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ORIGINAL ARTICLE

# Stimulated and unstimulated saliva progesterone in menopausal women with oral dryness feeling

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**Abstract** The aim of this study was to investigate the stimulated and unstimulated salivary progesterone in menopausal women with oral dryness (OD) feeling. A case–control study was carried out on 70 selected menopausal women aged 42–78 years with or without OD feeling (35 as cases with xerostomia and 35 as control without xerostomia), conducted at the Clinic of Oral Medicine, Tehran University of Medical Sciences (TUMS). Unstimulated and paraffin-stimulated saliva samples were obtained by expectoration. The salivary concentration of progesterone was determined with an enzyme immunoassay kit. Statistical analysis of the Student's *t* test was used. The mean stimulated and unstimulated whole saliva progesterone concentrations and unstimulated saliva flow, but not stimulated saliva flow rate, was significantly lower in the case than in the control. The results showed that subjects with dry mouth had decreased unstimulated saliva flow and salivary progesterone compared with those without dry mouth. Thus, salivary progesterone level appears associated with OD feeling in menopause.

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## Introduction

Menopause is a physiological process typically taking place in the fifth decade of life in women, involving permanent termination of menstruation [1]. It is the result of stable changes in the hormonal and reproductive functions of the ovaries. Menopause is accompanied by physical changes in the oral cavity [2]. Major oral symptoms of menopause are xerostomia and burning mouth [1]. Oral dryness (OD) feeling or xerostomia is a major complaint for many elderly individuals; it is a subjective feeling and does not reflect a dry mouth in up to one third of cases. It is coupled 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 [4–9].

Sex steroid hormones appear to play a considerable role in the physiology of the human oral cavity. It seems that oral soft tissues are sensitive to changes in female sex steroid blood levels. It has been shown that hormone replacement therapy (HRT) can reduce oral discomfort in menopausal women, further suggesting a role for female sex hormones in the maintenance of oral tissues [10–12]. We have recently studied the association between salivary 17β-estradiol and sensation of OD and found that salivary 17β-estradiol level in menopausal women with OD feeling is lower than in controls [8]. Subsequently, the aim of the present study was to examine the stimulated and unstimulated whole saliva progesterone concentration in menopausal women with or without OD feeling.

## Subjects and methods

### Subjects

The ethics committee of TUMS, Iran, approved the study protocol. Informed consent was obtained from all the participants.

Seventy menopausal women were asked to participate in a case–control study, conducted at the Clinic of Oral Medicine, Tehran University of Medical Sciences. The participants were aged between 42 and 78 years, had not had a menstruation cycle for at least 24 months, HRT for the previous 6 months, and were not taking any medication at the time of the study. Smokers, diabetics, obese patients (body mass index (BMI) >30 kg/m$^2$), patients with systemic diseases (including Sjogren's syndrome), oral candidiasis or with a bad oral health condition and periodontal disease were excluded. Thirty five participants who answered affirmatively to at least one of the questions related to xerostomia (Table 1) [7] formed the case group. In fact, all the participants in the case group answered affirmatively to at least three of the questions. Thirty five individuals who did not answer affirmatively to any of the questions in Table 1 formed the control group. The case and control groups were matched on age, duration of menopause, and BMI (Table 2).

### Saliva collection

Stimulated and unstimulated whole saliva were collected under resting conditions in a quiet room between 9 a.m. and 12 p.m., and at least 2 h after the last intake of food or drink. Unstimulated salivary samples were obtained by expectoration in the absence of chewing movements. For pre-stimulation, the women chewed a piece of paraffin of standard size. After 60 s 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 over a period of about 5 min. Stimulated and unstimulated whole saliva were collected 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 grams per minute, which is almost equivalent to milliliters per minute [13].

The samples were clarified by centrifugation (2,000 g; 10 min) and immediately stored at −20°C for later determination of progesterone concentration.

**Table 1** Questionnaire used for selection of subjects with xerostomia (OD 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** Characteristics of the cases and controls

| Characteristics | Women without OD feeling (control) | Women with OD feeling (case) |
|---|---|---|
| Age (years; mean ± SD) | 57.5±7.1 | 56.5±6.3 |
| Duration of menopause (years; mean ± SD) | 9.9±6.9 | 10.1±7.6 |
| BMI (kg/m$^2$; mean ± SD) | 24.8±2.9 | 25.9±2.0 |

### Analysis of saliva

Progesterone concentration was analyzed by ELISA technology using commercially available kits (DRG Instruments, GmbH, Germany).

