Serum and stimulated whole saliva parathyroid hormone in menopausal women with oral dry feeling

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Menopause is a physiological process typically occurring in the fifth decade of life in women, involving permanent cessation of menstruation.1 It is the result of irreversible changes in the hormonal and reproductive functions of the ovaries. Menopause is accompanied by physiological and sensorial oral changes in select individuals.2 The prevalence of oral symptoms was found to be significantly greater in menopausal women (43%) than in premenopausal females (6%).1 Major oral symptoms of menopause are xerostomia and burning mouth.1

Oral dryness (OD) or xerostomia is a major complaint for many elderly individuals; it is a subjective sensation, and does not reflect a dry mouth in up to one third of cases. It is associated with an unpleasant feeling in the mouth and throat.3 This complaint is more prevalent in menopausal women on medication, and is quite common also in those without disease or drug usage, unrelated to lowered salivary flow rates.4-6 We have recently studied the association between salivary calcium and sensation of OD,7 and found that salivary calcium level in menopausal women with OD is higher than in controls.

Calcium is of such major biological importance that at least 2 hormones and 1 vitamin are specifically concerned with the regulation of its metabolism. Calcium plays an indispensable part in the following physiological processes: mineralization of the skeleton, mineralization of teeth, control of excitability of membranes of nerves and transmission of impulses at synapses, control of impulse transmission at myoneural junctions, stabilization of cell membranes and adhesion between cells, activation of enzymes, and is involved in inflammation, secretion of hormones, synthesis of DNA in nuclei and of proteins in microsomes, cell division, and so forth.8 Parathyroid hormone (PTH) is

Objective. The aim of this study was to evaluate the correlation of severity of oral dryness (OD) with serum and saliva parathyroid hormone (PTH) and calcium levels, and to compare serum and stimulated whole saliva PTH and calcium between menopausal women with/without OD.

Methods. A case-control study was carried out in 76 (38 as case and 38 as control) selected menopausal women with/without OD conducted at the Clinic of Oral Medicine, Tehran University of Medical Sciences (TUMS). Xerostomia Inventory (XI) score was also used as an index of OD severity. Serum and saliva Ca++ concentrations were assessed colorimetrically by Arsenazo reaction. PTH concentration was analyzed by enzyme-linked immunosorbent assay (ELISA). Statistical analysis of Student t test and Spearman correlation was used.

Results. The mean saliva calcium and PTH concentrations and outputs, and serum PTH were significantly higher in the case group, compared with control. However, there were no significant differences in serum calcium concentration and stimulated saliva flow rate between groups. XI score correlated significantly with serum PTH (r = 0.387, P = .004), saliva concentration (r = 0.382, P = .002) and output (r = 0.346, P = .007) of PTH; and also with saliva concentration (r = 0.326, P = .013) and output (r = 0.315, P = .018) of calcium; but not with serum calcium and saliva flow rate.

Conclusion. OD severity correlated positively with serum and stimulated whole saliva PTH, and with saliva calcium levels in this group of menopausal women. Thus, salivary calcium and PTH levels appear associated with OD and menopause. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:806-810)
Table I. Questionnaire used for selection of subjects with xerostomia (oral dryness feeling)9

<table>
<thead>
<tr>
<th>Question</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Does your mouth feel dry when eating a meal?</td>
<td>Yes, No</td>
</tr>
<tr>
<td>2. Do you have difficulty swallowing any foods?</td>
<td></td>
</tr>
<tr>
<td>3. Do you need to sip liquids to help you swallow dry foods?</td>
<td></td>
</tr>
<tr>
<td>4. Does the amount of saliva in your mouth seem to be reduced most of the time?</td>
<td></td>
</tr>
<tr>
<td>5. Does your mouth feel dry at night or when you wake up?</td>
<td></td>
</tr>
<tr>
<td>6. Does your mouth feel dry during the daytime?</td>
<td></td>
</tr>
<tr>
<td>7. Do you chew gum or use candy to relieve oral dryness?</td>
<td></td>
</tr>
<tr>
<td>8. Do you usually wake up thirsty at night?</td>
<td></td>
</tr>
<tr>
<td>9. Do you have problems in tasting food?</td>
<td></td>
</tr>
<tr>
<td>10. Does your tongue burn?</td>
<td></td>
</tr>
</tbody>
</table>

