganisms and toxic materials that may present in the oral cavity (Squier & Kremer 2001). The soft tissues of the human oral cavity and esophagus are covered by a stratified squamous epithelium. The oral mucosa is tightly attached to the underlying collagenous connective tissue, called lamina propria (Squier & Kremer 2001). In the oral cavity, mucosal

regions differ from each other in their thickness, form, and state of epithelial maturation (Ten Cate 1994). In those regions that are subject to mechanical forces and associated with mastication (i.e., the gingiva and hard palate), the mucosa is covered by a keratinizing epithelium. In some regions of the oral cavity, aging causes slight thinning of the epithelium, with concomitant flattening of the epithelial-connective tissue interface (Williams & Cruchley 1994). In female patients aging has also been shown to decrease the permeability to water of the mucosa of the floor of the mouth (Hill 1994). Oral buccal epithelia and vaginal epithelia are microscopically similar. Thompson et al. (2001) observed that the patterns of surface keratinization and the distribution and appearance of the lipid lamellae in the intercellular spaces were similar in vaginal and buccal epithelial samples of postmenopausal women. The lipid composition of the two epithelia was similar, except for the cholesterol esters and glycosylceramides, which were more abundant in buccal epithelium. The response of oral epithelia to hormones and HT is not clear. However, in an earlier study Croley and Miers (1978) found that buccal smears of those patients with high estrogen levels had an increased number of superficial and keratinized epithelial cells, while those with higher progesterone levels had an increased number of intermediate epithelial cells and a decrease in the number of keratanized cells.

Consequently, changes like those seen in studies on vaginal micro-organisms might also be seen in the mouth microbiota. However, the oral lactobacilli strains differ from those in vaginal microbiota (Witkin et al. 2007). Postmenopausal women have a relative depletion of vaginal lactobacilli and an increase in vaginal *E. coli* compared with premenopausal women (Pabich et al. 2003). For example, it has been shown that HT restores the lactobacilli vaginal flora associated with a protective effect against urogenital infections (Devillard et al. 2004). Further, oral estriol was found to significantly increase lactobacilli in postmenopausal women with symptoms of vaginitis (Yoshimura & Okamura 2001). Whether corresponding changes are seen in oral microbiota after starting HT is not yet known.

3.3 Oral symptoms

According to the literature and clinical practice, pain and discomfort in the oral cavity are often due to local etiologic factors or are consequences of some systemic diseases, or side effect of medication. Oral discomfort is found in many menopausal women. Oral discomfort consists of various complaints including the sensation of DM, sensation of PM, changes in taste perception, and in some cases burning BMS (Ben-Aryeh et al. 1996).

3.3.1 Sensation of painful mouth (PM) and burning mouth syndrome (BMS)

Many menopausal women suffer from oral discomfort. DM and PM are prevalent in women of menopausal age. Also, but less frequently, BMS is diagnosed. It is important to distinguish between the terms PM and BMS as they represent two different symptoms or syndromes. However, until recently there has not been consensus concerning the definition of PM and BMS. Therefore there can be confusion in interpreting different data.

Recently Cho et al. (2010) reported that 67% of patients with xerostomia also had the sensation of PM and 57% had altered taste sensation as a xerostomia-associated complaint. PM has also been found to be associated a with reduced salivary flow rate (Lamey & Lamb 1988). Various denture flaws have been considered to be one of the primary factors causing sensation of burning in the mouth or PM (Main & Basker 1983) and mandibular dysfunction was found in 30 out of 150 PM patients in a study by Lamey & Lamb (1988). Diffuse gingival atrophy and oral ulcerations can be present with oral dryness and also cause PM (Forabosco et al. 1992). Other possible causative factors of PM are oral candidosis (Samaranayake et al. 1989; Wardrop et al. 1989), and systemic factors such as pernicious anemia (Hjørtin-Hansen & Bertram 1986), and other vitamin B-deficiencies (Lamey et al. 1986).

BMS is a chronic condition in which there is a burning sensation in the oral cavity. The main clinical feature of BMS is burning pain. In addition, altered taste sensations and DM are reported by these patients. The pain is described using terms such as burning, stinging, tickling or sticking sensation (Grushka 1987; Bergdahl & Bergdahl 1999; Tammiala-Salonen et al. 1993). This pain can be localized just to the tongue and/or lips but the pain can also involve the whole of the oral cavity and in most patients it occurs bilaterally (Grushka 1987; Bergdahl & Bergdahl 1999; Zakrewska 2005). In most cases the burning pain has continued for many months and the intensity of pain tends to increase towards the end of the day. The diagnosis of BMS can be made when the oral mucosa is normal on clinical examination and there are no identifiable oral lesions or laboratory abnormalities (Lamey & Lamb 1988; Lamey & Lewis 1989; Zakrewska et al. 2005).

The prevalence of BMS has been reported to be 2.6-11% in different groups of people (Basker et al. 1978). According to a prevalence study in Sweden by Bergdahl & Bergdahl (1999), BMS affects 3.7% of the general population, and is more common in women (5.5%) than in men (1.6%). In their study this difference between genders was particularly seen after menopause in women. In the

Finnish adult population the prevalence of BMS was found to be between 1-15%, depending on the criteria for diagnosis (Tammiala-Salonen et al. 1993).

Reviews have collected numerous etiological factors that have been proposed to be involved in BMS (Ship et al. 1995; Cibirka et al. 1997; Abetz & Savage 2009; López-Jornet et al. 2010). Still, thus far, according to Zakrewska (1995), the etiology of BMS remains an enigma. Pathogenic mechanisms, including the possible neuropathic origin, have recently been suggested as possible factors underlying BMS (Forssell et al. 2002; Zakrewska et al. 2005; Maltsman-Tseikhin et al. 2007). In a small Finnish study by Jääskeläinen et al. (1997), an abnormal blink reflex was found in chronic BMS patients. The presence of taste anomalies reported in BMS patients suggests abnormalities in an interaction between the taste perception and the nociceptive mechanism in the central nervous system (López-Jornet et al. 2010).

Antidepressants (Loldrup et al. 1989; Tammiala-Salonen & Forssell 1999), cognitive behavioral therapy (Bergdahl et al. 1995), analgesic mouthwash (Sardella et al. 1999), HT in postmenopausal women (Pisanty et al. 1975), antioxidants (Femiano et al. 2000; Femiano et al. 2004), and recently also acupuncture (Scardina et al. 2010) have been suggested as a treatments in the management of BMS patients. However, there are so far no standard treatment protocols for management of this disorder (Zakrewska et al. 2005).

Interestingly, like BMS with its unsolved etiology and difficulties in management of the symptom, vulvodynia is a chronic idiopathic pain syndrome with unknown etiology in vulva (Lotery et al. 2004; Paavonen 2006). Based of some recent case reports, BMS and vulvodynia may share a common cause (Gaitonde et al. 2002; Petruzzi et al. 2007; Sivelo & Rodrigues Nuñez 2010). It is not known how common "glossovulvodynia" is eg. that patients seldom report genital symptoms to the dentist. The symptoms described by the patients suffering of vulvodynia (burning, stinging, rawness or stabbing) (Paavonen 1995), mimic the specific symptoms described by BMS patients. Vulvodynia has been treated with using drugs effective against chronic neuropathic pain, or with cognitive-behavioral therapy, electromyographic biofeedback, and in some cases with vestibulectomy (Bergeron et al. 2001). These selected treatments suggest that, as has been proposed, this symptom is of neuropathic origin. However, management of vulvodynia remains poor and, like BMS, more studies on its treatment are needed according the treatment of vulvodynia (Paavonen 2006), as well as of BMS. However, in contrast to BMS, all adult female age groups suffer equally from vulvodynia.

3.3.2 Sensation of dry mouth (xerostomia)

The sensation of DM or xerostomia is defined as a subjective sensation of dryness in the mouth (Visvanathan & Nix 2010). Xerostomia is a major complaint for many elderly individuals (Wardrop et al. 1989; Närhi et al. 1999; Meurman & Rantonen 1994; Nederfors 2000). However, there is no convincing evidence that age alone is a significant cause of xerostomia (Eveson 2008). The prevalence of xerostomia is difficult to determine. According to a review article by Orellana et al. (2006), the prevalence of self-reported sensations of DM vary between 0.9% and 64.8%. Prevalence studies of xerostomia are difficult because of the heterogeneity of the patients. Also there are no standardized questions questions for diagnosing xerostomia. Xerostomia has various causes, and this symptom is often associated with an unpleasant feeling and other symptoms in the mouth and throat (Nederfors 2000; Thelin et al. 2008; CHO et al. 2010). As there is no doubt that xerostomia impairs the quality of life and also causes oral complications, it is important in clinical practice to recognize and distinguish between patients with subjective complaints and those who have evidence of salivary hypofunction or who are suspected of having some disease, in order to manage these conditions appropriately (Napenas et al. 2009).

3.4 Dental health

As stated by the WHO (2002b), a healthy oral unit consists of a functional, painless and infection-free oral cavity. Dental caries is a major oral health problem in 60-90% of schoolchildren worldwide, and it affects the majority of adults in most industrialized countries (Petersen 2003; WHO 2010). National data of the prevalence of dental caries are collected for 12-year-olds as part of the surveillance programme of WHO's Department of Noncommunicable Diseases, but there is no organized programme for the collection of national data on the oral health of older adults (WHO 2002a). In a large Finnish population study on adults, at least one decayed tooth with caries was detected in 32% of males and in 20% of women (Kansanterveyslaitos 2004).

3.5 Periodontal health

Periodontium is comprised of gingiva, periodontal ligament, root cementum, and alveolar bone. The main function of the periodontium is to attach the tooth to the bone tissue of the jaws and maintain the integrity of the surface of the masticatory mucosa of the oral cavity and protect the underlying tissues of the periodontium (Lindhe et al. 2006). Periodontal diseases are defined as a group of bacterial infectious and inflammatory diseases that result in the destruction of tooth-supporting tissue, and may eventually cause tooth and alveolar bone loss (Mealey & Rethman 2003). The results from population-based studies have shown that the pathogenesis and severity of periodontitis

are dependent on the presence of bacterial plaque and also on the susceptibility of the host (Offenbacher 1996; American Academy of Periodontology 2002) as well as on environmental and genetic factors (Kinane et al. 2006). Dental plaque can be defined as the soft deposits that form the biofilm adhering to the tooth surface and other hard surfaces in the oral cavity (Bowen 1976). The pathogenesis of plaque which is associated with gingivitis is due to bacterial accumulations (biofilms) on the surface of the teeth close to the gingiva (Haffajee & Socransky 1994). This biofilm is known to be resistant to normal host responses, and may initiate vascular changes in the gingival tissues, causing migration of polymorphonuclear leukocytes into the tissues and further into the gingival sulcus, finally causing loss of collagen just apical to the junctional epithelium lining the base of the gingival sulcus (Page et al. 1997). If the biofilm is disrupted by tooth brushing or by professional root scaling, the process is reversed and healing occurs (Kornman 1996). Without removal of the subgingival plaque, the condition may progress into periodontitis (Page 1991; Page et al. 1997).

Periodontal health indices are used in clinical practice to define a patient´s present gingival status as well as for following any changes in gingival status over time (Beck & Arbes Jr 2006). According to the international database (WHO, http://www.whocollab.od.mah.se/index.html) severe periodontitis is found in 5-15% of most populations. Results from a large Finnish national health survey reveal that periodontal diseases are a significant national dental health problem among dentate adults over 30 year of age. The prevalence of periodontitis was found to be 72% in men and in 57% in women (Kansanterveyslaitos 2004).

3.5.1 Periodontal microbiology

The mouth, being moist and having a high concentration and availability of different nutrients, is an ideal environment for the growth of micro-organisms. Also, as the temperature in the oral cavity is approximately 37^{o}C it offers a good environment for bacterial growth (Meurman et al. 2009). The presence of different surfaces favours attachment and colonisation of bacteria, which further grow to form microbial biofilms to cover the teeth and mouth mucosa. It has been shown that thick microbial plaques may form on teeth, where bacterial concentrations up to 10^9 colony forming units per mg have been detected (Li & Caufield 1995). The microbial communities are usually fairly stable throughout life, but the density of micro-organisms varies depending on a subject's health and disease status, oral hygiene practices, and diet (Li & Caufield 1995; Lee et al. 2006). By the beginning of the 21st century over 700 microbial species had been identified in the mouth (Paster et al. 2001; Kazor et al. 2003). In addition, a recent study reported signs of thousands of unidentified

micro-organisms in oral samples analysed with a molecular pyrosequencing technique (Keijser et al. 2008).

For oral diseases it is has been suggested that a small group of predominately Gram-negative, anaerobic or microaerophilic bacteria within the biofilm are often associated with periodontal disease initiation and progression (Page & Kornman 1997). Among those subgingival bacteria which are currently associated with periodontal diseases are the Gram-negative, strictly anaerobic bacteria species: *Porphyromonas gingivalis* (*P.g.*), *Prevotella intermedia* (*P.i.*), *Tannerella forsythia* (*T.f.)* (formely *Bacteroides forsythus*), *Treponema denticola* (*T.d.*), *Fusobacterium nucleatum* (*F.n.*) and the facultative/microaerobic bacteria *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* (*A.a.*) and *Campylobacter rectus* (American Academy of Periodontology 1996, 2005). The presence of different clonal types of these bacteria has been recognized. However, it is not known whether all clonal types are pathogenic (American Academy of Periodontology 2005). It is assumed that tissue destruction is caused when the population of the subgingival bacteria is high enough to overwhelm the host defense systems that prevent bacteria from migrating further subgingivally (Page et al. 1997).

3.6 Saliva

Saliva, a complex and versatile body fluid, is essential for oral health and functions (Lima et al. 2010; Thelin et al. 2008). Saliva is critical for oral function as it is responsible for lubricating and protecting all surfaces in the oral cavity. The main function of saliva is to hydrate and cleanse the mouth as well as protect the teeth through its buffering and remineralizing properties, and to provide anti-microbial activity (Thelin et al. 2008). Saliva also takes part in many other important functions such as mastication, speech, deglutition and gustatory sensitivity (Sreebny & Valdini 1988; Sreebny 2000; Mandel 1989; Lima et al. 2010).

