

Vitamin D status in recurrent aphthous stomatitis

Ewa Krawiecka¹, Zuzanna Ślebioda¹, Elżbieta Szponar¹, Anna Kowalska², Barbara Dorocka-Bobkowska¹

¹Department of Oral Mucosa Diseases, Poznan University of Medical Sciences, Poznan, Poland

²Department of Nucleic Acid Function, Polish Academy of Sciences, Poznan, Poland

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Abstract

Introduction: Recurrent aphthous stomatitis (RAS) is a common chronic inflammatory oral mucosa disease with an unknown cause. However, dysregulation of the immune response seems to play an important role in this disease.

Aim: To evaluate the vitamin D status in RAS patients and its effects on RAS severity, given the likely immunomodulatory function of vitamin D in the human organism.

Material and methods: Sixty-six patients with RAS and 66 controls were examined. Immunomodulatory or immunosuppressive treatment and other ulcerative oral diseases were used as exclusion criteria. The severity of RAS was assessed according to the clinical classification of the disease, the number of lesions per flare-up and the length of intervals between the attacks. The serum vitamin D level was established in each participant.

Results: The mean serum vitamin D (25(OH)D) levels were found to be 16.81 ng/ml in the study group and 19.22 ng/ml in the control group, with no statistically significant difference between the two groups. In the study group, 5 (7.6%) participants were diagnosed with the “normal” vitamin D levels, while 16 (24.2%) had “insufficient” levels and 45 (68.2%) had “deficient” levels. The corresponding distribution in the control group was 8 (12.1%), 18 (27.3%) and 40 (60.6%), respectively. There was no statistical significance in the difference of vitamin D deficits between the study and the control groups. No correlation was detected between the severity of RAS and the serum vitamin D level.

Conclusions: Vitamin D does not seem to be a trigger factor for RAS occurrence and does not appear to influence the severity of the disease in the studied group.

Key words: recurrent aphthous stomatitis, vitamin D, oral aphthous ulcer.

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosa diseases. It affects 5–25% of the population, with a predominance among women (particularly in the second and third decades of life) and in higher socio-economic groups [1, 2]. The condition is chronic and self-limiting in immunocompetent patients. It is characterised by recurrent onsets of shallow, clearly defined, round (or oval) and painful erosions (or ulcers) surrounded by an erythematous halo. Aphthous lesions are likely to occur on non-keratinized, non-attached oral mucosa [1–3]. Three clinical forms have been classified as minor aphthae (MiRAS), major aphthae (MaRAS) and herpetiform aphthae (HeRAS). MiRAS constitutes about 80–85% of all the cases and is characterised by 1–5 ero-

sions < 1 cm in diameter, healing spontaneously within 5–10 days without scarring, while MaRAS occurs as long-lasting ulcers exceeding 1 cm and leaving a scar. The herpetiform presentation consists of dozens of very small erosions that spread throughout the oral cavity, healing within 14 days without leaving a scar [1, 2, 4].

The exact cause of RAS remains unclear. However, dysregulation of the function of the immune system in genetically predisposed individuals is considered to be crucial in many cases in the aetiology of this entity. Both humoral and cellular types of the immunological response may be disturbed in patients with aphthae, resulting in e.g. activation of neutrophils and complementary ingredients, increased number of NK cells and B lymphocytes, disrupted CD4/CD8 ratio (< 1, especially in MaRAS), and elevated levels of T cell receptors in the peripheral blood [5–7]. Many authors have indicated the importance of increased production of pro-inflammatory Th1 type cytokines

Address for correspondence: Ewa Krawiecka DDS, Department of Oral Mucosa Diseases, Poznan University of Medical Sciences, 70 Bukowska St, 60-812 Poznan, Poland, phone/fax: +48 61 854 70 39/+48 61 854 70 27, e-mail: krawiecka.ewa@gmail.com

