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Role of Vitamin D supplementation in Immunomodulation and improvement of symptoms of patients with Chronic Spontaneous Urticaria

Mahmoud Mohamed El- Sayed, Fawzia Hassan Abo-Ali, Dina Sayed Sheha, Abeer Abdelhamid Eissa

Department of Internal Medicine, Faculty of Medicine, Ain Shams University

ABSTRACT

Background: Chronic Spontaneous Urticaria (CSU) is an allergic auto-immune disease with more than 6 weeks of continuous symptoms, it is known to trigger allergic wheal formations and angioedema. Vitamin D at optimal levels plays an important role in adjusting innate immunity thus People who has deficient or insufficient levels of serum vitamin D suffer from disturbance in immune system. Accordingly, studies have been established to explore the effect of vitamin D on CSU. Aim of the Study: To determine the effect of 12 weeks daily oral vitamin D supplementation [high (4,000 IU/d) versus low (600 IU/d) dose of orally administered vitamin D3] on Urticaria activity score (UAS-7), quality of life (QOL) and medication burden in patients with chronic spontaneous urticaria, and to assess the relationship between vitamin D levels and CRP in these patients. **Patients and methods:** This single blind randomized prospective study conducted to 50 patients with CSU, admitted to Ain shams hospital, 50 patients were divided into 2 groups according to the dose of vitamin D orally administrated to these subjects, the first was group A , patients have received vitamin D orally in High dose 4000 IU/Day compared to group B , which included 25 cases received oral vitamin D in a low dose concentration 600 IU/d , Patients has been followed up in 3 times at baseline (0 week) , 6 weeks and 12 weeks intervals. **Results:** Serum vitamin D levels in Group were higher than Group B (44.48±12.86 vs 34.45±5.43). Medication consumption was higher in group A compared to group B, thus favors orally low dose administration of vitamin D at first 6 weeks in the beginning of treatment course. UAS7 score in group A was better than Group B from baseline) to 6 weeks (P=0.*009 vs 0.239)* and from 6 weeks to 12 weeks. *(P= 0.*011 vs <0.0011). There was no significant difference in serum CRP between group A and group B as regards to CRP, furthermore there was no statistically correlation between 3 times intervals in group A and group B separately (12.71±1.47 vs 13.11±1.45). **Conclusion:** Improvement of both quality of life, and UAS7 score after receiving of High dose 4000 IU/d vitamin D orally in Group A, could benefit patients with CSU and decrease the complication of this disease. It was also found Serum Vitamin D level has no significant relation with C Reactive protein level, thus we couldn't relay on evaluation the chronicity of urticarial by measuring its value in serum blood with patients suffering from chronic spontaneous idiopathic urticaria.

Keywords: Vitamin D, Urticaria, auto-immune diseases, Immunomodulation.

INTRODUCTION

Chronic urticaria with or without angioedema (CU) is a common allergic skin condition associated with considerable morbidity and burden on health care expenditure. CU is defined as urticarial wheals occurring daily or almost daily and lasting longer than 6 weeks. It has been estimated that 10% to 20% of the population develop an acute episode of urticaria in their lifetime and 1% to 3% develop CU [(1)]. Through a comprehensive approach, cutaneous symptoms sometimes can be ascribed to drug, food, aeroallergen, contact allergen, or autoantibodies to the high-affinity IgE receptor or to free IgE. However, in most cases, the diagnosis remains idiopathic [(2)].

Chronic spontaneous urticaria (CSU) is an inflammatory disease, characterized by acute phase response (APR) and in many cases by the immune-activation. C-reactive protein (CRP) is a marker of systemic CSU activity, reflecting the systemic effects of inflammatory mediators associated with the disease, including IL-6 [(3)].

Treatment options are limited, and the mainstay of therapy is symptomatic control with antihistamines. Systemic corticosteroids, anti-leukotrienes, hydroxychloroquine, cyclosporine, dapsone, anti-IgE monoclonal antibody therapy, and other anti-inflammatory agents may be used, which themselves can pose substantial adverse events and cost. A potential alternative and safe immune-modulator is vitamin D [(4)].

Vitamin D has immunomodulatory properties and is able to suppress the inflammatory milieu, including IL-6 and CRP**.** In the clinical practice, vitamin D status is assessed by measurement of the circulating level of 25-hydroxyvitamin D [25(OH)D], considered as the best indicator of vitamin D status, including its [(5)].

Vitamin D plays key roles in innate and adaptive immunity through the stimulation of Toll-like receptors, increasing pro-inflammatory

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cytokine production, and possibly enhancing T helper type 2 responses. These mechanisms may explain the growing body of evidence connecting vitamin D to allergic diseases, including asthma, food allergies, and allergic rhinitis. As with many of the atopic diseases, there are conflicting data surrounding the effect that vitamin D has on the development of allergic skin diseases. Most of the studies to assess the impact of vitamin D on allergic skin diseases focus on atopic dermatitis (AD).

