Research Paper

Impact of Vitamin D_3 Supplementation on Recurrent Aphthous Ulcer

FATMA FATHI HUSSEIN[1]*, H. S. SADEK[1,2] AND LAMA HUSSEIN ELMARSSAFY[3]

Department of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Dentistry, Minia University, Damaris, Minya, Egypt, [1]College of Dental Medicine, Umm Al-Qura University, Mecca 24382, Kingdom of Saudi Arabia, [2]Department of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Dentistry, Cairo University, Giza 12613, Egypt, [3]Department of Oral Medicine, Periodontology and Oral Diagnosis, Department of Basic and Clinical Oral Sciences, College of Dental Medicine, Umm Al-Qura University, Mecca 24382, Kingdom of Saudi Arabia

Vitamin D is a well-known secosteroid hormone, which exerts multiple essential roles in bone physiology, cell growth and differentiation, the neuromuscular system, immunomodulatory functions and autoimmune diseases. Deficiency of vitamin D has been linked to many oral disorders including tooth decay, periodontitis, oral cancer, oral candidiasis, oral lichen planus and recurrent aphthous ulcers. Many studies have revealed a significant lower serum vitamin D level in recurrent aphthous ulcer patients compared with healthy volunteers. This proposes that vitamin D supplementation may reduce the severity of the lesion. To evaluate the efficacy of vitamin D_3 replacement on recurrent aphthous ulcer severity is the objective of the study. This study involved 65 patients with idiopathic minor recurrent aphthous ulcer and vitamin D deficiency/insufficiency. These patients received vitamin D_3 supplement over 1 y. Severity of the disease was delineated by the duration of episodes, the number of ulcers per attack and the frequency of recurrence. Severity parameters were compared before and after vitamin D_3 intake. In addition, 25-hydroxy vitamin D_3 concentrations were measured before and after administration of vitamin D_3 by enzyme-linked immunosorbent assay technique. We found a significant decrease in the frequency of attacks and the number of ulcers in each attack with p-value<0.05 following vitamin D replacement. Moreover, a highly significant lowering in the duration of episodes was detected with p-value<0.001. Gender specific analysis showed no statistically significance regarding severity parameters at any point of the study stages. Correlation studies revealed the only detectable correlation was between serum 25-hydroxy vitamin D_3 level at baseline and the number of ulcers before and after vitamin D correction. Vitamin D_3 supplementation has a safe and positive impact on improving the severity of recurrent aphthous ulcer with regards to the number of lesions, the frequency of recurrence and the duration of episodes, in patients who have vitamin D deficiency/insufficiency.

Key words: 25-hydroxy vitamin D_3, recurrent aphthous ulcer, minor recurrent aphthous ulcerations, vitamin D, vitamin D_3 supplementation

Vitamin D (VD) (Calcitriol) is a multifunctional lipid soluble ecosteroid hormone, besides its classical endocrine activity in the regulation of serum calcium and phosphate concentration, bone biology and growth[1,2]. VD also has a novel autocrine and paracrine actions by regulating cell proliferation, maturation and apoptosis, nervous system, cardiovascular system, immune system, antimicrobial peptides, glucose metabolism and even acts as antiaging factor[3-9]. Bioactivities of calcitriol are mediated through intracellular Vitamin D Receptor (VDR). Nearly all human tissues and cells express VDR[10].

The major endogenous source of calcitriol (>80 %) is obtained through the conversion of cutaneous 7-dehydrocholesterol to previtamin D_3 following solar ultraviolet B rays exposure. Previtamin D_3 is transformed to Vitamin D_3 (VD_3) (cholecalciferol) under the effect of heat-mediated process in the skin[11], the remaining 20 % of 25-Hydroxy (25-OH) VD_3 is acquired exogenously from diet and supplements. In the liver, VD_3 undergoes hydroxylation into calcifediol ($25(OH)D_3$), which is usually estimated to determine vitamin D status[11-13]. Further hydroxylation is carried out in the kidney where the biologically active VD

***Address for correspondence**
E-mail: fattma2003@yahoo.com

(1,25-hydroxycholecalciferol) is formed[11]. Also, hydroxylation of VD_3 takes place in the skin and lymph nodes[14].

