Original Article

Comparing Serum and Salivary Levels of Vitamin D in Patients with Recurrent Aphthous Stomatitis and Healthy Individuals

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KEY WORDS
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ABSTRACT

Statement of the Problem: Recurrent aphthous stomatitis (RAS) is the most prevalent ulcerative condition of the oral mucosa. Many studies have emphasized on immunologic factors as the reason of inducing RAS; however, the exact etiologic cause of RAS has not been identified yet. Vitamin D has an endocrine function and regulatory effects on the immune system. It has potential therapeutic effects on autoimmune diseases, psoriasis, and neoplasms. Vitamin D deficiency has been detected in some autoimmune diseases such as rheumatoid arteritis.

Purpose: The aim of the present study was to compare the serum and salivary levels of vitamin D in patients with RAS and healthy individuals.

Materials and Method: In this cross sectional study, patients with RAS, referring to the Department of Oral Medicine, Tabriz Faculty of Dentistry, were evaluated after taking medical history, clinical examinations, and completing an informed consent form. The serum and salivary vitamin D levels were compared between case (n=26) and control (n=26) groups.

Results: The mean serum vitamin D levels in the case and control groups were 33.0.7±12.41 and 50.89±9.30 (ng/dL), respectively, with a statistically significant difference ($p< 0.001$). On the other hand, the mean salivary vitamin D levels in the case and control groups were 17.36± 8.01 and 20.79±6.31 (ng/dL), respectively, with no statistically significant difference ($p= 0.09$). In addition, the correlation between the serum and salivary levels of vitamin D was 56%, being statistically significant ($p< 0.001$).

Conclusion: The serum levels of vitamin D in patients with RAS were significantly less than that in healthy individuals; however, there were no significant differences in salivary vitamin D levels between patients with RAS and healthy individuals. In addition, there was a significant and positive correlation between serum and salivary levels of vitamin D in all patients.

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Introduction

Recurrent aphthous stomatitis (RAS) is the most common ulcerative condition of the oral cavity mucosa, which is characterized by round and painful ulcers with an erythematous area surrounding the ulcers. The central areas of the ulcers are covered with a yellowish-brown pseudo membrane. [1-2] Generally, RAS appears with a burning sensation 2–48 hours before the ulcers appear on the buccal and labial mucosa and on the tongue. Involvement of the keratinized areas of the oral cavity such as the mucosa of the palate and gingiva is less common. [1-3]

Numerous factors such as trauma and microbial agents have been reported as possible causes inducing RAS in genetically susceptible individuals. The role of genetic is the best-defined cause of RAS. [4] Other causes include nutritional factors (such as a deficiency of folate and vitamin B), immunologic factors, psychosocial stresses, and food allergies. Nevertheless, the exact mechanism of RAS has not yet been clearly clarified. Currently, a large number of studies accentuate the immunologic factors as the etiological cause for inducing RAS. [5-10] Humoral immune reactions have an imperative role in immunopathogenesis of aphthous stomatitis. This reaction possibly occurs subsequent to cell-mediated immune reactions. [8-9] An imbalance has been reported in the immune system of patients with RAS compared to healthy individuals. [10]

Vitamin D has an endocrine function on immune system cells and is responsible for anti-inflammatory reactions and regulatory effects on the immune system. [11] In addition, it has potential therapeutic effects on autoimmune diseases, psoriasis, and neoplasms. [12] The basic mechanisms for the role of vitamin D in autoimmune diseases are still not very clear. Its deficiency has been reported in some autoimmune diseases such as rheumatoid arteritis, systemic lupus erythematosus, Type I diabetes mellitus, multiple sclerosis, inflammatory diseases of the intestine, thyroid autoimmune diseases (such as Hashimoto and Graves diseases), and autoimmune gastritis. [11-12]

Salivary test is a laboratory diagnostic test for identification of hormonal, immunologic, inflammatory, and infectious markers. Steroid hormones such as cortisol, genetic markers, and proteins such as enzymes and antibiotics are secreted into the saliva and can be measured. Several studies have reported similarities between the concentrations of some markers in the saliva and serum and some of these markers can be measured as diagnostic laboratory tests. [13-15]

Collecting venous blood sample is the most commonly used technique for the evaluation of blood markers. However, collecting venous blood sample and its transportation carries the risk of contamination. In addition, collecting salivary samples is a non-invasive technique and is easily accepted by patients. [13-14]

Considering the relationship between some of these markers in the saliva and serum and since there were no studies evaluating the salivary vitamin D levels in patients with RAS up to date, the present study was conducted to determine and compare salivary and serum levels of vitamin D in patients with RAS and healthy individuals.