Table II. The xerostomia inventory (XI)10

<table>
<thead>
<tr>
<th>Question</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I sip liquids to help swallow food.</td>
<td></td>
</tr>
<tr>
<td>2. My mouth feels dry when eating a meal.</td>
<td></td>
</tr>
<tr>
<td>3. I get up at night to drink.</td>
<td></td>
</tr>
<tr>
<td>4. My mouth feels dry.</td>
<td></td>
</tr>
<tr>
<td>5. I have difficulty in eating dry foods.</td>
<td></td>
</tr>
<tr>
<td>6. I suck sweets or cough lollies to relieve dry mouth.</td>
<td></td>
</tr>
<tr>
<td>7. I have difficulties swallowing certain foods.</td>
<td></td>
</tr>
<tr>
<td>8. The skin of my face feels dry.</td>
<td></td>
</tr>
<tr>
<td>9. My eyes feel dry.</td>
<td></td>
</tr>
<tr>
<td>10. My lips feel dry.</td>
<td></td>
</tr>
<tr>
<td>11. The inside of my nose feels dry.</td>
<td></td>
</tr>
</tbody>
</table>

**SUBJECTS AND METHODS**

**Subjects**

The Ethics Committee of Tehran University of Medical Sciences (TUMS), Iran, approved the study protocol. Informed consent was obtained from all participants.

A total of 120 menopausal women were asked to participate in a case-control study, conducted at the Clinic of Oral Medicine, TUMS, between 2007 and 2008. The participants were aged between 41 and 77 years, had not had a menstruation cycle for at least 24 months, and were not taking any medication at the time of the study. Smokers, obese patients (body mass index >24), patients with systemic diseases (including Sjogren’s syndrome), oral candidiasis, or with a bad oral health condition and periodontal disease were excluded. Of the 120 potential participants, 24 were excluded from the study based on these criteria (16 were eliminated owing to periodontal pocket depths more than 3 mm in multiple sites, 6 were excluded for obesity, and 2 for smoking). The remaining women were asked to answer a questionnaire with a list of symptoms associated with xerostomia (Table I). Thirty-eight answered affirmatively to at least one of the questions related to xerostomia,9 and formed the case group (mean ages ± SD 56.53 ± 7.27 years); in fact, all the participants in the case group answered affirmatively to at least 3 of the questions. Thirty-eight who did not answer affirmatively to any of the questions in Table I formed the control group (mean ages ± SD 58.24 ± 6.21 years). The remaining 18 were excluded in order to match case and control groups on the basis of age and duration of menopause. The 18 who were eliminated were done so without knowledge of the assay data; only the demographical factors were viewed with blinding to the assay data. Each participant also answered another questionnaire so that we could assess the severity of xerostomia (Table II). Xerostomia inventory (XI) score was determined as the severity of dry mouth feeling.10 The scores of responses were added to provide an XI score for each individual (the minimum possible score was 11 and the maximum possible score was 55).

**Sample collection**

Venous blood and saliva were collected simultaneously from each participant in the morning. Stimulated whole saliva was collected under resting conditions in a quiet room, between 9 AM and 12 PM, at least 2 hours after the last intake of food or drink. At the beginning and end of saliva collection, the time was recorded. For pre-stimulation, the women chewed a piece of paraffin of standard size. After 60 seconds of pre-stimulation, the participants were asked to swallow the saliva present in the mouth. Thereafter, whole saliva, stimulated by the same piece of paraffin, was collected in about 5 minutes into a preweighed, dry, deionized and sterilized plastic tube. The saliva-filled tubes were weighed, and the weight of the tubes subtracted. The flow rate was calculated in g/min, which is almost equivalent to mL/min.11

Blood specimens were obtained by venipuncture, collected in 10-mL glass vacuum tubes without additive, and allowed to clot. The blood and saliva were then centrifuged (2000g, 10 min) and the serum and supernatants of saliva were separated. Immediately after collection of saliva and serum, the specimens were
stored at −70°C for later determination of calcium and PTH concentrations.