Vitamin D Deficiency (VDD)/insufficiency are now more pervasive than ever and have become a widespread public health issue. Almost one billion people across the world are believed to have VDD ($25(OH)D_3$ level <20 ng/ml). On the other hand, about 50 % of population have VD insufficiency ($25(OH)D_3$ level≥20 and <30 ng/ml)[15,16]. Based on the crucial pathophysiological role of calcitriol, inadequate VD level has been reported in various musculoskeletal disorders, autoimmune diseases, hypertension, diabetes, respiratory and neurological diseases[16-18]. A low VD concentration has also increased the risk of any type of malignant lesions[19]. Regarding oral health, VDD has a negative effect including demineralization of enamel and dentine with increasing susceptibility to fracture and caries[20]. In addition, there is a reverse association with an increased severity of periodontitis, higher tooth loss and VD concentrations[21-24]. Furthermore, VDD is markedly more prevalent in patients with oral cancer, oral lichen planus and Recurrent Aphthous Ulcer (RAU)[25-32].

RAU (canker sore) is one of the most frequent oral ulceration which characterized by recurrent painful episodes and unclear multifactorial etiopathogenesis[33]. The lesion usually manifests as a self-limiting single or multiple oval, round or elliptical ulcers with central necrosis and bordered by well-circumscribed erythematous margin, mainly involving non-keratinized oral mucosa[34]. The onset of RAU is usually in childhood and adolescence with a slight female predominance[35]. Unlike herpetiform and Major Aphthous Ulcer (MaAU), Minor Aphthous Ulcer (MiAU) represents the most prevalent form and resolves in 7-10 d without leaving any scar[36]. The exact cause of RAU is undefined and may be attributed to an impressive array of multiple factors. The common triggering factors include genetic predisposition, local factors, infection, trauma, psychological stress, systemic diseases, allergy, hematological diseases, immunological defect, endocrine disorders, hormonal fluctuations and micronutrient deficiencies[37,38]. RAU is believed to be caused by a cell mediated immune defect characterized by alteration of Cluster of Differentiation, (CD) 4^+:$CD8^+$ T lymphocyte ratio and subsequent mucosal cytokines cascade dysfunction[39].

Micronutrients are defined as these magic substances that enhance the production of hormones, enzymes and other substances necessary for proper development and growth. A reduction in the concentration of these substances induce severe consequences[40]. The role of various micronutrients in RAU, such as (i.e., Vitamin B_{12}, VD, folic acid, iron and zinc), and their deficiencies have been subjected to several studies since the 1960s[29,30,41-47]. Recently, the association of RAU and VDD has been considered in several studies. These have revealed the presence of significantly reduced serum levels of VD in patients with RAU compared to healthy controls[29-32,48,49]. Consequently, VD replacement therapy may be beneficial in management of this lesion for patients who have inadequate $25(OH)D_3$ level. We set out to assess the effectiveness of VD_3 supplementation on RAU severity with regards to the duration of episodes, the frequency of recurrence and the number of lesions in each attack.

MATERIALS AND METHODS

The study was implemented over a period of 1 y. 65 patients had idiopathic Minor Recurrent Aphthous Ulcer (MiRAU) and VD deficiency/insufficiency who participated in our previous study to detect the relationship between RAU and serum VD level, were recruited in this current study[32]. All former patients were chosen from the out-patient clinic of College of Dental Medicine, Umm Al-Qura University in Mecca. All participants were given their written consent form after being informed about the objectives of the study, procedure and possible benefits and risks of participating in this clinical trial. The study protocol was approved by the Biomedical Research Ethics Committee of Umm Al-Qura University (Approval No. HAPO-02-K-012).