Saliva, as being a seromucous coating, is the principal defensive factor in the oral cavity. It lubiracates, protects oral tissues, act as a barrier against irritants by buffering and clearing, has antibacterial activity, and play a significant role in taste and digestion (Humphrey & Williamson 2001). Low salivary flow has detrimental effects on teeth and mouth mucosa. Reduced salivary flow enhances oral microbial colonization, in particular by yeast. These, in turn, are an infectious burden on the patient and may worsen overall systemic health (Guggenheimer & Moore 2003). Reduced salivary secretion is also a risk factor for increased caries (Dodds et al. 2005) and for a reduced saliva pH and higher numbers of acidogenic micro-organisms (Almståhl & Wikström

2003). Salivary secretion follows a diurnal rhythm and it depends on the general state of hydration but is mostly affected by systemic diseases and drugs (Scully & Bagan 2004). A problem with salivary studies is that salivary flow rate and concentration values have huge variations both within an individual and between subjects (Mandell 1980).

3.6.1 Composition

Saliva is produced by the salivary glands. The major salivary glands are the parotid glands, submandibular glands and sublingual glands. Minor glands are situated on the tongue, palate, lip, and buccal and labial mucosa (Ferguson 1999). In the salivary glands there are acinar cells, various duct system cells, and myoepithelial cells. The branched ductal system and the secretory end pieces (acini) are responsible for the saliva secretion from the salivary gland tissues. The duct system of the submandibular and parotid glands is well-developed and well–branched. It contains intercalated, striated and excretory ducts. Simple branched tubular glands are classed as minor salivary glands (Tenovuo & Lagerlöf 1994). The site of formation of the saliva is the acini which secrete the primary saliva and determine the type of secretion (mucous or serous) saliva produced from the different glands (Humphrey & Williamson 2001). The plasma-like isotonic primary saliva is secreted by acinar cells. The secreted fluid first passes through intercalated ducts to the striated ducts where ions are actively extracted, rendering this primary saliva progressively more hypotonic to enable it to pass down through excretory ducts and towards the mouth (Despopoulos & Silbernagl 1991; Smith 1996). The serous secretions are produced mainly from the parotid gland and mucous production from the minor glands. Mixed secretions of serous and mucous secretions are produced by the sublingual and submandibular glands (Edgar 1992). Whole saliva is a combination of fluids from the major and minor salivary glands along with gingival crevicular fluid (GCF), which contains oral bacteria and food debris (Edgar 1992; Dawes 2008). The major salivary glands contribute most of the secretion in terms of volume. However, minor gland secretions modify the properties of the saliva and thereby cause variation in the type of protection given by the secreted saliva. Minor glands also contribute the blood-group substances of the saliva (Humphrey & Williamson 2001).

Whole saliva is a dilute fluid that is almost 99% water (Humphrey & Williamson 2001). Organic and inorganic solid components are dissolved in the aqueous component and they vary widely from one individual to another. Variations in solid components occur even in the same individual over the course of the day (Lima et al. 2010). The inorganic part of saliva is composed of weak and strong ions. Na, K and Cl are the most important ions for maintaining the ionic strength of saliva.

Ca, Mg, and phosphates have an important role in mineralisation events. Epithelial cells lining the salivary ducts produce bicarbonate ions. When the salivary gland is stimulated, salivary flow rate increases and the production of bicarbonate increases. Bicarbonate acts as buffer and maintains the pH level of close to 7. Saliva is slightly acidic. The normal pH of saliva is 6-7. However, it can vary from 5.3 to 7.8 depending on the state of the flow (Humphrey & Williamson 2001). The organic part of saliva is composed mainly of amylase (α-amylase), antimicrobial salivary proteins, serum filtrates, nitrogenous products (urea and ammonia), and lipids such as cholesterols and fatty acids (Tenovuo & Lagerlöf 1994; Humphrey & Williamson 2001; Lima et al. 2010).

Almost 400 different proteins have been identified in saliva (Tenovuo & Lagerlöf 1994; Hofman 2001; Lima et al. 2010). In the saliva the most relevant proteins derived from the salivary glands are α-amylase, histatins, cystatins, lactoferrins, lysozymes, mucins and proline-rich proteins and statherin (Hofman 2001). Amylase is generally considered to be a reliable marker for serous cell function (Almståhl et al. 2001). The salivary proteins albumin and transferring are derived from plasma (Hofman 2001). Of the plasma proteins, albumin is the most abundant, accounting for more than 50% of all proteins in plasma. Although salivary albumin has been regarded as a serum ultra-filtrate (Oppenheim 1970) it may also diffuse into the mucosal secretions (Schenkels et al. 1995). Salivary albumin has been suggested to be a useful tool for diagnosting periodontitis. Higher salivary albumin concentrations have been found in patients with periodontits than in healthy dentate and edentulous patients (Takahashi et al. 2004) and in patients with gingivitis or periodontitis compared with healthy subjects (Henskens et al. 1993). Also albumin is selectively absorbed by different materials in the oral cavity and therefore it can attach to specific bacteria, and thus alter the composition of dental plaque (Kohavi et al. 1997). Salivary albumin levels have also been used as a marker for the degree of mucositis (Oppenheim 1970), and it has been suggested that albumin analyses may be useful in assessing the integrity of mucosal function in the mouth (Meurman et al. 1997b). Increasing albumin levels were seen in medically compromised patients whose general condition getting worse (Meurman et al. 2002) and also high concentration levels of albumin were detected in HIV-infected patients (Mellanen et al. 2001). Salivary albumin has also been used as a marker for salivary gland inflammation (Fox et al. 1985; Schiodt et al. 1992).

The immunological contents of saliva include secretory IgA (sIgA), IgG and IgM. IgA is produced by plasma cells in connective tissues and it is then translocated through the duct cells of the major and minor salivary glands to be added to the saliva (Humphrey & Williamson 2001). sIgA is a largest immunologic component of saliva (Bulkacz & Caranza 2006). The other immunoglobulins

in saliva are believed to enter the saliva mainly as components of GCF, and they are present in lower quantities than IgA (Humphrey & Williamson 2001). The IgG is mainly derived from serum, but a minor fraction may originate from local plasma cells when the gingivae are inflamed (Kaufman & Lamster 2000). It is not known whether locally produced and serum-derived IgM and IgG can also be transmitted paracellularly to saliva via oral mucosa.

Different components of saliva interact with each other and have important functions. Although they generally occur in small amounts and vary with changes in salivary flow, they continually take part in important and essential functions (Humphrey & Williamson 2001). One of these functions is the modulation of pH and buffering capacity by bicarbonates, phosphates, proteins and urea. Macromolecule proteins and mucins play roles in cleansing, aggregating, and/or attachment of oral microorganisms and contribute to dental plaque metabolism. Ca, P, and proteins work together as antisolubility factors and they also modulate demineralization and remineralisation. Immunoglobulins, proteins and enzymes are responsible for the antibacterial propeties of saliva (Humphrey & Williamson 2001).

3.6.2 Flow rate

The main factor affecting the salivary composition is the salivary flow rate (Tenovuo & Lagerlöf 1994). The flow rate is influenced by many factors including the degree of hydration, drug use, body position, previous stimulation, and circadian rhythm as well as the size of salivary glands (Dawes 2004). Salivary glands are innervated by parasymphathetic and symphathetic nerve fibers. These two types of nerve fibres produce different ratios of activation by different stimuli and the type of stimulation results in changes in consistency of the secreted saliva ie. more watery saliva or less watery saliva (Tenovuo & Lagerlöf 1994).

The normal average daily flow of whole saliva in adults varies from 1 to 1.5 litres (Edgar 1990; Humphrey & Williamson 2001). Lower estimates (0.6 L/24 h) have also been made for the normal daily production of saliva (Watanabe & Dawes 1988). When measuring and reporting salivary flow rates, the results are usually given as unstimulated and stimulated flow rates. Unstimulated whole saliva is the mixture of secretions which enter the mouth in the absence of exogenous stimuli such as chewing, whereas stimulated flow is secreted in response to masticatory stimulation, or to stimulation of vagal afferent fibers at the gastric end of the esophagus (Ganong 1989).

Dawes (1987) has provided a standardized protocol to measure the whole saliva flow rate. The generally accepted limit for a very low unstimulated whole saliva rate is ≤0.1 ml/minute (Sreebny & Valdini 1988; Edgar 1990). Values between 0.1 to 0.2 ml/min are considered to constitute low unstimulated flow rates, and values above those represent a normal unstimulated flow rate (Sreebny & Valdini 1988; Tenovuo & Lagerlöf 1994). Correspondingly, stimulated saliva flow rates below 0.1 ml/min are considered to be very low (Sreebny & Valdini 1988), and those below 0.7 ml/min are considered to be low (Sreebny & Valdini 1988; Edgar 1990; Närhi 1994). A wide variation among individuals has been found (Ghezzi et al. 2000) and ideally the saliva flow rate should be recorded as a base reference value after the age of 15 (Edgar 1990). However, there is no uniformly accepted reference value for saliva flow rates, and therefore clinical practice and recommendations vary (Ship et al. 1991b).

Reduced salivary flow enhances oral microbial colonization, in particular colonization of yeasts (Pajukoski et al. 2001). These factors, in turn, can cause an additional infection load in the patient and may worsen the systemic condition (Guggenheimer & Moore 2003). When sleeping, unstimulated saliva is the moisturizing and lubricating component of the oral defense system, and a decrease in its secretion may be indicated by dry mouth and burning mouth symptoms (Pajukoski et al. 2001).

The sensation of DM and its relation to minor gland saliva secretion has recently been studied (Eliasson et al. 2009). Eliasson et al. (2003) reported earlier that flow rate of labial gland saliva can increase, and the complaints of DM increase, in postmenopausal women treated with a low potency estrogen treatment. According to the study by Eliasson et al. (2009) the impaired saliva film retained on the oral surfaces seems to have important role in the sensation of oral discomfort, and the secretion of minor salivary glands may contribute considerable to this film, especially in the mucosa. Therefore these results indicate the importance of further studies on the role of minor salivary glands in DM (Eliasson et al. 2003; Eliasson et al. 2009).

3.6.3 The diagnostic use of saliva in normal dental practise

Saliva analysis has become important, and is useful for evaluating physiological and pathological conditions in humans in the fields of medicine and dentistry. The use of saliva has many advantages. It is easily obtainable, sample collection is non-invasive and easy, and storage is low cost (Lima et al. 2010). Due to the origin of saliva and its composition and functions, as well as its interactions with other organ systems, it is a useful tool for diagnosing various diseases (Lima et al.

2010). Saliva is also valuable for monitoring some drugs (Lillsund 2008), hormones and antibodies (Smith et al. 1991; Tabak 2001).

It is evident that, in dental practice, salivary measurements, at least salivary secretion rates and buffering capacity, should be used to supplement the anamnestic information and clinical findings with regard to prevention of dental caries (Tenovuo & Lagerlöf 1994). For normal dental practice the salivary bacterial counts are used for caries risk assessment. Dip slide tests are widely used and they are based on the identification and quantification of *Lactobacillus* species and mutans streptococci in the saliva. These bacteria are associated with caries development (Tenovuo & Lagerlöf 1994; Lima et al. 2010). Identifying those bacteria through saliva tests could strengthen preventive measures against caries development (Lenander–Lumikari & Loimaranta 2000). However, the tests that are currently available are useful for estimating caries activity due to bad dietary habits, and establishing the presence of infection and salivary yeasts (Larmas 1992).

In the majority of adult patient yeasts, mainly *Candida albicans*, are found to live commensaly in the oral cavity, mucosal surfaces being the primary reservoir (Odds 1988). Yeast colonization has also been demonstrated in prostheses, dental plaque and in periodontal pockets (Pizzo el al. 2002). However, a major factor associated with overgrowth of the yeast is a diminished host resistance as a result of some diseases (Rees 2006). A chair-side method (Oricult ®) has been developed for detecting yeast easily from the saliva in the clinical practise.

The use of saliva as a diagnostic and monitoring method for periodontal diseases has also been increasingly studied (Kaufman & Lamster 2000). Results from the population study by Könönen et al. (2007) showed that carriage of periodontal pathogens in saliva was common, since at least one of the studied pathogens was found in the saliva of 88% of the subjects. Monitoring the carriage pattern of periodontal pathogens from saliva samples at the general population level could therefore help in designing preventive strategies to control the acquisition of less beneficial members of the human oral microbiota (Könönen et al. 2007).

A problem with the reliability of using saliva samples to determine types and levels of compounds in circulation is that concentration of the biochemical compounds in plasma are defined with well documented reference values whereas oral fluid composition exhibits a wide variation both quantitatively and qualitatively. However, saliva is currently used in the measurement of some steroid hormones (Chiappin et al. 2007), although not all the hormones are found in saliva. To date,

there is no clear explanation for why some hormones can be found in saliva and some cannot (Gröschl 2009). Lipid-soluble steroids or amines are transfered more rapidly to the saliva than hydrophilic peptides, and hydrophilic conjugates are more likely transferred by ultrafiltration (Vining et al. 1983; Riad Fahmy et al. 1983; Gröschl 2009). Salivary concentrations of conjugated, lipid-insoluble hormones like cortisone, dehydroepiandrosterone sulphate (DHEAS), thyroxine and pituitary hormones are much lower than (1-20%) than the unbound plasma concentration, and therefore salivary analyses are of less value for these hormones (Vining et al. 1983; Vining & McGinley 1987; Read 1989) .

