Clin Oral Invest (2012) 16:767–773
DOI 10.1007/s00784-011-0573-4

ORIGINAL ARTICLE

Saliva in perimenopausal and early postmenopausal women. A 2-year follow-up study

Laura Tarkkila · Jussi Furuholm · Aila Tiitinen · Jukka H. Meurman

Received: 17 October 2010 / Accepted: 25 May 2011 / Published online: 3 June 2011

Abstract This study aims to investigate salivary flow and biochemical constituents of menopausal-age women with the hypothesis that women using hormone therapy (HT) might present better saliva values than non-users. Two hundred HT users and 200 non-users were selected at random from a cohort study of 3,173 peri- and postmenopausal women and invited to a 2-year clinical follow-up study. Clinical examination with saliva sampling was made at baseline and 2 years later. Salivary total protein, albumin, and immunoglobulin (Ig) concentrations were analyzed. Final material included 106 consistent HT users and 55 non-users. Backward logistic regression analysis was made to determine the risk factors for higher or lower than medium salivary protein values. No difference was seen in salivary flow rate, total protein, and IgA values between baseline and follow-up measurements or between the groups. Albumin, IgG, and IgM concentrations were significantly lower in the 2-year samples of the HT group when compared with baseline. IgA and IgM values were higher in the non-HT 2-year samples when compared with the corresponding HT samples. The only significant explanatory factor for higher than median salivary albumin concentration was the number of teeth both at baseline and 2 years later. HT possibly improved epithelial integrity since the concentrations of serum components albumin, IgG, and IgM decreased during the follow-up. HT as such does not seem to affect saliva, although it may modify it. The clinical relevance of these results needs to be assessed in future studies.

Keywords Saliva · Menopause · Hormone therapy

L. Tarkkila (✉) · J. Furuholm · J. H. Meurman
Institute of Dentistry, University of Helsinki,
PB 41, Helsinki 00014, Finland
e-mail: laura.tarkkila@helsinki.fi

A. Tiitinen
Department of Obstetrics and Gynaecology,
Helsinki University Central Hospital,
Helsinki, Finland

J. H. Meurman
Department of Oral and Maxillofacial Surgery,
Helsinki University Central Hospital,
Helsinki, Finland

Introduction

Menopause and the use of hormone therapy (HT) and their associations with saliva and oral health have not been systematically investigated. Some controversial studies exist on the effect of the use of HT on the occurrence of oral symptoms, such as the sensations of burning mouth, dry mouth, and temporomandibular joint disorders [1–4]. We have reported that the sensations of painful mouth or dry mouth were not associated with the use of HT in menopause age women in a questionnaire study on 3,173 women [5]. Neither did we observe any major differences in the oral health status of the women using or not using HT during 2 years of observation [6].

However, results are contradictory regarding the effect of use of HT on salivary flow rate [7–13]. Flow rates have been reported to be higher in perimenopausal women in comparison to postmenopausal women [11]. But the number of patients in these studies has been low.

Several authors have suggested that improved saliva secretion is one reason behind the positive findings in women who start using HT and report improvement in their quality of life including oral discomfort [8, 9, 11, 12]. More recently, a case–control study from Iran observed no

difference in stimulated salivary flow rates in non-medicated menopausal women with or without subjective xerostomia [14]. However, of salivary electrolytes analyzed, significantly higher calcium concentrations were detected in the women with xerostomia in comparison to those who did not report oral dryness. This was partly in contrast to findings from a smaller study where no effect of HT, alendronate and calcium supplementation was seen on salivary calcium concentrations, although flow rates slightly increased after the medication was started [15]. In our group, alendronate decreased resting salivary flow rate but otherwise HT or alendronate separately or in combination had no effect on oral health in elderly women with osteoporosis [16].

There seems to be no studies on salivary biochemical constitutes of women using or not using HT. We hypothesized that HT might improve epithelial integrity of the mouth mucosa known to contain estrogen receptors [17], which then would reflect in salivary constituents. We were particularly interested in salivary albumin, the serum ultrafiltrate in saliva. Salivary albumin concentration increases in patients systemically ill and/or with mucositis [18–21]. The study design was a 2-year follow-up study of a cohort of peri- and postmenopausal women invited to a free-of-charge community-based mammography screening [6].