Patient selection:

Inclusion criteria: Selected patients were of both sexes with 18-60 y age range; patients were diagnosed with at least three idiopathic MiRAUs/year; severity parameters of the disease were assessed at baseline and after 12 mo of receiving VD_3 by the most protracted episode duration, the number of ulcers/attack and the frequency of recurrence, reliant on the patient's report using standardized chart.

Exclusion criteria: Patient who had sufficient serum VD concentration ($25(OH)D_3$≥30 ng/ml); uses of any medical treatment for RAU in the last 3 mo; previous history of allergic reaction to any kind of VD preparations; history of taking any drugs that interact with VD_3 supplement including: Drugs that raise the serum level of VD (e.g., Estrogen Replacement Therapy (ERT), Isoniazid (INH), thiazide, multivitamin, calcium

and VD supplements), drugs that reduce serum VD level such antacids, sun blockers or sunscreen cream and anti-seizure medications (e.g., phenobarbital, phenytoin, primidone and valproic acid), bile acid sequestrants that reduce VD absorption (e.g., Cholestyramine, cholestipol, rifampin, mineral oil and orlistat), VD may interfere or reduce the action of atorvastatin and calcium channel blockers, VD may potentiate the action of digoxin, herbal medicines mainly Kava kava, St. John's wort (Hyperforin) which interfere VD metabolism; history of taking any medication which influences bone metabolism in the past 6 mo like glucocorticoids, Highly Active Antiretroviral Treatment (HAART), cytotoxic drugs, chemotherapy, bisphosphonates, anticoagulant (heparin, warfarin); patients with history of chronic liver, chronic renal, bone metabolic diseases, hyperparathyroidism, fibromyalgia, Behcet's syndrome, malabsorption diseases, short bowel, Human Immunodeficiency Virus (HIV), Diabetes Mellitus (DM) type 1, malignancies, thyroid disease, pregnancy as well as any blood disorders.

VD_3 replacement strategies:

For patients who had VDD ($25(OH)D_3$ of <20 ng/ml) initially received loading dose of 50 000 International Units (IU) of cholecalciferol (Biodal)* weekly for 8 w[50], followed by VD_3 dose reduction to a maintenance regimen generally 50 000 IU of cholecalciferol monthly for 10 mo.

For patients with VD insufficiency ($25(OH)D_3$ between 21-29 ng/ml) were replaced with 50 000 IU of cholecalciferol monthly for 12 mo[51].

Blood samples collection and serum VD levels measurement:

Blood specimens were collected before the administration of VD_3, after 2 mo following VD_3 replacement and at the end of the study to assess VD status. $25(OH)D_3$ concentrations were measured by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Statistical analysis:

Data from 65 subjects were recorded before and after treatment on excel spreadsheet and statistically analyzed using Statistical Package for the Social Sciences (SPSS) v. 21.0 for Windows Software. Descriptive data were illustrated as mean±Standard Deviation (SD) and range (minimum, maximum). While, analytical data were performed using paired t test, Analysis of Variance (ANOVA), student's test, correlation matrix and coefficient of correlation (Pearson's method). The probability values were significant at $p<0.05$.

RESULTS AND DISCUSSION

65 VD deficient/insufficient patients who had MiRAU, 36 female (55 %) and 29 male (45 %) were enrolled in this study. The mean patient's age was 28.923±10.638 with non-significant difference between female and male. Serum levels of $25(OH)D_3$ were analyzed at baseline, 2 and 12 mo, while the clinical outcomes were evaluated at baseline and 12 mo following VD_3 replacement. Descriptive statistics of all recruited subjects were recorded, tabulated and presented in Table 1.

