

Vitamin D Level among Egyptian Patients with Chronic Spontaneous Urticaria and Its Relation to Severity of the Disease

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Vitamin D has an important role in the immune system. Decreased serum vitamin D level is known to be associated with autoimmune and atopic diseases. This study aimed to assess vitamin D status in patients with chronic spontaneous urticaria (CSU) and its relation with severity of the disease. This case-control study was conducted on 22 patients and 20 age and sex matched controls. Patients were subjected to clinical assessment, routine laboratory examination: complete blood picture (CBC), erythrocyte sedimentation rate (ESR), hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), anti-thyroid antibody, anti-nuclear antibody (ANA), total immunoglobulin E (IgE), vitamin D assay and stool analysis, and special investigations including: skin prick test (SPT), autologous serum skin test (ASST) and urticaria activity score (UAS). Patients' mean age was 32.8 ± 13.17 years. The median and interquartile range of duration of illness was 2.5(1-4) years and of Ig E was 79(62-312) IU/ml. According to UAS; 14 (63.6%) had severe and 8 (36.4%) had moderate degree illness. The mean vitamin D level among the patients was 28.4 ± 9.09 nmol/L. Vitamin D level was significantly lower among patients in comparison to controls (28.4 ± 9.09 vs. 104.5 ± 76.8 , $t=4.4$ $P<0.01$). Vitamin D correlated negatively with IgE level ($r=-0.45$, $P<0.05$), meanwhile it was insignificantly correlated with age and duration of illness ($r=-0.117$ and 0.34 respectively, $P>0.05$). In conclusion; Low vitamin D level($r=0.45$) is associated with chronic spontaneous urticaria but has no relation with the severity of the disease.

Urticaria is characterized by sudden appearance of wheals and/or angioedema, defining wheals as a cutaneous swelling of variable size, almost invariably surrounded by a reflex erythema, with associated itching or, sometimes, a burning sensation, and of transient nature, with the skin returning to its normal appearance in usually 1 to 24 hours (Sánchez-Borges *et al.*, 2012). Urticaria can be classified on the basis of its duration; acute urticaria (AU) is characterized by the occurrence of hives and/or angioedema for, 6 weeks, whereas episodes lasting longer than 6 weeks are regarded as chronic urticaria (CU) (Greaves, 1995).

Vitamin D is essential for bone and mineral homeostasis, but vitamin D also regulates the growth and differentiation of multiple cell types and displays immunoregulatory and anti-inflammatory properties (Holick, 2007).

A role for vitamin D in allergic diseases gained attention after its potential causative role was proposed in anaphylaxis (Camargo *et al.*, 2007). Vitamin D deficiency also has been linked with difficult-to-control asthma (Sutherland *et al.*, 2010; Wu *et al.*, 2012). Vitamin D plays an important role in the balance between the innate and adaptive immune systems and may contribute to the etiopathogenesis of allergic diseases like chronic urticaria (CU) and atopic dermatitis (Chandrashekhar *et al.*, 2014). This study was designed to assess vitamin D status in patients with chronic spontaneous urticaria (CSU) and its relation to the severity of the disease.

Patients and Methods

This case-control study was conducted on 22 patients attended the allergy and clinical immunology clinic at Ain Shams University Hospital, and 20 age and sex matched controls. 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 (Zuberbier *et al.*, 2014); patients of either sex with chronic urticaria who complained of daily appearance of wheals for ≥ 6 months, age >18 years were included. Exclusion criteria included patients with secondary causes of urticaria, predominantly physical urticaria, history of other atopic diseases, and history of systemic corticosteroid or immunosuppressive drug use in the past 6 weeks and other systemic illnesses requiring treatment.

Patients were subjected to full clinical history and clinical assessment:

Laboratory Assessment

- 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°C until the time of assay. The investigations, CBC, ESR, HBsAg, HCV, ANA, antithyroid Abs, skin prick test, and ASST were needed to exclude secondary causes of urticaria. Patients must have normal CBC and ESR and negative as regards ANA, HBsAg, HCV, antithyroid Abs, stool analysis, ASST and skin prick test.
- 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). All patients were negative as regards antimicrosomal and antithyroglobulin autoantibodies.
- ANA was performed by indirect immunofluorescence assay using IMMCO Diagnostics (USA) on Hep-2 substrate. All patients were negative as regards ANA autoantibodies.
- Stool analysis to exclude parasitic infestation.
- 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 immunosorbent assay (ELISA) kit (Immundiagnostik 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 (Patel *et al.*, 2007).
- 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 (Berger, 2002).
- Autologous serum skin test (ASST) was performed by drawing 2 cc of venous blood in a non-heparinized test tube and leaving it to clot at room temperature; then the serum was separated by placing the blood in a centrifuge for 10 minutes at a velocity of 2000 RPM (revolution per minute). A volume of 0.05 mL of the patient's serum is then injected intradermally on the volar aspect of the patient's forearm at site not affected by a wheal in the last 24 hours; at the same time the same volume of histamine and normal saline are injected separately intradermally over the volar aspect of the forearm at least 5 centimeters away from the serum injection site; then we wait for 30 minutes

(15 minutes for the histamine) to see the response. The test is considered positive if there is a wheal and flare at serum injection site of a diameter (measured by taking the mean of the largest two diameters) at least 1.5 millimeters more than the wheal and flare induced at the control site (if any) (Vohra *et al.*, 2009).