3.7 Menopause

3.7.1 Definition

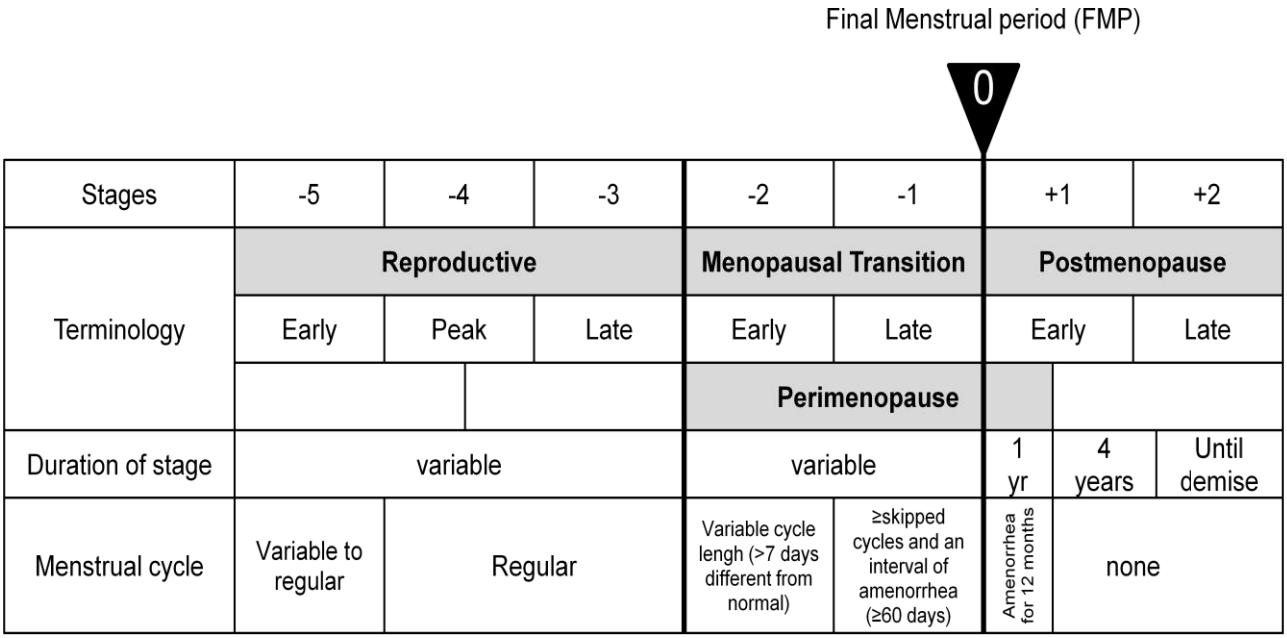
Natural menopause is defined as a spontaneous cessation of natural menstruation for 12 consecutive months at 45-55 years of age (mean 50-52) (McKinlay et al. 1992; Oldenhave et al. 1993; Gold et al. 2001). Formally, menopause is the moment of the final menstruation, directly preceded by the permanent cessation of ovarian follicular function (Morabia & Costanza 1998; Nelson 2008). The average age of a woman at menopause is 51 years (McKinlay et al. 1992; McKinlay 1996; Rutanen & Ylikorkala 2004).

Menopause results from reduced secretion of the ovarian estrogen, which takes place when ovarian follicles are depleted through genetically controlled apoptosis of the ovarian cells. During the menopausal transition the level of inhibin B originating from the follicles decreases and the levels of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase. As the level of FSH rises, small ovarian follicles temporarily undergo activation, and this leads to increases in circulating E2 levels at an early stage of the menopausal transition. However this occurs only at the period of transition and is not seen in all women. Nevertheless, hypoestrogenism is the ultimate outcome of menopause. With increasing age, the levels of FSH and LH continue to rise for several years, but in later menopause these levels decrease (Kase 2009).

WHO has defined three age stages of midlife age for women (Research on the menopause. WHO 1996): "1) Menopause is the year of the final physiologic menstrual period retrospectively designated as 1 year without flow (unrelated to pregnancy or therapy) in women aged ≥ 40 years. 2) Premenopause begins at ages 35 to 39 years; during this stage, decreased fertility and fecundity appear as the first manifestations of ovarian follicle depletion and dysfunction, despite the absence

of menstrual changes. 3*)* Perimenopause includes the period of years immediately before the menopause and the first year after the menopause." A model developed at the Stages of Reproductive Aging Workshop (STRAW) describes seven stages of reproductive ageing (**Figure 1**) (Soules et al. 2001).

Figure 1. Stages and nomenclature of normal reproductive aging in women (figure modified from Soules et al. 2001).



Diagnosis of menopause is complex as it can be made only retrospectively as menopause is defined as the onset of the last menstruation, followed by amenorrhea. Therefore the term "menopausal transition" has been used to refer to the first year of amenorrhea that marks the end of perimenopause and begins the postmenopause phase (Prior 1998; Martin & Mason 2008).

The term postmenopause is defined as the prolonged period of hypergonadotropic hypogonadism after menopause. Postmenopause is further divided into two different stages: early postmenopause, when estrogen is swiftly declining, and late postmenopause, when prolonged hypoestrogenism exists (Speroff & Fritz 2005). Climacterium consists of the transition period from fertility to infertility of which menopause (the last menstruation) as well as perimenopause and postmenopause are parts.

3.7.2 Consequences of menopause

3.7.2.1 Climacteric symptoms

The endocrine changes during perimenopause manifest as annoying clinical symptoms (Kase 2009). The symptoms which may occur before and/or within the first months of menopause are defined as immediate symptoms. Most characteristic for menopause are vasomotor symptoms such as hot flushes and night sweats, which are present in 75-80% of all women in menopausal age groups (Oldenhave 1993; Stearns et al. 2002; Deecher & Dorries 2007). Quality of life is greatly reduced because of VMS (Luoto 2009). Vasomotor symptoms are causally related to decreasing E2 concentrations, mainly in the serum and subsequently also in the hypothalamic temperature regulating centre (Rossmanith & Ruebberdt 2009). Immediate symptoms have become a leading reason for initiating HT in clinical practice (Lyytinen 2009).

Other symptoms which are commonly linked to the climacteric stage are mood swings, urogenital dryness, tiredness, joint and muscle pains, dizziness, irritability and insomnia (Kenemans et al. 2001; Stearns et al. 2002; Nappi & Lachowsky 2009). In a Finnish study, 46% of women aged 52-56 years had moderate or severe climacteric symptoms and only 5% were asymptomatic (Jokinen et al. 2003).

Also, oral symptoms such as sensation of DM and sensation of PM have been listed among symptoms that occur at menopause. In clinical practice, the majority of BMS patients seem to be at menopause or are postmenopausal women (Basker et al. 1978; Speciali & Stuginski-Barbosa 2008).

3.7.2.2 Osteoporosis

Data has established an association between low estrogen levels and bone loss (Waugh et al. 2009). Both bone–forming osteoblasts and bone-resorpting osteoclasts express estrogen receptors ERα and ERβ. This indicates that bone is a target for estrogen (Bord et al. 2001). It is known that estrogen reduces the activity of osteoclasts and increases their apoptosis, thus decreasing postmenopausal bone loss (Manolagas 2000). HT has shown a bone strengthening effect both in the spine and hip after 2 years of treatment (Wells et al. 2002). Estrogen use has been associated with a reduction of osteoporotic fractures of up to 40-59% (Farquhar et al. 2009).

According to the WHO criteria, osteoporosis is diagnosed when bone mineral density is at least 2.5 standard deviations below the average value for young and healthy women (T-score < -2.5). In Finland, it is estimated that approximately 400 000 people have osteoporosis, and annually 30, 000-40, 000 osteoporotic fractures are diagnosed (The Finnish Current Care guidelines, Finnish Medical Society Duodecim, www.kaypahoito.fi).

3.7.2.3 Cardiovascular diseases

Cardiovascular diseases (CVD) are rarely diagnosed in premenopausal women compared with age-matched men (Isles et al. 1992). According to results from Pepine et al. (2006) clinically significant CVD occurs in women approximately 10 years later than in men. However, menopause has been known to be associated with the incidence of CVD (Collins et al. 2007; Shaw et al. 2009). Menopause is known to induce hypoestrogenism and this has been suggested to be one of the explanatory factors for this association (Mendelsohn & Karas 2005).

3.8 Hormone therapy

3.8.1 General principles

Estrogen therapy to treat menopausal symptoms has been used worldwide for more than 80 years (Barrett-Connor 2003; Warren 2004; Stefanick 2005). However, already before 20th century treatments for the "climacterica" were described. Pills containing powder from derivates of dried cow ovaries were used until derivatives from human pregnancy urine were developed (Stefanick 2005). In 1942, products from the urine of pregnant mares were combined and developed for HT by Wyeth-Ayerst (Ltd. of Canada). This, the world's first conjugated estrogen medicine, named Premarin, was then approved by US Food and Drug Administration (FDA) for treating menopause (Stefanick 2005; http://en.wikipedia.org/wiki/Wyeth-Ayerst).

Natural human estrogens are E2, estrone (E1) and estriol (E3), and their conjugates, i.e. the sulfuric acid esters (sulfates) and glucuronic acid esters (glucuronides) (Kuhl 2005). E2 is considered the most potent of these. Estrogen-only therapy (ET) can be prescribed to hysterectomized women (Kuhl 2005) and it is used either orally or transdermally. In Europe the predominant estrogen for HT has been E2 whereas in the US conjugated equine estrogen (CEE; from mares urine) is most commonly used (Campagnoli et al. 2005; Kuhl 2005).

The use of ET in women with an intact uterus is associated with an increased risk of endometrial cancer (Smith et al. 1975; Ziel & Finkle 1975; Furness et al. 2009). Therefore, progestagen as a complement to oral or transdermal estrogen treatment (estrogen-progestagen therapy; EPT) is needed in non-hysterectomized women in order to protect the endometrium against hyperplasia and malignant transformation (Manson & Martin 2001).

Progestagens are divided into natural progesterone and synthetic progestagens (Schindler et al. 2008). In Europe a large variety of different progestagen products are available. In Scandinavia and the UK norethisterone acetate (NETA) and levonorgestrel (LNG) are more preferable used. The most common progestagen used in US is medroxyprogesterone acetate (MPA), while in Europe MPA is used to a lesser extent (Campagnoli et al. 2005). MPA is known to have some antiestrogenic, androgenic and glucocorticoid properties which may counteract the beneficial effects of estrogen (Kuhl 2005).

Synthetic steroid tibolone, various phytoestrogens, testosterone and selective estrogen-receptor modulators (SERM) are used under some conditions as alternatives to traditional HT (Kuhl 2005; Campagnoli et al. 2005). Of these, only tibolone is effective and useful in treating vasomotor symptoms.

Human fetal steroid estetrol (E4) was previously considered to be a weak estrogen, but recent research has demonstrated that it is an effective estrogen agonist with estrogen antagonistic effects on the breast in the presence of E2 (Coelingh Bennink et al. 2008). Based on its pharmacokinetic properties, especially its slow elimination, long half-life and oral bioavailability, it seems potentially suitable as a drug for human use as an adjuvant in HT (Visser & Coelingh Bennink 2009).

3.8.2 Use of hormone therapy

Women are treated with HT not only to avoid climacteric symptoms but also to protect them from CVD and osteoporosis (Grodstein & Stampfer 1995; Seeman et al. 1995). However, as evidenced by numerous studies, endogenous hyperestrogenism is associated with an elevated risk for breast cancer, therefore it is expected and also it has been confirmed that also the use of exogenous female sex hormone may increase the risk for breast cancer (Collins et al. 2005; Lee et al. 2005; Lyytinen 2009). Recently the use of HT has received much publicity when the results from large population studies, The Heart and Estrogen/Progestin Replacement Study (HERS) of 2763 postmenopausal

USA women with CVD and average age of 67 years in 1998, the USA study Women's Health Initiative (WHI) of 16,608 postmenopausal women (aged 50 to 79 years) in 2002, and a study of 1,084,110 UK women aged 50-64 years, the Million Women Study (MWS) in 2003, were published. These studies suggested that the benefits of using HT for the prevention of diseases other than menopause-associated symptoms may not be warranted (Hulley et al. 1998; Rossouw et al. 2002; Beral 2003). Before the publication of the results from HERS, it was recommended that postmenopausal women consider taking HT based on the diverse beneficial effects such as the reduction in risk of osteoporosis and heart disease (Ballard 2002). After the results from the trials (HERS, WHI, MWS), the European Medicines Agency (EMEA) recommended that HT should only be used for severe menopausal symptoms over short periods (EMEA 2003).

As a result of these studies and recommendations, the use of HT has declined markedly since 2002 in many countries, including Finland (Lawton et al. 2003; Hersh et al. 2004; Tiihonen et al. 2007). Recent experimental and clinical studies have indicated that the effects of HT depend on the estrogen and progesterone/progestin formulation, dosage, mode of administration, patient's age, associated diseases, and duration of treatment (Harman 2006; Caufrietz 2007).

However, there is no doubt that many women clearly benefit from the use of HT, which may also have implications in the oral cavity (Friedlander 2002). HT at the lowest effective dose is now recommended as an established treatment for healthy, recently postmenopausal women suffering with disturbing moderate to severe hot flushes (Rutanen & Ylikorkala 2004; International Menopause Society Consensus Statement 2009). According to further analysis of WHI data it was revealed that women who started HT within 10 years from the onset of menopause had a reduced risk of coronary heart disease, compared with those who started HT later than 10 years of onset of menopause (Rossouw et al. 2007).

In Finland in 2008, various forms of HT were used by a total of 352, 600 postmenopausal women (Paakkari et al. 2008). According to The Finnish Medical Society and the Academy of Finland 2004, the use of HT can be continued as long as it improves the quality of life, and therefore there is no upper limit defined for the duration or the age of women using HT.

3.8.3 Estrogen receptors (ERs)

Estrogens are the main female sex steroids. They are known to control and regulate many cellular processes including growth, differentiation and function of reproductive as well as non-productive tissues and systems. The most important estrogenic hormone, E2, is synthesized in the ovary and in the other tissues by aromatization of testosterone (Hollihn 1997; Gruber et al. 2002). E2 is involved in the control of sexual behaviour and reproduction functions, differentiation of several tissues and organs, modulation of inflammation, and brain and cardiovascular function (Gruber et al. 2002; Deroo & Korach 2006). Estrogens are also suggested to be involved in the regulation of tissues such as bone, tooth and periodontium (Hollihn 1997; Mariotti 1994). In the non-pregnant female, E1 and E2 are the main forms of estrogen. E3 is is the main estrogen during pregnancy. Unbound estrogens are diffused from the bloodstream into their target tissues. The biological effects of estrogens are based on genomic mechanisms that are mediated by interactions and with activation by the nuclear ERs in these target tissue cells (Jensen et al. 1972; Ray et al. 2002; Marino et al. 2006). Two different subtypes of ERs have been identified so far: ERα (Greene et al. 1986) and ERβ (Kuiper et al. 1996). ERα has been found in commonly known estrogen-target tissues (mammary glands and the endometrium), whereas ERβ has also been detected in more unexpected target tissues such as colonic (Campbell-Thompson et al. 2001) and prostatic epithelia (Kuiper et al. 1996). However, endocrinologists now have a target to develop specific agonists, which can selectively activate/inactivate either ERα or ERβ as it seems that in those tissues where both reseptors are expressed, they seem to work to counteract each other (Morani et al. 2008). Also, strong evidence has been recently presented demonstrating that ERβ has a role in estrogen signalling, along with its protective role in the epithelial-mesenchymal transition and in atherosclerosis as well as in regulation of cell proliferation in the colon (Zhao et al. 2010).

