

Serum and Stimulated Saliva 25-hydroxy Vitamin D in Menopausal Women with Xerostomia

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ORIGINAL PAPER

SUMMARY

Aim: The aim of this study was to evaluate serum and stimulated whole saliva 25-hydroxy vitamin D (25-(OH)D) levels in women with/without oral dryness (OD) feeling. **Methods and materials:** A case-control study was carried out in 91 selected menopausal women aged 47 – 77 years with or without OD feeling (45 as case and 46 as control) conducted at the Clinic of Oral Medicine, Tehran university of medical sciences. Paraffin-stimulated saliva samples were obtained by expectoration. The serum and saliva 25-(OH)D concentrations were measured by ELISA. Statistical analysis of Student's t-test was used. **Results:** No significant difference was found in serum and stimulated whole saliva 25-(OH)D concentrations between the two groups. **Conclusions:** It seems that there is no relationship between OD feeling and stimulated whole saliva or serum 25-(OH)D in menopausal women.

Keywords: 25-hydroxy vitamin D; Menopause; Oral dryness feeling; Stimulated whole saliva; Serum

1. INTRODUCTION

Menopause is a unique section of the book of a woman's life and any woman who reaches the middle age reads this part (1). Physiologically it is characterized by cessation of menstrual cycles for 12 months or more. The significant drop of serum estrogen level may lead to the long list of different manifestation of menopause including xerostomia, oral dysesthesia, taste alterations, atrophic gingivitis, gingivostomatitis and etc (2, 3, 4).

Xerostomia is an annoying subjective feeling in the mouth and pharynx which may be unrelated to the decreased salivary flow rate in nearly one third of xerostomic patients (2, 5, 6, 7). Based on the author's past study, increased salivary calcium, estrogen and parathyroid hormone levels are objective finding significantly correlate to the subjective complaint of oral dryness in menopausal women (2, 5, 7). Calcium plays a major role in human physiology, from DNA synthesis to teeth mineralization and due to its importance there are hormones and biologically designated processes that keep calcium metabolism within normal physiological limits (8). Parathyroid hormone, calcitonin, 1,25-dihydroxycholecalciferol, estrogen, progesterone and cortisol are some hormones that regulate calcium metabolism (9). It is reasonable to assume that high salivary calcium level in saliva of menopausal women would correlate to changes of hormones contributing to the metabolism of this ion.

In the liver, vitamin D is converted to 25-(OH)D, and then to the more active metabolite 1,25-(OH)₂D in the kidneys. 1,25-(OH)₂D facilitates the entry of calcium to

the intestinal cells against the ions gradient (9). It is shown that 1,25-(OH)₂D has a role in calcium dependant salivary protein exocytosis and vitamin D deficiency may reduce parotid secretion (10, 11).

As saliva calcium level is high in menopausal women with OD and 1,25-(OH)₂D contribute in calcium metabolism, we decided to evaluate the serum and salivary levels of 25-(OH)D as precursor of 1,25-(OH)₂D in menopausal women with/without xerostomia.

2. MATERIAL AND METHODS

2.1. Subjects

The ethic committee of Tehran University of Medical Sciences (TUMS) Iran approved the study protocol. Informed consent was obtained from all participants. Menopausal women who had not had a menstruation cycle for at least 12 months were asked to participate in this case – control study, conducted at the Clinic of Oral Medicine, TUMS, in 2008 and 2009. Smokers, Obese patients (body mass index ≥ 30 kg/m²), patients taking xerogenic medical agents, patients with certain systemic diseases (including Sjogren's Syndrome), oral candidiasis or unfavorable oral health conditions such as poor oral hygiene and periodontal diseases (pocket depth more than 3mm), were excluded.

Based on these exclusion criteria, 91 women (47 – 77 years old) were submitted to the study. With the help of a questionnaire (Table 1) asking about symptoms associated with xerostomia, 45 subjects (mean age \pm S.D 55.4 \pm 7.1 years) who had at least one positive answer to questionnaire with a list of symptoms associated with xerostomia (Table 1) entered the case group (12), and another 46 women who

had no positive answers, formed the control group (mean age \pm S.D 56.3 ± 6.5 years).

2.2. Saliva collection

Stimulated whole saliva was collected under resting conditions in a quiet room, between 10 a.m. and 12 p.m., at least 90 minutes after the last intake of food or drink. Duration of saliva sample collecting was recorded by a stopwatch. Pre-stimulation were accomplished by chewing a piece of standard size paraffin. After 60 s of pre-stimulation, the participants were asked to swallow the saliva pooled in the mouth. Thereafter, whole stimulated saliva was collected for about 5 minutes 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 (ng / ml) multiplied by saliva flow rate (ml / min). The samples were clarified by centrifugation (2500 g, 10 min) and immediately stored at -20°C for later determination of 25-(OH)D .

2.3. Analysis of saliva

25-(OH)D concentration was analyzed by ELISA technology using commercially available kits (DRG Instruments GmbH, Germany).

2.4. Statistical analysis

For statistical analysis, the data are presented as a mean \pm S.E.M. The two-tailed Student's unpaired t-test was used to compare salivary flow rates, serum and saliva 25(OH)D levels between case and control groups. $P < 0.05$ was considered statistically significant.