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(IL-2, IL-12, TNF- α , IFN- γ) and decreased anti-inflammatory Th2 type cytokines (IL-4, IL-5, IL-10, IL-13) and transforming growth factor β (TGF- β) in the pathogenesis of RAS, which may be a risk factor for autoimmunisation [5, 8, 9]. The presence of antibodies for different antigens of the epithelium in RAS patients also suggests an autoimmune character of the disease [10]. The improper immune response is activated by undefined trigger factors. Some of the modifying factors include local trauma, hormonal imbalance, haematinic deficiencies, coeliac disease, bacterial and viral antigens, smoking cessation, and even some food and drug preservatives [1, 2, 11]. Hyperactivity of the immune system results in a non-specific inflammation in the affected tissues, with neutrophils being predominant in the ulcerated area in the immediate phase, as well as massive leukocyte infiltration in the ulcer-surrounding area (consisting mainly of T lymphocytes, but also including B lymphocytes, macrophages and monocytes). Dilatation of blood vessels is also observed in the histological picture [12, 13].

Vitamin D is a steroid hormone. There are two pre-hormone forms, 25(OH)D; vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol), which are converted to the active form, 1,25-dihydroxyvitamin D (1,25(OH)₂D or calcitriol) by subsequent hydroxylation reactions in the liver and kidneys. Vitamin D₂ is provided by dietary sources (oily fish and fortified dairy products). Vitamin D₃ is synthesized in the skin on exposure to sunlight [14]. Cutaneous synthesis is the main source of vitamin D in the human organism. The role of vitamin D in calcium-phosphorus homeostasis and bone metabolism is well established, but recently an increasing body of evidence suggests that vitamin D affects the function of the immune system [14–16]. The biological effects are due to mediation by the ligand-activated vitamin D receptor (VDR). Vitamin D receptor has been found in most of the immune system cell types, including antigen-presenting cells (APCs), such as macrophages and dendritic cells, and T-cells. Vitamin D suppresses antigen presentation, proliferation of T-cells and production of antibodies by B-cells. It stimulates monocyte differentiation and synthesis of an active form of vitamin D in macrophages [14–16]. The profile of secreted cytokines is altered by vitamin D; production of Th1 cytokine type is decreased and production of Th2 type is increased. The immunomodulatory effect of this vitamin has raised an interest in its possible role in aetiology of immunodependent entities. It has been suggested that the vitamin D deficiency may be a risk factor for autoimmunisation [17–20]. Recent studies have linked vitamin D deficiency with autoimmune diseases, such as type 1 diabetes, psoriasis, rheumatoid arthritis and lupus erythematosus [16, 21–25].

The role of vitamin D as a modifier of immunologically conditioned entities of the oral cavity, including RAS, may be of considerable importance. As described above, the disruption of both types of immunological response:

humoral and cellular is observed in this condition [5, 6]. Due to its biological role via VDRs, vitamin D affects the action of numerous immune cells and its deficiency may also lead to the autoimmunisation. Vitamin D modifies the profile of secreted Th1 and Th2 cytokines [17, 18]. The autoimmunologic background of RAS has been already suggested. The enhanced production of pro-inflammatory Th1 type cytokines and the decreased production of anti-inflammatory Th2 type cytokines and TGF- β have been defined as a risk factor for autoimmunisation in RAS by many authors [7–9, 13]. Vitamin D activates the innate immunologic response mechanisms via Toll-like (TLR) membrane receptors. One such outcome is the enhanced production of antibacterial proteins: cathelicidins and defensins [26]. In addition, significantly increased salivary human neutrophil peptide-1 (HNP-1) concentrations in comparison to healthy controls were observed in RAS and Behçet's syndrome (IBD) [27]. Furthermore, several studies have shown a potential link between two syndromes with recurrent aphthous ulcers involved, BD and periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome [19, 28–31]. To the best of our knowledge, only one study on the potential role of vitamin D in RAS occurrence has been published to date [32]. The results suggest that vitamin D is a potential trigger factor in this disease.

Aim

The aim of the study was to determine the vitamin D status in RAS patients, and the effects on the disease severity.

