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ORIGINAL ARTICLE

A comparative study of vitamin D serum levels in patients with recurrent aphthous stomatitis

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KEYWORDS

Recurrent aphthous stomatitis;
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Abstract *Background:* Recurrent aphthous stomatitis (RAS) is characterized by recurrent, painful, shallow, round or oval oral ulcers surrounded by inflammatory halos.

Aim of the work: To investigate the status of vitamin D in RAS patients and the relationship between vitamin D levels and the severity of RAS.

Patients and methods: In this cross sectional study 46 patients with idiopathic minor RAS (MIRAS), and 49 age and sex matched healthy controls were included. Patients with at least 3 minor aphthous oral ulcers in a year without any known cause for RAS were determined as idiopathic minor recurrent aphthous stomatitis. The severity of RAS was assessed by the number of oral aphthous ulcers in each attack and the frequency of attacks. 25-Hydroxy vitamin D [25(OH) D] levels were measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method in patients and control groups.

Results: The mean 25(OH) D level in the MIRAS group was lower than the control group (12.1 ± 7.7 ng/dl vs. 27.4 ± 9.7 ng/dl, $p = 0.0001$). Insufficiency and deficiency of 25(OH) D in the recurrent aphthous stomatitis groups were more common than those in the control group (Vitamin D sufficient, Vitamin D insufficient and Vitamin D deficient ($p < 0.0001$ for all the cases)). No correlation was found between the serum levels of 25(OH) D and the duration of RAS, the number of oral aphthous ulcers in each attack, and the frequency of attacks ($p < 0.05$ for all the cases).

Conclusions: Our study suggests that deficiency of vitamin D may be a trigger factor for RAS. © 2014 Production and hosting by Elsevier B.V. on behalf of Egyptian Society of Rheumatic Diseases.

1. Introduction

Recurrent aphthous stomatitis (RAS) is characterized by recurrent, painful, shallow, round or oval oral ulcers surrounded by inflammatory halos. RAS is the most frequent ulcerative diseases of the oral mucosa [1] and may affect up to 25% of population [2–5]. Three clinical forms of RAS have

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been described: minor RAS (MiRAS), major RAS (MaRAS), and herpetiform ulcerations [6]. MiRAS is the most common form, in which the ulcers are less than 1 cm in diameter and heal without scar. Major ulcers are over 1 cm in diameter, take longer to heal and often heal with scar. Herpetiform ulcers manifest as recurrent crops of dozens of small ulcers throughout the oral mucosa.

The pathogenesis of RAS is not clear, but immune dysregulation may play an important role [7–17]. Many studies have disclosed the involvement of acquired immunity in the pathogenesis of RAS. Hayrinen-Immonen et al. showed a dense infiltration of lymphocytes and monocytes adjacent to the damaged cells in oral aphthous lesions [18,19]. Initially, CD4+ T cells are predominant but during the ulcerative stage CD8+ become predominant. The local release of pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-2 (IL-2) and interferon- γ represents a dominant Th1 response in RAS [20]. Keratinocyte death is thought to be mediated by the cytotoxic T-cells and the cytokines like TNF produced by these leukocytes and other inflammatory cells [21]. Like Behcet's disease (BD), the role of the $\gamma\delta$ T-cells in the pathogenesis of RAS has been introduced [22]. The alteration of innate immune systems is also involved in the pathogenesis of RAS. A recent study showed a higher expression of Toll-like receptor 2 (TLR2) and a lower expression of TLR3 and TLR5 in the oral mucosa of patients with RAS [23]. BD which is characterized by the attacks of RAS, genital ulcers, skin lesions and uveitis share some common immunopathogenic mechanisms with RAS. However, it is not clear why lesions in RAS are limited to the oral cavity, while BD is a multisystem disease.

A growing body of evidence suggests that vitamin D has immunomodulatory effects and plays an important role in the pathogenesis of T-cell-mediated autoimmune diseases such as rheumatoid arthritis (RA) [24–26] and systemic lupus erythematosus (SLE) [27–32]. Recent studies also showed the role of vitamin D deficiency in the pathogenesis of BD [33–36]. To the best of our knowledge, no study has been performed about the status of vitamin D in RAS.