Materials and Method

The protocol of the current cross sectional study was approved by the Ethics Committee of Tabriz University of Medical Sciences under the code IR.TBZMED.REC. 1395.419. The patients were thoroughly informed about the study. They signed informed consents before being included in the study. During the study, no therapeutic interventions were made and the patients' data were kept confidential. No costs were inflicted on patients for carrying out laboratory tests.

To determine the sample size, the results of a study by Khabbazi *et al.* [16] were employed by considering a 30-unit difference in the serum and salivary vitamin D levels between the patients and the healthy individuals. The sample size was calculated (n=27) in each group at α=0.05 and a study power of 80%.

The inclusion criteria were affliction with RAS based on clinical manifestations (individuals who have aphthous lesions in their oral cavity at least three times each year), age range of 18–60 years.

The exclusion criteria were use of medications that decrease the serum levels of vitamin D (including vitamin D supplements, calcium and calcium channel blockers), active infections (such as hepatitis, HIV ,and tuberculosis), use of medications for the treatment of RAS in recent two months, conditions that change vitamin D levels (such as hyperthyroidism), pregnancy, chronic renal and hepatic diseases, malabsorption, con-

ditions that produce ulcers similar to aphthous ulcers (including cyclic neutropenia, Behçet's syndrome and gastrointestinal diseases such as Crohn disease and ulcerative colitis), use of medications (such as streptomycin, tetracycline and gold salts, which result in lichenoid drug reactions that are mistaken with aphthous lesions). [16]

In the present study, 26 patients with RAS, referring to the Department of Oral Medicine, Tabriz Faculty of Medicine and 26 healthy individuals were evaluated after taking medical histories, clinical examinations and completing the required checklists, and after the individuals signed informed consent forms. One patient was excluded from the study due to lack of cooperation to submit a salivary sample. A total of 5 mL of blood sample was taken from each patient; in addition, a 2-mL salivary sample was collected from each subject. Vitamin D total (25-hydroxy vitamin D) kit was used with the electrochemiluminescence technique to determine and compare salivary and serum levels of vitamin D between the healthy individuals and those with RAS.

Data were analyzed with independent samples t-test, using SPSS 17. Statistical significance was set at $p< 0.05$.

Results

The mean age of the subjects was 39.80±11.74 years, with mean ages of 38.80±12.02 (an age range of 21–58 years) and 40.80±11.60 (an age range of 22–55 years) in the case and control groups, respectively. Based on the results of independent t-test, there was no statistically significant difference between the mean age of the two groups ($p= 0.54$, CI=-8.58 to 4.58).

33(63.5%) individuals were male and 19(36.5%) individuals were female. 16(61.5%) and 17(65.4%) individuals were male and 10(38.5%) and 9(34.6%) were female in the case and control groups, respectively. Based on the results of Chi squared test, there was no statistically significant difference between the two groups considering the gender ($p= 0.77$).

The mean serum level of vitamin D in the subjects was 41.98±14.10, with 33.07±12.41 and 50.89±9.30 (ng/dL) in the case and control groups, respectively (Figure 1).

In addition, the mean salivary level of vitamin D in the individuals was 19.07±7.35, with 17.36±8.01 and 20.79±6.31 (ng/dL) in the case and control groups, respectively (Figure 2).

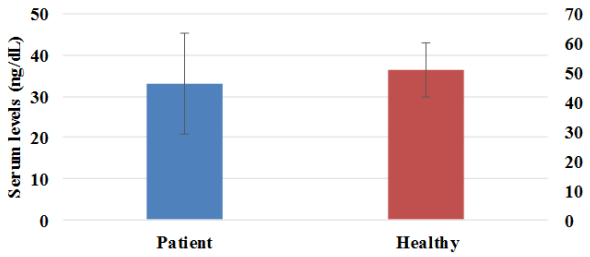

Figure 1: Comparison of the mean serum levels of vitamin D between the patients with RAS and healthy controls

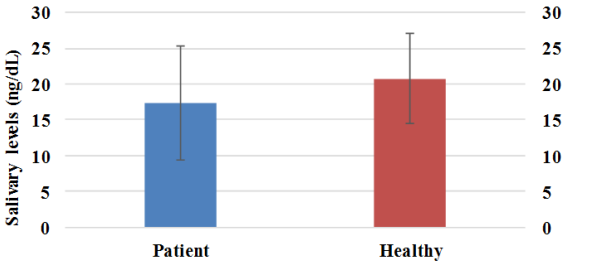

Figure 2: Comparison of the mean salivary levels of vitamin D between the patients with RAS and healthy controls

Normal distribution of data was analyzed with Kolmogorov-Smirnov test, which showed that all the serum and salivary vitamin D levels and the individuals' ages were distributed normally ($p> 0.05$). Hence, independent t-test was employed to compare these values between the patients with RAS and healthy individuals.