Current data demonstrate the importance of screening for vitamin D deficiency measured by serum concentration of 25(OH) D in chronic urticaria patients. In addition, such observations may have certain therapeutic implications. Interestingly, it has been demonstrated that in patients suffering from idiopathic chronic urticaria, isolated pruritus, and rash with low 25(OH)D level, the symptoms resolution is often possible with oral supplementation of vitamin D [(6)].

To date very few randomized, prospective trials have investigated the role of vitamin D supplementation in patients with CU and whether vitamin D supplementation decreases CU symptoms in patients with vitamin D insufficiency.

PATIENTS AND METHODS

The study is a prospective, randomized, single blind study. This observational case control study was conducted on 50 patients, attended the allergy and clinical immunology clinic at Ain Shams University Hospital, An informed consent was taken from all participants prior to enrollment in this study which was approved by the Ain Shams Medical Research Ethics Committee. All the patients were diagnosed according to EAACI/GA2LEN/EDF/WAO Guideline [(7)].

Patients of either sex with chronic urticaria who complained of daily appearance of wheals for ≥6 weeks divided into 2 groups:

- **The first group** including 25 patients with a daily administration of 4000 IU of vitamin D (High dose group1)
- **The second group** including 25 patients receiving 600 IU of vitamin D daily for 12 weeks (Low dose group 2)

Patients were subjected at 3 time intervals, the first was baseline before administrating Vitamin D in both groups, a second was after 6 weeks intervals, and a third was after 12 weeks intervals, both 6 and 12 weeks intervals were after administrating vitamin d with low or high dose in both groups.

Inclusion Criteria

Subjects were included if they have physician-diagnosed chronic urticaria and/or angioedema (CUA). CUA is defined by having urticarial wheals (hives) and/or angioedema (dermal swelling) on a daily or almost daily for more than 6 weeks. Patients with CUA also having signs of dermatographism and/or delayed-pressure urticaria were included in the study. Subjects with history of intolerance to non-steroidal anti-inflammatory drugs were included but warned not to take this drug class (acetaminophen will be allowed instead).

Exclusion Criteria

1. Subjects with a pure physical or allergic urticaria, and/or hereditary and acquired angioedema (C1 esterase inhibitor deficiency). These subjects will be excluded as the etiology of their disease is known.
2. Pregnant or lactating women. All child-bearing women will be asked (verbally and on the questionnaire) if they are pregnant or lactating. If they answer yes, they will be excluded. As there is no risk or harm to the pregnant or lactating woman, a urine pregnancy test will not be used.
3. Subjects with any clinically significant abnormality in biochemistry testing, and/or hypercalcemia (calcium > 10.3 mg/dl) or renal insufficiency (GFR< 50 ml/min).
4. Subjects with a history of primary hyperparathyroidism, renal tubular acidosis, sarcoidosis, granulomatous disease, or malignancy.
5. Physical urticaria acquired or hereditary angioedema, Calcium level > 10.3 mg/dl, Glomerular filtration rate <50.
6. Pregnancy and lactation.

Clinical analysis and Investigations

A. Clinical analysis

I. The clinical records of the subjects were examined for age, sex, disease duration.
II. The urticaria activity score (UAS) of each patient, which was recorded in the medical records, was also reviewed. Using the UAS, which was estimated according to the number of wheals and pruritus intensity based on the EAACI/GA2LEN/EDF guidelines, the patients' disease severity levels were graded as mild (0~14), moderate (15~29), and severe (30~42).
III. Quality of life (Q2oL).
IV. E.N.T. & dental examination.

B. Laboratory analysis

In all cases, Each patient underwent the following tests;

- Routine laboratory tests (complete blood count, C-reactive protein (CRP), urine analysis, ESR, calcium, phosphorus, blood sugar, liver function tests and kidney function tests)
- Stool (for parasites),
- Hepatitis serology (HBVsAg, HCV Ab)
- Antinuclear antibody (ANA)
- Antithyroid microsomal antibodies
- Serum levels of 25-(OH)D_3
- Thyroid function tests.
- Total IgE
- ASST
- Skin brick test

Patients must have normal CBC and ESR and negative as regards ANA, HBsAg, HCV, antithyroid Abs, stool analysis, ASST and skin prick test.

Laboratory parameters, including serum levels of 25-(OH)D_3, eosinophil, calcium, phosphorous, alkaline phosphatase and total IgE, as well as for the results of the ASST.

C. Radiological analysis

- Chest X-ray
- Abdominal ultrasonography.