The molecular structure of non-steroidal estrogens allows them also bind to the ERs. However, they do not fit exactly in the ligand-binding domain of the receptor protein, and this incomplete binding prevents the correct interaction necessary for induction of the normal biological function induced by E2. These structure-dependent characteristics and the existence of these two different ERs, ERα and ERβ, are the basis of the development of SERMs. The effects of phytoestrogens are also due to their binding to ERs, but their estogen activity differs largely from that of E2. It has been discovered that steroid and non-steroid molecules with less similarity to the structure of E2 have lower binding affinity for the ERs, and this dissimilarity correlates with their biological effect (Kuhl 2005). It is known that ER-mediated transcriptional activity is modulated by the actions of other nuclear receptors (Min et al. 2002).

Endorcine-disrupting chemicals are xenobiotics that can affect the healthy functioning of a variety of tissues by acting as potent estrogens by non-genomic signalling pathways, or by interfering with binding actions of physiological estrogens (Watson et al. 2010). The mechanism by which estrogen action is modulated by the environmental xenobiotics and their effects on human reproductive tissues are under investigation worldwide by various research teams (Crews & McLachalan 2006; Brucker-Davis et al. 2010)**.**

3.8.4 Sex steroid hormones and mouth

Oral tissues are well vascularized, which means that the steroid hormone supply is good. Salivary glands and oral soft tissues contain sex steroid hormone receptors, which are typical of steroid responsive tissues. Earlier binding studies have shown that oral tissues are able to bind estrogen (Vittek et al. 1982; Dimery et al. 1987; Forabosco et al. 1992). Later immunohistochemical studies have shown the location of these receptors in oral mucosa, gingiva and salivary glands (Laine et al. 1993, Ozono et al. 1992, Välimaa et al. 2004). Välimaa et al. (2004) demonstrated expression of ERβ subtype within oral epithelium and suggested a direct physiological role of ERβ to the oral mucosal, gingival and salivary gland function. In addition to blood circulation human oral mucosa and gingiva are exposed to steroid hormones by saliva. However, estrogen effects are mediated by both types of ERs in oral region. In contrast to salivary glands and oral soft tissues temporomandibular joint (TMJ) expresses ERα (Puri et al. 2009). In a recent study by Ribeiro-Dasilva et al. (2009) suggested that a polymorphism in the ER may increase the risk of women developing TMJ.

The steroid hormones detected in the saliva are thought to reflect the free, not protein bound, hormone concentration in blood (Hofman 2001). Hormones transfer from the blood to the saliva through the capillary endothelia and through the glandular epithelia (Gröschl 2009). The salivary level is a valuable clinical tool for those lipid-soluble hormones like cortisol (to show hypersecretion of endogenous cortisol), E2 (prediction for premature birth; a test-method approved by the FDA) and progesterone (Vining & McGinley 1986; Tivis et al. 2005).

3.9 Oral health, menopause and hormone therapy

3.9.1 Oral symptoms in menopause

In 1946 Ziskin and Moulton described a typical patient with oral symptoms as being a menopausal woman (Ziskin & Moulton 1946). In further studies, Massler (1951) found that patients with subjective oral symptoms were all at the same stage of menopausal transition. In his studies, 93% of the women complained of sensation of PM, 72% of abnormal taste sensation, and 90% of cancerphobia (Massler 1951). Wardrop et al. (1989) studied the relationship between oral discomfort and menopause in 149 women. They observed that the prevalence of oral discomfort was significantly higher in perimenopausal and postmenopausal women (43%) than in premenopausal women (6%). Their results also showed an association between oral discomfort and psychological symptoms in menopausal women. An Israeli group reported a high prevalence of oral discomfort in women attending a menopause clinic with a highly significant odds ratio (OR up to 8.03) between systemic and oral complaints of menopause. They also observed a significantly altered salivary composition in the symptomatic women, pointing to sympathetic activation due to psychological stress (Ben-Aryeh et al. 1996).

3.9.2 Oral symptoms and hormone therapy

Approximately two-thirds of the menopausal women with oral discomfort but without oral clinical observed abnormalities, found that oral symptom were relieved with the use HT (Wardrop et al. 1989). In Italy, the efficacy of HT was investigated in 27 postmenopausal patients with oral discomfort and in 47 postmenopausal women with no oral discomfort. The patients were treated with CEE and MPA. In that study HT did relieve oral symptoms in 15 of 27 patients who had reported symptoms (Forabosco et al. 1992). However, in this study HT had no effect on oral cytology in the 40 symptom-free postmenopausal women when compared with a control group of 47 postmenopausal women who had no oral symptoms and were not treated. The researchers further found nuclear ERs in 8 of 10 randomly selected patients with symptoms who responded to HT, but this result was not found in two patients who did not benefit from the therapy. The authors suggested that oral discomfort may be related to steroid hormone withdrawal only in some postmenopausal women and that HT may improve the clinical and cytologic features only in some patients (Forabosco et al. 1992). The same group also reported earlier that HT improved subjective and objective symptoms in 12 out of 22 patients treated with estriol and in 7 out of 10 patients treated with CEE plus NETA, suggesting that estrogen deficiency can be considered as a possible

cause of oral discomfort in some postmenopausal patients and that HT may indeed improve subjective symptoms of these patients (Volpe et al. 1991).

Yalcin et al. (2006) observed in a 2-year clinical follow-up study on 348 Turkish women from 44-65 years of age of whom 23% were using HT, that self-assesed sensation of DM was significantly less frequent among the hormone users than non-users (48.8% vs. 68.3%, $p<0.05$). Hakeberg et al. (1997) reported in their study on middle-aged and elderly women that female sex hormones were one of the predictors for occurrence of PM. They interpreted this result to indicate problems due the climacteric period.

A questionnaire study by Jansson et al. (2003) observed more frequent xerostomia among postmenopausal women who did not use HT than in women using HT or in premenopausal women of the same age. The authors suggested that some of these oral symptoms might be related to estrogen deficiency, but that oral symptoms do not develop until some years after menopause. It may also be that women with the most severe symptoms decided to use HT and use of HT decreased their symptoms to the same level as in non-users (Jansson et al. 2003).

However, attempts to relieve menopausal symptoms such as hot flushes with medication may even worsen symptoms of the mouth. Grady et al. (2007) reported in a randomized controlled 6-week trial that, compared with placebo, antidepressant sertraline was ineffective in reducing the frequency of hot flushes but caused a number of unpleasant side effects, including dry mouth.

3.9.3 Periodontal health and menopause

Female gender–related hormonal conditions, such as pregnancy- and puberty-associated gingivitis are known as temporary periodontal diseases (American Academy of Periodontology 2005; Armitage 1995). A number of studies have linked menopause with periodontal conditions. Osteoporosis and alveolar bone loss and periodontitis in addition to menopause are often assessed together which make that literature difficult to analyse. There is a lack of studies linking only menopause or an estrogen-deficient state to susceptibility to periodontal disease (Mascarenhas et al. 2003; Kinane et al. 2006). However, systemic bone loss may be a risk indicator for periodontal destruction (Tezal et al. 2000), and increased rates of bone mineral density loss after menopause are associated with greater risk of tooth loss (Krall et al. 1994). Hence, prevention and treatment of osteoporosis after menopause may also have broader oral health consequences (Krall et al. 1994; Eviö et al. 2006).

3.9.4 Periodontal health and hormone therapy

Targets for potent sex hormones such as androgens, estrogen and progesterone have been localised in periodontal tissues (Gornstein et al. 1999). As stated in a review by Mascarenhas et al. (2003) a number of studies have shown that changes in periodontal conditions might be associated with variations in sex hormone levels. Recently Haas et al. (2009) reported that the prevalence of periodontitis was significantly greater in postmenopausal women not using HT than premenopausal women. Postmenopausal women using HT and premenopausal women had similar periodontal status. Prior to that study Norderyd et al. (1993) showed that postmenopausal women (aged 50 to 64 years) taking ET exhibited lower proportion of gingival bleeding units than an age-matched control group. However, Lopez-Marcos et al. (2005) found no significant effect on periodontal health parameters in a study group of 210 women receiving or not receiving HT. In contrast to these results, a study in 330 postmenopausal Japanese women showed that estrogen may promote tooth retention by strengthening the periodontal attachment surrounding the teeth, but not by increasing oral bone height or decreasing oral bone porosity (Taguchi et al. 2004). In their results the duration of estrogen use was significantly associated with the number of remaining teeth (Taguchi et al. 2004). The same group investigated also the affect of ER and vitamin D receptor gene polymorphisms and observed that certain gene polymorphisms indeed were associated with tooth loss (Taguchi et al. 2003).

Meisel et al. (2008) in Germany found in a population-based epidemiological study of 4290 participants that postmenopausal women who were using estrogen had higher number of teeth than men of the same age group. In addition women without hormone treatment had less teeth than other women in this study. This finding is in agreement with results from the Women´s Health Study from the United States were among the 42,171 postmenopausal women the overall risk of tooth loss was 24% lower in current HT users than in non-users (Grodstein et al. 1998).

3.9.5 Saliva and menopause

The composition of female saliva varies during different hormonal stages, such as during menstrual period and pregnancy (Tenovuo et al. 1981; Laine et al. 1988). In menopausal women the composition of saliva and decreased saliva flow seem to be estrogen-dependent (Leimola-Virtanen et al. 1997a; Bardow et al. 2001). However, medications are the major reason for impaired salivary function. Steroid hormones can be assessed in saliva samples and their salivary concentrations correlate with those in the blood serum (Vining & McGinley 1986; Tivis et al. 2005).

Salivary flow rate and the composition of saliva have been investigated in 42 menopausal women with/without xerostomia (21 cases, 21 controls) in a study from Iran (Agha-Hosseini et al. 2007). The results showed that the mean Ca-concentration in paraffin stimulated saliva was significantly higher in subjects with xerostomia than in controls (Agha-Hosseini et al. 2007). Recently Agha-Hosseini et al. (2009) reported a case-controlled study on 76 non-medicated menopausal women, with an age range of 41-77 years old. The authors analysed salivary E2 concentrations and observed significantly lower E2 concentrations and stimulated salivary output among the 38 symptom cases than in the 38 controls. However, there did not find any significant correlation between stimulated flow rate and the severity of the self-assessed xerostomia in the women (Agha-Hosseini et al. 2009). This result was consistent with earlier studies by other investigators (Närhi 1994; Ben-Aryeh et al. 1996; Agha-Hosseini et al. 2007). In contrast to these observations, Streckfus et al. (1998) showed that premenopausal women had higher salivary flow rates than postmenopausal women. They compared stimulated salivary flow rates between three groups of females who were healthy Caucasian individuals from Baltimore, USA. The groups were divided according to their menopausal status. The study groups were premenopausal women (n=51), with a mean age of 39 years, perimenopausal women (n=26), with a mean age of 48 years, and postmenopausal women (n=76) with a mean age of 69 years. They found that premenopausal women had higher salivary flow rates than postmenopausal women whereas no differences were found in salivary flow rates between women using HT or women not using HT (Streckfus et al. 1998).

3.9.6 Saliva and hormone therapy

Several authors who have shown that women who start using HT report improvement in their quality of life including less oral discomfort have suggested that improved saliva secretion is one reason behind the positive finding (Laine & Leimola-Virtanen 1996; Leimola-Virtanen et al. 1997a; Friedlander 2002; Eliasson et al. 2003). In a Swedish study, Eliasson et al. (2003) investigated the flow rate from minor salivary glands and the secretion rate and buffer capacity of whole saliva in 18 postmenopausal women (61-76 years) prior to and during one year of low potency estrogen, ie. estriol (E3) use. HT caused a significant increase in the labial saliva flow and complaints of DM decresed. However, in this study increased stimulated whole saliva flow and better buffer capacity were seen in both groups, indicating that an eventual HT effect on saliva flow is not that straightforward at all (Eliasson et al. 2003). A study from Turkey investigated saliva in a group of menopausal women (n=14) (average age 50.7 years) with osteoporosis treated with HT, and an equal number of non-menopausal controls (average age 42.4 years). They observed that the salivary flow rates decreased during the menopausal period and increased after HT use, while salivary pH,

electrolytes and calcium concentrations were not affected (Yalcin et al. 2005). However, Sewon et al. (2000) showed a decrease in salivary Ca-consentrations in 15 healthy menopausal (47-67 years) women treated with HT.

The effect of HT on salivary peroxidase and IgA, IgG, and IgM, and on total protein in whole saliva was observed in 19 postmenopausal and 8 perimenopausal women by Leimola-Virtanen et al. (1997a). The mean concentrations of salivary IgA and IgG showed a significant time-related decrease (3 and 5 months after estrogen treatment) in both groups. The salivary IgM concentration in perimenopausal women also showed a significant time-related decline. Total IgA output per minute increased in perimenopausal women whereas it decreased in postmenopausal women. These results indicate that the composition of saliva in post- and perimenopausal women seems to be estrogen-dependent as regards these defensive factors of the mouth (Leimola-Virtanen et al. 1997a). However, HT seemed to have no effect on the amount of the total salivary bacteria in either perimenopausal or postmenopausal women (Leimola-Virtanen et al. 1997a).

HT may thus improve both the quantity of saliva and the quality of salivary gland function in peri- and postmenopausal women (Laine & Leimola-Virtanen 1996). However, Ship et al. (1991a) showed in their study in the USA on 43 healthy premenopausal and postmenopausal females that there were no alterations in the quantity of saliva, suggesting that among healthy women salivary gland function is not significantly influenced by menopause or HT.

These partly conflicting results need to be confirmed in larger studies as data on the effect of menopause on saliva is based on small patient numbers and no randomized controlled trials exist on the effect of HT on salivary secretion and composition (Elliasson et al. 2003; Meurman et al. 2009). It is also worth noting that doses of hormones that have been used earlier vary in formulation in different studies, and that hormone doses to relieve climacteric symptoms were higher in the past than those presently recommended for such symptoms (Nelson 2008).

4. THE HYPOTHESIS AND AIMS OF THE STUDY

This investigation was based on the working hypothesis that there are differences in oral health between women using HT and those not using HT.

The aim of this study was to investigate oral health and oral symptoms in women living in Helsinki, Finland who were at menopause (50- to 58-years-old) in years 1997 to 2001. The primary purpose was to explore whether women in this cohort who were using HT differed from non-HT users in dental habits, self-assessed oral and general health, oral symptoms and the oral health parameters studied. A questionnaire study and a clinical study were done to achieve these aims. A two-year follow-up study was carried out to evaluate possible changes in dental habits and self-assessed oral symptoms during the follow-up time.

