Salivary flow, testosterone, and femur bone mineral density in menopausal women with oral dryness feeling

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Objectives. We compared salivary flow, serum and saliva testosterone, and femur bone mineral density (BMD) of menopausal women with or without xerostomia.

Study Design. A case/control study was performed on 60 selected menopausal women with or without xerostomia. BMD and testosterone concentration were measured by a dual-energy x-ray absorptiometry system and enzyme-linked immunosorbent assay method, respectively.

Results. Multinomial logistic regression demonstrated that low saliva flow rate (odds ratio [OR] = 22.8, 95% confidence interval [CI]: 5.4, 96.8), low femur BMD (OR = 6.0, CI: 1.8, 20.0), high stimulated saliva testosterone (OR = 5.2, CI: 2.0, 18.9), high unstimulated saliva testosterone (OR = 3.8, 95% CI: 1.6, 12.3), and high serum testosterone (OR = 2.7, CI: 1.1, 7.2) were associated with an increased risk of xerostomia in menopausal women.

Conclusions. High serum and salivary testosterone and low femur BMD and saliva flow were associated with xerostomia. Of these factors, low salivary flow seems to be the most important element in the perception of dry mouth. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:612-616)

By 2030, an estimated 47 million women will be undergoing menopause each year.[1] One of the most common disorders in menopause is osteoporosis. Osteoporosis is characterized by low bone mass and microarchitectural decline of bone tissue, resulting in increased bone fragility and fracture risk. Xerostomia is another common disorder in menopause, but the exact cause of this sensation has not been definitively recognized.[2-5]

A change in salivary composition is a possible cause of xerostomia in menopausal patients. Our previous studies showed that lower salivary estrogen and progesterone and higher cortisol and parathyroid hormone (PTH) in saliva and serum are associated with xerostomia in postmenopausal women with xerostomia[2,3,6-8]; because of the effects of estrogen, PTH, and cortisol in bone turnover, lower bone mineral density (BMD) in these patients is to be expected. Recently we have found that menopausal women who lose BMD may experience xerostomia.[9]

Systemic bone loss results from a decline in estrogen levels in menopause.[10] In women, the function of estrogens is well established, but the function of androgens is not.[11] Androgens enhance bone formation.[12] Evidence suggests that testosterone alone is more effective than estrogen–testosterone or estrogen therapy for the management of somatic and psychological symptoms in menopausal patients, and it appears safe at pharmacologic doses.[13] Other studies have shown that a single assay of serum testosterone was not valuable in the diagnosis of androgen deficiency. Incidence/severity of symptoms and treatment of androgen deficiency did not correlate with free or total serum testosterone levels.[13] In contrast, Orozco et al. reported that high levels of salivary testosterone are associated with higher lumbar BMD in premenopausal healthy women of the same age and with normal levels of serum testosterone. They suggested that this hormone in saliva could be more sensitive in detecting its active form and did not necessarily directly reflect the free concentrations in serum.[11]

Saliva has a number of benefits compared with blood for the evaluation of sex steroids: it can be easily collected by noninvasive methods and does not require special storage, and the steroid concentrations measured exclude the fraction that is bound to serum protein and is therefore biologically unavailable.[14] Our

Statement of Clinical Relevance

Low salivary flow, high serum and salivary testosterone, and low femur bone mineral density are associated with the perception of xerostomia in menopausal women.

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Received for publication May 13, 2012; returned for revision Nov 9, 2012; accepted for publication Nov 17, 2012.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2012.11.014

Table I. Questionnaire used for selection of subjects with xerostomia

1. Does your mouth feel dry when eating a meal?
2. Do you have difficulties swallowing any foods?
3. Do you need to sip liquids to aid in swallowing dry foods?
4. Does the amount of saliva in your mouth seem to be reduced most of the time?
5. Does your mouth feel dry at night or on waking?
6. Does your mouth feel dry during the daytime?
7. Do you chew gum or use candy to relieve oral dryness?
8. Do you usually wake up thirsty at night?
9. Do you have problems in tasting food?
10. Does your tongue burn?

Response options: yes/no.

Table II. The Xerostomia Inventory (XI)

I sip liquids to help swallow food.
My mouth feels dry when eating a meal.
I get up at night to drink.
My mouth feels dry.
I have difficulty in eating dry foods.
I suck sweets or cough lollies to relieve dry mouth.
I have difficulties swallowing certain foods.
The skin of my face feels dry.
My eyes feel dry.
My lips feel dry.
The inside of my nose feels dry.