TABLE 1: CLINICAL PARAMETERS BEFORE AND AFTER VD_3 SUPPLEMENTATION

	Minimum	Maximum	Mean±SD
Age	18	60	28.923±10.638
$25(OH)D_3$ ng/ml-M_0	5.3	28.1	19.309±5.061
$25(OH)D_3$ ng/ml-M_2	27.9	40.1	34.523±2.572
$25(OH)D_3$ ng/ml-M_{12}	31.7	41.3	35.698±2.425
Number of ulcers-M_0	1	4	1.462±0.752
Number of ulcers-M_{12}	1	3	1.338±0.538
Frequency-M_0	3	24	5.000±4.077
Frequency-M_{12}	3	22	4.646±3.356
Duration-M_0	7	10	7.354±0.623
Duration-M_{12}	6	8	7.077±0.407

Serum 25(OH)D_3 level:

Response of total serum 25(OH)D_3 values to VD_3 replacement over time, showed highly significant elevation of the mean serum 25(OH)D_3 concentration when the 2 mo (34.523±2.572) and 12 mo (35.698±2.425) measurements were compared to the starting (baseline 19.309±5.061) value ($p<0.001$). The same level of significance was observed between the 2 mo and 12 mo data. There was an 84.9 % improvement in mean serum VD level from the baseline interval till the end of the study (Table 2-Table 4 and fig. 1).

Clinical findings before and after treatment were shown below. Regarding the number of ulcers, the clinical findings indicated a significant decrease in number of ulcers/attack with p-value<0.05 following VD replacement. Exactly, the same statistically significant value was observed for the frequency (number of attacks/year). Moreover, a highly significant lowering in duration of episodes was detected with p-value<0.001 (Table 4 and fig. 2).

Upon comparing the results between female and male, female showed a significant low mean serum VD value (17.664±5.355) more than male (21.352±3.860) at baseline p-value<0.05. Furthermore, we observed a high significant elevation in the mean serum 25(OH)D_3 level in male more than female at 2 mo following VD_3 administration with p-value<0.001. Notwithstanding that the VD_3 supplement was more efficient in rising 25(OH)D_3 concentration in female by 89.8 % in comparison to male (67.50 %) after 2 mo. On the contrary, 25(OH)D_3 mean value displayed no significant difference between them at the end of the study. As for the severity parameters, there was no detectable significant difference along the whole studied period with p-value>0.05 (Table 5).

Correlation between 25(OH)D_3 serum level and severity parameters of RAU is shown below. Severity of MiRAU was set by the number of ulcers per attack, frequency of recurrence and duration of episodes. The only mild to moderate inverse correlation was noticed between VD serum level at baseline and the number of ulcers before and after treatment with p-value<0.05 (Table 6 and Table 7, fig. 3 and fig. 4).

TABLE 2: COMPARISON BETWEEN SERUM 25(OH)D_3 LEVEL AT BASELINE AND 2 MO AFTER TREATMENT

	0 mo	2 mo	T-test
	Mean±SD	Mean±SD	p value
25(OH)D_3 ng/ml	19.309±5.061	34.523±2.572	0.000000

TABLE 3: COMPARISON BETWEEN SERUM 25(OH)D_3 LEVEL AT 2 AND 12 MO AFTER TREATMENT

	2 mo	12 mo	T-test
	Mean±SD	Mean±SD	p value
25(OH)D_3 ng/ml	34.523±2.572	35.698±2.425	0.000000

TABLE 4: COMPARISON OF DIFFERENT CLINICAL PARAMETERS BEFORE AND AFTER VD_3 SUPPLEMENTATION

	0 mo	12 mo	T-test
	Mean±SD	Mean±SD	p value
25(OH)D_3	19.309±5.061	35.698±2.425	0.000000
Number of ulcers	1.462±0.752	1.338±0.538	0.005149
Frequency	5.000±4.077	4.646±3.356	0.003073
Duration	7.354±0.623	7.077±0.407	0.000060