The specific aims of the present study were:

1. To determine using a standardized questionnaire the use of HT and the prevalence of self-assessed climacteric and oral symptoms such as sensation of pain (excluding toothache) and dry mouth (**Study I**).

2. To examine dental health clinically in a two-year follow-up study using WHO methods, and to evaluate self-assessed oral symptoms and dental habits by using a structured questionnaire (**Study II**).

3. To determine periodontal conditions in a two-year follow-up study the based on the clinical and radiological findings, including microbiological evaluations of the periodontopathogens, *A. a.*, *P.g.*, *T.f.*, *P.i.* and *P.n.* by polymerase chain reaction (PCR) (**Study III**).

4. To analyse salivary flow rates, total proteins, albumin, and IgA, IgG and IgM concentrations in a two-year follow-up study the (**Study IV**).

5. SUBJECTS AND METHODS

This study was planned in co-operation with the Institute of Dentistry, University of Helsinki, the Department of Gynecology, Helsinki University Central Hospital, and the Helsinki City Health Department. The study plan was approved by the ethics committee of the City of Helsinki (ethical permit #53 1.4.1997). All subjects attending signed an informed consent agreement according to the Declaration of Helsinki.

5.1 Subjects

Study I

Subjects were recruited from the women who attended a community mammography screening programme organized by the Helsinki City Health Department. Every year the health department invites and offers a free of charge mammography screening for all women citizens who are 50-, 52-, 54-, 56- and 58- year of age. Yearly, approximately 20, 000 women are offered mammography. The response rate to the offer for screening has been around 77-85%. For the years 1997-1998, when this questionnaire study was conducted, the response rate for screening was 83% in 1997 and 86% in 1998 (communication, Dr. Jaakko Koskela). Altogether at the time of our study 24, 476 women participated in the communal mammography screening.

When arriving at the mammography screening every fifth woman received our study questionnaire on oral symptoms. The women filled in the questionnaires at the mammography clinic and returned the forms to the nurse. Altogether 3182 women returned a filled-in form. Only 9 forms were filled in inappropriately and were excluded from the data analysis of the study. Therefore altogether 3173 completed questionnaires were used for further analyses. The questionnaire response rate was 65%.

Studies II, III and IV

Of these 3173 women, 40 who had reported in the questionnaire that they were using HT and other 40 not using HT were randomized from each of the five age cohorts (50-, 52-, 54-, 56- and 58- years). These randomly selected women were invited for further clinical and radiographic examinations at the Institute of Dentistry, University of Helsinki. Altogether 400 women were invited by a mailed invitation letter. One reminder letter was send to those who did not respond to the first invitation. 249 women attended our baseline clinical study (response rate=62%).

Two years later those women who participated in our baseline study were re-invited to the follow-up clinical examination. The study protocol followed the same model as at baseline. One reminder letter was send to those who did not response to our first invitation. Altogether 193 women participated in the follow-up study (response rate=78%).

5.2 Study protocols

Study I

The structured questionnaire consisted of 36 questions on oral and general health.

Studies II, III and IV

Randomly selected women (from the selected age cohorts) were invited participate in the follow-up study. In the invitation letter women were asked to avoid eating, drinking and smoking for two hours before the appointment time. In the invitation letter it was pointed out that the free-of-charge examination would be non-invasive, and also that no dental treatment would be available or offered.

When the women arrived at the dental clinic they were first given a questionnaire which consisted of the same questions asked in the primary questionnaire study. However, some additional questions on menopause status, and duration and type of used HT were included in this second questionnaire. This structured questionnaire consisted altogether of 51 questions on oral and general health (**Appendix**). After filling out the questionnaire, a panoramic tomography (PTG) of the jaws was taken. PTG was therefore available in the clinical examination to support clinical decision making.

All clinical examinations and collection of the samples, and preparing the samples for the further biochemical analyses, as well as coding all the questionnaires for statistical analyses, were carried out by the same dentist (author L.T).

The examination for each woman was carried out in the same order for everyone. At first, unstimulated and stimulated saliva flow rates were measured and recorded. Stimulated saliva was collected for further biochemical analyses. Dip slide tests were used to measure yeast counts and buffer capacity. Dental and periodontal status was recorded. Also, if according to the questionnaire the patient reported current sensation of PM, the cause of the pain was evaluated. After the

examination, the principal findings on dental status were explained to each patient, and if requested a written form as well as copy of the PTG was given to the patient to take to her own dentist.

249 women participated in the baseline in our follow-up study. According to our inclusion and exclusion criteria, eleven women not using HT were excluded from the data because they were still menstruating regularly. Those excluded were statistically significantly ($p<0.001$) younger but otherwise they did not differ from the women included. Hence, after the baseline study, results for 238 women were available for the baseline statistical analyses.

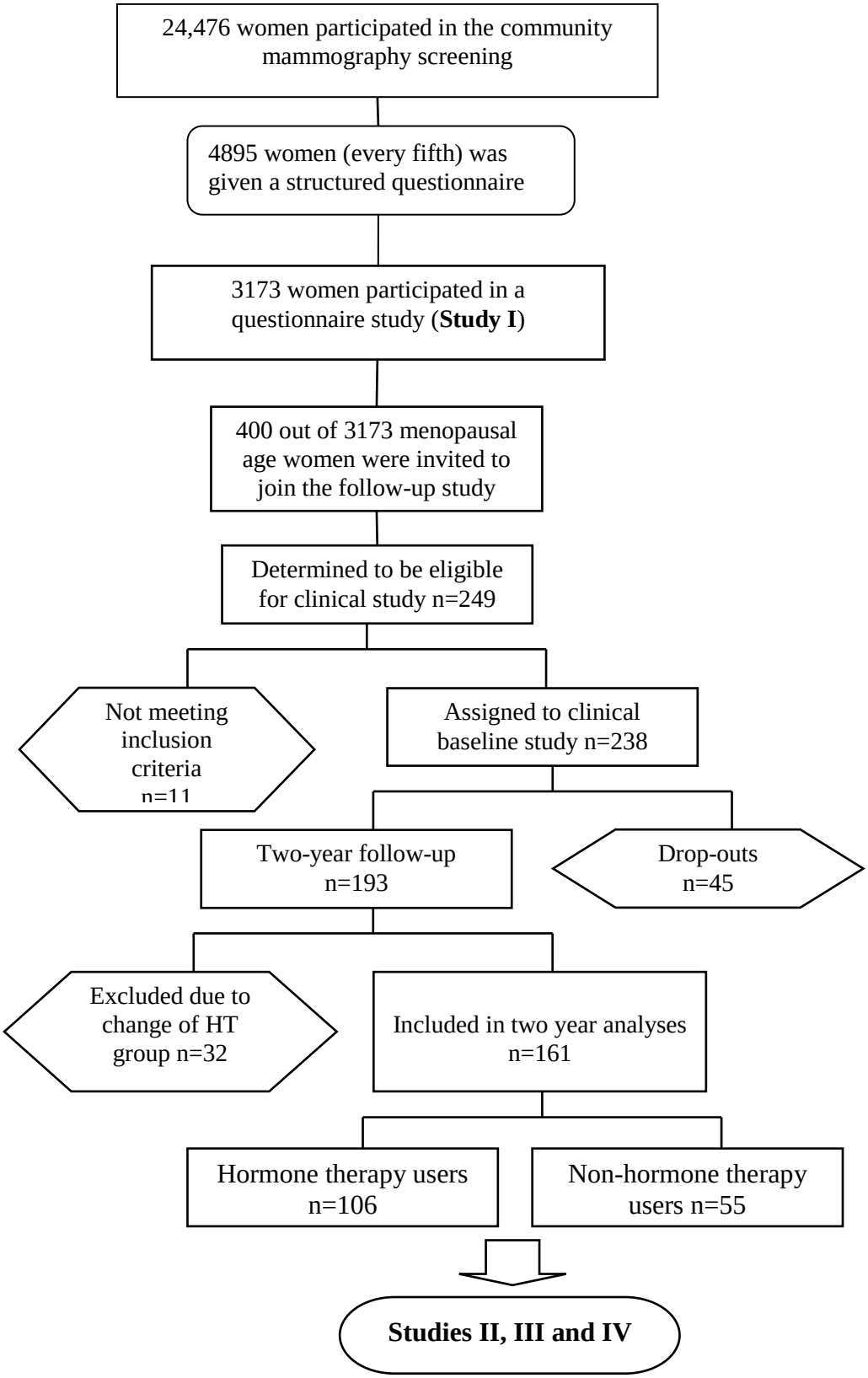
HT had have been used for at least 6 months already in the beginning of the investigation in order for a woman to be regarded as an HT subject. Among these 238 women 138 were HT users and 100 non-users.

Of those 238 women who attended the baseline study 193 participated in the 2-year-follow-up study. Of those participating in the follow-up, 32 women had changed their hormonal medication (started or stopped) during the intervening two years and were not included in the final statistics. Finally, statistical analyses of the results of this follow-up study were carried out for 161 women from which 106 women used HT during the two year interval and 55 women did not use. The study profile is illustrated in the flow-chart in **Figure 2.**

Inclusion criteria as a HT user for the final analysis were:
1) HT had been used for at least 6 months prior to the baseline study
2) The HT was used during the intervening two years

Exclusion criteria for final analysis were:
1) Regular menstruation of non-HT users at baseline
2) Changing from user to non-user of HT during the two year follow-up or *vice versa.*

Figure 2. Flow-chart of the study.



5.3 Measurements

5.3.1 Questionnaire

The structured questionnaire for **Study I** consisted of 36 questions and the questionnaire for the follow-up study **(Studies II, III and IV**) consisted of 51 questions. Questions concerned systemic medication, menstrual history (disturbances, menopause-age), climacteric symptoms and the use of HT and the type of HT, as well as oral symptoms and self-assessed general and oral health. Subjects were also asked about dental habits (daily tooth brushing and flossing) and frequency of dental check-ups. Use and duration of prescribed drugs as well as illnesses diagnosed by a physician were asked about with open ended questions. The other questions were mainly multiple-choice.

5.3.2 Oral symptoms

For the baseline study, oral symptoms were determined in the questionnaire with questions such as: "do you have sensation of pain in your mouth (excluding toothache)?", and "do you have a sensation of dry mouth?" The multiple choice answers were: "1"; yes, "2"; yes previously, but not now, "3"; never. If PM or DM were reported at present, detailed questions on these conditions and treatment attempts followed in the questionnaire. Self-assessed oral health was also queried. Responses for multiple choice questions, and coding thereafter for statistical analyses were:
"1" = good, "2"= fairly good, "3" = moderate, "4" = fairly bad, and "5"= bad.

For the 2-year follow-up study the same questionnaire was used. If reporting PM in questionnaire, the cause was evaluated during the clinical examination. The corresponding coding of clinical evaluation was: "1" indicating irritation by a removable prosthesis, "2" for malocclusion , "3" for periodontal problem, "4" for candida infection, "5" for lichenoid reaction, "6" no reason found (sensation of PM/BMS), "7" other oral mucosa lesion (in which case the subject was sent for further examination), and "8" for cortisone inhaler- induced irritation. For diagnosing the candida infection, results from the dip slide test were used. For codes 5, 6 and 7, if a biopsy was needed the subject was referred to the Department of Oral and Maxillofacial Diseases of Helsinki University Central Hospital.

5.3.3 Clinical dental recordings

Clinical examinations were done according to the WHO criteria (WHO 1997). PTG was taken to support clinical findings. For dental status, the DMFT index was used. Decayed teeth (DT) and filled teeth (FT) indices were separately recorded. Teeth were classified as carious whenever one or more surfaces were assessed as needing restoration due to decay. Clinically detected retained roots were included in the DT score. Teeth with fractures or lost fillings without clinically observable caries were not categorized as carious. Because not all women remembered their history of dental treatment, all the missing teeth, whether they were extracted or congenitally missing, were calculated in the M-score of the index. Following the WHO criteria for the DMFT-index, the third molars were not taken into account. However, all erupted natural teeth, including third molars, were included with the DT and FT counts, and in the scores of the total number of teeth.

5.3.4 Periodontal recordings and analyses

5.3.4.1 CPITN

To describe periodontal status, the WHO CPITN index was used. The periodontal examination involved evaluations of all surfaces of the teeth. The highest score per sextant was recorded and used in the analyses. According to the WHO Third edition of "Oral Health Surveys - Basic methods", Geneva 1987 CPITN is defined as follows:

Recording

Codes in descending order of severity are:

4: pocket > 6 mm

3: pocket 4 or 5 mm

2: calculus felt during probing.

1: bleeding observed, directly or by using a mouth mirror, after sensing with a probe.

0: healthy

The number of ≥ 6mm gingival pockets was recorded separately and “periodontitis” was diagnosed if at least one sextant had a CPI score of 3 or higher.

The presence of the periodontal bacterial species *A.a., P.g., T.f., T.d., P.i.* and *P.n.* was determined using PCR methods. The PCR method has been described in detail by Wahlfors et al. (1995) and Meurman et al. (1997a).

Subgingival plaque samples were taken from the four deepest periodontal sites in every patient. If no ≥ 4 mm pockets existed or there were less than four ≥ 4 mm pockets in the subject the rest of the plaque samples were taken from the first premolars. Before sampling the supragingival sites, plaque was gently removed with a cotton swab and the site of collection was isolated with cotton rolls and dried. Thereafter, the subgingival plaque samples were taken with a sterile curette and placed into a micro-centrifuge tube containing 0.5 ml distilled water. The pooled samples were then centrifuged and 5 µl aliquots of supernatant were added to the PCR reaction mixtures.

5.3.5 Salivary analyses

Flow rates for unstimulate and stimulated saliva were measured as well as the buffer capacity of the saliva. The saliva collection was collected at the beginning of the clinical study in order to avoid the stimulus from the clinical examination. The subjects were asked to avoid eating, drinking and smoking two hours before the examination. Unstimulated saliva was collected for 3 minutes with the free flowing method. For the stimulated saliva, a 1-gram piece of paraffin wax was given to the women to chew. The collection time for paraffin stimulated saliva was 3.5 minutes. Saliva collected during the first 30 seconds was discarded (for details see Meurman & Rantonen 1994). To determine the salivary buffer capacity, the Dentobuff Strip®-method (Orion Diagnostica Ltd., Espoo, Finland) was used. It was recorded according to the manufacturer's guidelines (score 1: final-pH >6; score 2: final-pH 4.5-5.5; or score 3; final-pH less than 4.5). Yeast was analysed by use of the Dentocult CA® dip slide method (Orion Diagnostica). This method mainly detects *Candida albicans*. The samples for the yeast analyses were taken with a sterile spatula from the tongue surface. After incubation for 5 days at 37°C, the colony densities were recorded and classified into three groups of colony-forming units (CFU) as instructed by the manufacturer (score 1: 0 to 20 CFU; score 2: 21-50 CFU; or score 3: more than 50 CFU).