1.1. The aim of the work

Given the likely importance of vitamin D in the pathogenesis of RAS, the aim of this study was to investigate the status of vitamin D in patients with RAS and the relationship between vitamin D levels and the severity of RAS.

2. Patients and methods

In this cross sectional study 46 patients with idiopathic MiRAS, and 49 age and sex matched healthy controls were included. Patients with at least 3 minor aphthous oral ulcers in a year without any known cause for RAS were determined as idiopathic MiRAS. RAS was diagnosed by an expert physician according to the clinical characteristics of ulcers. The study design was approved by the Ethics Committee of the Tabriz University of Medical Sciences (TUOMS). Patients with MiRAS and healthy controls after explaining the aims of the study and obtaining written informed consents in accordance with the Declaration of Helsinki were recruited to the study. The study group was selected consecutively from the

outpatient clinic of connective tissue disease research center between February 2013 and April 2013. Exclusion criteria were the treatment with vitamin D supplements, steroids, colchicine, disease modifying anti-rheumatic drugs (DMARDs), and anti-convulsant drugs in the past 6 months; the use of anti-solar creams; pregnancy; chronic renal failure, liver disease, thyroid and parathyroid disorders; malnutrition; BD and fibromyalgia. The severity of RAS was assessed by the number of oral aphthous ulcers in each attack and the frequency of attacks. The duration of RAS and frequency of attacks were documented on patient self-reporting.

Blood samples of the MiRAS group were obtained between February 2013 and April 2013 and blood samples of the control group were obtained in April 2013. Separated serums were collected and stored at -20°C until laboratory tests were performed.

Vitamin D status was determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method. According to the serum 25(OH) D level, each given participant was defined as Normal ($30 < 25(\text{OH}) \text{ D} < 100 \text{ ng/dl}$), Insufficient ($10 \leq 25(\text{OH}) \text{ D} \leq 30 \text{ ng/dl}$), or Deficient ($10 \leq 25(\text{OH}) \text{ D} \leq 30 \text{ ng/dl}$). Blinding was employed (test versus control samples) prior to the ELISA analysis.

2.1. Statistical analysis

Statistical analyses were performed by the SPSS software version 18.0 (SPSS Inc, Chicago, IL). The Kolmogorov–Smirnov test was used to evaluate data distribution. Results are expressed as median (minimum–maximum values), or mean $\pm$ SD and Mann–Whitney *U* test or independent *t* test was used, as appropriate, to assess the significance of any differences between the two groups. All correlations were evaluated using the Spearman test and the statistical significance was set at $p < 0.05$.

3. Results

Demographic and clinical characteristics of both study groups are presented in Table 1. The mean 25(OH) D level in the RAS group was lower than that in the control group (Fig. 1). Patients and control group classification according to Vitamin D status is presented in Table 2. Insufficiency and deficiency of 25(OH) D in the RAS groups were more common than those in the control group (Table 2). The levels of 25(OH) D were correlated to the clinical characteristic of RAS. No correlation was found between the serum levels of 25(OH) D and the duration of RAS ($r = 0.079$; $p = 0.690$), the number of oral aphthous ulcers in each attack ($r = 0.024$; $p = 0.870$), and the frequency of attacks ($r = 0.187$; $p = 0.225$) in this group.

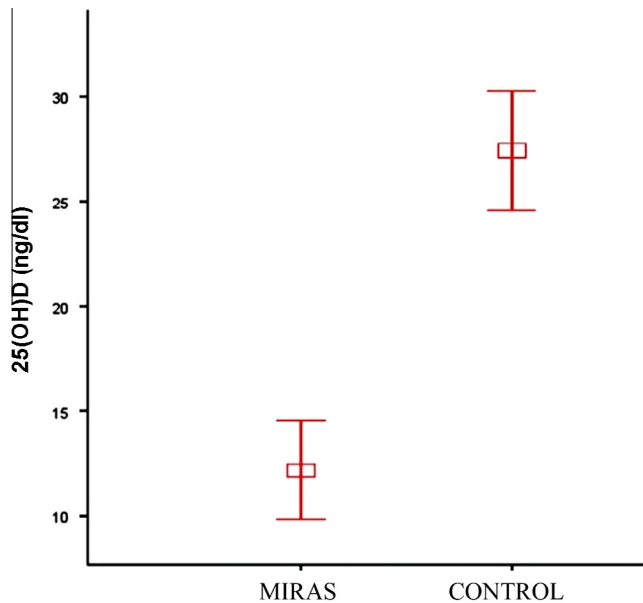
4. Discussion

Our study showed that 25(OH) D in the patients with MiRAS is lower than that in the control group. However, there is no relation between 25(OH) D levels and the clinical characteristic of MiRAS. This is the first study that compares 25(OH) D in the patients with RAS and the normal population and that investigates the correlation between vitamin D status and RAS.