Subjects and methods

Patients

The study was carried out at the Institute of Dentistry, University of Helsinki, Finland. The patients were collected from a sample of 3,173 menopausal-age women who participated in mammography screening at the City of Helsinki Health Department. The age cohorts of the women were 50, 52, 54, 56, and 58 years. Forty women were selected at random from each of the 5 age cohorts who reported the use of HT, and 40 women who did not use HT. This resulted in 80 women from each age cohort, altogether, 400 women who were then invited to attend the baseline clinical examination. Of these, 249 women were included in the present study. Inclusion criterion for the HT group was at least 6-month use of the therapy before the onset of the study.

Eleven women were dropped out because they were still menstruating regularly at the start of the study. The women excluded were statistically significantly younger ($p<0.001$) but otherwise did not differ from the women included. Hence, 238 were available for statistics. Of these, 193 continued in the study, while 45 discontinued for various reasons. However, 32 women had changed their medication during the follow-up and were not included in the final statistics. After the final exclusion, the age between the groups could not be matched, but there was no difference in the mean age between the groups as given in Table 1. Finally, statistical analyses of the results of this follow-up study were carried out on 161 women. Of them, 106 subjects had used HT during the 2-year follow-up, while 55 women had no HT medication. The study profile is illustrated in the flowchart in Fig. 1.

Questionnaire

A structured questionnaire was given to all the subjects prior to the clinical examinations. The questionnaire comprised multiple-choice questions on general health, climacteric complaints, self-assessed general and dental health, and information about the last visit to a dentist. Use and duration of prescribed medications, as well as illnesses diagnosed by physician, were asked with open questions. The questionnaire comprised basically the same questions as in the original enquiry by Tarkkila et al. [5] on the 3,173 women.

Saliva sampling and analyses

The subjects were asked to avoid eating, drinking, and smoking 2 h before the examination. Saliva samples were taken before the clinical examinations in the morning or afternoon to avoid diurnal variation. Flow rates of resting and stimulated saliva were measured. Resting saliva was collected for 3 min with the free-flowing method. For the stimulated saliva, a 1-g piece of paraffin wax was given to the women to chew. Collection time for stimulated saliva was also 3 min. During this time, the subject chewed the paraffin wax at a constant rate (about once a second). Saliva collected during the first 30 s, however, was discarded [22]. Salivary total protein, albumin, and immunoglobulin A, G, and M concentrations were analyzed. Total protein was analyzed with the colorimetric Lowry method; albumin was analyzed according to Webster et al. [23] and the immunoglobulin concentrations according to Lehtonen et al. [24]. All analyses were made in duplicates with serum standards and appropriate controls. For details, see Mellanen et al. [25].

Statistical analyses

Descriptive statistical analyses were made using the Student's t test or the Mann–Whitney U test, and the χ^2 test when appropriate. Differences between the HT users and non-users were studied. The women who had started using HT or stopped the medication were excluded for the final analyses. Basic characteristics of the women who were dropped out or themselves discontinued for various reasons were analyzed with respect to those who were followed-up using cross tables and t test; no significant differences were found

Table 1 Descriptive baseline data of the subjects according to the use of hormone therapy (HT)

