

Serum levels of vitamin D in patients with recurrent aphthous stomatitis

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Conflict of interest

None declared

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Abstract

Background. Recurrent aphthous stomatitis (RAS) is one of the most common recurrent ulcerations in the oral mucosa, the etiology of which has not been elucidated; the immune system dysfunction may play an important role in the pathogenesis of RAS. The anti-inflammatory and regulatory role of vitamin D in the functioning of the immune system is well-documented.

Objectives. This study aimed to evaluate and compare the serum levels of vitamin D between patients with RAS and healthy controls.

Material and methods. In this case–control study, 43 patients with minor RAS and 43 healthy controls were included. Two groups were matched in terms of age and sex. Blood samples were obtained from all participants. The serum levels of vitamin D were measured with the use of the enzyme-linked immunosorbent assay (ELISA) in patient and control groups. The data was analyzed using the SPSS for Windows software, v. 17.0, with the independent samples *t* test and the Mann–Whitney test. A *p*-value of <0.05 was considered statistically significant.

Results. The mean serum level of vitamin D in the control group was significantly higher than that in the case group (22.59 ± 16.06 ng/mL vs 13.19 ± 8.19 ng/mL, respectively; $p = 0.002$).

Conclusions. The serum levels of vitamin D are lower in patients with RAS in comparison with healthy controls.

Key words: vitamin D, immune system, recurrent aphthous stomatitis

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Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common recurrent ulcerations in the oral mucosa. It mostly occurs in societies with a high socioeconomic status and affects 5–25% of the population.¹ The lesions are self-limiting. Pain and oral dysfunction are the most important complications of chronic inflammatory lesions.²

Recurrent aphthous stomatitis is classified into 3 clinical forms: minor; major; and herpetiform ulcerations, with the minor form being the most common.² Multiple factors, including genetic and hematologic disorders, immunological deficiency, stress, and trauma, are known as predisposing for RAS.^{3,4} Many researchers have evaluated the role of the immune system dysfunction in aphthous stomatitis. According to their studies, at the early stages of RAS, CD4⁺ T lymphocytes are predominantly involved, and at the ulcerative stages – CD8⁺ T lymphocytes.⁵ An increase in Th1 pro-inflammatory cytokines and a decrease in Th2 anti-inflammatory cytokines in the pathogenesis of RAS are confirmed.^{6,7} Recent studies have shown the role of vitamin D in skeletal disorders, cardiovascular diseases, cancers, and autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus and type 1 diabetes mellitus.^{8–10} The anti-inflammatory and regulatory role of vitamin D in the functioning of the immune system is well-documented.⁹ Vitamin D receptor (VDR) is found in most cells of the immune system, including antigen-presenting cells (APCs). Serum 25(OH)D is considered the most accurate marker for vitamin D status. It is well known that the measurement of 25(OH)D levels is the best indicator of the vitamin D status in humans.^{10,11}

1,25(OH)-vitamin D3 is the metabolically active form of vitamin D which inhibits the maturation of dendritic cells, and shifts the balance of the differentiation pathway from Th1 and Th17 into Th2 and regulatory T cells, respectively. The inflammatory response is increased by Th1 and decreased by Th2. Therefore, vitamin D modulates the immune system through re-establishing the balance between the immune system components.^{11–13}

However, the role of vitamin D in the pathogenesis of RAS is not clear, and contradictory results have been reported. Öztekin and Öztekin showed a decreased serum level of vitamin D in patients with RAS,¹⁰ whereas Krawiecka et al. reported no significant difference between patients with RAS and healthy individuals in terms of serum vitamin D levels.⁷

Objectives

Therefore, the current study aimed to compare the serum levels of vitamin D in RAS patients and healthy individuals.

Material and methods

The participants in this case–control study were referred to the Department of Oral and Maxillofacial Medicine of the School of Dentistry at Babol University of Medical Sciences in Iran (Ethics committee code: IR.MUBABOL.HRI.REC.1397.204).

The sample size for each group ($n = 43$) was calculated with a 95% confidence level, a study power of 80%, and by considering a 5-unit difference in the serum levels of vitamin D between the 2 groups.