All the patients were divided into several subgroups, according to the urticarial activity score (UAS), glucocorticoids therapy response as well as serum 25(OH)D concentration.

Sample processing

- Venous blood (8 ml) was withdrawn from each patient where, 5 ml was placed in EDTA tube for performing complete blood count (CBC) and erythrocyte sedimentation rate (ESR) and 3 ml of blood was collected in plain vacutainers for analysis of ANA, vitamin D and total Ig E. Serum samples were stored at -20 ºC until the time of assay.
- Complete blood picture (CBC) was done using Coulter counter (T660).
- Erythrocyte sedimentation rate (ESR, mm/h) was assessed with the Westergren method.
- Viral markers HBsAg and HCV Ab.
- Anti-thyroid antibody was performed by indirect immunofluorescence assay using *Inova* Diagnostics (USA).

ANA

- ANA was performed by indirect immunofluorescence assay using IMMCO Diagnostics (USA) on Hep-2 substrate. All patients were negative as regards ANA autoantibodies.

Serum total IgE

- Serum total IgE concentration (IU/mL) was evaluated using the Total IgE enzyme immunoassay (ELISA) kit (DRG International Inc., USA), according to the instructions of the manufacturer. The minimum detectable concentration was 5 IU/mL. The normal limit of total IgE was 100 IU/ml.

Vitamin D assay

- Serum 25(OH)D concentration was analyzed using an enzyme linked immune sorbent assay (ELISA) kit (Immunodiagnostic AG, Bensheim, Germany).The assay could detect 25(OH)D concentrations as low as 6.4 nmol/L. The intra-and inter-assay coefficients of variation for the ELISA were both 7.0%. 25(OH)-D was studied rather than the more active form (1, 25- dihydroxyvitamin D [1, 25(OH)2D]), because reported associations with disease activity have been shown to be stronger for 25(OH)-D [8].
- Serum 25(OH)D concentration was measured with the use of an automated direct electro-chemiluminescence immunoassay (Elecsys, Roche Diagnostic, Mannheim Germany) with the detection limit of 3.0 ng/ml. Sufficient vitamin D concentration was defined as ≥30 ng/ml.

25 (OH)D - 25-hydroxyvitamin D

1. Insufficiency (≥20 ng/ml but <30 ng/ml)
2. Deficiency (<20 ng/ml)
3. Severe deficiency (<10 ng/ml)

Skin prick test

Puncturing the skin with a calibrated lancet (1 mm) held vertically, or a hypodermic needle or blood lancet at an angle of 45°, and introducing a drop of diluted allergen.

All patients were also administered skin prick with one drop of histamine as positive control and one drop of normal saline as negative control. An itchy wheal should develop at the histamine puncture site within 10 minutes. Test solutions are standardized to give a mean wheal diameter of 6 mm. The maximum or mean diameter of the wheals to various locally prepared allergen extracts including mites, moulds, animal epithelia (contain both hair and the outer epidermal layer of skin), and mixed pollens extract were read at 15 minutes. A wheal of 3 mm or more in diameter was considered to represent a positive response (indicating sensitization to the allergen) and was excluded from the study. The negative control is of value due to the fact that it excludes the presence of dermographism i e: the phenomenon of skin

whealing occurring at sites of trauma, friction with clothing, or scratching, which if present makes the tests difficult to interpret [9].

Assessment of activity and quality of life (QoL) in CSU

Quantitative measurement of CSU activity is useful to monitor disease and the effects of medications and other interventions. In the absence of biochemical markers or objective parameters, patient reported outcomes (PRO) are used. Parameters such as extent of rash, severity of symptoms, and quality of life (QOL) impact are recorded.

Baiardini *et al.* [10] in a GA2LEN position paper recommended the use of the urticaria specific quality of life assessment CU-Q2oL due to its availability in a number of languages, and due to an urticaria specific QOL assessment being more sensitive to changes in symptoms but no references were given.

Original (Italian) CU-Q2oL questionnaire

The Italian version of the CU-Q2oL questionnaire comprises 23 items categorised into six domains: pruritus (two items), impact on daily activities (six), sleep problems (five), limitations (three), look (five), and swelling (two).1 For each item, patients were asked to choose between five response values (scored 0–4) indicating the intensity of each item in the last 2 weeks. A total summed score across all items was calculated and transformed into scores ranging from 0 to 100, with a score of 100 indicating the worst HRQoL impairment.

RESULTS

Statistical Methods

Data were analyzed using SPSS© Statistics version 24 (SPSS© Corp., Armonk, NY, USA).

Normally distributed numerical variables were presented as mean ± SD and inter-group differences were compared using the independent-samples Student t test. Skewed numerical data were presented as median and interquartile range and between-group differences were compared using the Mann-Whitney test.