	Baseline			2-year follow-up			Significance between baseline and follow-up in HT group	Significance between baseline and follow-up in no HT group
	HT (106)	No HT (55)	*P* value	HT (106)	No HT (55)	*P* value		
Mean age	55.4±2.7	55.9±2.4	Ns.	57.4±2.7	57.9±2.4	Ns.	–	–
Current smoker	23 (22%)	11 (20%)	Ns.	19 (18%)	8 (15%)	Ns.	Ns.	Ns.
Current climacteric symptoms	16 (15%)	24 (44%)	***	14 (13%)	19 (35%)	***	Ns.	Ns.
Satisfactory self-assessed dental health	65 (61%)	28 (51%)	Ns.	63 (59%)	31 (56%)	Ns.	Ns.	Ns.
Sensation of dry mouth	24 (24%)	20 (36%)	Ns.	23 (22%)	15 (29%)	Ns.	Ns.	Ns.
Sensation of painful mouth	11 (11%)	7(13%)	Ns.	6 (6%)	1 (1%)	Ns.	Ns.	Ns.
Regular medication								
Use of cardiovascular drugs[a]	29 (27%)	14 (26%)	Ns.	39 (37%)	14 (26%)	Ns.	Ns.	Ns.
Use of neurological drugs[b]	7 (7%)	10 (18%)	*	5 (5%)	9 (16%)	*	Ns.	Ns.
Use of respiratory drugs[c]	7 (7%)	4 (7%)	Ns.	15 (14%)	4 (7%)	Ns.	Ns.	Ns.
Use of analgesics and antipyretics	7 (7%)	1 (2%)	Ns.	5 (5%)	5 (9%)	Ns.	Ns.	Ns.
Allergy medication	4 (4%)	0 (0%)	Ns.	0	0	–	Ns.	Ns.
Estrogen therapy	49 (47%)			47 (47%)			Ns.	
Progestin therapy	2 (2%)			1 (1%)			Ns.	
Combination therapy	53 (51%)			53 (53%)			Ns.	
Diagnosed illnesses								
Cardiovascular disease	19 (18%)	10 (18%)	Ns.	25 (24%)	11 (20%)	Ns.	Ns.	Ns.
Psychiatric disease	1 (1%)	2 (4%)	Ns.	1 (1%)	3 (6%)	Ns.	Ns.	Ns.
Asthma	8 (8%)	3 (6%)	Ns.	12 (11%)	3 (6%)	Ns.	Ns.	Ns.
Rheumatic disease	8 (8%)	2 (4%)	Ns.	6 (6%)	3 (6%)	Ns.	Ns.	Ns.

Percentage of subjects in each group is in parentheses

Ns. statistical difference not significant

*$p<0.05$; **$p<0.01$; ***$p<0.001$

[a] Includes antidepressant drugs, tranquilizers, sedatives, and antipsychotic agents

[b] Includes diuretics, antihypertensive agents, nitrates, digitalis, and anti-arrhythmic agents

[c] Mainly medication for asthma

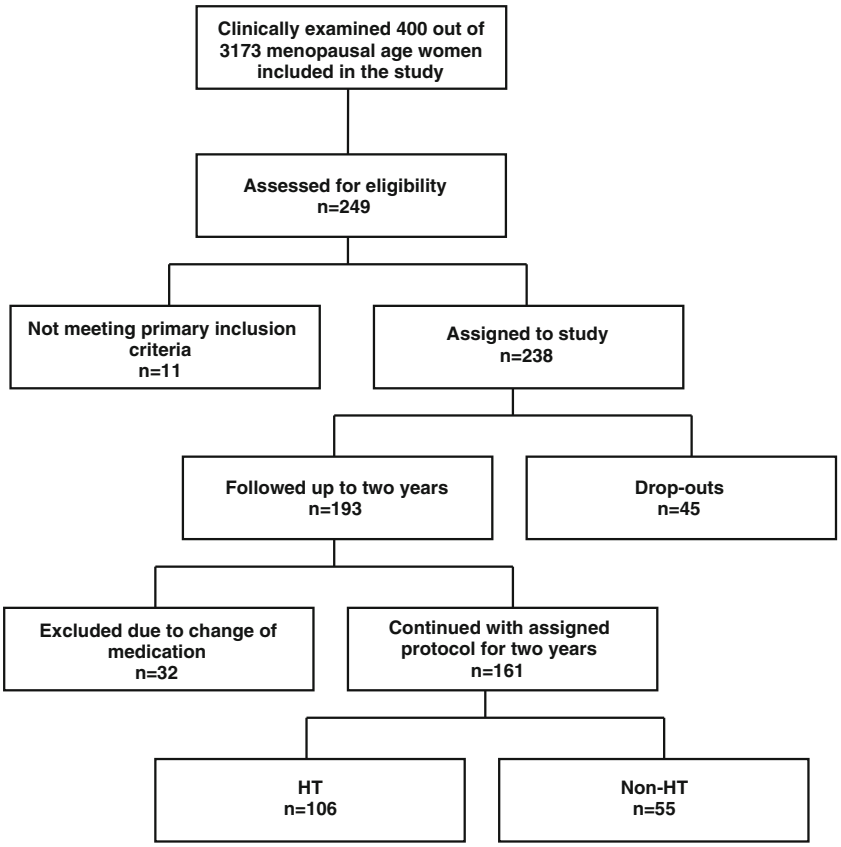

Fig. 1 Study profile. *HT* hormone therapy. The original cohort of 3,173 women was invited for free-of-charge community-based mammography

(except the younger age of those initially excluded due to menstruation). Multiple logistic regression analysis was used to investigate the higher than median salivary protein values by simultaneously controlling variables such as use of HT, smoking, numbers of general diseases and medications, self-assessed oral health, and variables of the recorded oral health status. Backwards elimination was used to control correlation between confounders. The model estimates were used to calculate odds ratios (OR) and 95% confidence intervals (CI). Statistical significance was set at $p<0.05$. SPSS version 17.1 (Chicago, IL, USA) was used.