Material and methods

The study group consisted of 66 patients from the Oral Mucosa Diseases Department suffering from RAS (24 males and 42 females), aged 19–63 years, with a mean age of 34.15 \pm 12.26 years. The diagnosis was made on the basis of a characteristic RAS clinical picture during an examination and the history of a regular mode of recurrence of the lesions. The clinical type of RAS was characterised as MiRAS, MaRAS and HeRAS. The severity was determined by the number of aphthae per flare-up (3 or less than 3; more than 3) and by the frequency of recurrences, based on the patients' self-report. The latter classification was proposed by Bagan *et al.*; type 1 disease is characterized by the intervals between the flare-ups of over 3 months, while in the type 2 disease, the flare-ups occur at one to 3 months' intervals. In type 3, aphthous lesions are present almost continuously [4]. The control group consisted of 66 participants with no evidence and/or clinical history of RAS (16 males and 50 females), aged 20–65 years, with a mean age of 32.05 \pm 12.40 years. The exclusion criteria included the presence of an ulcerative oral mucosa disease other than RAS and current immunosuppressive or immunomodulatory treatment.

Blood samples were collected from all the study participants during autumn and winter months (September – April). The serum was separated and stored at -20°C prior to analysis. Total 25-hydroxyvitamin D (25(OH)D) levels were determined by electro-chemiluminescence binding assay (ECLIA) that has been shown as effective as radioimmunoassay (RIA) or high-performance liquid chromatography (HPLC) [33]. According to the serum vitamin D level, each participant was classified as “normal” (30–50 ng/ml), “insufficient” (20–30 ng/ml), or “deficient” (< 20 ng/ml).

The study design was approved by the local Ethics Committee. Written informed consent was obtained from all the study participants.

Statistical analysis

Statistical analysis was performed by the means of Statistica software and statistical significance was set at $p < 0.05$. The χ^2 , Mann-Witney and Kruskal-Wallis tests were used in the statistical analysis.

Results

The mean serum vitamin D (25(OH)D) levels were found to be below the normal range (< 30 ng/ml) in

both the study (16.81 ± 8.45 ng/ml) and the control (19.22 ± 10.44 ng/ml) groups, with no statistically significant difference between the groups ($p = 0.2073$). The age and sex structure of the study and control groups with respect to the mean vitamin D serum levels is shown in Table 1.

In the study group, only 5 (7.6%) participants were found to have normal vitamin D levels, while 16 (24.2%) were insufficient and 45 (68.2%) were deficient. The corresponding results for the control group were 8 (12.1%), 18 (27.3%) and 40 (60.6%), respectively. No statistically significant difference was evident between the normal level, insufficiency or deficiency of vitamin D in both groups ($p = 0.5758$).

No correlation was detected between the severity of RAS and serum vitamin D levels. In the mildest clinical RAS type, MiRAS, the mean serum vitamin D levels were similar to those of the combined more severe MaRAS and HeRAS types ($p = 0.1517$). Vitamin D levels were the lowest in the most severe type 3 RAS, based on the frequency of recurrences. However, the result was not statistically significant ($p = 0.0749$). No correlation was observed between the vitamin D levels and the number of lesions per flare-up ($p = 0.9151$). Vitamin D status and RAS types are shown in Table 2.

Table 1. Age and sex structure in the study and control groups with respect to the mean vitamin D serum levels

Variables	RAS group		Control group	
	n (%)	Mean 25(OH)D [ng/ml]	n (%)	Mean 25(OH)D [ng/ml]
Total	66 (100)	16.81	66 (100)	19.22
Age [years]:				
19–34	42 (63.6)	16.36	50 (75.8)	18.64
35–49	14 (21.2)	16.23	7 (10.6)	19.22
50–65	10 (15.2)	16.81	9 (13.6)	18.13
Gender:				
Male	24 (36.4)	16.23	16 (24.2)	18.60
Female	42 (63.6)	16.81	50 (75.8)	19.22

RAS – recurrent aphthous stomatitis, 25(OH)D – 25-hydroxyvitamin D.