Salivary total protein, albumin, and IgA, IgG and IgM concentrations were analysed. Total protein was analysed with the colorimetric Lowry method, albumin was analysed according to Webster (1977), and the immunoglobulin concentrations according to Lehtonen et al. (1984). All analyses were made in duplicate with serum standards and appropriate controls. For details, see Mellanen et al. (2001).

5.4 Statistical analyses

Descriptive statistical analyses were made using the Student *t* test or the analysis of variance (ANOVA), Mann-Whitney *U*-test, and the χ^2 test when appropriate. Differences between the HT users and non-users were studied.

For the baseline study (**Study I**) the multinomial logistic regression (LR) model was used to estimate the coefficients for proportional risk for PM and DM. The final model estimates were used to calculate odds ratios (OR) and 95% CIs. The estimations were done by using LIMDEP's logit–subroutines.

For statistical analyses for the follow-up study (**Studies II, III and IV**), multiple logistic regression models were used to analyse the independent effects of the studied background variables on the probabilities of the dependent variables. Backwards elimination was used to control correlation between confounders. The model estimates were used to calculate odds ratios (OR) and 95% confidence intervals (CI). In all studies (**I, II, III and IV)**, a probability value (*p*) of less than 0.05 was considered to represent a significant difference. The SPSS for Windows versions 15, 17 and PASW Statistics 18 were used in analyses for **Studies II, III and IV**.

6. RESULTS

Detailed results are given in the original publications and only the main results are presented here.

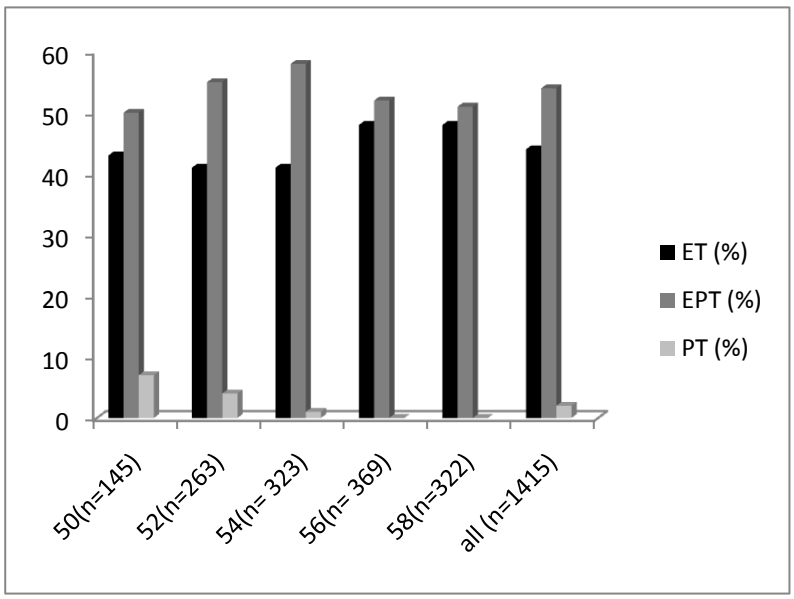
6.1 Oral symptoms and hormone therapy (Studies I, II, III and IV)

Study I

Results were obtained from 3173 women who responded to the questionnaire study. HT was used by 46.8% (n=1486), and 53.2% (n=1687) were non HT users. The distribution of the type of HT in different age groups by 1415 HT users are given in **Figure 3.** 69 women did not specify the type of HT they were taking, but they were included in present study as HT users.

As there were a few non-responses on the questions concerning PM or DM, the total numbers of observations used in analyses was 2946 for PM and 2973 for DM. PM was reported by 8.2% (n=259) and DM by 19.9% (n=631) of the total sample, respectively. There was no significant difference in the occurrence of PM or DM between the HT users and non users. The prevalence (%/n) of sensation of PM and DM in the selected variables for statistical analyses is more detailed seen in **Study I**. According to logistic regression analyses, climacteric complaints significantly correlated with the occurrence of PM (p=0.000) and DM (p=0.000) irrespective of the use of HT.

Figure 3.
Distribution of the type of HT (%) used by the studied age cohorts (50-58 years) (**Study I**).



ET = Estrogen-only therapy
EPT= Estrogen progestagen therapy
PT = Progestagen only therapy

Studies II, III and IV

For the statistical analyses, the study population for the follow-up study consisted of 161 women. Out of them 66% (n=106) were HT users who had used hormone therapy on average for 3.9 years (SD 3.3). At the baseline, estrogen-only therapy (ET) was used by 48% (n=51), Estrogen-progestagen therapy (EPT) by 50% (n=53) and progestin-only therapy (PT) by 2% (n=2). Corresponding results for the follow-up were 49% (n=49) for ET, 50% (n=53) for EPT, and 1% (n=1) for PT. There was no statistical difference in the distribution of the HT used during the two year follow-up time.

In the questionnaire prior to the clinical examination PM was reported by 10% (n=11) of the HT users at the baseline and by 5.7% (n=6) two years later. For the non-HT users, corresponding figures were 12.7% (n=7) and 1.8% (n=1). 22.6% (n=24) of HT users complained of DM at the baseline and 21.6% (n=23) did so at the follow-up. For non-HT users, respective results for DM were 36.4% (n=20) and 28.8% (n=15). Descriptive data on the oral symptoms is given in **Table 1.**

Table 1.
Oral symptoms of the follow–up study subjects divided in groups according to the use of hormone therapy (HT) (**Studies II, III and IV**). (Pearson's chi-square for categorical variables, Student's t test and Mann-Whitney *U* test for continuous variables; independent samples t test for cross-sectional study, and paired samples t test for prospective study).

	Baseline			**Two-year follow-up**				
	HT (106)	**Non-HT (55)**	***p*-value**	**HT (106)**	**Non-HT (55)**	**Significance between groups**	**Significance between baseline and follow-up in HT group**	**Significance between baseline and follow-up in non-HT group**
Mean age	55.4 ± 2.7	55.9 ± 2.4	Ns.	57.4 ± 2.7	57.9 ± 2.4	Ns.		
Current climacteric symptoms	16 (15%)	24 (44%)	0.000	14 (13%)	19 (35%)	$p < 0.001$	Ns.	Ns.
Satisfactory self-assessed dental health	65 (61%)	28 (51%)	Ns.	63 (59%)	31 (56%)	Ns.	Ns.	Ns.
Sensation of dry mouth	24 (23%)	20 (36%)	Ns.	23 (22%)	15 (27%)	Ns.	Ns.	Ns.
Sensation of painful mouth	11 (10%)	7 (13%)	Ns.	6 (6%)	1 (2%)	Ns.	Ns.	Ns.

Ns. = Statistical difference not significant.

6.2 Dental and periodontal health and hormone therapy (Study II)

According to a baseline questionnaire, HT users had visited a dentist less than year ago more often than non-users ($p<0.05$). At the follow-up, this difference no longer existed. Tooth brushing at least twice daily was reported by 76% vs. 75% of HT users/non–users at the baseline and by 79% vs. 69% at the follow up, respectively. There was no statistical difference between the groups in this respect (data not shown).

The average DMFT index was 20.1 in the whole study group. There was no difference in DMFT index values between HT users and non-users. In the HT group, the FT index score increased significantly during the follow-up ($p<0.05$). At baseline, self -assessed oral health was considered satisfactory by 61% of HT users and by 51% of non-users, and at the follow up by 59% and respectively 56%. These differences were not statistically significant. Descriptive data on dental and periodontal findings is given in **Table 2.**

Table 2.

Dental and periodontal findings in subjects using or not using hormone therapy (HT). Data is presented as means ± SD, or frequencies (n) with percentages (%). (Pearson's chi-square for categorical variables, Student's t test and Mann-Whitney *U* test for continuous variables; independent samples t test for cross-sectional studies, and paired samples t test for prospective testing).

	HT (106)		**Non-HT (55)**		**Significance**
	Baseline	**2-year follow-up**	**Baseline**	**2-year follow-up**	
Number of teeth	25.0 ± 5.5	24.6 ± 5.6	23.5 ± 7.5	23.3 ± 7.5	Ns.
DMFT	20.1 ± 4.3	20.9 ± 4.3	20.1 ± 4.7	20.2 ± 4.7	Ns.
DT	0.4 ± 0.9	0.3 ± 0.7	0.5 ± 1.0	0.6 ± 1.3	Ns.
FT	16.9 ± 5.3	17.6 ± 4.9	15.5 ± 6.3	15.6 ± 6.1	$p < 0.05$
Subjects with periodontitis	83 (79%)	74 (71%)	43 (80%)	41 (76%)	Ns.
Number of ≥6mm deep gingival pockets	0.9 ± 1.7	1.1 ± 2.1	1.0 ± 1.7	1.2 ± 1.9	Ns.

DMFT= Decayed missing filled teeth- index
DT= Decayed teeth
FT= Filled teeth
Ns.= not significant

At baseline the percentage of women with periodontitis (defined as at least one sextant with a CPITN score of 3) was 79% in the HT group and 80% in the non-HT group. Two years later the respective figures were 71% and 76%. The mean numbers of ≥ 6 mm deep periodontal pockets were 0.9 ± 1.7 at baseline vs. 1.1 ± 2.1 two years later in the HT group, and 1.0 ± 1.7 vs. 1.2 ± 1.9, respectively, in the non-HT group. These differences were not statistically significant (**Table 2**).

6.3 Periodontal microbiota and hormone therapy (Study III)

The prevalence of periodontal pathogens at baseline and follow-up was assessed by PCR (**Table 3).** Based on statistical analyses, *A. a.* was found to be more prevalent in women with cardiovascular disease (OR 5.48, 95% CI 1.04 - 29.77; p<0.05). In both groups using stepwise logistic regression analyses the main explanatory factor in the model for the occurrence of *P.g., P.i., T.f.* and *T.d.* was the existence of deep periodontal pockets.

Table 3.
Positive PCR findings for periodontal bacteria in the women at baseline and two years later. (Pearson's chi-square test)**.**

Bacterium *	HT		Non-HT	
	Baseline	2-year follow-up	Baseline	2-year follow-up
A.a.	1/95 (1.1%)	4/95 (4.2%)	2/40 (5.0%)	5/40 (12.5%)
P.g.	20/95 (21.1%)	9/95 (9.5%)*	6/40 (15.0%)	3/40 (7.5%)
P.i.	33/95 (34.7%)	21/95 (22.1%)	12/40 (30.0%)	10/40 (25.0%)
P.n.	58/95 (61.1%)	57/95 (60.0%)	23/40 (57.5%)	21/40 (52.5%)
T.f.	44/95 (46.3%)	31/95 (32.6%)	24/40 (60.0%)	18/40 (45.0%)
T.d.	27/95 (28.4%)	32/95 (33.7%)	13/40 (32.5%)	16/40 (40.0%)

*A.a. = *Aggregatibacter actinomycetemcomitans*; P.g. = *Porphyromonas gingivalis*;
P.i. = *Prevotella intermedia*; P.n. = *Prevotella nigrescens*; T.f. = *Tannerella forsythia*;
T.d. = *Treponema denticola*
*p<0.05 (baseline vs. follow-up)

6.4 Saliva and hormone therapy (Study IV)

In the final study (**Study IV**) pair-wise saliva values were obtained from 106 HT users and 55 non-HT users. No differences were seen in salivary flow rates between or within the groups. In the HT group a very low (<0.1 ml/min) unstimulated flow-rate was obtained in 5.6% (n=6) at the baseline and in 2.8% (n=3) at the two-year follow-up. Respective values for non-HT group were 5.8% (n=3) and 3.8% (n=2). For stimulated saliva flow rate the corresponding values were 0% and 0% for the HT group and 1.8% (n=1) and 0%, respectively. Positive yeast counts were observed in 61% (n=63) of HT users and in 60% (n=32) of non-users (Ns.), and at the follow-up the corresponding numbers were 37% (n=39) and 35% (n=19) (Ns.). Results of the salivary flow, buffer capacity and yeast tests are seen in **Table 4**.

Salivary albumin, IgG and IgM concentrations decreased statistically significantly in the HT group during the two-year observation period. A one-way repeated measures ANOVA was conducted to compare levels of salivary flow rate, total protein, albumin, IgA, IgG and IgM levels at baseline and 2-year follow-up. The means and standard deviations are presented in **Table 4**. There was a statistically significant effect for time for consecutive variables: The values were; for albumin: Wilks'Lambda=0.869, F(1, 94)=14.16, p=0.0003, partial eta squared=0.131, for IgG: Wilks'Lambda=0.947, F(1, 94)=5.235, p=0.0024, partial eta squared=0.053, and for IgM: Wilks' Lambda=0.863, F(1, 94)=14.92, p=0.0002, partial eta squared=0.137. The logistic regression analysis showed that the only statistically significant explanatory factor for higher than median salivary albumin concentrations was the number of teeth of the subject. The other study parameters were not found to correlate with the salivary protein values.

Table 4.

Baseline and follow-up (2 years) salivary flow rates, yeast counts, buffer capacity and biochemical constituents [(Means ± SD or n (%)]. (Pearson's chi-square test for class variables, and Student's t test for continuous variables in cross-sectional study, and ANOVA for repeated measures for the prospective study).