Based on the results of independent t-test, there was a 17-unit difference in the mean of serum levels of vitamin D between the subjects with RAS and healthy controls, which was statistically significant ($P<0.0001$, CI= 11.711 to 23.929).

Based on the results of independent t-test, there was a 3-unit difference in the mean of salivary vitamin D levels between the subjects with RAS and the healthy controls, with a higher level in healthy controls; however, the difference was not statistically significant ($p= 0.09$, CI= -7.447 to 0.587).

Pearson's correlation coefficient was used to evaluate the correlation of salivary and serum levels of vitamin D in subject with RAS and healthy controls. The correlation was reported to be 56%, which was significant ($p<0.001$). Considering the positivity of Pearson's correlation coefficient and the significant of level of <0.001, it was concluded that the salivary level of vita-

min D increased with an increase in its serum level.

Discussion

Based on the results, there was no significant difference in mean age and gender between case and control groups. Since age and gender can be considered as a factor affecting the serum and salivary levels of vitamin D, the absence of a significant difference between the two groups might be considered as the absence of the effect of age and gender on measurements. The mean serum level of vitamin D in the healthy controls was higher than that in patients' group and it was statistically significant. This finding is consistent with the results of similar studies, which have shown that the serum levels of vitamin D in RAS patients were less than that in healthy individuals. Khabbazi *et al.* [16] showed that patients with RAS exhibited lower vitamin D serum levels compared to healthy individuals. A similar study by Lalla *et al.* [17] showed that treatment with multivitamin, compared to placebo, resulted in a significant decrease in the number of RAS attacks.

On the other hand, the relationship between the serum levels of vitamin D and other autoimmune diseases indicates the important role of this vitamin in the prevention of such conditions. For example, in the study enrolled by Khabbazi *et al.,* [18] the results showed that the mean serum level of vitamin D in patients with Behçet's disease was lower than that in control individuals. In addition, vitamin D deficiency was more prevalent in patients compared to healthy controls. [18]

Hamzaoul *et al.*, [19] have also reported a lower serum level of vitamin D in patients with Behçet's disease compared to healthy individuals. In addition, subjects with active Behçet's disease exhibited a lower serum level of vitamin D compared to those with inactive Behçet's disease. [19]

In a study conducted by Watad *et al.,* [20] the serum levels of vitamin D in patients with lupus were lower than that in healthy controls. Vondra [21] represented that vitamin D deficiency can be considered as one of the risk factors for autoimmune thyroiditis. Stagi *et al.*, [22] reported the vitamin D levels in patients with PFAPA syndrome were significantly lower than the control group. In addition, following vitamin D replacement in case group, there was a significant decrease in disease symptoms and signs. [22]

There was a positive and significant correlation between salivary and serum levels of vitamin D in the present study; in other words, the salivary level of vitamin D increased with an increase in serum vitamin D level. In all participants, the salivary levels of vitamin D (19.07±7.35) were about 50% lower than the serum levels (41.10±14.10). Although there was a 3-unit difference in the means of salivary vitamin D levels between the subjects with RAS and the healthy controls, with a higher level in healthy controls, the difference was not statistically significant. Saliva is mostly secreted from parotid gland and previous studies reported that parotid is a vitamin D dependent exocrine organ. [23-24] Glijer *et al.* [24] conducted a study on the effect of vitamin D deficiency on parotid saliva of rats .They reported that exertion of water and electrolytes was dependent on vitamin D[24]. Hayakawa *et al.* [25] revealed that microsomal Ca++ pump activity in parotid gland of rats was reduced after administration of vitamin D. Agha-Hosseini *et al.* [23] evaluated serum and stimulated salivary vitamin D in menopausal women with xerostomia. They concluded that the lack of statistical difference between stimulated salivary flow rates in case and control groups might be attributed to the sufficiency of vitamin D in parotid glands that permit the normal secretion of stimulated serous saliva. [23] Therefore, given the normal mean vitamin D serum level in the present study population (> 30 ng / dL [26]) and the adequacy of vitamin D in the parotid gland, normal secretion of saliva is expected. Perhaps this issue justifies the reduction of the vitamin D levels in saliva in contrast to its serum levels and consequently the absence of statistical difference between salivary vitamin D in case and control groups.