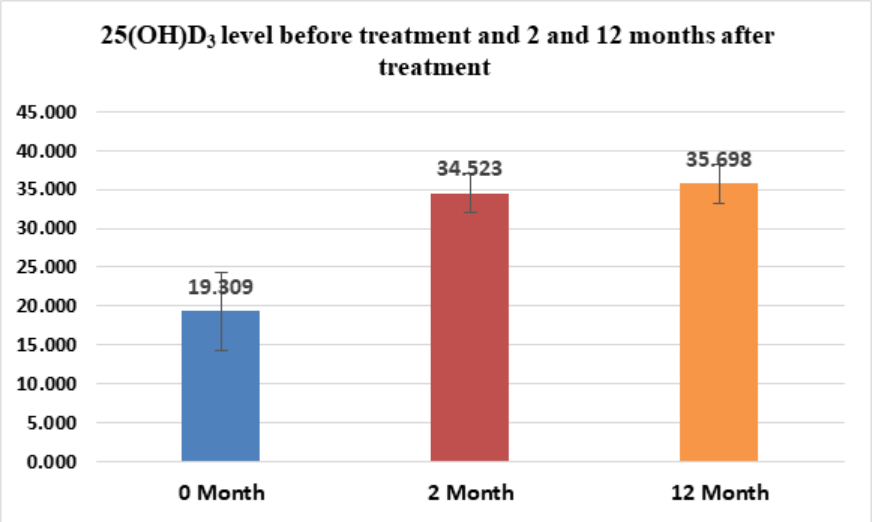

Fig. 1: Serum VD level at different stages of treatment

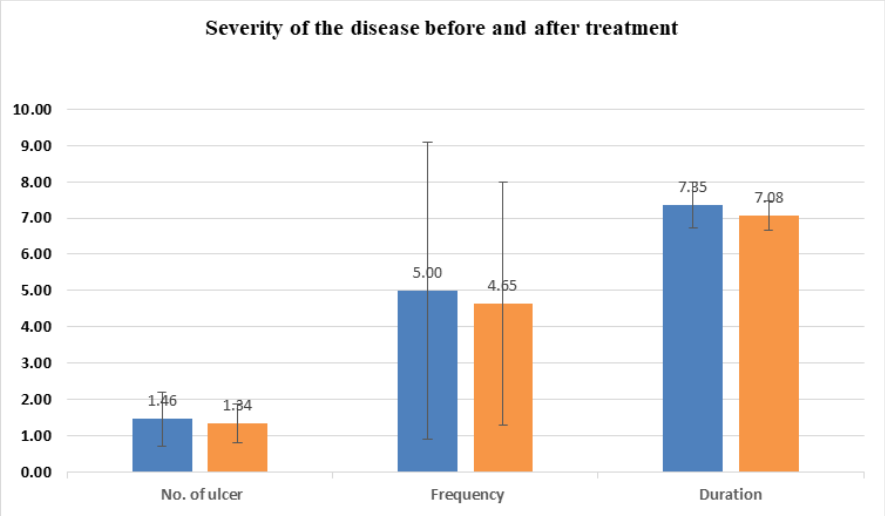

Fig. 2: Clinical outcomes before and after treatment, (■) 0 mo; (■) 12 mo

TABLE 5: COMPARISON OF DIFFERENT CLINICAL PARAMETERS BETWEEN FEMALE AND MALE BEFORE AND AFTER VD_3 SUPPLEMENTATION

	Female	Male	T-test
	Mean±SD	Mean±SD	p value
$25(OH)D_3$-M_0	17.664±5.355	21.352±3.860	0.001394604
$25(OH)D_3$-M_2	33.522±2.491	35.766±2.117	0.000136865
$25(OH)D_3$-M_{12}	34.700±2.108	36.938±2.239	5.28451E-05
Number of ulcers-M_0	1.583±0.806	1.310±0.660	0.07344099
Number of ulcers-M_{12}	1.417±0.554	1.241±0.511	0.097131469
Frequency-M_0	4.833±3.745	5.207±4.515	0.358284828
Frequency-M_{12}	4.528±2.893	4.793±3.904	0.377052646
Duration-M_0	7.333±0.535	7.379±0.728	0.385044835
Duration-M_{12}	7.056±0.410	7.103±0.409	0.320556246

TABLE 6: CORRELATION BETWEEN 25(OH)D_3 SERUM LEVEL AT BASELINE AND SEVERITY PARAMETERS OF RAU AT DIFFERENT STAGES OF THE STUDY