Individuals with RAS, aged 15–40 years, were included in the case group. An aphthous ulcer was diagnosed based on the clinical manifestation (1 or 2 minor RAS ulcers, <1 cm in diameter, located in the buccal or labial mucosa) and at least 3 periods of minor RAS per year. The control group consisted of healthy individuals, and was matched in terms of age and sex with the case group.

The exclusion criteria for both case and control groups were as follows: any systemic disease; malnutrition; smoking; oral lesions other than RAS; the consumption of vitamin D and calcium supplements in the last 6 months; pregnancy; and lactating.

The participants were informed of the study design and provided written informed consent. Their medical history and clinical examination were recorded in the checklist.

Blood samples were collected by 1 laboratory technician from all participants in the laboratory of the Shahid Yahya Nejad hospital in Babol, Iran, during spring and summer (April–September). A total of 5 cc of blood was drawn from each patient. Then, serum was separated from whole blood; it was centrifuged at $2,500 \times g$ for 10 min and was kept at -80°C until the analysis was performed.

The measurement of vitamin D level was performed by means of the enzyme-linked immunosorbent assay (ELISA), using a laboratory kit (Cat. No. EUROIMMUN, EQ. 6411-9601; PerkinElmer, Lübeck, Germany) based on the present protocol of the kit.

All test steps were conducted using Elisys Uno, a fully automated ELISA analyzer (HUMAN, Wiesbaden, Germany).

According to serum vitamin D level, each participant was classified as ‘normal’ (30–50 ng/mL), ‘insufficient’ (20–30 ng/mL) or ‘deficient’ (<20 ng/mL).

Finally, the data was analyzed with the independent samples *t* test and the Mann–Whitney test, using the SPSS for Windows software, v. 17.0 (SPSS, Inc., Chicago, USA), with the significance level set at 0.05.

Results

In this study, out of 86 individuals, 43 individuals (11 men and 32 women) were in the case group and 43 individuals (8 men and 35 women) were in the control group, and the 2 groups were matched in terms of sex ($p = 0.600$).

The mean age was 32.56 ± 7.93 years in the case group and 33.74 ± 7.07 years in the control group, and there was no statistically significant difference between the groups in this respect ($p = 0.460$).

The mean serum level of vitamin D in the control group was 22.59 ± 16.06 ng/mL and in the RAS patients, it was 13.89 ± 8.19 ng/mL. Vitamin D level in the control group was significantly higher than in the case group ($p = 0.002$) (Fig. 1).

Vitamin D categories in both groups are shown in Table 1. There was a statistically significant difference between the groups according to vitamin D categories ($p = 0.032$).

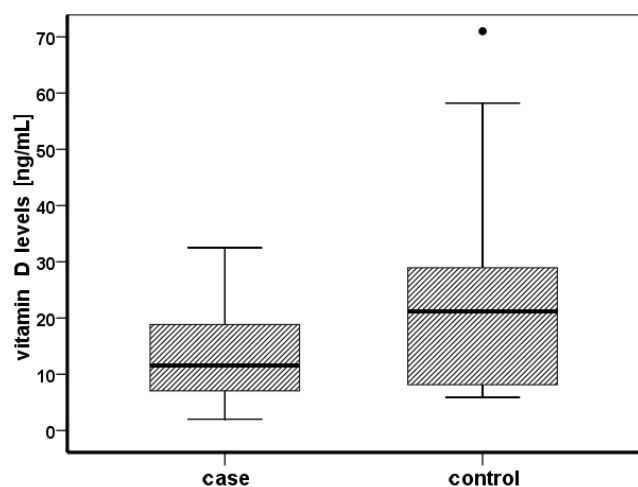


Fig. 1. Comparison of 25(OH)D concentrations between recurrent aphthous stomatitis (RAS) patients and the control group ($p = 0.002$)
Data presented as mean (M) \pm standard deviation (SD).

Table 1. Vitamin D categories in the study groups

25(OH)D category	Case group	Control group	p -value
Vitamin D-deficient	32 (74.4)	21 (48.8)	0.032*
Vitamin D-sufficient	9 (20.9)	12 (27.9)	
Vitamin D-normal	2 (4.7)	10 (23.3)	

Data presented as number (percentage).

* statistically significant.

Discussion

In the present study, the serum levels of vitamin D in patients with minor RAS were lower than those in healthy individuals.