	HT (106)			**Non-HT (55)**				
	Baseline	**2-year follow-up**	***p*-value**	**Baseline**	**2-year follow-up**	***p*-value**	**Significance between groups in baseline**	**Significance between groups in follow-up**
Unstimulated salivary flow rate (ml/min)	0.6 ± 0.4	0.7 ± 0.4	Ns.	0.6 ± 0.4	0.6 ± 0.4	Ns.	Ns.	Ns.
Stimulated salivary flow rate (ml/min)	2.0 ± 0.9	2.0 ± 0.8	Ns.	1.7 ± 0.8	1.8 ± 0.8	Ns.	Ns.	Ns.
Positive yeast count	63(61%)	32(60%)	p<0.01	39(37%)	19(35%)	p<0.01	Ns.	Ns.
Buffer capacity			Ns.			Ns.	Ns.	Ns.
High	85 (90%)	95(91%)		42 (86%)	45(87%)			
Medium	7 (7%)	8 (8%)		6 (12%)	6 (12%)			
Low	2 (2%)	1 (1%)		1 (2%)	1 (2%)			
Total protein (mg/ml)	1.46 ± 0.5	1.47 ± 0.4	Ns.	1.53 ± 0.4	1.55 ± 0.4	Ns.	Ns.	Ns.
Albumin (µg/ml)	306 ± 183	243 ± 115	p<0.001	311 ± 168	299 ± 222	Ns.	Ns.	Ns.
IgA (µg/ml)	26.0 ± 15.1	24.7 ± 12.8	Ns.	31.6 ± 16.9	32.6 ± 20.9	Ns.	Ns.	p<0.05
IgG (µg/ml)	20.1 ± 15.8	17.1 ± 13.1	p<0.05	22.8 ± 20.3	20.4 ± 22.6	Ns.	Ns.	Ns.
IgM (µg/ml)	1.51 ± 1.2	1.18 ± 1.1	p<0.001	1.93 ± 1.6	1.66 ± 1.6	Ns.	Ns.	p<0.05

Ns.= not significant

7. DISCUSSION

7.1 Rationale for the study

During menopause women go through biological changes, particularly in their secretion of female sex steroid hormones, with reduction in estrogen levels being especially pronounced (Brace & McCauley 1997). The transition to menopause can occur over a 10–15 year period (Morrison et al. 2006). As a variety of climacteric symptoms characterize the menopausal period, some women at this time live with expectations of worsening health (Matthews 1992). Many menopausal women also seem to suffer from oral discomfort (Meurman et al. 2009).

Women using HT generally experience satisfactory relief of vasomotor symptoms, such as hot flushes, sweating, palpitation and/or sleeping problems, and also urogenital problems, such as vaginal dryness, itching, and dyspareunia (Notelovitz et al. 2000; MacLennan et al. 2001; Stearns et al. 2002; Rutanen & Ylikorkala 2004; Nelson 2008). However, women not only take HT to avoid climacteric symptoms, but also to protect themselves from cardiovascular diseases and osteoporosis, and also for increased well-being (Seeman et al. 1995; Herrington et al. 2000; Thunell et al. 2005; Jalava-Broman et al. 2008). We did not ask in our questionnaire whether HT users had chosen to take HT to avoid climacteric symptoms or for some other reason. It is known that estrogens also influence oral health in a number of ways and saliva undergoes changes depending upon the levels and type of these hormones (Yalcin et al. 2005). In support of our study hypothesis there is no doubt that many women clearly benefit from the use of HT, which may also have effects in the oral cavity (Friedlander 2002; Rusanen & Ylikorkala 2004).

Our hypothesis also rested on the idea that those women who had chosen to take HT in the first place were women with better health and health habits in general (Hemminki et al. 1993; Matthews et al. 1996). As our study was carried out prior to the controversial reports from HERS and WHI on HT we consistently hypothesized that HT users would also have better oral health than those women who do not use HT. HT users had been characterized as well-educated, slim women who had never smoked (Egeland et al. 1988; Cauley et al. 1990; Topo et al. 1991). Therefore we logically thought that the oral health of HT users would be better than that of non-users. However, in the capital city area of Helsinki where the present study was conducted, demographic differences in employment, social class, or level of education did not exist between HT users and non users in previous studies (Topo et al. 1995).

When the results from large population studies and controlled trials were published after the onset of our present series of studies (Hulley et al. 1998; Herrington et al. 2000; Manson et al. 2003; Hsia et al. 2006) the number of users of HT decreased (Kase 2009). Despite the fact that recent experimental and clinical studies have indicated that the effects of HT depend on the estrogen and progesterone/progestin formulation, dosage, mode of administration, patient's age, associated diseases, and duration of treatment (Harman 2006; Caufriez 2007) some women using HT have worries and fears concerning adverse reactions to HT which might affect decision making regarding its use (Tiihonen et al. 2007). However, our investigation on oral health and hormone treatment was carried out before the results from these randomized trials (HERS and WHI) were published.

7.2 Evaluation of the methods

For the present study we succeeded in collecting a representative sample of woman at the age of their climacterium. We did not analyse the effect of the use of HT on oral health, thus we compare the groups of women using HT or not using HT. This study was carried out from 1997 to 2001. At that time approximately 200,000 women, aged 45 or more, used HT in Finland (Rutanen & Ylikorkala 2004).

In both studies questionnaires were used. Response rates according to the original study populations (**Study I** n=3176, and **Studies II-IV** n=400) are generally acceptable (65% in **Study I** and 62% (baseline) and 48% (follow-up) **Studies II-IV**). Comparable response rates (63%) were achieved in a Finnish questionnaire study on HT (Tiihonen et al. 2007) and (53%) in a study of self–medication for upper gastrointestinal symptoms (Sihvo & Hemminki 1997). For statistical analyses we had to exclude a few subjects who had changed their hormonal medication during the follow-up period. The exclusion did not have any effect on the final statistical results. The characteristics of the women who were dropped out from the final statistical analyses or who themselves discontinued for various reasons were analysed with respect to those who were followed-up using cross tables and t tests; no significant differences were found. It was impossible to characterize the dental and oral health of the non-responders of our clinical study. We can only speculate that maybe the non-responders were those with poor oral health and poor self-assessed oral and general health. This free-of-charge dental check-up was thought to be a good incentive to attend our study. However, in support of our hypothesis that women using HT are more interested in their health than non-users, more HT users responded at the baseline to our invitation to attend our study than non-users (138 HT users vs. 100 non-users).

We did not include questions on social status or education in our questionnaire; however, it can be assumed that the large population cohorts originally included (n=3173) effectively leveled off bias in this regard. Using a questionnaire may cause difficulties in defining the actual prevalence of symptoms or poor dental habits: they may be even more common among populations than surveys indicate (Ahlberg 2008). We therefore conducted the clinical follow-up study to verify the prevalence of oral symptoms reported in the first questionnaires.

A questionnaire was also used at the follow-up, and the subjects were informed of this in the invitation letter; therefore, more precise questions were able to be added to the questionnaire. Nevertheless, despite the request to check their current regular medication before attending the examination, a considerable number of women still did not remember all their medications when filling in the questionnaire. Therefore we had to categorize the medication fairly widely, especially concerning the use of neurological and psychiatric medications and also cardiovascular medications. To verify menopause is difficult by using a questionnaire. Thus our results are based on self-assessed menopausal stage. Use of any medication (nature of drugs) was not associated with oral health variables, however.

7.3 Prevalence of oral symptoms and hormone therapy

7.3.1 Sensation of PM and BMS

We observed in our questionnaire study that the prevalence of PM was 8.2% of the total sample. There was no difference between users and non-users of HT in the prevalence of PM. In addition, there was no difference in incidences of use of other medications between those who had PM and those who did not have PM (data not shown). According to logistic regression analyses, climacteric symptoms were found to be predictive of PM, and the use of HT increased the occurrence of PM. We thus concluded that PM seemed to be associated with climacteric symptoms in general and that the use of HT did not prevent the PM. However, there is nonetheless no certainty as to whether HT had given some relief from oral symptoms or not as this result was based only on questionnaire. It may also be that women with the most severe symptoms decided to use HT.

At the clinical follow-up study, women received the questionnaire prior to the clinical examination. According to the questionnaires, PM was reported by 9.4% of the HT users at baseline and by 5.7% two years later. For the non-HT users, the corresponding numbers were 12.7% and 1.9%. However, after clinical evaluation of reported PM, among HT users a code of “6” (no reason found for

sensation of PM/BMS) was only found for in 4.7%, and at the follow-up for 2.8%. For the non-HT users, respective values were 7.2% at the baseline, and 0% at the follow-up. As mentioned earlier, using a questionnaire may cause difficulties in defining actual prevalence: symptoms may be under- or over-estimated, as for the patient it is difficult to distinguish PM from any other organic disease in the oral cavity. This was also seen in our clinical evaluation of the cases of PM reported in questionnaire.

Abnormal sensations in the oral cavity such as BMS present a difficult diagnosis for many clinicians. Diagnostic criteria are not consistent and the etiology of BMS is known to be multifactorial and poorly understood by dentists and physicians (Maltsman-Tseikhin et al. 2007; Abetz & Savage 2009). It seems likely that both physiological and psychological factors play a role in BMS, but the interaction between these and the relative significance of each remains largely speculative and remains an enigma (Abetz & Savage 2009). BMS mainly affects postmenopausal women and, because of this, it has been postulated that menopause, the cessation of menopause, or hormonal disruption is a precipitating factor. However, according to Abetz & Savage (2009) women with BMS do not have more hormonal abnormalities than those without.

The prevalence of BMS has been estimated to be 0.7% - 4.5% in the general population (Basker et al. 1978; Locker & Grushka 1987; Lipton et al. 1993), although higher figures have been presented (Tammiala-Salonen et al. 1993). These prevalence values are similar to those in our study for PM without any clinically observable explanation. However, number of those cases in our study was very small, and we did not specify the burning sensation as it has been stated for BMS (Zakrewska et al. 2005), therefore we did not give any BMS diagnoses to our subjects.

7.3.2 Sensation of dry mouth (xerostomia)

In our questionnaire study DM was reported by 19.9% of subjects. The use of HT did not correlate with the occurrence of DM. However, 34% of those suffering from climacteric symptoms reported also having DM. The women with climacteric complaints or those who assessed their general health as bad had a significantly increased risk for the occurrence of DM ($p=0.000$).

For the follow-up study, DM was complained by 28% at the baseline and by 24.3% at the two-year follow-up. There was no significant difference between the HT users and non-users in reporting DM. The selection of the covariates used in our studies may be criticized, as they did not adequately take into account all possible systemic and/or psychological factors. Nevertheless, the study was

planned to evaluate the occurrence of self-assessed symptoms and in regard of the use of HT. Another weakness was the drop-out of subjects which was done due to women changing their HT status during the 2 years of follow-up. They were then excluded from the statistical analyses.

Nederfors et al. (1997) estimated the prevalence of xerostomia in their study to be 21% and 27% in men and women, respectively. Similarly, Bergdahl (2000) reported the prevalence of subjective oral dryness to be 22% among 20 to 69 year old men and women when asked by questionnaire. A questionnaire study on pre- and postmenopausal women at 53-54 years of age showed the prevalence of xerostomia to be 12.2% - 18%, irrespective of the use of HT (Jansson et al. 2003). Our results on DM and PM are based on self-assessed sensations and not on clinical examinations, therefore these results provide valuable information on self-assessed oral well-being of the women in this study cohort.

7.4 Oral health parameters and hormone therapy

According to the follow-up clinical study presented her, the dental health of the group of menopausal-aged women studied was fairly good. The relatively high mean DMFT index score is understandable as in the youth of these cohorts (born in 1940-1948) dental treatment in Finland comprised mainly extractions of the teeth or restorative treatment with large amalgam fillings. The number of teeth was higher than reported in Finnish national health survey (Kansanterveyslaitos 2004), where the mean number of teeth was reported to be 21.5, and in women aged 55-64 years was 15.8. In our study mean number of teeth in both groups was over 23 with no differences between the groups or during follow-up.

Until the year 2001, Finns born before 1956 had not been eligible for any nationwide subsidized dental care in adulthood. In Helsinki, for practical reasons, due to a shortage of oral health professionals, subsidized dental care covered only those born in 1963 and later until the year 2001. The women attending our study were all living in Helsinki at the time of the study. Therefore the majority of the study patients had used private dentists for their dental appointments instead of community health center clinics. As far as we know, there are no previously published data on the dental status of women of these age groups in general. The weakness of the study was the fairly short two-year follow-up period where big changes in oral health parameters are not to be expected. However, the periodontal conditions may alter within this time period if the treatment is appropriate. But to see changes in occurrence of dental caries, this two-year follow-up time is too short. It is well known that that caries is associated with reduced salivary flow (Nauntofte et al.

2003), and dramatic increase in caries activity is mainly seen in individuals with no or very low saliva flow rate for example in individuals taking medication with hyposalivation as a side effect, and in patients with Sjögren´s syndrome (Atkinson & Fox 1992; Ravald & List 1998). In our study the salivary flow rates were normal, and only a few very low salivary flow rates measured measured, therefore no dramatic change in caries were expected to occur. In contrast to our results Yalcin et al. (2006) found in their two-year follow up study of 348 women (aged 44-65) that the women using HT had lower DMFT, DMFS and CPI values than non-users. For practical reasons we used only DMFT-index in our study to describe the dental status. However, DMFS-score which count the number of surfaces which are decayed (D), missing (M), and filled (F), would have given a more precise picture of the current dental status, and probably shown more differences between groups than use of DMFT did (Manji & Fejerskov 1994). When using DMFS or DMFT, it is recommended that the M-score is excluded when reporting affected surfaces, and therefore we measured DT and FT separately.

However, according to the measured CPITN results, there was a high prevalence of periodontitis in our study cohort. Although the reliability of CPITN in diagnosing periodontal disease may be criticized, it is a practical tool for population studies (Unell et al. 2000). The index was originally developed for the purpose of assessing periodontal treatment needs in a population (Ainamo et al. 1982; Ainamo & Ainamo 1994), but it has been recommended for prevalence studies and assessments of the severity of periodontal disease (Cutress et al. 1987). In the present study the number of ≥6 mm deep gingival pockets was included in the statistical analyses, which gave more precise information on the severity of periodontal disease. According to a large Finnish national health survey, the prevalence of periodontitis measured by CPITN among 45 to 64 year old women was 62% (Kansanterveyslaitos 2004). Periodontitis was diagnosed when at least one pocket was measured to be ≥ 4mm (CPITN of score 3). In our study 79% of HT users at baseline and in 71% at the follow-up had CPITN scored of 3 or more. The values for non-HT users were 80% vs. 76%, respectively. The CPITN method for evaluating periodontitis has been criticized for overestimating periodontitis in young and healthy subjects and underestimating it in elderly subjects (Gaengler et al. 1988; Baelum et al. 1993).

Severe periodontits (CPITN score of 4) is diagnosed when there are at least one gingival pocket ≥ 6mm deep. In Finnish national health survey (Kansanterveyslaitos 2004) the prevalence of severe periodontitis was 17% in the 45-54 year old age group, and 23% in the 55-64 year old age group. To define the severity of periodontal status in our study, we recorded the numbers of ≥ 6 mm deep

periodontal pockets separately. The mean numbers of ≥ 6 mm deep periodontal pockets were 0.9 ± 1.7 at baseline vs. 1.1 ± 2.1 two years later in the HT group, and in non-HT group 1.0 ± 1.7 vs. 1.2 ± 1.9, respectively. These results indicate that in our study cohort the prevalence of severe periodontitis was lower than reported in the Finnish national health survey.