Categorical variables were presented as number and percentage. Fisher's exact test was used to compare nominal data and the chi-squared test for trend to compare ordinal data. Correlation was tested using the Spearman rank correlation. The correlation coefficient (Spearman rho) is interpreted as follows:

Correlation coefficient	*Strength of correlation*
<.2	Very weak
.2 –.39	Weak
.4 –.59	Moderate
.6 –.79	Strong
.8 – 1	Very strong

P-value <.05 was considered statistically significant.

Table 1. Demographic characteristics of both study groups

Variable	***Group A (n=25)***	***Group B (n=25)***	***p-value***
Age (years)	40 ± 16	32 ± 6	**.020¶**
Gender			.769§
M	8 (32.0%)	10 (40.0%)	
F	17 (68.0%)	15 (60.0%)	
BMI (kg/m^2)	30.8 ± 5.8	24.1 ± 0.5	**<.001¶**
Smoking	5 (20.0%)	4 (16.0%)	1.000§
Disease duration (weeks)	11 ± 5	30 ± 5	**<.001¶**
Associated angioedema	18 (72.0%)	15 (60.0%)	.551§
Family history of chronic urticaria	10 (40.0%)	9 (36.0%)	1.000§

Data are presented as mean ± SD or number (%).
¶Unpaired t test.
§Fisher's exact test.

This table showed no significant difference between 2 groups regarding gender, Education, smoking , associated angioedema or family history of chronic urticaria. However, mean age of Group A was significantly higher than Group B. Mean value of BMI was significantly higher in Group A compared to Group B (31, 24 kg/m^2) respectively. Disease duration was significantly lower in group A than group B.

Table 2. Laboratory findings in both study groups

Variable	*Group A (n=25)*	*Group B (n=25)*	*p-value*
ESR (mm/h)	25 ± 3	25 ± 3	.962¶
Eosinophil count (cells/µl)	128 ± 27	125 ± 26	.662¶
Serum IgE (kU/l)	137 ± 8	155 ± 14	**<.001¶**
Positive thyroid antibodies	10 (40.0%)	5 (20.0%)	.217§
Positive ANA	7 (28.0%)	14 (56.0%)	.085§
Positive SPT	14 (56.0%)	22 (88.0%)	**.025§**

Data are presented as mean ± SD or number (%).
¶Unpaired t test.
§Fisher's exact test.

Table 2 shows no significant difference between both groups regarding ESR, Eosinophil count, thyroid antibodies & ANA. However, mean value of Serum IgE in Group A was significantly lower than Group B (137 , 155 kU/l) respectively and numbers of Positive SPT was significantly lower in Group A than Group B (14, 24) respectively.

Table 3. Vitamin D level in both study groups

	Group A (n=25)	**Group B (n=25)**	**P value**
Baseline vitamin D level (ng/ml)	27.8±1.22	38±1.8	**<.001**
Vitamin D level (ng/ml) at 6 Weeks	47.20±2.08	38.2±1.6	**<.001**
Vitamin D level (ng/ml) at 12 Weeks	58.44±1.87	27.16±1.43	**<.001**
	P value	**P value**	
Vitamin D levels at Baseline versus 6 weeks	**<.001**	.681	
Vitamin D levels at Baseline versus 12 weeks	**<.001**	**<.001**	
Vitamin D levels at 6 weeks versus 12 weeks	**<.001**	**<.001**	

Data are presented as mean ± SD.
¶Unpaired t test.

Table 3 shows : Mean value of Baseline Vitamin D in Group A was significantly lower than Group B (28, 38 ng/ml) respectively, mean value of Vitamin D at 6 weeks was significantly higher in Group A compared to Group B (47, 38 ng/ml) respectively and Vitamin D at 12 weeks mean value was significantly higher in Group A than Group B (58, 27 ng/ml) respectively.

Table 4: Mean level of serum vitamin D from baseline, 6 weeks, 12 weeks and number of cases as regards to serum vitamin D ranges from (24-28), (25-29) and 30 ng/ml

	Group A			**Group B**		
Vitamin D Mean	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
	28.00	48.00	59.00	39.00	39.00	28.00
Serum Vit D ng/ml	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
(24-28) ng/ml						24(96%)
(25-29) ng/ml	23(92%)					
30 ng/ml	2(8%)					1(4%)

Table 4 shows mean level of serum vitamin D from baseline, 6 weeks and 12 weeks in Group A as follows (28, 48, 59 ng/ml) compared to Group B (39, 39, 28 ng/ml). As regards to number of cases, level of serum vitamin D at a range (24-28) ng/ml Group B showed 24(96%) at 12 weeks , moreover 23(92%) of cases at baseline at a range of (25-29 ng/ml) at baseline in group A , meanwhile 2(8%) cases at baseline at 30 ng/ml serum vitamin D in Group A compared to 1(4%) case at 12 weeks in Group B.