Results

At baseline, the HT users had used their current HT on average for 3.9 years (SD 3.3). In the final material, pair-wise saliva values were obtained from 106 HT users and 55 non-HT users, respectively. Descriptive data of the study subjects are given in Table 1. Results of the salivary analyses are given in Table 2. Albumin, IgG, and IgM concentrations decreased statistically significantly in the HT group during the 2-year observation. On the group level, mean salivary IgA and IgM values at the 2-year follow-up investigation were higher in the non-HT women when compared with those taking HT. No other differences were seen in any other salivary parameters between or within the groups. Hence, also the salivary flow rate values remained stable in both groups throughout the follow-up, and no difference was observed between the HT and non-HT groups in this regard.

Of the whole study group, 27% ($n=63$) reported having climacteric symptoms. Of the HT users, 17% ($n=24$) and of the non-users, 39% ($n=39$) reported having climacteric symptoms ($p<0.001$).

The logistic regression analysis showed that the only statistically significant explanatory factor for higher than median salivary albumin concentration was the number of teeth of the subject. This result was the same both at baseline and 2 years later as given in Table 3. Salivary albumin concentration was used as a proxy for oral epithelial integrity. The other study parameters were not found to correlate with the salivary protein values in this perspective. Nevertheless, the interesting variables taken into the statistical model are given in the table.

Discussion

In our previous study, we showed that women reporting current climacteric complaints were at higher risk of having uncomfortable sensations also in their mouth [5]. As

Table 2 Salivary flow rates and protein concentrations at baseline and 2 years later in women using or not using hormone therapy (HT)

	HT (106)			Non-HT (55)			Significance between groups in baseline	Significance between groups in follow-up
	Baseline	2-year follow-up	*P* value	Baseline	2-year follow-up	*P* value		
Resting flow (ml/min)	0.6±0.4	0.7±0.4	Ns.	0.6±0.4	0.6±0.4	Ns.	Ns.	Ns.
Stimulated flow (ml/min)	2.0±0.9	2.0±0.8	Ns.	1.7±0.8	1.8±0.8	Ns.	Ns.	Ns.
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	<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	<0.05	22.8±20.3	20.4±22.6	Ns.	Ns.	Ns.
IgM (μg/ml)	1.51±1.2	1.18±1.1	<0.001	1.93±1.6	1.66±1.6	Ns.	Ns.	$p<0.05$

Means with standard deviation. Number of subjects in each group is in parentheses

Ns. not significant

expected, the difference in reported climacteric complaints was significant between the HT users and non-users so that HT decreased the symptoms ($p=0.000$). Eliasson et al. [8] reported a significant increase in labial gland secretion in 18 postmenopausal women after receiving 1-mg estrogen daily for 1 year. A majority of the women also reported improvement in their feeling of dry mouth. In our present study, however, we did not measure labial secretion but collected resting and stimulated saliva samples according to the clinical examination routine of our institute. We observed no difference in these values between the users and non-users of HT or with respect to the 2-year follow-up. This is in contrast to earlier observations also from Finland. Namely, Hietala et al. [10] observed in a 2-year study higher salivary flow rate values in HT users than non-users. Similarly, in the study by Laine and Leimola-Virtanen [11], a significant increase in salivary flow rate was observed 5 months after starting HT in 27 perimenopausal and postmenopausal women. Sewon et al. [26] also reported a significant increase in salivary flow rate in 16 women receiving HT for 5 months. In our study, the salivary secretion rate values did not correlate statistically significantly with the reported climacteric symptoms.