As definitions of periodontal disease in epidemiologic studies lack uniformity the findings from different research groups are not always readily interpretable (Borrell & Papapanou 2005). Recently a review was published by König et al. (2010) on periodontal health in Europe. According to the review CPITN was assessed in most studies (König et al. 2010), although the limitations of this widely used index are well recognized (Savage et al. 2009). In our study for practical reasons the protocol had to be restricted according to periodontal status measuring, and therefore the use of CPITN was most convenient for us.

However, contrary to the expectations based on our study hypothesis, there was no difference in dental or periodontal health between women using HT or not using HT.

Every fifth women in our cohort was a current smoker. Smoking is known to be a major risk factor for periodontitis, affecting the prevalence, extent, and severity of periodontal disease (Novak & Novak 2006). There was no difference in the number of smokers on a percentage basis between HT users and non-user groups, both in the questionnaire and in the follow-up study. According to our logistic regression analyses in questionnaire study (**Study I**), smoking was a significant risk factor for DM. Smoking was also used as one covariate in logistic model when investigating risk factors for higher than median salivary protein values (**Study IV**). Regarding periodontal status and smoking, our data is not sufficient to allow us to make statistical conclusions.

Based on present knowledge of periodontal diseases and mechanism of tissue destruction, the role of matrix metalloproteinases (MMP) are known to play a significant role in the regulation of cellular communication and immune functions by processing bioactive molecules, including cytokines, hormones and growth factors (Sorsa et al. 2004). MMPs are involved in physiological processes during tissue development, remodeling, and wound healing (Uitto et al. 2003; Sorsa et al. 2006). MMP-8 is one of the central biomarkers in the connective tissue breakdown of periodontitis (Sorsa et al. 2004; Sorsa et al. 2006). MMP-8 levels detected by immunofluorometric assay (IMFA) in GCF have shown to be a good aid in diagnosing periodontal disease (Mäntylä et al. 2003; Mäntylä et al. 2006). Salivary MMP-8 has also been detected by IMFA (Gursoy et al. 2010). There

is no doubt that the lack of the information on MMP-8 levels is a weakness on our results concerning the periodontal status of the studied women.

7.5 Periodontal microbiota and hormone therapy

If the subject is healthy, oral microbiota do not pose a systemic threat. Dental and/or oral mucosal diseases can lead to bacteraemia of oral origin, however (Meurman et al. 2009). Anatomically, the oral cavity is the only part of the human body where hard tissues directly penetrate soft tissue epithelium: teeth surrounded by the gingival epithelium and periodontal connective tissue extend from alveolar bone to the oral cavity and may open the parenteral space to the outer environment. Even chronic low-grade exposure to bacteria of oral origin may then have systemic consequences, including cardiovascular diseases. In this context the association between periodontal disease and atherosclerotic diseases has been much discussed in literature (for reviews, see: Meurman et al. (2004) and Pussinen & Mattila (2004). Pussinen et al. (2007) suggested that systemic exposure to *P.g.* may predispose to incident stroke. The progression of atherosclerosis is also modified by female sex hormones (Collins et al. 2007). Therefore the role of oral microbiota is also of interest in further studies.

Sex hormones have long been considered to play an influential role in periodontal tissues and periodontal disease progression (Sooriyamoorthy & Gower 1989; Mascarenhas et al. 2003; Brennan et al. 2007; Carillo-de-Albornoz et al. 2010). However, there are hardly any data on the effect of female sex hormones on oral microbiota in menopausal or postmenopausal women. There are more data on the effects of oral contraceptives and pregnancy on oral micro-organisms (Jensen et al. 1981; Klinger et al. 1998; Raber-Durlacher et al. 1994; Laine 2002).

Our **Study III** was based into on the known associations between periodontal bacteria and systemic diseases (Meurman et al. 2004) and the interactions between HT use and general health (Manson et al. 2003). We thought that HT might affect periodontal infections, as reflected in the occurrence of specific bacteria. We thus assumed consistently that the more health-conscious HT users would less frequently harbour periodontal pathogens when compared with non-users at the end of this two-year study.

However, in our clinical study the strongest explanatory factor for harbouring the periodontal bacteria investigated was the existence of deep periodontal pockets in both the HT and non-HT groups. This result as such came as no surprise since deep periodontal pockets and furcation

involvement are obviously the characteristic sites harboring periodontal pathogens. Also, self-assessed poor oral health status showed an association with harbouring of the bacteria investigated. However, only the less harmful *P.n.* species, which are mainly associated with gingivitis (Lee et al. 2006), were statistically linked with self-assessed poor oral health. *P.n.* is also frequently isolated from periodontally healthy sites, like the *P.i.* species (Paster et al. 2001). Since we used pooled plaque samples, there are no data from individual periodontal pockets. Our aim was to assess the overall association of the HT use and periodontal microbiota rather than monitor single pockets in this respect. In our study in regards the effect of reported systemic diseases on the studied microbiological results, *A.a.* was found to be more prevalent in women with cardiovascular disease. No other bacterial species was associated statistically with any other systemic disease reported (asthma or rheumatic diseases). However, in general the number of these cases was only a few individuals, so the finding on *A.a.* probably has no clinical relevance.

7.6 Salivary analysis and hormone therapy

HT has been reported to ameliorate dry mouth sensations, which are very common in elderly women (Hakeberg et al. 1997; Leimola-Virtanen et al. 1997a; Wardrop et al. 1989; Friedlander et al. 2002; Eliasson et al. 2003). The salivary flow rate has been shown to decrease during the menopausal period (women of age 50.7 years compared to non-menopausal women at an age of 42.4 years) but to increase after the start of HT with alendronate and calcium supplementation in younger postmenopausal women (Yalcin et al. 2005). The authors concluded that this was the effect of HT, but they did not study the effects of alendronate separately (Yalcin et al. 2005). However, in a study on the effects of alendronate and HT on elderly women, there was a significant decrease in the mean unstimulated salivary flow in the alendronate group, which of course was not a desirable result from the clinical point of view (Eviö et al. 2006).

According to standardized methods for saliva flow rate measurement it has been shown that unstimulated saliva tests should be perfomed at fixed time-points or over limited time interval early in the morning (Flink et al. 2005) in order to obtain reliable results (Tenovuo & Lagerlöf 1994). In our present study, the study subjects were given to clinical examinations at varying times. Although they were asked to avoid eating, drinking and smoking two hours prior to the appointment time, we could not avoid stimulus effects prior to those two hours that day. This uncontrolled time of salivary flow measurement and collection undoubtedly caused higher values to be obtained for unstimulated flow rates and to some extent also for paraffin stimulated saliva flow rates.

No differences were observed in measured unstimulated and stimulated saliva flow rates between HT users and non-users, and between the baseline and follow-up study. However, we measured clearly higher paraffin stimulated whole saliva flow rate values than reported earlier in healthy women (Heintze et al. 1983; Sevón et al. 2008) and also higher unstimulated flow rates than reported earlier (Heintze et al. 1983; Yeh et al. 1998). These high stimulated flow rates may also explain the high frequency of "high" buffer capacities that we found. Stimulation is known to affect the carbonic acid/bicarbonic acid system in saliva (Bardow et al. 2000). Unfortunately we did not measure the pH of the unstimulated or stimulated saliva, which would have given us more information on the saliva collected than using only a dip-slide test on stimulated saliva.

The frequency of positive yeast counts in the saliva of our study patients at baseline was also higher than reported previously for "normal" people (Odds 1988). However, at follow-up, the frequency of positive yeast counts was lower in both groups, being 35% of HT users and 37% of non-users, which are normal prevalences (Odds 1988). An explanation for such a clear drop in the frequency of positive yeast counts in both groups is that after the baseline study, dip slide test scores of 3 (indicating a high yeast count; more than 50 CFU) were reported to the patients. Therefore most of those with high dip-slide scores had been treated for their high yeast colonization before the follow-up study testing. We did not define the role of removable dentures on positive yeast counts although the presence of dentures is a known risk factor for yeast colonization. This is of course an error in this study.

In our study a statistically significant decrease was seen in the mean salivary IgG, IgM and albumin values of the HT users while no such effect was seen among the non-users. These findings may reflect an improvement in mucosal integrity among the HT users, since albumin, IgG and IgM in saliva have been suggested to be serum ultrafiltrates to the mouth and, consequently, their decreased concentrations may indicate improved mucosal and gingival health in the oral cavity (Meurman et al. 2002, Aviv et al. 2009). However, according to present knowledge, salivary IgG and IgM are primarily derived from GCF or as serum infiltrate (Kaufman & Lamster 2000; Van Nieuw et al. 2004). In our logistic model the only explanatory factor for higher than median salivary albumin concentrations was the number of teeth of the subject. This supports the GCF origin of albumin. In both groups of the present study the other protein concentrations analysed stayed at the same level during the 2-year follow-up but IgA and IgM values were significantly higher in the non-HT group. However, as pointed earlier, we found quite high stimulated salivary flow rates, and they may also partly explain the immunoglobulin and albumin results in the HT group. The clinical relevance of

these results needs to be assessed in future studies. In addition, we did our analyses only on whole saliva sample whereas the minor salivary gland secretions have been shown to be rich in IgA (Shiba et al. 1980).

In the study by Johnson (2005) in African-American postmenopausal women, salivary IgA concentrations were significantly higher than in Caucasian women, thus suggesting racial differences in this parameter. In the present series of studies all the women were Caucasian, which is strength of these results. Also, the Finnish population is racially homogenous. However, in contrast to our results, salivary immunoglobulin concentrations were found to decrease 5 months after starting HT while salivary peroxidase and total protein output increased in the longitudinal study by Leimola-Virtanen et al. (1997b). It must be kept in mind that we did not measure the effect of estrogen treatment nor did take into account the type of hormone therapy used (ie. ET, EPT, PT).

Natural variation in saliva parameters in general has to be taken into account when interpreting results on saliva constituents (Dawes 1987); this particularly needs to be emphasized in salivary hormone assessments (Ostrowska et al. 2001; Chatterton et al. 2005; Tivis et al. 2005). However, Patacchioli et al. (2006) observed in their study on stress reactions analysed by repeated salivary cortisol measurements that menopause was not associated with an impairment of circadian fluctuations of the cortisol concentrations. In the present investigation we did not analyse any hormone concentrations, however. It is also worth mentioning the need of standardizing the collection of saliva, as shown by Laine et al. (1999). They observed in a group of menopausal women that repeated collection resulted in significantly increased flow rates over seven weeks of observation. In our study, the interval time between the collections was two years, so it is improbable that there was any effect in this regard.

8. CONCLUSIONS

In general, the present findings showed that 50 to 58 year old women living in Helsinki have fairly good oral and dental health. These observations are partly explained by the fact that life expectancy has increased and also the number of dentate people is increasing. Today the standard of dental treatment allows people to retain their own teeth but it seems that many periodontal problems still occur.

Prevalence numbers for sensation of PM and DM reported in the questionnaires of the present study were in consistent with previously published data on the prevalence of these symptoms. However, clinical examination clarified that some PM symptoms were caused by treatable factors. Interestingly, our results in Study I showed that the occurrence of PM and DM seemed to be associated with climacteric symptoms in general, but the use of HT did not reduce the prevalence of oral symptoms studied. This was in contrast to our original study hypothesis.

Further studies with longer follow-up time are needed to evaluate the effect of HT on oral health parameters. The role of HT in ameliorating oral symptoms is still controversial. It seems that the effect of HT is highly individual so that some women with menopause-related symptoms benefit from HT while others do not. It is also probable that different hormone preparations have different effects in this regard. However, so far there are no randomized controlled studies to answer these questions.

Although not a population study still the present study gives new information about the correlation of the use of HT with oral symptoms in women of menopausal age living in Helsinki, Finland. When planning optimal HT for menopausal women, oral symptoms are a factor that may be considered by the patient and her gynecologist.

9. SUMMARY

Based on results of the present series of studies the following conclusions can be drawn:

1. A sensation of PM was reported by 8.2% and 19.9% complained of a sensation of DM, (xerostomia). The occurrence of PM and DM seemed to be associated with climacteric symptoms in general. Use of HT did not have any effect on this association. Poor self-assessed general health increased the risk for PM and DM (**Study I**).

2. Dental health, according to the DMFT index, did not differ between the HT users and non-users during the two-year follow-up time. However, during the follow-up, filled teeth index increased in the HT group ($p<0.05$), which may indicate a more health-conscious attitude in this group. Self-assessed oral health was considered satisfactory by 61% of HT users at baseline and by 59% at the follow up, for non-users respective values were 51% vs. 56%. These differences were not statistically significant (**Study II**).

3. The use of HT did not correlate with periodontal conditions based on the clinical examinations. Periodontitis was defined as at least one sextant had a CPITN score of 3. In HT group periodontitis was diagnosed at baseline in 79% and at two-year follow-up in 71%. The respective figures for non-HT users were 80% and 76%. Mean number of ≥ 6 mm deep periodontal pockets were 0.9 ± 1.7 at baseline vs. 1.1 ± 2.1 two years later in the HT group, and 1.0 ± 1.7 vs. 1.2 ± 1.9, respectively, in the non-HT group. These results concerning periodontal status indicate a higher prevalence of periodontitis but lower prevalence of severe periodontitis than reported in the Finnish national health survey in adults of this age group.

According to microbiological PCR evaluations of periodontopathogens, *P.g.* decreased in the HT group during the follow-up ($p<0.05$). Further studies are needed to determine the clinical relevance of this result. At baseline, positive yeast counts in the saliva of our study patients were higher than reported earlier in normal healthy population. The patients were informed when their yeast values were high. At follow-up, the frequency of positive yeast counts was at normal levels (**Study III**).

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4. During the two-year follow-up time the salivary albumin, IgG and IgM concentrations decreased slightly in HT users. This may indicate improvement in soft tissue integrity. However, more specific methods are needed to evaluate changes in oral mucosa and gingiva. There was no difference between the salivary flow rates in HT users and non-users (**Study IV**).

In general, the present findings showed that 50 to 58 year old women living in Helsinki, Finland, have fairly good oral and dental health, and they were interested in their oral health. In contrast to our study hypothesis, in our present study cohort there was no difference in the studied oral and dental health parameters between the women using HT and not using HT.

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