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ORIGINAL ARTICLE

Unstimulated whole saliva 25-hydroxycholecalciferol in patients with xerostomia in menopausal women

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Abstract
Background and aim The aim of this study was to compare unstimulated whole saliva 25-hydroxycholecalciferol (25(OH)D) in menopausal women with and without oral dryness (OD) feeling, and evaluate the relationship between saliva 25(OH)D and severity of OD feeling.
Methods A case–control study was carried out on 70 selected menopausal women aged 41–77 years with or without OD feeling (35 as case and 35 as control) conducted at the Clinic of Oral Medicine, Tehran University of Medical Sciences. Unstimulated saliva samples were obtained by expectoration. Xerostomia inventory (XI) score was used as an index of OD feeling severity. The saliva 25(OH)D concentration was measured by ELISA. Statistical analysis of Student's t test and Spearman correlation was used.
Results The mean saliva 25(OH)D level was significantly higher in the case group (897.1 ± 128.9 pg/ml), compared with control (156.7 ± 43.4 pg/ml; $P < 0.05$). XI score correlated significantly with saliva 25(OH)D concentration ($r = 0.457$, $P < 0.001$).
Conclusions It seems that the level of salivary 25(OH)D concentration may be higher in menopausal women with OD feeling than in the control group, and there is a positive correlation between OD feeling severity and unstimulated whole saliva 25(OH)D.

Keywords 25(OH)D · Menopause · Oral dryness feeling · Unstimulated whole saliva

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Introduction

Saliva is a complex organic fluid which has multiple effects on maintaining a healthy environment in the oral cavity [1]. A simple effect is hydration of oral mucosa that provides a comfortable feeling in the mouth. Xerostomia is known as a subjective feeling of oral dryness that can be due to objective hyposalivation or merely an altered perception.

Generally, 14–46 % of people suffer from dry mouth. This is a common complaint among the elderly population, mostly caused by medication [2, 3]. Also, menopause has a strong correlation with oral dryness feeling [4–7]. To determine whether the reported xerostomia is objective or not, one should measure the saliva flow rate. Flow rates lower than 0.1 ml/min for unstimulated, and 0.7 ml/min for stimulated saliva, are attributed to the hypofunction of salivary glands [8–10].

According to available publications that mostly measured the stimulated whole saliva flow rate (stimulated by chewing or sour flavors), dry mouth feeling may be the result of changes in saliva composition, not flow rate changes [1, 4, 8, 11–13].

According to our previous studies, the level of salivary calcium concentration seems to be higher in menopausal women with xerostomia than in the control groups [11, 13]. Calcium is a component of many physiological processes such as synaptic transmission, stimulation potential of

skeletal or heart muscular cells, platelet aggregation, coagulation, hormones secretion and regulation of cell division. The transcellular route of calcium movement across the epithelial cell has been proposed to occur by facilitated diffusion, vesicular transport, and tunneling through the endoplasmic reticulum or a combination of three routes [14]. Its metabolism is dependent on a number of hormones or biologically active factors. With this background, a series of research studies were performed to evaluate the hormonal changes that might affect the calcium metabolism and OD feeling. It has been shown some hormones such as estrogen [12], progesterone [15, 16], parathyroid hormone [13] and cortisol [17] appear differ between the xerostomic women and the control subjects.

25 (OH) vitamin D [25(OH)D], a precursor of a hormonally active form, 1,25-dihydroxyvitamin D, which is the major circulating form of vitamin D and is present in saliva [18, 19] has a direct effect on isolated intestinal epithelial cells with regard to calcium handling [14]. It promotes calcium transport in duodena [20]. $1,25(OH)_2D$ increases the uptake of calcium from the intestinal cells against the ions gradient [21]. It has been shown that $1,25(OH)_2D$ influences the calcium-dependant salivary protein exocytosis, and vitamin D deficiency may reduce the parotid secretion [22, 23]. 25(OH)D is thought to exert its effect by a similar mechanism to that of the $1,25(OH)_2D$ [14, 20].

As saliva calcium and PTH levels appear to be high in menopausal women with OD and 25(OH)D contributes to calcium and PTH metabolisms, we decided to evaluate whether unstimulated whole saliva 25(OH)D level correlates with severity of OD feeling, and to compare unstimulated whole saliva 25(OH)D of menopausal women with and without oral dryness (OD) feeling.

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.

Seventy menopausal women were asked to participate in a case–control study, conducted at the Clinic of Oral Medicine, TUMS. The participants were aged between 41 and 77 years, had not had a menstruation cycle for at least 12 months, or hormone replacement therapy (HRT) for the previous 6 months; they were also not taking any medication at the time of the study. Smokers, diabetics, obese patients (body mass index >30 kg/m^2), patients taking xerogenic medical agents, patients with systemic diseases (including Sjogren's syndrome), oral candidiasis or with a bad oral health condition and periodontal disease (pocket depth more than 3 mm) were excluded.