Table 5. Mean CRP level in both study groups

	Group A (n=25)	Group B (n=25)	P value
Baseline CRP (mg/dl)	12.92±1.73	13.52±1.50	.197
CRP mg/dl at 6 Weeks	12.88±1.39	12.96±1.43	.842
CRP mg/dl at 12 Weeks	12.32±1.22	12.84±1.38	.163
	P value	**P value**	
CRP at Baseline versus 6 weeks	.929	.183	
CRP at Baseline versus 12 weeks	.162	.102	
CRP at 6 weeks versus 12 weeks	.137	.763	

Data are presented as mean ± SD,¶Unpaired t test.

No significant difference between 2 groups regarding Baseline CRP, CRP at 6 weeks and CRP at 12 weeks.

Moreovere, there were no statistically significant difference in CRP at baseline versus 6 weeks, baseline versus 12 weeks and from 6 weeks to 12 weeks in Group A and in Group B.

Table 6: Relationship between levels of serum vitamin D in Group A and CRP values

Group A	Serum vitamin D 25(OH)D (ng/ml)	CRP = C reactive protein (mg/d	P value
baseline	27.8±1.22	12.92±1.73	**<.001**
6 weeks	47.20±2.08	12.88±1.39	**<.001**
12 weeks	58.44±1.87	12.32±1.22	**<.001**
Total	44.48±12.86	12.71±1.47	

Data are presented as mean ± SD, ¶Unpaired t test.

This table shows significant difference in group A between serum vitamin D and CRP as regards to baseline, 6 weeks and 12 weeks.

Table 7: Relationship between levels of serum vitamin D in Group B and CRP values

Group B	Serum vitamin D 25(OH)D (ng/ml)	CRP = C reactive protein (mg/dl)	P value
baseline	38±1.8	13.52±1.50	**<.001**
6 weeks	38.2±1.6	12.96±1.43	**<.001**
12 weeks	27.16±1.43	12.84±1.38	**<.001**
Total	34.45±5.43	13.11±1.45	

Data are presented as mean ± SD, ¶Unpaired t test.

This table shows significant difference in group B between serum vitamin D and CRP as regards to baseline, 6 weeks and 12 weeks.

Table 8. UAS-7 score in both study groups

Variable	Group A (n=25)	Group B (n=25)	P value
Baseline UAS-7			.777
Mild	0 (0.0%)	0 (0.0%)	
Moderate	14 (56.0%)	15 (60.0%)	
Severe	11 (44.0%)	10 (40.0%)	
UAS-7 at 6 weeks			.553
Mild	0 (0.0%)	1 (4.0%)	
Moderate	20 (80.0%)	16 (64.0%)	
Severe	5 (20.0%)	8 (32.0%)	
UAS-7 at 12 weeks			**.030**
Mild	3 (12.0%)	1 (4.0%)	
Moderate	20 (80.0%)	16 (64.0%)	
Severe	2 (8.0%)	8 (32.0%)	
	P value	**P value**	
UAS-7 score at Baseline versus 6 weeks	**.009**	.239	
UAS-7 score at Baseline versus 12 weeks	.086	.239	
UAS-7 score at 6 weeks versus 12 weeks	**.011**	**<.001**	

Data are presented as number (%), ¶Chi-squared test for trend.

Table 8 shows no significant difference in UAS 7 score between both groups at baseline and after 6 weeks of vitamin D intake. However at 12 weeks UAS-7 score was significantly lower in Group A compared to Group B indicating better improvement of CSU in this group.

Table 9. CU-Q2oL score in both study groups

	GroupA (n=25)	**GroupB (n=25)**	**P value**
Baseline CU-Q2ol	16.96±7.541	16.36±7.76	.783
CU-Q2ol at 6 Weeks	14.56±6.04	14.4±7.10	.932
CU-Q2ol at 12 Weeks	12.60±5.67	14.4±7.10	.327
	P value	**P value**	
CU-Q2ol at Baseline versus 6 weeks	.220	.356	
CU-Q2ol at Baseline versus 12 weeks	**.025**	.356	
CU-Q2ol at 6 weeks versus 12 weeks	.243	1.00	

¶Unpaired t test.

Table 9 shows that at baseline, 6 & 12 weeks there was no statistically significant difference in CU-Q2ol between both groups. And however, there was significant difference in CU-Q2ol in Group A when comparing baseline to 12 weeks (14.7, 12.6) respectively.