**Calcium and PTH assays**

Serum and whole saliva Ca$^{2+}$ concentrations were assessed colorimetrically by Arsenazo reaction$^{12}$ using affiliated kits (ZiestChem Diagnostics, Tehran, Iran). PTH concentration was analyzed by enzyme-linked immunosorbent assay (ELISA) technology using commercially available kits (Biosource, Nivelles, Belgium).

**Statistical analysis**

For statistical analysis, the data are presented as a mean ± SEM. The 2-tailed Student unpaired $t$ test was used to compare saliva flow rate, saliva and serum PTH, and Ca$^{2+}$ levels between case and control groups.

The Spearman correlation analysis was used to identify any correlation between XI score and the salivary and serum components. $P$ less than .05 was considered statistically significant.

**RESULTS**

Student $t$ test showed that there was no significant difference in stimulated whole salivary flow rate (mL/min) between the case (0.37 ± 0.03, $n = 38$) and the control (0.35 ± 0.03, $n = 38$, $P = .64$) groups.

There were significant differences in serum concentration and stimulated whole saliva concentration and salivary output of PTH between the groups (Fig. 1, A). The serum ($P = .004; n = 76$) and the saliva PTH concentration ($P < .001; n = 76$) and also salivary output of PTH ($P < .001; n = 76$) were significantly higher in cases compared with the controls.

According to the catalog of manufacturer PTH kit, normal range of serum PTH concentration is 16 to 46 pg/mL. It is less than 6.4 pg/mL in hypothyroidism and higher than 106 pg/mL in hyperparathyroidism. In our study, ranges of serum PTH concentration were 5.4 to 117.0 pg/mL in the control group ($n = 1$, lower than 6.4 pg/mL) and 2, higher than 106 pg/mL) and 11.2 to 127.0 pg/mL in the case group ($n = 3$, higher than 106 pg/mL).

There was no significant difference in serum calcium concentration between the groups ($P = .8$; Fig. 1, B). However, the saliva Ca$^{2+}$ concentration was significantly higher in the case group than in the control ($P = .007$; Fig. 1, C). The individuals in the case group also showed a higher saliva Ca$^{2+}$ output than the individuals in the control group ($P = .003; n = 76$, Fig. 1, C).

Spearman correlations were performed to see if relationships existed between severity of oral dry feeling (XI score) and saliva and serum components ($n = 76$). XI score correlated significantly with serum ($r = 0.387$, $P = .004$) and saliva ($r = 0.382$, $P = .002$) PTH concentrations, saliva PTH output ($r = 0.346$, $P = .007$), and saliva calcium concentration ($r = 0.326$, $P = .013$) and output ($r = 0.315$, $P = .018$). However, there was no significant correlation between XI score and serum calcium concentration ($r = 0.007$, $P = .96$), and between XI score and stimulated whole saliva flow rate ($r = -0.052; P = .67$).

**DISCUSSION**

Oral dryness is a major complaint for many elderly individuals, and is strongly associated with the menopause. The exact mechanisms that cause sensation of OD in menopausal women have not been firmly established. In this study, the relationship between saliva and serum calcium and PTH levels, and OD in menopausal women was investigated. We found that serum and saliva PTH and salivary calcium levels are significantly higher in menopausal women suffering from oral dryness. It also appears that OD severity...
correlates with serum and stimulated levels of whole salivary PTH and salivary calcium.