Response options: never (score of 1), hardly (2), occasionally (3), fairly often (4), and very often (5).

previous studies have shown that the saliva and serum levels of estradiol are lower in menopausal women with xerostomia than in control groups.[7] Because estrogens are produced from androgens, it seems that these processes may be impaired. Therefore, the purpose of the current study was to evaluate whether serum and saliva testosterone levels and BMD correlate with severity of xerostomia and to compare serum and saliva testosterone levels, BMD, and salivary flow of menopausal women with and without xerostomia.

MATERIAL AND METHODS

Only women who were menopausal were enrolled in the current study. The study excluded smokers, obese patients (body mass index >30 kg/m^2), patients taking xerostomic drugs, patients with certain systemic diseases (including diabetes, Sjogren's syndrome, etc.), patients with oral candidiasis or unfavorable oral health conditions such as poor oral hygiene and periodontal diseases (pocket depth more than 3 mm), and patients under corticosteroid or hormone replacement therapy. The study was approved by the ethics committee of Tehran University of Medical Sciences.

Menopausal patients who had been referred to the Clinic of Oral Medicine, Tehran University of Medical Sciences, and had none of the exclusion criteria were recruited. Subjects who had at least 3 positive responses to a questionnaire with a list of symptoms associated with xerostomia (Table I) formed the case group (women with xerostomia), and women who had no positive responses formed the control group (women without xerostomia).[8] Specifically, women with at least 3 positive responses were entered in the case group (with xerostomia) because some questions (questions 2 and 9) are not specific for xerostomia and may be observed in other disorders. We selected patients in this manner until 30 subjects were entered in each group and all subjects gave their consent to participate. The mean age of women was 54.57 ± 0.92 years in the case group and 56.80 ± 1.41 years in the control group. No subject had had a menstruation cycle for at least 24 months.

To assess the severity of xerostomia, a second questionnaire (xerostomia inventory [XI]) was completed by both groups (Table II).[9,15] Each response was graded as follows: 1, never; 2, hardly ever; 3, occasionally; 4, fairly often; and 5, very often. The response scores were added, resulting in an XI score for each individual that demonstrated the severity of xerostomia (XI score). These scores ranged from 11 to 55. An XI score higher than 19 was considered severe.

Sample collection

Stimulated and unstimulated whole saliva was collected under resting conditions in a quiet room between 10 AM and 12 PM and at least 90 minutes after the last intake of food or drink. Unstimulated salivary samples were obtained by expectoration in the absence of chewing movements. Prestimulation was accomplished by chewing a piece of standard-size paraffin; after 60 seconds, the participants were asked to swallow the saliva present in the mouth. Both stimulated and unstimulated saliva was collected for about 5 minutes in a preweighed, dry, deionized and sterilized plastic tube. By subtracting the empty tube weight from the weight of the saliva-filled tube, the saliva sample weight was determined to calculate the salivary flow rate. The flow rate was calculated in grams per minute, which is almost equivalent to milliliters per minute.[9]

Blood specimens were obtained by venipuncture, collected in 10-mL glass vacuum tubes without additive, and allowed to clot. The blood and saliva were centrifuged (2500 *g*, 10 minutes), and the serum and supernatants of saliva were separated and immediately stored at -80°C for later determination. All samples were assayed within 3 months of storage.

BMD assessment

Measurements of BMD were performed at the neck of the femur with the use of a dual-energy x-ray absorp-

tiometry system (Hologic Discovery, Research Center of Diabetes and Metabolic Disease, Shariati Hospital, Tehran, Iran). One clinician performed these measurements for all patients using the same technique. BMD was presented as grams per centimeter squared, *T* score, and *Z* score. Scores indicate the amount by which one's BMD varies from the mean. Negative scores indicate lower bone density and positive scores indicate higher bone density. The *T* score is the relevant measure when screening for osteoporosis and is the BMD at the site when compared with the young, normal reference mean. The *T* score represents a comparison of a patient's BMD with that of a healthy 30 year old of the same sex and ethnicity. This value is used in postmenopausal women and in men over the age of 50 years because it better predicts risk of future fracture. The *Z* score is a comparison with the age-matched normal reference and is usually used in cases of severe osteoporosis. The *Z* score represents the number of standard deviations by which a patient's BMD differs from the average BMD of individuals of their same age, sex, and ethnicity. This value is used in premenopausal women, in men under the age of 50, and in children. It is most useful when the score is less than 2 standard deviations below the normal. In this setting, it is helpful to scrutinize for coexisting illnesses that may contribute to osteoporosis, such as glucocorticoid therapy, hyperparathyroidism, or alcoholism.[16]

Analysis of saliva and serum

Enzyme-linked immunosorbent assay was applied to measure the serum and saliva concentrations of testosterone duplicated using enzyme-linked immunosorbent assay kits from Diagnostics Biochem Canada, Inc. (Ontario, Canada). The kit was designed to assay serum specimens. Determination of testosterone levels was carried out according to the manufacturers' instruction. The assay had a lower limit of sensitivity of 22 pg/mL. Intra- and interassay coefficients of variation were <9.6% and 8.5%, respectively. The control sample of the kit had a testosterone concentration of 760 pg/mL, which must be read at 650 ± 150 pg/mL.