- Urticaria Activity Score (UAS): According to EAACI/GA2LEN/EDF/WAO Guideline(Zuberbier *et al.*, 2014);daily intensity of pruritus (range: 0 none to 3 severe) and number of hives ratings (range: none [=0 points], <10 [=1 point], 10–50 [=2 points], or >50 per day [=3 points]) are summed to create a daily UAS score (range: 0–6 points/day).Daily UAS scores are summed over a week to create the UAS7 (range: 0–42).According to the sum of the UAS-7 in one week, Mild urticaria: UAS7=7-15, Moderate urticaria: UAS7=16-27, Severe urticaria: UAS7=28-42 (Stull *et al.*, 2014)

Statistical Analysis

Data was collected, tabulated and statistically analyzed using SPSS version 15. Data were expressed as Mean \pm SD for quantitative parametric measures in addition to median percentiles for quantitative non-parametric measures and both number and percentage for categorized data. Mann-Whitney test was used for comparison between two independent groups for non-parametric data. Independent t -test was used for comparison between two independent groups for parametric data .Paired t test was used for comparison between two dependent groups for parametric data. Correlation between two variables was done using Spearman correlation coefficient. Two tailed P value of >0.05 was insignificant, $P<0.05$ was significant

Results

The study included 22 patients; 10 (45.5%) were males. Their mean age was 32.8 ± 13.17 years. The median and IQR of duration of illness was 2.5(1-4) years.

According to UAS; 14 (63.6%) were severe and 8 (36.4%) were moderate degree. The mean vitamin D level was 28.4 ± 9.09 and median and IQR of IgE level was 79(62-312) (Table 1).

Table 1. Socio-demographic data, duration of illness, UAS, vitamin D and IgE level of the patients

Male n (%)	10 (45.5%)
Age years (mean \pm SD)	32.8 ± 13.17
Duration of illness years (median and IQR)	2.5 (1-4)
Vitamin D nmol/L (mean \pm SD)	28.4 ± 9.09
IgE IU/mL (median and IQR)	79 (62-312)
UAS7	
Severe n (%)	14 (63.6%)
Moderate n (%)	8 (36.4%)

IgE: immunoglobulin E, UAS: urticaria activity score, n; number, SD: standard deviation IQR: interquartile range

Vitamin D level was significantly lower among patients in comparison to control (28.4 ± 9.09 vs. 104.5 ± 76.8 respectively, $t=4.4$ $P<0.01$) (Figure 1).

Vitamin D negatively correlated with IgE level ($r=-0.45$, $P<0.05$) (Figure 2), meanwhile no correlation was found between it and age and duration of illness ($r=-0.117$ and 0.34 respectively, $P>0.05$).

No association was found between vitamin D level and severity of the urticaria. There was insignificant differences between patients with severe and moderate grade as regards vitamin D (28.14 ± 8.83 and 28.87 ± 10.13 respectively, $P>0.05$).

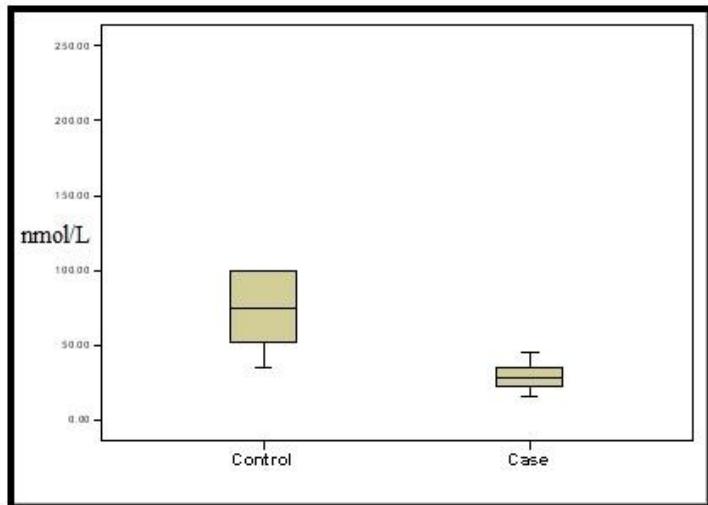


Figure 1. Vitamin D level difference between case and control groups.