No study has evaluated the salivary levels of vitamin D in patients with RAS up to date. However, similar studies with different purposes have been conducted. Karincaoglu *et al.* [27] determined and compared the salivary and serum levels of antioxidative enzymes in patients with RAS and healthy controls and reported no significant difference in the salivary concentrations of these enzymes, including superoxide dismutase and catalase.

In a different study performed by Saral *et al.,* [28] the serum levels of antioxidative vitamins (A, C and E) and lipid peroxidases were evaluated in patients with

RAS. The serum and salivary levels of vitamins A, C and E were lower in patients with RAS compared to healthy controls. The serum and salivary levels of malodialdehyde (MDA) was significantly higher in patients with RAS compared to healthy controls [28]. Aziz *et al.* [9] determined the total salivary capacity of antioxidants in patients with RAS and reported no significant differences between the patients with RAS and healthy individuals.

Numerous factors such as trauma, microbial agents, nutritional factors (deficiency of folate and vitamin B), immunologic factors, psychosocial stresses, and food allergies have been reported as possible causes of RAS, particularly in genetically susceptible individuals. Though the exact mechanism of RAS has not been clearly explicated yet, several studies highlight the immunological elements as the major etiologic factors for inducing RAS. [5-7] The potential role of vitamin D in modifying immune responses and discovery of vitamin D receptors (VDRE) on macrophages, dendritic cells, and active B and T lymphocytes has been associated with the discovery of the role of $1,25(OH)_2D_3$ in the proliferation, differentiation and function of these cells. These cells can express cyp27B1 gene for immune responses. [11, 30] The vital role of $1,25(OH)_2D_3$ in the regulation of the immune response via prevention of some autoimmune diseases has been shown in previous studies. [11, 30] Therefore, given the regulatory effect of vitamin D on the immune system and based on the results of the present study, it can be concluded that the lower serum levels of vitamin D might be considered as possible factor for inducing RAS, especially in genetically susceptible patients. Nevertheless, for definitive conclusions, further studies are recommended with larger population and considering the relationship between vitamin D and the severity and frequency of lesions. The results of such studies might help prevent and find new treatment modalities with the use of vitamin D supplements so that the antioxidative system in patients with RAS can be strengthened.

Conclusion

The serum levels of vitamin D in subjects with RAS were significantly less than that in healthy individuals. There was a significant and positive correlation between vitamin D serum and salivary levels. There were no significant differences in salivary vitamin D levels between patients with RAS and healthy individuals.

Conflict of Interest

None declared.

References

[1] Akintoye SO, Greenberg MS. Recurrent aphthous stomatitis. Dent Clin North Am. 2014; 58: 281-297.

[2] Cui RZ, Bruce AJ, Rogers RS 3rd. Recurrent aphthous stomatitis. Clin Dermatol. 2016; 34: 475-481.

[3] Stoopler ET, Musbah T. Recurrent aphthous stomatitis. CMAJ. 2013; 185: E240.

[4] Akintoye SO, Greenberg MS. Recurrent Aphthous Stomatitis. Dental clinics of North America. 2014; 58: 281-297.

[5] Preeti L, Magesh K, Rajkumar K, Karthik R. Recurrent aphthous stomatitis. J Oral Maxillofac Pathol. 2011; 15: 252-256.

[6] Chattopadhyay A, Shetty KV. Recurrent aphthous stomatitis. Otolaryngol Clin North Am. 2011; 44: 79-88.

[7] Gallo Cde B, Mimura MA, Sugaya NN. Psychological stress and recurrent aphthous stomatitis. Clinics (Sao Paulo). 2009; 64: 645-648.

[8] Scully C, Porter S. Oral mucosal disease: recurrent aphthous stomatitis. Br J Oral Maxillofac Surg. 2008; 46: 198-206.

[9] Slebioda Z, Szponar E, Kowalska A. Etiopathogenesis of recurrent aphthous stomatitis and the role of immunologicaspects: literature review. Arch Immunol Ther Exp (Warsz). 2014 Jun; 62: 205-215.