The women were asked to answer a questionnaire with a list of symptoms associated with xerostomia (Table 1). Thirty-five answered affirmatively to at least one of the questions related to xerostomia [11], and formed the case group (mean age ± SD 56.6 ± 7.5 years); in fact, all the participants in the case group answered affirmatively to at least three of the questions. Thirty-five who did not answer affirmatively to any of the questions in Table 1 formed the control group (mean age ± SD 58.1 ± 6.2 years). The case and control groups were matched by age and duration of menopause.

Each participant also answered another questionnaire to assess the severity of xerostomia (Table 2). Xerostomia inventory (XI) score was determined as the severity of dry mouth feeling [12, 13]. 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). The responses to each question were marked as follows: 1 = never, 2 = hardly, 3 = occasionally, 4 = fairly often and 5 = very often.

Table 1 Questionnaire used for selection of subjects with xerostomia (oral dryness feeling)

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 and no

Table 2 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
Response options: never (scoring 1), hardly (2), occasionally (3), fairly often (4) and very often (5)

Saliva collection

Unstimulated whole saliva was collected under resting conditions in a quiet room, between 10 a.m. and 12 p.m., at least 90 min after the last intake of food or drink. The participants were asked to swallow the saliva pooled in the mouth. Then, whole unstimulated saliva was collected for about 5 min by spitting into a pre-weighed, dry, de-ionized and sterilized plastic tube. By subtracting the empty tube weight from the saliva filled one, saliva sample weight was determined to calculate the salivary flow rate. The flow rate was calculated in g/min, which is almost equivalent to ml/min. 25(OH)D output was calculated as its saliva concentration (pg/ml) multiplied by saliva flow rate (ml/min). The samples were clarified by centrifugation (2,500*g*, 10 min), and immediately stored at −20 °C for later determination of 25(OH)D.

Analysis of saliva

25(OH)D concentration was analyzed by ELISA technology, performed in duplicate using commercially available kits (DRG Instruments GmbH, Germany). Vitamin D-binding protein in samples has been precipitated with further treatment with the test. Therefore, total 25(OH)D (free and bound) concentration was assessed.

Statistical analysis

For statistical analysis, the data are presented as a mean ± SEM. The two-tailed student's unpaired *t* test was used to compare salivary 25(OH)D level between case and control groups. The Spearman correlation analysis was used to identify any correlation between XI score and the salivary 25(OH)D concentration. Receiver operating characteristic (ROC) analysis was used to determine cut-off point for salivary 25(OH)D between patients and healthy individuals. $P < 0.05$ was considered statistically significant.

Results

Student's unpaired *t* test showed that there was a significant difference between the case and control groups concerning unstimulated whole salivary flow rate (Fig. 1a). It was lower in the case (range 0.1–0.83 ml/min) than in the control group (range 0.1–1.04 ml/min) ($P < 0.01$).

There was a significant difference in unstimulated whole saliva 25(OH)D concentration between the case and control groups (Fig. 1b). They were higher in the case than in the control group ($P < 0.05$). There was also a significant difference in saliva 25(OH)D output between the case (242.4 ± 46.7) and control (52.1 ± 15.2) groups ($P < 0.05$).

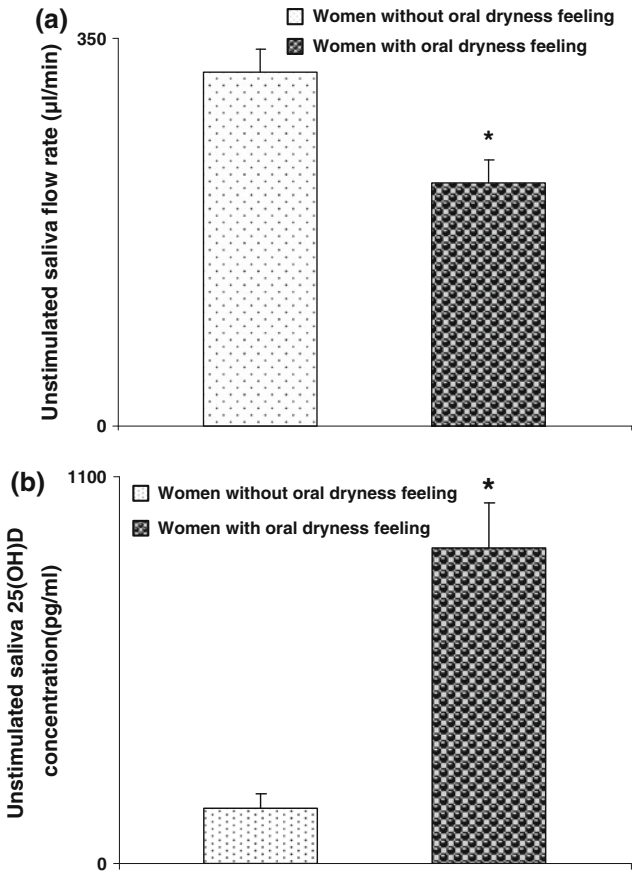

Fig. 1 Unstimulated whole saliva **a** flow rate and **b** 25(OH)D concentration (mean ± SEM) in menopausal women with/without oral dryness feeling. Data are expressed as mean ± SEM. *$P < 0.05$

Furthermore, an optimal cut-off point of 290 pg/ml between two groups was determined using ROC analysis. The AUC was 0.98 (asymptotic significance $P = 0.001$). In particular, the cut-off value was associated with 0.87 prognostic sensitivity and 0.96 negative predictive values.