It was also observed that medication usage in both study groups according to treatment at baseline at 12 weeks, there was no statistically difference between 2 study groups regarding H1RA, LTRA, H1RA-H2RA, H1RA-LTRA and Prednisone , except significant difference in number of patients as regard H2RA , number was lower in group A compared to group B (8, 16) respectively.

Table 10. Allergy pill usage (medication score) in both study groups

Variable	***Group A (n=25)***	***Group B (n=25)***	***p-value¶***
Baseline allergy pill usage (pills/day)	2 (2 – 3)	3 (2 – 3)	**.016**
Allergy pill usage at 6 weeks (pills/day)	1 (1 – 2)	1 (1 – 2)	.190
Allergy pill usage at 12 weeks (pills/day)	2 (2 – 3)	2 (2 – 3)	.773

Data are presented as median (interquartile range), ¶Mann-Whitney U test.

This table showed no significant difference between both groups regarding medications usage at 6 weeks and medications usage at 12 weeks. However median value of baseline medications usage in Group A was significantly lower than Group B (2, 3) pills/day respectively.

Table 11. Change in medication score in both study groups

Variable	***Group A (n=25)***	***Group B (n=25)***	***p-value¶***
Change in medication score at 6 weeks (difference in pills/day from baseline)	-1 (-1 to 0)	-2 (-2 to -1)	**.006**
Change in medication score at 12 weeks (difference in pills/day from baseline)	0 (0 to 0)	0 (-1 to 0)	.075

Data are presented as median (interquartile range), ¶Mann-Whitney U test.

This table shows no significant difference between both groups regarding Change in medication score at 12 weeks. However, median value of Change in medication score at 6 weeks in Group A was significantly higher than Group B (-1, -2) pills/day respectively.

Table 12. Improvement in medication score in both study groups

Variable	***Group A (n=25)***	***Group B (n=25)***	***p-value¶***
Medication score at 6 weeks			**.007**
Unchanged	21 (84.0%)	11 (44.0%)	
Improved	4 (16.0%)	14 (56.0%)	
Medication score at 12 weeks			.110
Unchanged	25 (100.0%)	21 (84.0%)	
Improved	0 (0.0%)	4 (16.0%)	

This table shows improvement in medication score in both study groups, through 6 weeks and 12 weeks intervals, medication score at 6 weeks showed significant difference in unchanged and improved numbers of patients in Group A versus Group B , the number of unchanged patients was higher in Group A compared to Group B (21, 11) respectively. While number of improved patients was lower in group A

compared to Group B (4, 14) respectively. However there was no significant difference between 2 study groups regarding medication score at 12 weeks.

Table 13. Correlation between vitamin D level and measures of disease activity at baseline

		Baseline vitamin D level
ESR	*Correlation coefficient*	-.081
	p-value	.575
Eosinophil count	*Correlation coefficient*	.070
	p-value	.629
Serum IgE	*Correlation coefficient*	**.740****
	p-value	**<.001**
Baseline CRP	*Correlation coefficient*	.257
	p-value	.072
Baseline UAS-7	*Correlation coefficient*	-.007
	p-value	.960
Baseline CU-Q2oL	*Correlation coefficient*	.003
	p-value	.982
Baseline allergy pill usage	*Correlation coefficient*	.264
	p-value	.064

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation between vitamin D level and measures of disease activity at baseline, the table shows no significant correlation between vitamin D level, ESR, Eosinophil count, Baseline CRP, Baseline UAS-7, Baseline CU-Q2oL and Baseline allergy pill usage. There was positive correlation between vitamin D level and Serum IgE level.

Table 14. Correlation between vitamin D level and measures of disease activity at 6 weeks and 12 weeks

		Vitamin D at 6 weeks	***Vitamin D at 12 weeks***
CRP	*Correlation coefficient*	0.011	-0.254
	p-value	0.937	0.075
UAS-7	*Correlation coefficient*	-0.035	-0.22
	p-value	0.81	0.126
CU-Q2oL	*Correlation coefficient*	0.114	-0.195
	p-value	0.432	0.175
% of change in CU-Q2oL score	*Correlation coefficient*	0.088	-0.048
p-value	*p-value*	0.708	0.738
Allergy pill usage	*Correlation coefficient*	0.185	0.015
p-value	*p-value*	0.199	0.92
Change in medication score	*Correlation coefficient*	-.374**	0.253
	p-value	0.007	0.077

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation between vitamin D level and measures of disease activity at 6 weeks, shows no significant difference between vitamin D level and CRP, UAS-7, CU-Q2oL, % of change in CU-Q2oL score and Allergy pill usage. There was negative correlation between vitamin D level and change in medication score at 6 weeks. This table also shows no significant correlation between vitamin D level and CRP, UAS-7, CU-QOL, pill usage and % of change in both CU-QOL and medication use after 12 weeks.