Table 1 Demographic data and characteristics of the minor recurrent aphthous ulcers.

Characteristics mean $\pm$ SD	MiRAS	Control	<i>p</i> value
Age (years)	33.4 $\pm$ 9.8	34.1 $\pm$ 10.1	NS
Sex (Female/Male)	18/28	19/30	NS
Disease duration (years)	8.2 $\pm$ 7.5	–	–
Frequency of attacks/month	1.2 $\pm$ 1	–	–
Number of aphthous ulcers/attack	1.9 $\pm$ 1	–	–

MiRAS, minor recurrent aphthous ulcer; NS, non-significant.

**Figure 1** Comparison of the 25(OH) D concentrations between minor recurrent aphthous stomatitis (MiRAS) patients and the control group (12.1 $\pm$ 7.7 ng/dl vs. 27.4 $\pm$ 9.7 ng/dl, *p* = 0.0001).**Table 2** Vitamin D status in the minor recurrent aphthous ulcer patients and control.

Vitamin D status <i>n</i> (%)	MiRAS (<i>n</i> = 46)	Control (<i>n</i> = 49)	<i>p</i> value
Normal level	2 (4.3)	17 (34.7)	0.0001
Insufficient	27 (58.7)	29 (59.2)	0.0001
Deficient	17 (37)	3 (6.1)	0.0001

According to the serum 25(OH) D level, each given participant was defined as: Normal (30 < 25(OH) D < 100 ng/dl), Insufficient (10 $\leq$ 25(OH) D $\leq$ 30 ng/dl), or Deficient (25(OH) D < 10 ng/dl). MiRAS, minor recurrent aphthous ulcer.

Many studies showed that vitamin D has an important regulatory role in the function of immune system [37–39]. It affects many aspects of the innate and adaptive immune system. Vitamin D decreases antigen-presenting activity of macrophages to lymphocytes by down regulation of the MHC II and costimulatory receptor expression [40]. It also inhibits the differentiation of monocytes into dendritic cells (DCs) and the T-cell stimulatory activity of DCs [41–43]. Vitamin D decreases IL-12 and increases IL-10 production [43,44].

The result is the inhibition of Th1 and Th17 cell development [44]. The immune modulatory effect of 1,25(OH)₂ D3 through down-regulation of the expressions of TLR2 and TLR4 was demonstrated in human monocytes in vitro model. Do et al. showed that 25(OH) D levels inversely correlated with the expressions of TLR2 and TLR4 in patients with BD [33]. Macrophages and activated lymphocytes express vitamin D receptors (VDR) [45]. Stimulation of VDR on macrophages activates them and increases the production of TNF and IL-1, while stimulation of lymphocytes VDR inhibits T cell proliferation and production of cytokines. Vitamin D deficiency has been found to be associated with several immune mediated diseases, including RA, SLE, BD and inflammatory bowel diseases [46], insulin dependent diabetes mellitus [47], and multiple sclerosis [48]. Epidemiological evidence also indicates an association between the vitamin D deficiency and an increased incidence of immune mediated diseases. Cutolo et al. showed a relationship between circannual vitamin D serum levels and disease activity in RA in northern and Western Europe [49]. In another study, Hyppönen et al. reported that vitamin D supplementation reduces the risk of diabetes mellitus type 1 [50].

The complex role of vitamin D in the regulation of immune system function, deficiency of vitamin D in autoimmune disorders, and epidemiological studies suggest that the deficiency of vitamin D is an important environmental factor in the pathogenesis of immune mediated disorders. The relationship between the immune system dysregulation and the pathogenesis of RAS, and the results of our study may also prove the role of vitamin D deficiency in the pathogenesis of RAS.

In conclusion, the results of the present study suggest that deficiency of 25(OH) D may be a trigger factor for RAS.

Conflict of interest

None.

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