	25(OH)D_3-M_0	
	R	p value
Number of ulcers-M_0	-0.206125	0.040614
Number of ulcers-M_{12}	-0.236274	0.018509
Frequency-M_0	-0.085416	0.400515
Frequency-M_{12}	-0.091342	0.368509
Duration-M_0	-0.107063	0.291477
Duration-M_{12}	0.152031	0.132973

TABLE 7: CORRELATION BETWEEN 25(OH)D_3 SERUM LEVEL AT 12 MO AND SEVERITY PARAMETERS OF RAU AT DIFFERENT STAGES OF THE STUDY

	25(OH)D_3-M_{12}	
	R	p-value
Number of ulcers-M_0	-0.087905	0.386879
Number of ulcers -M_{12}	-0.107298	0.290412
Frequency-M_0	-0.005847	0.954193
Frequency-M_{12}	-0.018883	0.852819
Duration-M_0	0.031380	0.757812
Duration-M_{12}	0.011196	0.912411

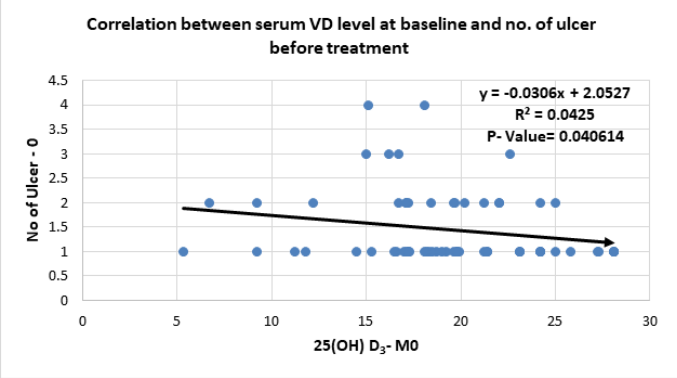

Fig. 3: Correlation between VD serum level and number of ulcers before treatment

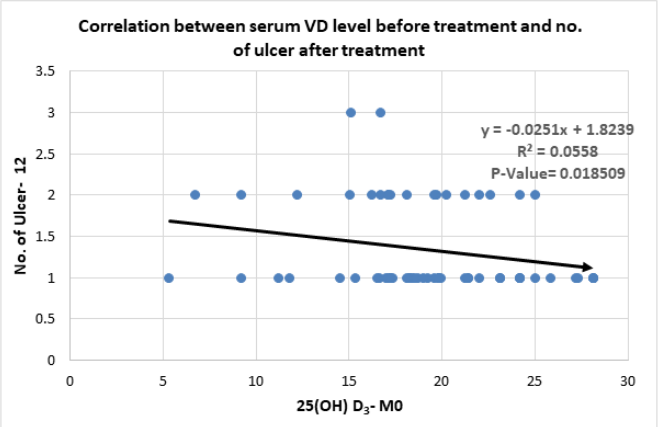

Fig. 4: Correlation between VD serum level at baseline and number of ulcers after treatment

VD is the principle organic lipophilic calcitriol precursor that regulates intestinal calcium absorption[52] and renal reabsorption, to maintain musculoskeletal health[53,54]. Activated VD has a fundamental immunomodulatory role in different inflammatory and T-cell mediated autoimmune diseases[55] through regulation and modulation of innate and acquired immune response[56]. VDD has been linked with many oral diseases and carries a high risk of oral treatment failure[57]. The association between the RAU incidence and VD status has been studied numerous times[58]. Whilst several studies have found a significant relationship between recurrent aphthous stomatitis and low serum level of VD[29-32,48,49,59,60], others have failed to reveal a significance[61,62].