Our results showed that differences in stimulated whole saliva flow rate between menopausal women, either with or without OD were not significant. Our data also indicated that there was no significant correlation between stimulated saliva flow rate and severity of oral dryness in menopausal women. The results of this study support the observation by many authors that dry mouth may exist in the presence of an apparently sufficient amount of stimulated saliva. It seems that OD may be unrelated to lowered stimulated whole salivary flow rates.

It has been shown that elderly people have significantly elevated salivary calcium compared with younger people. Consistent with our previous study, the results showed that subjects with OD had significantly higher stimulated whole saliva calcium concentration and output compared with the control group. In addition, a positive correlation between stimulated whole saliva calcium levels and severity of OD in menopausal women was also observed. Therefore, it is possible that PTH may also be an important factor associated with OD in menopausal women.

There are many factors and hormones that play a role in general calcium turnover. PTH is an important hormone in calcium turnover. Our data showed that patients with sensation of OD had significantly more concentration and output of stimulated whole saliva PTH. There was also a significant positive correlation between salivary PTH levels and OD in menopausal women. The results also indicate that serum PTH level is significantly higher in OD individuals, and a positive correlation exists between serum PTH levels and severity of OD. Therefore, it is possible that PTH may also be a cause in incidence of OD in menopausal women. This study, to our knowledge, is the first to show an association between a subjective complaint of dry mouth and an increase in serum and stimulated saliva PTH of menopausal women.

In the current study, salivary PTH levels were higher than those detected on the serum. The large amounts detected in saliva compared with serum suggest that PTH may be selectively concentrated in saliva, so that the salivary level was higher than the serum level.

We have demonstrated that serum and saliva 17β-estradiol were significantly lower in patients with OD, compared with control individuals in menopausal women. Furthermore it has been shown that the composition of saliva in menopausal women is estrogen dependent. In addition, the concentration of calcium in submandibular saliva is low during ovulation, when the estrogen level is high, and appears to be lower during pregnancy than in labor. A decrease in female hormones, especially 17β-estradiol, suppresses intestinal absorption of calcium, which leads to elevated concentrations of serum PTH and enhanced bone resorption, and may increase saliva calcium and PTH levels. Furthermore, estrogens prevent osteoporosis by inhibiting the stimulatory effects of certain cytokines on osteoclasts, so menopausal women have been considered at risk of periodontitis because of osteoporosis of the alveolar bone. Patients who have salivary calcium concentrations over the mean have more gingivitis than those with values lower than the mean. The suggested mechanism would be that low levels of estrogen in menopausal women with OD may affect general calcium and PTH turnover and their salivary concentrations. As estrogen induces calcium absorption in the intestine and precipitates it in bones, it seems that because of the low level of estrogen in menopausal women with OD, the plasma calcium level oscillates downward, causing elevation of serum PTH. As a result, PTH increases plasma calcium through its effect on bones. However, the plasma calcium level is regulated by many factors, which prevents the serum calcium level from increasing significantly in OD patients, therefore it is possible that elevated calcium is excreted by saliva or urine. These women may exhibit hypercalciuria and osteoporosis.

This study addressed the question of whether elevated salivary calcium level in menopausal women with OD was related to factors involved in calcium turnover such as PTH, so we did not assay the bone density or urinary calcium of participants. Serum estrogen levels were not measured that could have provided insight into the degree of estrogen deficiency as well as correlations between the calcium and PTH values obtained. Our research had not planned for day-to-day collection of saliva and serum sample, because we anticipated and experienced resistance from the study participants especially in the control group, so we took only one sample. There were other limitations to this study, e.g., this was a cross-sectional study and longitudinal studies may find similar or different results. Further studies are needed to evaluate whether menopausal women with OD suffer from osteoporosis or hypercalciuria, and the role of estrogen in these relationships.

**CONCLUSION**

OD severity correlated positively with serum and stimulated whole saliva PTH, and with saliva calcium levels in this group of menopausal women. Thus, salivary calcium and PTH levels appear associated with OD and menopause.
REFERENCES


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