Statistical methods

Student's unpaired *t* test was used to compare the serum and salivary components and femur BMD in women with and without oral dryness comparisons. Spearman correlation analysis was used to identify any correlation of XI score with BMD, testosterone, or saliva flow rate levels. Odds ratios (OR) and 95% confidence intervals (CI) were obtained through multinomial logistic regression. $P < 0.05$ was considered statistically significant.

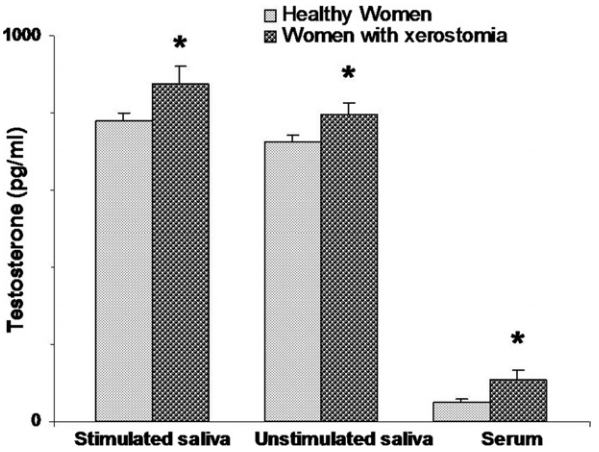

Fig. 1. Concentrations of testosterone in serum, stimulated, and unstimulated saliva of women with or without xerostomia. *$P < 0.01$. Data are presented as means ± SEM.

Table III. Bone mineral density (BMD), *T* score, and *Z* score (means ± SEM) in menopausal women with or without xerostomia

	Group	*Mean*	*P value*
Femur *T* score	With xerostomia	−1.49 ± 0.12	0.024
	Without xerostomia	−0.93 ± 0.22*	
Femur *Z* score	With xerostomia	−0.52 ± 0.13	0.013
	Without xerostomia	0.13 ± 0.22*	
Femur BMD (g/cm^2)	With xerostomia	0.69 ± 0.01	0.03
	Without xerostomia	0.75 ± 0.03*	

T score was used to compare the BMD of women with that of the normal young reference. *Z* score was used to compare the BMD with that of an age-matched control.
*$P < 0.05$ according to Student's unpaired *t* test.

RESULTS

There were no significant differences between the 2 groups in body mass index, age, or years after menopause. Student's unpaired *t* test showed that whole unstimulated salivary flow rate was lower in the case group (0.23 ± 0.01) than in the control group (0.35 ± 0.01; $P < 0.05$), but the differences in stimulated salivary flow rate between the case (0.35 ± 0.02) and control groups (0.37 ± 0.02) were similar. Seven patients in the case group had unstimulated saliva flow rate <0.2 mL/min, but none of the participants had unstimulated saliva flow rate <0.1 mL/min.

Mean testosterone concentrations were significantly higher in the serum ($P = 0.036$), stimulated saliva ($P = 0.025$), and unstimulated saliva ($P = 0.006$) of women suffering from xerostomia compared with controls (Figure 1). Mean femur BMD, *T* score, and *Z* score are shown in Table III. The BMD, *Z* score, and *T* score were significantly lower in women with xerostomia ($P \leq 0.03$).

Multinomial logistic regression demonstrated that low saliva flow rate (OR = 22.8, 95% CI: 5.4, 96.8), low femur BMD (OR = 6.0, CI: 1.8, 20.0), high stimulated saliva testosterone (OR = 5.2, CI: 2.0, 18.9), high unstimulated saliva testosterone (OR = 3.8, 95% CI: 1.6, 12.3), and high serum testosterone (OR = 2.7, CI: 1.1, 7.2) were associated with increased risk of xerostomia in menopausal women.

Spearman correlation was performed to determine whether any relationship existed between the severity of xerostomia (XI score) and *T* score and *Z* score. XI score had a significant negative correlation with femur *T* score ($r = -0.3$, $P = 0.048$) and also with femur *Z* score ($r = -0.400$, $P = 0.008$). In addition, the mean XI score significantly correlated with serum ($r = 0.394$, $P = 0.034$) and unstimulated ($r = 0.477$, $P = 0.008$) and stimulated ($r = 0.406$, $P = 0.005$) saliva testosterone levels. XI score also significantly correlated with unstimulated saliva flow rate ($r = -0.468$, $P = 0.039$) but not with stimulated saliva flow rate ($r = -0.108$, $P = 0.413$).