Table 2. Vitamin D status according to RAS types

RAS type	N	Mean vitamin D level [ng/ml]	SD [ng/ml]	Minimum [ng/ml]	Maximum [ng/ml]	P-value
MiRAS	51	17.62	8.85	3.0	45.82	0.1517
MaRAS + HeRAS	15	14.06	6.41	5.68	26.53	
Type 1	23	15.64	7.74	6.53	37.88	0.0749
Type 2	26	19.60	9.25	3.0	45.82	
Type 3	17	14.12	7.18	5.22	30.0	
3 or less lesions/flare ups	54	16.88	8.62	3.0	45.82	0.9151
More than 3 lesions/flare ups	12	16.50	7.97	7.35	30.0	

RAS – recurrent aphthous stomatitis, MiRAS – minor aphthae, MaRAS – major aphthae, HeRAS – herpetiform aphthae, type 1 – intervals between the flare-ups of over 3 months, type 2 – flare-ups observed after 1 to 3 months, type 3 – lesions observed almost continuously.

Discussion

The exact etiopathogenetic mechanism of RAS remains unclear. The disruption of both humoral and cellular types of immunological response may occur in patients with aphthae. The enhanced expression of the Th1 gene cluster in comparison to the Th2 cluster in patients with RAS has been demonstrated [5]. The increased activity of Th1-type immune response accompanied by the decreased anti-inflammatory Th2 type cytokines and TGF- β levels are typical features of autoimmune diseases. Also the antibodies for different antigens of the epithelium found in RAS patients indicate the role of autoimmunisation in the etiopathologic process [10]. VDRs are present in most of the immune system cell types, therefore the immunomodulating effect of this hormone is indisputable. The profile of secreted cytokines is altered by vitamin D, hence the production of Th1 cytokine type is decreased and the production of Th2 type is increased. Vitamin D deficiency may be a risk factor for autoimmunisation [17–20].

Our study showed no significant difference in vitamin D levels between RAS patients and the control group. We also found no correlation between vitamin D status and the severity of RAS (number of lesions per flare-up, frequency of the attacks, and clinical type of lesions). To the best of our knowledge, only one study has been published on the relationship between RAS and vitamin D. The findings were contrary to our results. Khabbazi *et al.* examined a smaller group: 46 patients suffering from an idiopathic MiRAS and 49 controls [32]. The mean 25(OH)D level in peripheral blood was found to be lower in the control group than in the RAS group (12.1 ± 7.7 ng/ml vs. 27.4 ± 9.7 ng/ml). In the present study we found a similar prevalence of the vitamin D insufficiency and deficiency in both the study and the control groups, while in the aforementioned work these conditions were found to be more common in the RAS group (58.7% and 37%) than in the control group (59.2% and 6.1%). Unlike our study, the deficiency has been defined as a vitamin D level lower than 10 ng/ml, and the insufficiency has been defined as 20–30 ng/ml. However, Khabbazi *et al.* found no correlation between the 25(OH)D status and the clinical characteristics of MiRAS (mean frequency of attacks per month and mean number of lesions per onset).

It is worth noting that in both studies, the mean serum 25(OH)D levels were lower than optimal (< 30 ng/ml) in both the case and control groups. The studies were conducted in different geographic regions (Poland and Iran), but at a similar time of the year (autumn – winter). Vitamin D deficits are common in Central and Eastern Europe, especially in autumn-winter months when sunlight exposure is too low to stimulate sufficient cutaneous vitamin D synthesis [34–36]. In the Middle East countries, including Iran, serum 25(OH)D levels were reported to be less than optimal despite the lower latitude and

high insolation. Possible reasons for this situation may include traditional clothing, a lifestyle that avoids sun exposure, skin pigmentation and diet [36–38].

The literature published over the past two decades indicates an increasing awareness of the pleiotropic effects of vitamin D in the human organism. Vitamin D deficiency is recognised as a worldwide health problem [35, 36]. It may be a significant risk factor not only for rickets or osteomalacia, but also for many other diseases. 25(OH)D deficiency may result in a higher incidence of cancer, cardiovascular, metabolic or autoimmune diseases [39, 40].