### 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 flow rates and saliva concentration of progesterone between the case and control groups. $P<0.05$ was considered statistically significant.

## Results

The Student's unpaired *t* test showed that there was a significant difference between the case and control groups concerning unstimulated whole salivary flow rate. It was lower in the case than in the control group ($P<0.05$; Fig. 1b). However, the differences in stimulated saliva flow rate between the case and control group was not significant (Fig. 1a).

There were significant differences in stimulated ($P=0.002$; Fig. 2b) and unstimulated ($P=0.023$; Fig. 2a) whole saliva progesterone concentration between the groups. The progesterone concentrations were lower in the case group than in the control group.

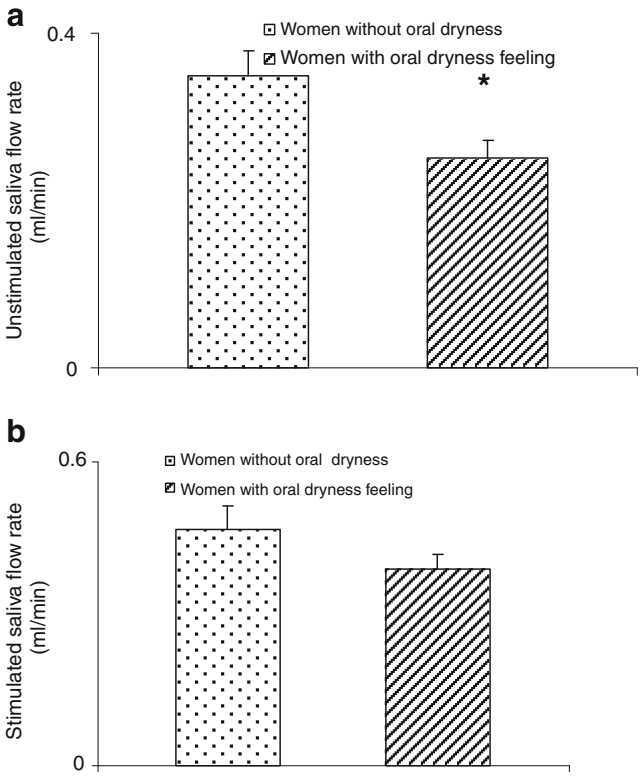


**Fig. 1** Unstimulated (**a**) and stimulated (**b**) whole saliva flow rate (means ± SEM) in menopausal women. *$P<0.05$