3. RESULTS

There was no significant difference between case and control groups concerning stimulated salivary flow rate (0.38 ± 0.03 vs. 0.34 ± 0.03 ; $P = 0.17$ respectively). No significant difference was found between case and control groups regarding serum 25-(OH)D concentration (Fig 1a) and also stimulated salivary 25-(OH)D concentration or output (Fig 1b).

4. DISCUSSION

Subjective feeling of oral dryness in menopausal women was the matter of a great deal of studies (7,13-17). Oral dryness (OD) is upsetting specially while eat-

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 awakening?
 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 1. Questionnaire used for selection of subjects with xerostomia (oral dryness feeling).

ing dry foods. Since it appears not to be related with the stimulated salivary flow rates as we showed also in this study, a reasonable approach to find the underlying cause is to compare the composition of saliva in menopausal women with or without OD. Our previous studies reveal a higher concentration of calcium in serum and saliva of menopausal women with OD than control subjects (5, 7). One of the important hormones that regulate the calcium homeostasis in human body is $1,25\text{-(OH)}_2\text{D}$. It helps the absorption of calcium ions from intestinal mucosa in response to daily metabolic needs for calcium (9). A drop in serum calcium concentration causes increased parathyroid hormone (PTH) which in turn, stimulates the synthesis of $1,25\text{(OH)}_2\text{D}$ from renal tissue. In the current study a higher concentration of serum 25-(OH)D was found in subjects with OD in comparison with the control group, but it was not statistically significant. This way, it may come to one's mind that serum calcium concentration should be within normal limits too; which is in agreement with previous studies on serum calcium levels in menopausal females with dry mouth (2, 5).

As we know, PTH is also contributing in calcium homeostasis, cooperating $1,25\text{-(OH)}_2\text{D}$. PTH is an stimulator of alpha-1 hydroxylase enzyme, which synthesizes $1,25\text{(OH)}_2\text{D}$ from its precursor 25-(OH)D (9). Therefore, increased levels of PTH may lead to increased production and release of $1,25\text{-(OH)}_2\text{D}$. In contrast, cortisol excess, leads to decreased intestinal absorption of calcium and inhibition of renal calcium re-absorption (9, 18, 19). It has been suggested that reduced intestinal absorption may partly be due to decreased $1,25\text{-(OH)}_2\text{D}$ synthesis (18). Increased PTH and cortisol serum concentration in menopausal women with

OD has been shown in some studies (6, 7, 20). As we know that synthesis of $1,25\text{-(OH)}_2\text{D}$ is stimulated by PTH and inhibited by increased levels of cortisol, it is not unexpected that serum 25-(OH) $_2$ D concentration shows no significant changes in menopausal women with OD as a result of counteraction between increased PTH and cortisol concentrations.

Glijer et al (10) in their study carried on the effect of vitamin D deficiency on the parotid sa-

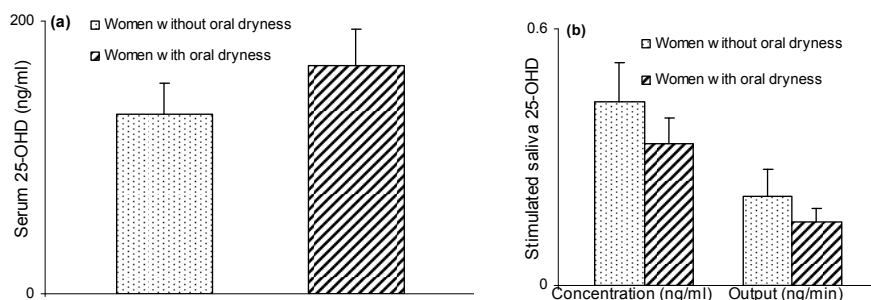


Figure 1. Serum (a) and Stimulated saliva concentration and output (b) of 25-OH D in case and control groups

liva of rats, indicated that calcium entry to the acinar cells is independent of vitamin D but exertion of water and electrolytes is dependant to vitamin D and mediated by calcium. Later the results were confirmed by another study, done by Peterfy and colleagues (21). Actually parotid is an vitamin D dependant exocrine organ that needs the vitamin for the synthesis of proteins contribute in maintenance and circulation of intracellular calcium (10, 22, 23). Hayakawa et al showed that microsomal Ca⁺⁺-pump activity in rat parotid gland decreased by the administration of vitamin D, in vitamin D deficient rats which indicate the role of vitamin D in maintenance of intra cellular calcium turnover (24).

Stimulated saliva is a watery solution, mostly secreted from parotid. Finding no significant difference between stimulated salivary flow rates in case and control groups of this study may be attributed to the sufficiency of calcium and vitamin D in parotid glands that allow the normal production of stimulated serous saliva.

The current study, to best of our knowledge, is the first one that investigates the relation of salivary and serum 25-(OH)D in menopausal women with or without oral dryness feeling.

5. CONCLUSION

It seems that the level of serum and saliva 25-(OH)D may be maintained in normal limits and have no relationship with the oral dryness feeling in menopausal women.

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