Therefore, the possible action of vitamin D as a modifying factor in RAS seemed to be worth considering, because despite multi-centred, international studies, relatively little is known about RAS etiopathogenesis. The biologic effects of vitamin D including its modification of both the innate and acquired immune system and its influence on the cytokine profile suggest the potential role of this hormone in the development of the disease [17–19].

Although there has been only one previous study published on the vitamin D status in RAS, other studies have been reported on syndromes associated with RAS such as BD and periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome.

BD is a systemic disorder, resulting in vasculitis with endothelial dysfunction. The diagnostic criteria include aphthous stomatitis, recurrent genital ulcerations, ocular lesions (uveitis and a retinal vasculitis) and skin lesions (erythema nodosum) or a positive pathergy test [19, 28, 29]. The origin of BD is not fully understood, but immunological disorders have been implicated, with T-cell mediated immunoresponse playing an important role [19, 28, 29].

Karatay *et al.* conducted the first study on vitamin D serum levels in 32 patients suffering from BD, and 31 controls. They reported considerably lower 25(OH)D levels in BD patients than in the controls ($p < 0.001$) [29]. Ganeb *et al.* examined 42 BD patients and 41 controls, and confirmed lower vitamin D levels in those suffering from BD. They concluded that there was no significant correlation between vitamin D serum levels and the disease duration ($p = 0.6$), but reported a negative association between the vitamin D levels and Behçet's Disease Current Activity Form (BDCAF) score [28]. Faezi *et al.* studied a large group of 112 BD patients and 112 healthy controls and concluded that although the vitamin D serum levels were lower in the study group, the prevalence of vitamin D deficiency was more common in the control group [19].

PFAPA syndrome is an autoinflammatory disorder, primarily affecting pre-school children. It is characterised by recurrent fever episodes of 3–6 days duration. They are accompanied by cardinal signs (aphthous stomatitis, pharyngitis, cervical adenopathy) and systemic symptoms (headache, fatigue, vomiting, skin rash etc.)

[30, 31]. Stagi *et al.* found the 25(OH)D levels to be significantly lower in PFAPA patients than in the controls ($p < 0.0001$) and concluded that all PFAPA patients had inadequate vitamin D levels. They also claimed that vitamin D supplementation significantly reduced febrile flares and their duration [30]. The data confirmed the results of Mahamid *et al.*, showing that vitamin D deficiency may be a significant risk factor for PFAPA occurrence and recurrences [31].

The activation of the pathways that produce antibacterial proteins: cathelicidins and defensins, which occurs via VDRs is another potential link between vitamin D action and RAS aetiology. Significantly increased HNP-1 salivary concentrations in comparison to healthy controls were observed in RAS and BD. In the examined subjects, the defensin concentrations were also elevated during the exacerbations in comparison to the periods of their disease remission [27].

Since the role of Th1 cytokines in the pathogenesis of RAS has been suggested by many authors, studies have been carried out on certain cytokine gene polymorphisms present in RAS patients. An association has been detected between e.g. TNF- α polymorphism, one of the IL-1 β and particular IL-1 α gene polymorphisms and the higher risk of RAS development [41]. Given the presence of vitamin D receptors in the immune system cells, a likely role of VDR gene polymorphisms in RAS aetiology has been suggested. However, Bazrafshani *et al.* did not confirm the association of RAS and known VDR gene polymorphisms. However, it does not preclude the role of VDR in RAS pathogenesis which requires further investigation [42].

Conclusions

In the present study, there was no relationship demonstrated between the vitamin D serum levels and RAS occurrence. A higher incidence of vitamin D deficits in RAS patients than in the controls was not confirmed. Vitamin D does not seem to be a trigger factor for RAS occurrence and does not seem to influence the severity of the disease in the examined group. However, previous studies on vitamin D status in RAS and RAS-related syndromes, such as BD and PFAPA, have shown a positive correlation. Further investigations on large RAS groups should be undertaken to determine a possible vitamin D role in the pathogenesis of this entity.

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Conflict of interest

The authors declare no conflict of interest.

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