Salivary immunoglobulin concentrations were found to decrease during 5 months after starting HT, while salivary peroxidase and total protein output increased in the study by Leimola-Virtanen et al. [12]. 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 improvement in mucosal integrity among the HT users since albumin, IgG, and IgM in saliva are serum ultrafiltrates to

Table 3 Results of backwards logistic regression analysis with higher than medium salivary albumin concentration as the dependent variable and several background variables as explanatory factors

Baseline				
	Significance	OR	95% CI	
Number of teeth	0.048	1.078	1.001	1.162
HT	0.552	0.790	0.363	1.720
Smoking	0.365	0.665	0.275	1.607
Self-assessed oral health	0.292	1.500	0.705	3.191
Number of medications	0.382	1.269	0.744	2.166
Number of diseases	0.673	0.855	0.413	1.771
2-year follow-up				
	Significance	OR	95% CI	
Number of teeth	0.005	1.138	1.039	1.246
HT	0.290	0.648	0.290	1.447
Smoking	0.963	0.977	0.363	2.630
Self-assessed oral health	0.068	2.045	0.949	4.404
Number of medications	0.743	1.133	0.537	2.389
Number of diseases	0.189	0.583	0.261	1.304

the mouth, and subsequently, their decreased concentrations may indicate improved mucosal health in the oral cavity [18, 27]. Nevertheless, an earlier study by Pisanty et al. [28] did not find any specific effects of female sex hormones on buccal mucosa after topical application of estrogen ointment with or without progesterone. But in this study, an improvement of salivary secretion was recorded together with alleviation of subjective oral complaints [28]. However, in the 1970s, the HT combinations used were different from modern therapy. Finally, it should be emphasized that dental status among the women during the 2-year follow-up stayed fairly stable, and thus, the effect of the number of teeth and gingival and periodontal inflammation did not affect the results [6]. The number of teeth as such associated statistically significantly with salivary protein values.

Nagler and Hershkovich [29] in their study on saliva of elderly vs. young individuals observed a distinct age-related difference in salivary components mainly so that a reduction was seen in the flow rates and mean chemical concentrations except for total protein, albumin, and salivary immunoglobulin values. Hence, not only the reduction in flow rates but also alterations in salivary composition may explain oral discomfort often reported by elderly individuals and particularly among menopausal-age women [9]. As said, no decrease in the mean salivary flow rates was observed in our study and also the differences in salivary mean values between the age cohorts included were not statistically significant.

In both groups of the present study, the other protein concentrations analyzed stayed on the same level during the 2-year follow-up, but IgA and IgM values were significantly higher in the non-HT group. Salivary IgA concentrations were significantly higher also in African–American postmenopausal women in comparison to Caucasian women in the study by Johnson [30], thus suggesting racial differences in this parameter. In the present study, all the women were Caucasian, and the Finnish population is racially homogenous, which is the strength of the present investigation.

The well-known natural variation of saliva parameters in general has to be taken into account when interpreting the results on saliva constituents [31]. This has been particularly emphasized in salivary hormone assessments [32–34]. On the other hand, Patacchioli et al. [35] observed in their study on stress reactions analyzed by repeated salivary cortisol measurements that menopause was not associated with an impairment of circadian fluctuations of the cortisol concentrations. However, in the present investigation, we did not attempt to analyze any hormone concentrations. According to the guidelines for salivary flow rate measurements, it has been shown that unstimulated saliva tests should be performed at fixed time-points or over limited time interval early in the morning in order to obtain reliable results [36]. In the present study, saliva samples were taken at the clinical examinations, and strict appointment times could not be followed. This is the weakness of the study. However, large diurnal variation was avoided by scheduling the individual appointments either in the morning or afternoon. The subjects had also been asked to avoid eating, drinking, and smoking 2 h prior to the appointment time, but there was no way of avoiding eventual stimuli prior to those 2 h. Finally, it should be mentioned that Laine et al. [37] observed in a group of menopausal women that repeated collection resulted in significantly increased flow rates in 7 weeks of observation. However, in our study, the time lag between the collections was 2 years, so it is improbable that there was any effect in this regard.

We did not include questions on social status or education into our questionnaire, but it can well be assumed that the large population cohorts originally included (n= 3,173) effectively leveled off bias in this regard [5]. Also, despite the request to check the current regular medication before attending the examination, considerably many 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 the cardiovascular medication. However, when the saliva values were analyzed with respect to the drugs used daily, no statistically significant differences were seen between or within the groups.