DISCUSSION

Chronic spontaneous urticaria (CSU) is an immune-inflammatory disease, characterized by acute phase response (APR) and immune activation. There has been increasing evidence showing that vitamin D deficiency/insufficiency is associated with increased incidence and/or severity of immune-inflammatory disorders [(11)].

Vitamin D is a "hormone" having important immunomodulatory and immunoregulatory properties. In vitamin D deficient conditions, disrupted mucosal and skin

complex integrity and intercurrent infections may act synergistically with allergenic exposure to amplify sensitization risk. There has been emerging data to show that vitamin D can enhance the anti-inflammatory effects of glucocorticoids and potentially be used as adjuvant therapy in steroid-resistant severe asthma.

The goal of the present study was to determine the effect of daily oral intake of vitamin D3 supplementation [high (4, 000 IU/d) versus low (600 IU/d) dose for 12 weeks on Urticaria activity score (UAS-7), quality of life (QOL) and medication burden in patients with CSU , and to assess the relationship between vitamin D levels and CRP in these patients.

This study was conducted on 50 patients recruited from outpatient clinic in Ain Shams University hospital; patients were divided into 2 groups :

1) Group A: Administered a dose with 4000IU/day
2) Group B : Administered a dose with 600IU/day.

Serum 25(OH)D concentration was measured using an automated direct electro-chemiluminescence immunoassay (Elecsys, Roche Diagnostic, Mannheim Germany) with the detection limit of 3.0 ng/ml. Sufficient vitamin D concentration was defined as ≥30 ng/ml, Insufficiency (≥20 ng/ml but <30 ng/ml), Deficiency (<20 ng/ml) and Severe deficiency (<10 ng/ml) [12].

Although both groups were matched regarding gender, group A was statistically significantly older in age as compared to group B & showed higher BMI. The disease duration was statistically significantly longer in Group B.

Serum vitamin D level

Serum vitamin D level in group A was insufficient (28) at baseline while in group B serum vitamin D level was sufficient (38) at baseline and the comparison was significant.

The current study reported significant difference between 2 study groups as regards serum vitamin D level. The baseline level of vitamin D in group A was lower than group B (28, 38 ng/ml) respectively, However vitamin D level in group A was (47, 38 ng/ml) higher than group B (58, 27 ng/ml) respectively at 6 & 12 weeks.

Group A was insufficient (28 ng/ml) & became adequate at 6 & 12 weeks respectively (47, 58 ng/ml). However group B was adequate (38, 38 ng/ml) at baseline & 6 weeks respectively & became insufficient (27 ng/ml) at 12 weeks.

There was significant increase in group A in levels of serum vitamin D, from baseline, which show insufficiency (27 ng/ml), compared to 12 weeks follow up, which show sufficiency (59 ng/ml).

There was significant decrease in group B in serum vitamin D levels, from baseline which show sufficiency (38 ng/ml), compared to 12 weeks follow up, which show insufficiency (28 ng/ml).

After 3 months there was significant increase of serum vitamin D level in group A which was sufficient (59 ng/ml) compared to with group B which show insufficiency (28 ng/ml)

As regards vitamin D status in patients with CSU at baseline, Many studies reported insufficiency in vitamin D :

Sindher *et al.* [13] reported deficiency of vitamin D in patients with Chronic urticaria cases at base line, in a group administered vitamin D orally with a dose 2000 IU/D

As regards to the effect of vitamin D supplementation after 3 months :

Oguz Topal *et al.* [14] showed sufficiency of vitamin D in patients with Chronic urticaria cases after 3 month follow up, in a group administered vitamin D orally with a dose 10.000 IU/D

UAS7 score:

Regarding UAS7 score, was negatively correlated in both groups, indicating improvement in severity of chronic spontaneous urticaria after administration oral vitamin D in 6 and 12 weeks follow up.

Therefore the improvement in UAS7 score was recorded regardless of the dose of vitamin D administration through the initial 6 weeks, in mild and moderate cases the improvements in group B was higher than group A, however in severe cases the improvements in group A higher than group B.

In group A , there was significant improvement in cases of CSU from baseline to 6 weeks and 12 weeks , the values were (11, 5, 2) respectively. However mild and moderate show no improvement at 3 times intervals with significant differences.

Cu-Q2ol score

As regards Cu-Q2ol score, group A showed significant improvement in CSU from baseline to 12 weeks, the values were (17, 13) respectively (P value = 0.025)

There was no significant differences between both study groups, the same as there was no significant correlations at group A times intervals from baseline to 6 weeks, from 6 weeks to 12 weeks, and in group B, there was no significant difference at 3 times intervals.