RAU is the most common painful oral lesion that can impair speech, daily nutrition, oral hygiene and life quality[63,64], hence the main objectives of RAU treatment are to relief pain, reduce the duration of episodes, decrease the number of ulcers and increase lesion-free periods. Eradication of etiological predisposing factors is the prime concern. So far, the etiopathogenesis of RAU is indefinite; however, both innate and acquired immune dysregulation and inflammatory processes caused by several triggers may enhance the development of RAU. This concept has been confirmed in large-scale bioinformatics analysis[65,66]. Altered immunoregulatory balances involve T-lymphocytes infiltration that mediates local epithelial cells destruction[67], higher serum immunoglobulins levels, boost in antibody-dependent cell-mediated cytotoxicity and defects in lymphocyte subpopulations with decreased numbers of T-suppressor/inducer cells, increased T-helper/inducer cells and suppressed response to mitogens[68,69]. T helper type 1 (Th1) hyperimmune response stimulates the production of Interleukin 2 (IL-2), tumour necrosis factor alpha (α) and interferon-gamma (γ). These pro-inflammatory mediators promote inflammatory process that proceed ulceration[70,71]. In view of the foregoing, low VD level correction may be beneficial for RAU, due to the VD immunomodulatory role on both innate and adaptive immune response and suppression of the inflammatory response by inhibiting Th1 pro-inflammatory cytokines and increasing Th2 anti-inflammatory cytokines like IL-4, IL-5, IL-10 and IL-17[55,72].

Nowadays, the most common commercial preparations and fortified food of VD are VD_2 (ergocalciferol) and VD_3 (cholecalciferol)[73]. Meta-analysis and Tripkovic *et al.*[74,75] demonstrated that VD_3 is more proficient in restoring serum $25(OH)D_3$ than equivalent VD_2. This observation may be attributed to a higher rate of VD_3 hydroxylation[76] and a higher affinity of VD_3 metabolites for VD-Binding Protein (VDBP)[77] which lead to longer half-life and decreased rate of circulatory clearance[78] suggesting the fact that cholecalciferol remain biologically active and maintain serum $25(OH)D_3$ status[79,80]. For these reasons, we recommended VD_3 administration in our study.

In this study, we followed the current strategies for VD_3 replacement in adult patients. Patients with a serum $25(OH)D_3$ concentration >20 ng/ml were initially treated with 50 000 IU of VD_3/week for 8 w. VD status was tested 2 mo after completion the course to ensure serum $25(OH)D_3$ repletion. Once replete, VD_3 was administered at a maintenance dose of 50 000 IU/mo, as the majority of these patients were more susceptible to VDD and would likely require life-long therapy. Patients with a $25(OH)D_3$ serum level between 21-29 ng/ml were administered only a maintenance dose of 50 000 IU/mo[81]. de Niet *et al.* proved that higher dose of VD_3 (50 000 IU) administered monthly speed up serum $25(OH)D_3$ repletion than daily dose of 2000 IU[82]. Monitoring the success of VD_3 replacement is the key to ensure sufficiency and avoid toxicity. The first follow-up of VD status should not be earlier than 8-12 w following the start of treatment[83].

The response of total $25(OH)D_3$ concentration to VD_3 administration was the primary endpoint used to determine VD repletion. VD status was measured at different intervals through this study to fulfill this point. We observed a significant rise in the $25(OH)D_3$ serum level throughout all study periods (p value<0.001) with an 84.9 % improvement from the baseline interval till the end of the study. This highly significant responsiveness may be owing to proper dose, type of VD (cholecalciferol) and low initial $25(OH)D_3$ level (19.309±5.061 ng/ml).

Mazahery *et al.* found that baseline $25(OH)D_3$ level, body mass index, duration, dose and type of VD are the most important factors affecting the response to the administered dose of VD[84]. Baseline $25(OH)D_3$ value markedly affects bioavailability of VD because VD hepatic hydroxylation may be a saturable process. VD status has a significant converse relation with initial $25(OH)D_3$ level with the greatest increase in response has been seen in subjects with the lowest baseline $25(OH)D_3$ value[85]. Our results are consistent with the study conducted by Bacon *et al.* who concluded that large bolus dose of VD_3 rapidly and safely repletes

$25(OH)D_3$ in deficient elderly subject [<50 nmol/l (<20 ng/ml)] at 1 mo while monthly dosing of 50 000 IU of VD_3 takes about 3 mo to attain similar effect[86].