Finally, we conclude that in contrast to our study hypothesis, there was no significant difference in salivary flow rates between the HT users and non-users in menopausal-age women. However, during the 2-year observation, salivary albumin, IgG, and IgM concentrations decreased significantly in the HT users which finding may indicate improvement in mucosal integrity. Of the background variables investigated, only the number of teeth emerged significant in the regression model both at baseline and 2 years later when the salivary albumin concentrations were analyzed as a proxy for mucosal health. The HT users of our study had used the current therapy for only approximately 4 years. More data are therefore needed for assessment of the long-term effects of HT on oral health.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Forabosco A, Criscuolo M, Coukos G, Uccelli E, Weinstein R, Spinato S, Botticelli A, Volpe A (1992) Efficacy of hormone replacement therapy in postmenopausal women with oral discomfort. Oral Surg Oral Med Oral Pathol 73:570–574

2. Hakeberg M, Berggren U, Hägglin C, Ahlqwist M (1997) Reported burning mouth symptoms among middle-aged and elderly women. Eur J Oral Sci 105:539–543
3. Hatch JP, Rugh JD, Sakai S, Saunders MJ (2001) Is the use of exogenous estrogen associated with temporomandibular signs and symptoms? J Am Dent Assoc 132:319–326
4. Wardrop RW, Hailes J, Burger H, Reade PC (1989) Oral discomfort at menopause. Oral Surg Oral Med Oral Pathol 67:535–540
5. Tarkkila L, Linna M, Tiitinen A, Lindqvist C, Meurman JH (2001) Oral symptoms at menopause-the role of hormone replacement therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 92:276–280
6. Tarkkila L, Furuholm J, Tiitinen A, Meurman JH (2008) Oral health in perimenopausal and early postmenopausal women from baseline to 2 years of follow-up with reference to hormone replacement therapy. Clin Oral Investig 12:271–277
7. Ben Aryeh H, Gottlieb I, Ish-Shalom S, David A, Szargel H, Laufer D (1996) Oral complaints related to menopause. Maturitas 24:185–189
8. Eliasson L, Carlen A, Laine M, Birkhed D (2003) Minor gland and whole saliva in postmenopausal women using a low potency oestrogen (oestriol). Arch Oral Biol 48:511–517
9. Friedlander AH (2002) The physiology, medical management and oral implications of menopause. J Am Dent Assoc 133:73–81
10. Hietala EL, Heikkinen J, Vaananen HK, Larmas M (1993) Effect of postmenopausal estrogen treatment on some diagnostic salivary variables. Ann N Y Acad Sci 694:286–288
11. Laine M, Leimola-Virtanen R (1996) Effect of hormone replacement therapy on salivary flow rate, buffer effect and pH on perimenopausal and postmenopausal women. Arch Oral Biol 41:91–96
12. Leimola-Virtanen R, Helenius H, Laine M (1997) Hormone replacement therapy and some salivary antimicrobial factors in post- and perimenopausal women. Maturitas 27:145–151
13. Streckfus CF, Baur U, Brown LJ, Bacal C, Metter J, Nick T (1998) Effects of estrogen status and aging on salivary flow rates in healthy Caucasian women. Gerontology 44:32–39
14. Agha-Hosseini F, Mirzaii-Dizgah I, Moghaddam PP, Akrad ZT (2007) Stimulated whole salivary flow rate and composition in menopausal women with oral dryness feeling. Oral Dis 13:320–323
15. Yalcin F, Gurgan S, Gurgan T (2005) The effect of menopause, hormone replacement therapy (HRT), alendronate (AL), and calcium supplementation on saliva. J Contemp Dent Pract 6:10–17
16. Eviö S, Tarkkila L, Sorsa T, Furuholm J, Välimäki M, Ylikorkala O, Tiitinen A, Meurman JH (2006) Effects of alendronate and hormone replacement therapy, alone and in combination, on saliva, periodontal conditions and gingival crevicular fluid matrix metalloproteinase-8 levels in women with osteoporosis. Oral Dis 12:187–193
17. Välimaa H, Savolainen S, Soukka T, Silvoniemi P, Mäkelä S, Kujari H, Gustafsson JA, Laine M (2004) Estrogen receptor-beta is the predominant estrogen receptor subtype in human oral epithelium and salivary glands. J Endocrinol 180:55–62