DISCUSSION

Our results showed that unstimulated saliva flow rate was lower in menopausal women suffering from xerostomia. This finding is consistent with other reports.[17] Our data also indicate that low unstimulated saliva flow rate correlated with the severity of xerostomia in menopausal women. These data should be interpreted with the knowledge that only 7 of our cases had an unstimulated whole saliva flow rate lower than 0.2 mL/min but higher than 0.1 mL/min. Thus, our cut point for hyposalivation is a potential issue[18,19]; however, the onset of xerostomia occurs when the total salivary flow rate is reduced to just less than 50% of normal.[20] In contrast, it has been reported that dry mouth may exist in the presence of an apparently sufficient amount of stimulated saliva.[6-8,21,22] Despite the controversy in this area, our data indicate that our group of menopausal women with xerostomia suffered from reduced unstimulated salivary flow rate, and low saliva flow rate could be the cause of xerostomia in menopausal women.

One of the most common disorders in menopause is osteoporosis. Appropriate management of osteoporosis includes early diagnosis of the disease, before significant bone loss. Therefore, complementary tests are needed to assess serum and urinary markers for bone turnover.[16] Androgens enhance bone formation, and studies have shown that testosterone in saliva could be more sensitive in detection.[11,12] In this study, the salivary and serum testosterone concentrations as well as the BMD of menopausal women with and without xerostomia were investigated. We found that the testosterone level in stimulated and unstimulated saliva and serum of menopausal women with xerostomia was significantly higher than that in women without xerostomia. To the best of our knowledge, this is the first study to evaluate salivary testosterone in menopausal women with xerostomia. Also, we found that the BMD of the femur is significantly lower in menopausal women with xerostomia. A salivary composition change is a possible cause of xerostomia in menopausal patients. This could be caused by lower estrogen and progesterone and higher cortisol and PTH in the saliva and serum of postmenopausal women with xerostomia.[2,3,6-8] Additionally, we found that menopausal women who lose BMD may also experience xerostomia[10]; these hormones affect bone turnover.

Saliva is a complex fluid containing a variety of enzymes, hormones, antibodies, antimicrobial constituents, and growth factors, many of which enter the saliva from the blood by passing through the spaces between cells via transcellular or paracellular routes. Therefore, most compounds found in the blood are also present in saliva, and thus saliva is alternative to serum in reflecting the physiological state of the body, including emotional, hormonal, nutritional, and metabolic variations.[23] In the current study, salivary testosterone levels were higher than those detected in the serum. This finding is consistent with that of Baxendale et al., who reported on salivary and unbound plasma testosterone in women,[24] and with our previous study of salivary and serum CA125 levels in women with breast cancer.[25]

In this study the stimulated, unstimulated, and serum amount of testosterone in menopausal women with xerostomia was significantly higher than that in women without xerostomia, and the BMD of the femur was significantly lower in menopausal women with xerostomia, as was lumbar BMD, which was reported previously.[9] Orozco et al.[11] found that salivary testosterone was a positive independent predictor of lumbar BMD, but not in femoral bone. Our results indicate apparent disagreements with Orozco's findings. Higher serum and salivary testosterone are associated with lower BMD in both femur and lumbar bones. These differences may be the result of the different study populations (premenopausal women versus menopausal women) and study design. However, greater femur bone loss, as well as lumbar, in menopausal women with xerostomia may also suggest more rapid or destructive bone loss than in women without xerostomia. Our previous findings about hormonal status in menopausal women with xerostomia suggest this idea. On the other hand, as a result of estrogen deficiency, compensatory processes try to produce more testosterone to create estrogen by aromatization, but for reasons that are not clear, this conversion is not possible. Hence, the

serum and salivary amounts of testosterone are higher in menopausal women with xerostomia who have more severe estrogen deficiency. However, we believe that one important limitation in our study, and in many other studies in menopausal women, is the failure to divide menopause into surgically induced and natural menopause. Also, the small sample size in this study limits broad of interpretation of the data.

Finally, multinomial logistic regression showed that high serum testosterone, high stimulated and unstimulated saliva testosterone, low saliva flow rate, and femur BMD were all associated with an increased risk of xerostomia in menopausal women. Because the OR for unstimulated saliva flow rate was higher than the other variables, it seems that saliva flow rate may be the most important factor in the perception of dry mouth.

CONCLUSIONS

Based on these results, low salivary flow, high serum and salivary testosterone, and low femur BMD are associated with xerostomia in menopause. Salivary flow in menopausal women seems to be an important factor in the perception of dry mouth.

This study was supported by the Dental Research Center, School of Dentistry, Tehran University of Medical Sciences.

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