In the current study, gender-specific analysis revealed significant rise in the mean $25(OH)D_3$ level for male more than female, 2 mo after the onset of VD_3 supplementation. The gender effect can be indirectly attributed to either more physical activity, increased sunlight exposure, more outdoor activities, less adipose tissue, clothing style in male[87] or sample size effect. However, VD_3 supplement was more efficacious in boosting $25(OH)D_3$ concentration in female by 89.8 % in comparison to male (67.50 %) since female showed lower/suboptimal baseline serum 25(OH) VD levels (17.664±5.355) *vs.* male (21.352±3.860). Lack of this significance at the end of the study indicates both male and female attained near serum $25(OH)D_3$ sufficiency.

In our previous study[32], patients with MiRAU displayed deficient/insufficient serum level of $25(OH)D_3$, which correlated with the number of ulcers and frequency of episodes, indicating a possible benefit of using VD for these patients. As far as we know, the current clinical trial may be the first study to provide data showing the impact of VD_3 replacement on severity parameters of RAU. The present findings show marked decrease in severity parameters, following low VD correction, shedding light on the possible immuno-modulatory therapeutic effect of VD_3 on RAU patients.

It was hard to compare our results with those of other studies due to different study design, sample sizes, dose and type of VD and follow-up periods. In a controlled clinical trial, Bratel *et al.* found that LongoVital (herbal based multivitamins including VD) significantly decreased the number of ulcers as well as the duration of pain[88]. Similarly, Pederson *et al.* evinced the significant role of LongoVital on lessening the recurrence of RAU[89]. On the contrary, Lalla *et al.* revealed that the use of Recommended Daily Intake (RDI) vitamins containing vitamin B complex, A, C, D and E for RAU on a regular basis over 1 y had no effect on reducing the number of lesions and duration of episodes[63]. Noteworthy, the initial $25(OH)D_3$ level has not been addressed in the three aforementioned studies.

Moreover, in RAU related disorders where RAU is one of the main diagnostic criteria as Behcet's Disease (BD) and Periodic Fever, Aphthous Stomatitis, Pharyngitis and Cervical Adenitis (PFAPA), few trials with respect to VD correction have been published. The findings of Adeeb *et al.* suggested that VD may potentially down regulate the inflammatory response in BD patients and recommended VD supplementation to assess whether it confers protective benefits or not[90]. However, in Aslan *et al.* study, who detected that VD has no efficacy on the activity and clinical manifestations of BD[91]. In the study by Stagi *et al.* the positive effect of PFAPA patients with deficient/insufficient serum level of $25(OH)D_3$ to 400 IU/d of VD for approximately 7 mo was reported. They observed a significant shortening of mean duration of episodes from 4.3 to 2.3 d in 36 % of cases and reduction in the number of febrile episodes[92]. Furthermore, a case of 32 mo old girl with PFAPA syndrome and insufficient VD serum level (23.7 ng/ml) was reported by Rodes *et al.* They noticed the outcomes of using 400 IU of cholecalciferol until achieving normal level (30 ng/ml), resulting in reduction in the severity and interval between the episodes from episode/6-8 w to only two mild attacks over the following 12 mo[93].

As regard to correlation, we detected the only significant inverse correlation was between the $25(OH)D_3$ serum level at baseline and the number of lesions before and after VD_3 supplementation (p-value<0.05). Even the fact that, the correlation between VD concentration and the number of ulcers and frequency of recurrence were recorded in our previous study[32], we observed loss of this correlation with frequency before treatment in the current study. This observation may be explained by exclusion of patients who had MiRAU with sufficient VD level in the present study resulting in more smaller sample size.

VD_3 supplementation seems markedly, improves the severity of RAU regarding the number of lesions, frequency of recurrence and duration of episodes, proving a safe promising intervention for the treatment of RAU patients who have low serum level of VD.

Conflict of interests:

The authors declared no conflict of interest.

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