The intra- and inter-assay CVs of the ELISA for 25(OH)D were 10.7 and 11.8 %, respectively. The limitation of the ELISA test requires that samples with 25(OH)D concentration greater than 400 ng/ml should be diluted and re-essayed.

Spearman correlation was performed to see if any relationship existed between severity of oral dryness feeling (XI score) and salivary concentration of 25(OH)D. There was a significant positive correlation between XI score and unstimulated whole saliva concentration of 25(OH)D (rs = 0.457, $P < 0.001$) and also between XI score and salivary output of 25(OH)D (rs = 0.433, $P < 0.01$). There were no significant correlations between age and salivary concentration of 25(OH)D (rs = 0.17, $P > 0.05$) or XI score (rs = 0.05, $P > 0.05$).

Discussion

Oral dryness is a major complaint for many elderly individuals, and is strongly associated with the menopause [6, 7, 24]. The exact mechanisms that cause sensation of OD in menopausal women have not been firmly established. In this study, the relationship between unstimulated whole saliva 25(OH)D and OD in menopausal women was investigated. We found that unstimulated saliva 25(OH)D level is significantly higher in menopausal women suffering from oral dryness. It also appears that OD severity correlates with salivary 25(OH)D.

We also found that unstimulated salivary flow rate was significantly lower in menopausal women with OD feeling in comparison with the women without OD feeling, which was in agreement with the other studies [7, 17]. As flow rate lower than 0.1 ml/min for unstimulated whole saliva is considered hypo-salivation, or true oral dryness [10], the measurements were higher than the lower limit of normal flow rate and could not be considered as true hypo-salivation. In addition, xerostomia can occur in spite of the existence of correct glandular function and normal salivary flow rates and the mean onset of xerostomia occurs when the total salivary flow rate is reduced to just less than 50 % of normal [25]. It can be concluded that menopausal women with OD feeling suffer from reduced salivary flow rate in unstimulated conditions.

In this study, we found a cut-off value of 290 pg/ml for salivary 25(OH)D between women with/without OD feeling. This cut-off level derived from ROC analysis may provide an appropriate threshold value for salivary 25(OH)D to be used as a tool for decision making about occurrence of xerostomia. However, comprehensive studies with adequate number of participants should be done to define standard reference values for salivary 25(OH)D.

The main advantage of saliva is that it presents non-invasive, stress-free and real-time repeated sampling where blood collection is either undesirable or difficult. Additionally, no special training or equipment is needed and subjects can conveniently collect samples themselves, if required [26]. A number of steroid hormones circulate in human plasma bound to specific binding globulins. Some of these steroids have also been shown to be present in saliva [27]. They can enter saliva by rapid diffusion through the acinar cells [28]. It has been suggested that the salivary concentration of them reflects the free level of plasma steroids not bound to specific globulin. Vitamin D metabolites [25(OH)D and $1,25(OH)_2D$] are thought to exert their effects by a similar mechanism to that of the steroid hormones [27].

In the liver, vitamin D is converted to 25(OH)D. It is converted in the cells of the proximal tubules of the kidneys to the more active metabolite $1,25\text{-}(OH)_2D$. The formation of $1,25\text{-}(OH)_2D$ in the kidneys, which is catalyzed by 1α-hydroxylase, is facilitated by parathyroid hormone (PTH) [21].

It has been shown that subjects with OD have significantly higher stimulated whole saliva calcium concentration and output compared with the control group [11]. There are many factors and hormones that play a role in general calcium turnover. Parathyroid hormone, $1,25\text{-}(OH)_2D$ and 17β-estradiol are important hormones in calcium turnover. We have previously demonstrated that OD severity correlates positively with serum and stimulated whole saliva PTH [13], and negatively with stimulated whole saliva 17β-estradiol [12]. 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 [29], and may increase saliva calcium and PTH levels. The suggested mechanism would be that low levels of estrogen in menopausal women with OD may affect general calcium, PTH and 25(OH)D 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, and production of $1,25(OH)_2D$ from kidneys. Since the saliva calcium, 25(OH)D and PTH concentrations are significantly high in menopausal women suffering from OD feeling, we suppose that the increase of 25(OH)D may be due to a decrease in production of $1,25(OH)_2$ D in the kidneys. Evaluating serum 25(OH)D, calcium, and PTH and serum and salivary of 1,25(OH)D concentrations were as limits of the study and would be reasonable topics for future studies and would help us to have a better understanding of the underlying pathophysiology.

Conclusion

It seems that the level of salivary 25(OH)D concentration may be higher in menopausal women with OD feeling than those without, and there is a positive correlation between OD feeling severity and unstimulated whole saliva 25(OH)D.

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Conflict of interest The authors declare that there is no conflict of interests.

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