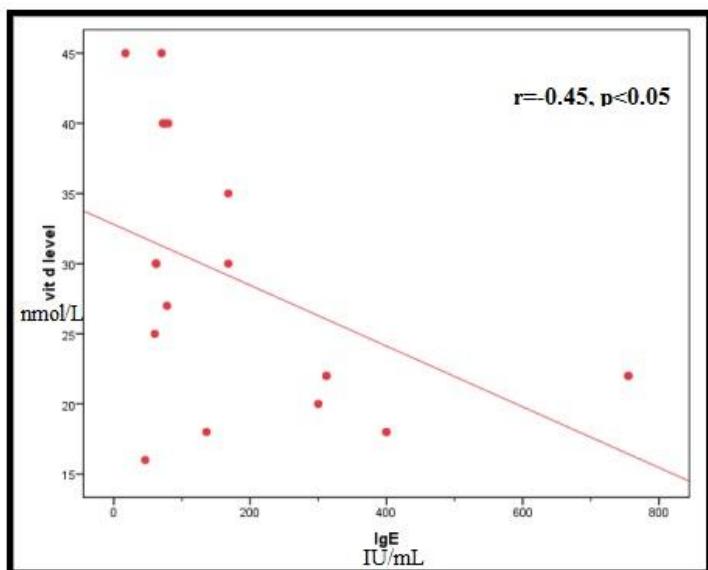


Figure 2. Negative correlation between vitamin D & IgE.

Discussion

Vitamin D regulates the immune system both directly and indirectly leading to multiple cell type differentiation, and in turn, modulation of inflammation (Holick, 2007). These cells (antigen presenting cells, B cells, and T cell) express vitamin D receptors and therefore, play role in sensitization of active vitamin D.

This results in regulation of innate and adaptive immunity (Aranow, 2011). Studies presented role function of vitamin D in different allergic skin diseases. In atopic dermatitis, vitamin D plays an important role in stimulation and regulation of cathelicidin, an important antimicrobial peptide in skin, which on deficiency leads to breakdown of

the skin barrier and exposing the immune system to stimulate and react with external allergens (Hata *et al.*, 2008). Also Benson *et al.* (2012) studied role of vitamin D in different allergic skin diseases. Few studies concentrated on role of vitamin D in urticaria.

Thorp *et al.* (2010) performed a study on vitamin D in chronic spontaneous urticaria in comparison with allergic rhinitis and showed that vitamin D was lower in those with CSU but didn't prove the high prevalence between the two groups. Grzanka *et al.* (2014) decided to perform a similar study on CSU in comparison with healthy control and proved the significant difference and showed high prevalence of vitamin D deficiency in CSU. In the present work, we came in strong agreement with Grzanka *et al.* (2014) but we found that the vitamin d deficiency didn't correlate with duration or severity of the disease. Unfortunately most of our patients were moderate and severe and maybe inclusion of mild cases should have been considered. Surprisingly, Boonpiyathad *et al.* (2014) also showed that vitamin D deficiency didn't correlate with symptoms and severity of urticarial and added that vitamin D shouldn't be used as a biomarker for severity of disease. On the contrary, Chandrashekhar *et al.* (2014) demonstrated that vitamin D deficiency correlated with disease severity. The difference in Chandrashekhar study is that they included different etiologies of CSU in the study. On the other hand, we excluded all causes of CSU to try to prove vitamin D as an independent factor in CSU. Interestingly several studies came up with clinical trial of vitamin D in CSU and found marked improvement of symptoms and decrease in severity (Rorie *et al.*, 2014).

As regards B cell function, vitamin D suppresses B cell differentiation, proliferation, and therefore, inhibits immunoglobulin secretion (Chen & Lipsky, 2007). First proposed by Heine *et al.* (2002), they showed

decrease of Ig E production on administration of vitamin D *in vitro*. Later on, Hyppönen *et al.* (2009) showed a non-linear association between levels of vitamin D and total IgE. Furthermore, Hartmann *et al.* (2011), specifically investigated B cell function on targeting vitamin D receptors and they showed that inhibition of B cell allergic response occurred. In the present study, we proved an inverse correlation between vitamin D levels and total IgE studied groups. This may clarify role of vitamin D in immune and allergic control of CSU. Limitation to our study is the number of patient studies on larger scale is recommended. Also follow up of vitamin D deficient patient should be considered after vitamin D supplementation. New insights on role vitamin D on T regulatory cell in CSU have been mentioned (Chandrashekhar *et al.*, 2014) so we recommend further studies on effect of vitamin D on T cell.

In conclusion, Low vitamin D level is strongly associated with chronic spontaneous urticaria but has no relation with the severity of the disease.

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