Improvement of both quality of life, and UAS7 score after receiving of High dose 4000 IU/d vitamin D orally in Group A, could benefit patients with CSU and decrease the complication of this disease.

Goetz *et al.* [15] in a study of oral administration of vitamin D with a dose 50.000 IU/D , they were reported complete clinical resolution of urticaria at follow up time 8-12 weeks

Sindher S.B. *et al.* [13] reported that after oral administration of vitamin D with a dose 2000 IU/D, there was improvement in patients with chronic Urticaria

Oguz Topal *et al.* [14] showed significant improvement of CSU regarding symptoms and quality of life at a dose of 10.000 IU vitamin D orally administered for 3 month follow up

Likewise, **Thuerk** [16] in a study included 42 patients with CSU , divided into 2 groups , the first received vit. D with a dose 4000 IU/D (high dose group) compared to the other lower dose group with a dose 600 IU/D , they reported significant improvement in UAS7 & and Q2Ol in high dose group compared to the other group.

Pramyothin and Holick [12] declared no significant inverse correlation between disease severity and serum vitamin D concentrations.

Medication burden

According to data, As regard to medication score, at 12 weeks, group A was lower than group B in medication score, (0, -1) respectively the result was near significant (P value =0.07). However median value of Change in medication score at 6 weeks of group A was significant lower than group B (-1, -2) pills/day respectively. There was Improvement in medication score in both study groups, through 6 weeks, medication score at 6 weeks showed significant difference in unchanged and improved numbers of patients in group A versus group B , the number of unchanged patients was higher in group A compared to group B (21, 11) respectively. While number of improved patients was lower in group A compared to group B (4, 14) respectively.

We recommend supplementation of oral vitamin D with initial dose 600 IU/Day at first 6 weeks, which followed by high dose 4000 IU/day in the next 6 weeks, can be used as additive medication to antihistaminic or corticosteroids medication used in treatment of CSU cases to decrease medication burden in patients by 12 weeks. This could lower the side effects of medication and lower its consumption which lead to decrease the cost.

In contrast to our study **Oguz Topal *et al.*** [14] in another study included 42 patients with CSU , divided in 2 groups the first received 4000 IU/D and the second received 600 IU/D , vitamin D was added to triple therapy (EC trizine, Rantidine and Mntelukast) in a written action plan. They showed no significant improvement in medication usage in high dose group compared to low dose group.

Relations between serum vitamin D levels and CRP

This study demonstrated that there was significant difference between both study groups regarding 3 times intervals baseline, 6 weeks and 12 weeks. Although in this study CRP showed no significant values between both study groups and follow up from baseline to 6 weeks, from baseline to 12 weeks and from 6 weeks to 12 weeks.

Grzanka *et al.* [1] in a study revealed the relation between CRP and Serum vitamin D levels in patients suffering from chronic spontaneous urticaria, the result showed no significant correlation between both entities.

In contrast to our study outcome, **Boonpiyathad *et al.*** [17] reported significant relation between serum vitamin D level and chronic urticaria at a daily dose 20.000 IU/d from vitamin D for 6 weeks. Likewise, **Rorie *et al.*** [4] declared the significance of screening patients with CSU for serum vitamin D levels, who they reported lowering of serum vitamin D levels in patients with CSU.

LIMITATIONS OF THE CURRENT STUDY

This study includes limited numbers of cases & short duration of follow up as well as absence of a third group administered a placebo.

Future studies on a larger scale with longer follow up periods are required to validate role of vitamin D administration to treat patients with CSU.

CONCLUSION AND RECOMMENDATION

In the present study, we could found sufficient body of evidence suggesting that Oral vitamin D in high dose (4000 IU/d) can significantly help in alleviating and relieving symptoms, severity of disease UAS7 score , Q2ol , Lowering total consumption of medication pills , decreasing the amount of CRP and improvement of serum vitamin D over 12 weeks follow up.

We recommend supplementation of oral vitamin D with initial dose 600 IU/Day at first 6 weeks, which followed by high dose 4000 IU/day in the next 6 weeks, can be used as additive medication to antihistaminic or corticosteroids medication being used in treatment of CSU cases and decreasing medication burden of patients by 12 weeks follow up could lower from the side effects of medication and lower its consumption led to decrease the cost that could be achieved.

Improvement of both quality of life, and UAS7 score after receiving of High dose 4000 IU/d vitamin D orally in Group A, could benefit patients with CSU and decrease the complication of this disease.

Serum vitamin D level has no significant relation with C Reactive protein level, thus we couldn't relay on evaluation the chronicity of urticarial by measuring its value in serum blood with patients suffering from chronic spontaneous idiopathic urticarial.

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