[10] Chavan M, Jain H, Diwan N, Khedkar S, Shete A, Durkar S. Recurrent aphthous stomatitis: a review. J Oral Pathol Med. 2012; 41: 577-583.

[11] Turhanoğlu AD, Güler H, Yönden Z, Aslan F, Mansuroglu A, Ozer C. The relationship between vitamin D and disease activity and functional health statusin rheumatoid arthritis. Rheumatol Int. 2011; 31: 911-914.

[12] D'Aurizio F, Villalta D, Metus P, Doretto P, Tozzoli R. Is vitamin D a player or not in the pathophysiology of autoimmune thyroid diseases? Autoimmun Rev. 2015; 14: 363-369.

[13] Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta. 2007; 383: 30-40.

[14] Lima DP, Diniz DG, Moimaz SA, Sumida DH, Okamoto

AC. Saliva: reflection of the body. Int J Infect Dis. 2010; 14: e184-e188.

[15] Venkatapathy R, Govindarajan V, Oza N, Parameswaran S, Pennagaram Dhanasekaran B, Prashad KV. Salivary creatinine estimation as an alternative to serum creatinine in chronic kidneydisease patients. Int J Nephrol. 2014; 2014: 742724.

[16] Khabbazi A, Ghorbanihaghjo A, Fanood F, Kolahi S, Hajialiloo M, Rashtchizadeh N. A Comparative Study of Vitamin D Serum Levels in Patients with Recurrent Ap hthous Stomatitis, The Egyptian Rheumatologist. 2014; 37: 133–137.

[17] Lalla RV, Choquette LE, Feinn RS, Zawistowski H, Latortue MC, Kelly ET, et al. Multivitamin therapy for recurrent aphthous stomatitis: a randomized, double-masked, placebo-controlled trial. J Am Dent Assoc. 2012; 143: 370-376.

[18] Khabbazi A, Rashtchizadeh N, Ghorbanihaghjo A, Hajialiloo M, Ghojazadeh M, Taei R, et al. The status of serum vitamin D in patients with activeBehcet's disease compared with controls. Int J Rheum Dis. 2014; 17: 430-434.

[19] Hamzaoui K, Ben Dhifallah I, Karray E, Sassi FH, Hamzaoui A. Vitamin D modulates peripheral immunity in patients with Behçet's disease. Clin Exp Rheumatol. 2010; 28(4 Suppl 60): S50-S57.

[20] Watad A, Neumann SG, Soriano A, Amital H, Shoenfeld Y. Vitamin D and Systemic Lupus Erythematosus: Myth or Reality? Isr Med Assoc J. 2016; 18: 177-182.

[21] Vondra K. Vitamin D and autoimmune thyroid diseases. Vnitr Lek. Fall; 62(9 Suppl 3): 121-125.

[22] Stagi S, Bertini F, Rigante D, Falcini F. Vitamin D levels and effects of vitamin D replacement in childrenwith periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. Int J Pediatr Otorhinolaryngol. 2014; 78: 964-968.

[23] Agha-Hosseini F, Mirzaii-Dizgah I, Mirjalili N. Serum and stimulated saliva 25-hydroxy vitamin D in menopausal women with xerostomia. Materia Socio-Medica. 2011; 23: 4.

[24] Glijer B, Peterfy C, Tenenhouse A. The effect of vitamin D deficiency on secretion of saliva by ratparotid gland in vivo. J Physiol. 1985; 363: 323-334.

[25] Mitsuo H, Hidefumi A, Naoto T, Yoshimitsu A, Hisashi T. Vitamin D-mediated decrease of Ca2+-pump activity in the rat parotid gland. International Journal of Biochemistry. 1983; 15: 1175-1178.

[26] Holick MF. Vitamin D status: measurement, interpretation, and clinicalapplication. Ann Epidemiol. 2009; 19: 73-78.

[27] Karincaoglu Y, Batcioglu K, Erdem T, Esrefoglu M, Genc M. The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. J Oral Pathol Med. 2005; 34: 7-12.

[28] Saral Y, Coskun BK, Ozturk P, Karatas F, Ayar A. Assessment of salivary and serum antioxidant vitamins and lipidperoxidation in patients with recurrent aphthous ulceration. Tohoku J Exp Med. 2005; 206: 305-312.

[29] Azizi A, Shah Siah S, Madhani A. Comparison of amount of salivary total antioxidant in patients with recurrent aphtous stomatitis. Journal of Dental Medicine. 2012; 25: 14-18.

[30] Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013; 5: 2502-2521.