18. Avivi I, Avraham S, Koren-Michowitz M, Zuckerman T, Aviv A, Ofran Y, Benyamini N, Nagler A, Rowe JM, Nagler RM (2009) Oral integrity and salivary profile in myeloma patients undergoing high-dose therapy followed by autologous SCT. Bone Marrow Transplant 43:801–806
19. Helenius LM, Meurman JH, Helenius I, Kari K, Hietanen J, Suuronen R, Hallikainen D, Kautiainen H, Leirisalo-Repo M, Lindqvist C (2005) Oral and salivary parameters in patients with rheumatic diseases. Acta Odontol Scand 63:284–293
20. Hirasaki S, Yamazaki T, Shiba K (2005) Changes in salivary components by drug administration in patients with heart diseases. J Med Dent Sci 52:183–188
21. Vesterinen M, Ruokonen H, Leivo T, Honkanen AM, Honkanen E, Kari K, Lindqvist C, Meurman JH (2007) Oral health and dental treatment of patients with renal disease. Quintessence Int 38:211–219
22. Meurman JH, Rantonen P (1994) Salivary flow rate, buffering capacity, and yeast counts in 187 consecutive adult patients from Kuopio, Finland. Scand J Dent Res 102:229–234
23. Webster D (1977) The immediate reaction between bromcresol green and serum as a measure of albumin content. Clin Chem 23:663–665
24. Lehtonen OP, Grahn EM, Stahlberg TH, Laitinen LA (1984) Amount and avidity of salivary and serum antibodies against *Streptococcus mutans* in two groups of human subjects with different dental caries susceptibility. Infect Immun 43:308–313
25. Mellanen L, Sorsa T, Lahdevirta J, Helenius M, Kari K, Meurman JH (2001) Salivary albumin, total protein, IgA, IgG and IgM concentrations and occurrence of some periodontopathogens in HIV-infected patients: a 2-year follow-up study. J Oral Pathol Med 30:553–559
26. Sewon L, Laine M, Karjalainen S, Leimola-Virtanen R, Hiidenkari T, Helenius H (2000) The effect of hormone replacement therapy on salivary calcium concentrations in menopausal women. Arch Oral Biol 45:201–206
27. Meurman JH, Rantonen P, Pajukoski H, Sulkava R (2002) Salivary albumin and other constituents and their relation to oral and general health in the elderly. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 94:432–438
28. Pisanty S, Rafaely B, Polishuk W (1975) The effect of steroid hormones on buccal mucosa of menopausal women. Oral Surg Oral Med Oral Pathol 40:346–353
29. Nagler RM, Hershkovich O (2005) Relationships between age, drugs, oral sensorial complaints and salivary profile. Arch Oral Biol 50:7–16
30. Johnson RB (2005) Racial differences in salivary sIgA concentrations in postmenopausal women. Spec Care Dentist 25:145–149
31. Dawes C (1987) Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. J Dent Res 66:648–653
32. Chatterton RT Jr, Mateo ET, Hou N, Rademaker AW, Acharya S, Jordan VC, Morrow M (2005) Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women. J Endocrinol 186:77–84
33. Ostrowska Z, Kos-Kudla B, Marek B, Swietochowska E, Gorski J (2001) Assessment of the relationship between circadian variations of salivary melatonin levels and type I collagen metabolism in postmenopausal obese women. Neuro Endocrinol Lett 22:121–127
34. Tivis LJ, Richardson MD, Peddi E, Arjmandi B (2005) Saliva versus serum estradiol: implications for research studies using postmenopausal women. Progr Neuro Psychopharmacol Biol Psychiatr 29:727–732
35. Patacchioli FR, Simeoni S, Monnazzi P, Pace M, Capri O, Perrone G (2006) Menopause, mild psychological stress and salivary cortisol: influence of long-term hormone replacement therapy (HT). Maturitas 55:150–155
36. Flink H, Tegelberg Å, Lagerlöf F (2005) Influence of the time of measurement of unstimulated human whole saliva on the diagnosis of hyposalivation. Arch Oral Biol 50:553–559
37. Laine M, Pienihakkinen K, Leimola-Virtanen R (1999) The effect of repeated sampling on paraffin-stimulated salivary flow rates in menopausal women. Arch Oral Biol 44:93–95