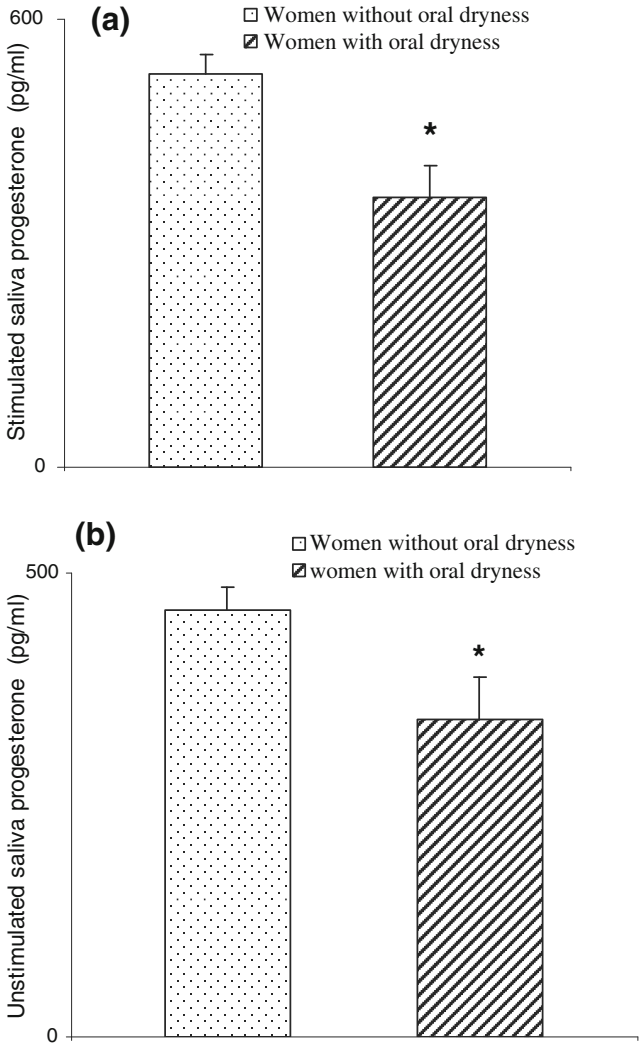


**Fig. 2** Stimulated (**a**) and unstimulated (**b**) whole saliva concentrations of progesterone (means ± SEM) in menopausal women. *$P<0.05$

## Discussion

Oral mucosa is known to be sensitive to changes in hormonal balance, especially to changes in female sex steroids [14]. In addition, OD feeling is a major complaint for many aged persons and strongly coupled with menopause [13–17]. The exact mechanisms that mediate the feeling of OD in menopausal women have not been determinedly recognized. In this study, the relationships between OD feeling and saliva progesterone and also flow rate levels in menopausal women were investigated, and we found that unstimulated saliva flow rate and stimulated and unstimulated saliva progesterone levels, but not stimulated saliva flow rate, are significantly lower in menopausal women suffering from OD.

Our results showed that differences in stimulated whole saliva flow rate between menopausal women, either with or without OD were not significant. 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 [6–9, 18]. It seems that OD may be unrelated to lowered stimulated whole salivary flow rates.

We 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 study by Yalcin et al. [16]. Saliva collected without any stimulus is a more reliable indicator of reduced salivary flow rate than the stimulated saliva [19]. It can be concluded that menopausal women with OD feeling suffer from reduced salivary flow rate in unstimulated conditions. However, it may alleviate in a stimulated state.

Data from the present study show that the mean concentration of stimulated and unstimulated whole saliva progesterone are significantly lower in patients with OD feeling compared with the control group. Therefore, it is possible that progesterone may be a cause in the 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 a decrease in stimulated and unstimulated saliva progesterone of menopausal women.

The female hormones influence many physiological and psychological functions. They exert their influence by

binding to specific intracellular receptors that belong to a superfamily of ligand-activated transcription factors that regulate growth, differentiation, and development of cells. The receptors are zinc finger proteins sharing a highly conserved DNA-binding domain that interact with hormone-response elements, and modulate the transcription of the target gene. Sequences for hormone-response elements have been identified in the promoters of many genes, including adhesion molecules, cytokines, growth factors, degradative enzymes, and extracellular matrix components. The formation and activity of sex hormone receptor complexes thereby play an important part in cell interactions, inflammation and connective tissue break down. Thus, while it is generally considered that the main targets of sex hormones are sex-associated tissues, they are clearly also capable of influencing gene activities in the cells of many other tissues [20].

The composition of saliva in menopausal women is sex hormone-dependent [21], and hormonal changes may affect the composition of saliva. Hormonal replacement therapy has been reported to reduce the complaints of dry mouth feeling resulting in improved oral well-being [7, 10, 11, 16, 21–24]. It was shown that during hormone treatment, the saliva flow is significantly increased and the complaints of dry mouth reduced [11]. In addition, the stimulated whole saliva flow rate is increased after combined estradiol and progesterone replacement therapy [11].

Some evidence suggests a positive relationship between ovarian hormone modifications and changes in the oral mucosa [10]. Previous studies have also suggested that oral symptoms are more prevalent in menopausal women than in women in other age groups [25]. It seems that steroid hormone withdrawal might be a cause of incidence of OD feeling in menopausal women. The results of this study support the observation by many authors of an association between oral discomfort and symptoms of sex hormonal deficiency seen in menopausal women.

In conclusion, we found that subjects with dry mouth had decreased unstimulated saliva flow and salivary progesterone compared with those without dry mouth. Thus, salivary progesterone level appears associated with OD feeling in menopause.

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

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