Few studies have evaluated the possible relationship between RAS and serum vitamin D level. In a study by Öztekin and Öztekin,¹⁰ similar to Bahramian et al.'s research,¹⁴ patients with RAS had vitamin D deficiency. According to Nalbantoğlu and Nalbantoğlu, the serum levels of vitamin D in children with RAS, aged 3–12 years, were decreased.¹⁵ The results of the above-mentioned studies are in line with our results, thus supporting the thesis that there is a relationship between vitamin D level and RAS.

On the contrary, Krawiecka et al. reported different results, implying no significant correlation between the serum level of vitamin D and RAS.⁷

The level of 25(OH)D was measured in all studies assessing serum vitamin D.

It is well documented that 25(OH)D is the most appropriate marker to assess vitamin D status in humans.¹¹ According to the confounding effect of age and sex on the serum level of vitamin D, matching for these 2 variables in both study groups decreases the bias; thus, in the current study, the effects of confounders were controlled.

The contradictory results obtained by Krawiecka et al. could be due to the lack of matching for sex.⁷

Lower serum levels of vitamin D in RAS patients as compared to the control group in Krawiecka et al.'s study might have been statistically significant if gender had been matched.⁷

In this study, the mean serum level of 25(OH)D was 13.89 ± 8.19 ng/mL in the RAS group and 22.59 ± 16.06 ng/mL in the control group, which was reported lower by Öztekin and Öztekin,¹⁰ and higher by Bahramian et al.¹⁴ and Nalbantoğlu and Nalbantoğlu.¹⁵

Although there are various methods to assess the serum levels of vitamin D, including chemiluminescent immunoassay (CLIA), high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA), there is no standard established. This variety of measuring methods may result in reporting different serum levels of vitamin D.^{16,17}

In addition, differences in the measured serum level of vitamin D between the studies might be due to gender, age, geographical region, seasonal changes, diet, ethnicity, and culture.^{16,17}

Vitamin D deficiency may be related to a higher melanin content in the skin and the use of extensive skin coverage. Hence in Middle East countries, including Iran, serum 25(OH)D levels are usually low.^{7,18,19}

In the United States, 47% of African American infants and 56% of Caucasian infants have vitamin D deficiency, while in Iran, Turkey and India, over 90% of infants have vitamin D deficiency.¹⁹

Since RAS etiology is not clear and several factors are thought to contribute, there is no standard and definite treatment for it. Therapeutic options include local analgesics, corticosteroids and immune modulators, which are non-specific and administered based on the patient's symptoms.^{10,20}

Some studies have investigated the therapeutic role of various vitamins in treating aphthous stomatitis.

Lalla et al. showed that vitamin supplements (A, B1, B2, B3, B5, B6, B9, B12, C, D, and E) had no effect on the number and duration of aphthous lesions.²⁰ However, Pederson et al. reported multivitamin to be effective in treating RAS, even when the serum levels of vitamin D were appropriate.²¹

A number of researchers suggested the role of innate and acquired immunity in the pathogenesis of RAS, which leads to the activation of neutrophils and the complement system, an increase in the number of NK cells and B lymphocytes, and an imbalanced CD4⁺/CD8⁺ ratio.^{4,22,23}

There are shreds of evidence supporting the regulatory role of vitamin D in the cellular and humoral immunity function. Most cells of the immune system, including APCs (e.g., macrophages, dendritic cells and T cells) express the hormone VDR.^{11,24}

The biological effects of vitamin D, including the regulation of innate and acquired immunity as well as its influence on the cytokine profile, imply the role of this hormone in RAS progression.^{11,24}

A decrease in the serum level of vitamin D is reported in other autoimmune disorders, such as Behçet's disease, the periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome, inflammatory bowel disease (IBD), rheumatoid arthritis, and autoimmune disorders of thyroid.^{7,8,10}

Limitations

In the current study, the severity of aphthous lesions was not assessed. Therefore, further research with larger sample sizes is needed to investigate the intensity of aphthous lesions.

In addition, clinical trial studies to evaluate the potential therapeutic and protective role of vitamin D in RAS are warranted.


Conclusions

With our findings, the serum levels of vitamin D in patients with RAS were significantly lower than those in healthy individuals.


Given the regulatory effects of vitamin D on the immune system, and having considered the results of the current study, it can be concluded that low serum levels of vitamin D might be a probable etiologic factor for RAS, especially in genetically susceptible patients.


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