

Letter to the Editor

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25-Hydroxy vitamin D levels in chronic urticaria and its correlation with disease severity from a tertiary care centre in South India

Keywords: autoimmune urticaria; chronic urticaria; 25-hydroxy vitamin D; interleukin-17; T-helper cells; transforming growth factor- β .

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To the Editor,

25-Hydroxy 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. Recently, there has been a report of a patient with long lasting CU with vitamin D deficiency showing marked improvement in urticaria, after vitamin D supplementation [1].

Studies have demonstrated that CU has mainly a T-helper (Th)0 or mixed T-helper-1(Th1)/T-helper-2(Th2) profile. Both an excess in T-helper-17 (Th17) function or increased numbers of Th17, and defects in regulatory T (Treg) function or reduced numbers of Treg cells, may contribute to the systemic inflammation seen in CU [2]. It has been recently reported that 25-hydroxy vitamin D is a negative acute phase reactant [3]. In addition, inverse relationships between 25-hydroxy vitamin D levels and transforming growth factor- β (TGF- β) levels have also been established [4].

Hence, the purpose of the present research was to evaluate the 25-hydroxy vitamin D, interleukin-17 (IL-17) and TGF- β 1 levels in the serum of patients with CU, in comparison with healthy controls and to identify their association with disease severity in patients with CU.

This was a hospital-based, cross sectional study done in 45 patients with CU who presented to the dermatology clinic of our institute and 45 age- and gender-matched healthy controls, provided that they met all inclusion criteria and none of the exclusion criteria and that they gave informed consent. This study was approved by the Institute Ethics Committee, JIPMER, Puducherry, India.

The exclusion criteria for cases and controls were the following: acute urticaria, age <18 years and >40 years, on any medication or supplementation for the past 3 months other than anti-histamines which had to be stopped for 1 week prior, physical urticarias, urticarial vasculitis, pregnant women, obesity (BMI >25 kg/m²), dyslipidaemia, diabetes, pre-existing cardiovascular disease, hypertension, smokers, cerebro-vascular accidents, malignancies, hepatic and renal disease and inflammatory cutaneous disorders. Recruitment of cases and controls was done simultaneously in the summer over a period of 3 months.

All study participants were residents of Puducherry (11.9310° N, 79.7852° E) or adjacent to it, in India with Fitzpatrick skin type V. The weather is hot and humid throughout the year. The disease severity was assessed using the urticaria severity score (USS) [5]. Autologous plasma skin test (APST) was done in all patients with CU to screen for autoreactivity as described by Asero et al. [6] after stopping antihistamines for a week. Those with a positive APST were diagnosed to have autoimmune urticaria.

Estimation of IL-17 (Ray Biotech Inc., Norcross GA, USA), TGF- β 1 (Ray Biotech Inc.) and 25-hydroxy vitamin D (Euroimmun AG, Lubeck, Germany) were done from serum samples using commercially available ELISA kits. ELISA assays for cases and controls were run simultaneously

as a single batch. The lower detection limit of 25-hydroxy vitamin D was 1.8 ng/mL. Those with 25-hydroxy vitamin D levels <20 ng/mL were labelled as deficient and those between 20 and 30 were labelled as insufficient. Any value >30 ng/mL was labelled normal.

Statistical analysis was performed using IBM SPSS statistics version 20 for Windows. Student's t-test or Wilcoxon rank sum test was used for comparison of means. The data was expressed as mean±standard deviation. Spearman's correlation was used to assess correlation between the levels of 25-hydroxyvitamin D, inflammatory markers and USS. Analysis was carried out at 5% level of significance and $p < 0.05$ (two tailed) was considered as statistically significant.

There were no significant differences in baseline characteristics like age (30.2 ± 8.2 years in cases vs. 29.0 ± 7.1 years in controls), gender (35.5% males and 64.5% females in cases vs. 35.5% males and 64.5% females in controls) and BMI (23.08 ± 1.45 kg/m² in cases and 22.74 ± 1.53 kg/m² in controls) between the two groups. In the CU group, 27 were APST-positive and 18 were APST-negative. Among the cases with CU, 25-hydroxy vitamin D deficiency was seen in all study participants. Among the controls, 16 (35.55%) cases were deficient, 18 (40%) were insufficient and 11 (24.44%) had normal 25-hydroxy vitamin D levels. There was a significant lowering of mean 25-hydroxy vitamin D levels between cases with CU and controls (12.7 ± 2.7 vs. 24.3 ± 13.5 ng/mL, $p < 0.0001$). We also observed significantly raised levels of IL-17 (48.12 ± 6.95 vs. 5.1 ± 1.57 ng/mL)

and TGF- β 1 (55.05 ± 6.23 vs. 31.1 ± 5.84 ng/mL) in cases with CU, as compared to controls ($p < 0.0001$) (Figure 1A). Erythrocyte sedimentation rate (ESR) was significantly higher in the CU group, as compared to controls (16.57 ± 8.73 vs. 12.82 ± 3.59 mm/h, $p = 0.03$).

Mean USS was significantly higher in the APST-positive group, in comparison to the APST-negative group (5.3 ± 1.24 vs. 2.72 ± 1.07 , $p < 0.0001$). The mean 25-hydroxy vitamin D was significantly lower (11.1 ± 2.1 vs. 15.1 ± 1.3 ng/mL), whilst mean IL-17 (58.8 ± 5.2 vs. 49.4 ± 1.5 ng/mL) and TGF- β 1 levels (52.5 ± 3.9 vs. 41.4 ± 4.9 ng/mL) were significantly higher in the APST-positive group, in comparison to the APST-negative group (Figure 1B) ($p < 0.0001$).

In patients with CU, we observed a significant negative correlation between 25-hydroxy vitamin D levels and USS ($r = -0.95$, $p < 0.0001$), whilst cytokines-TGF- β 1 and IL-17 correlated positively with USS ($r = 0.95$, $p < 0.0001$ and $r = 0.95$, $p < 0.0001$, respectively). In both cases and controls, we found a significant negative correlation between 25-hydroxy vitamin D levels and IL-17 ($r = -0.9$, $p < 0.0001$), 25-hydroxy vitamin D levels and TGF- β 1 ($r = -0.9$, $p < 0.0001$) and 25-hydroxy vitamin D levels and ESR ($r = -0.3$, $p = 0.002$) (Figure 2). We also observed a significant positive correlation between IL-17 and TGF- β 1 levels in both cases and controls ($r = 0.9$, $p < 0.0001$).

25-Hydroxy vitamin D has been suggested as an immunomodulator of allergic skin diseases because of its role in regulating cathelicidin expression in the skin of patients with atopic dermatitis [7]. In a study performed

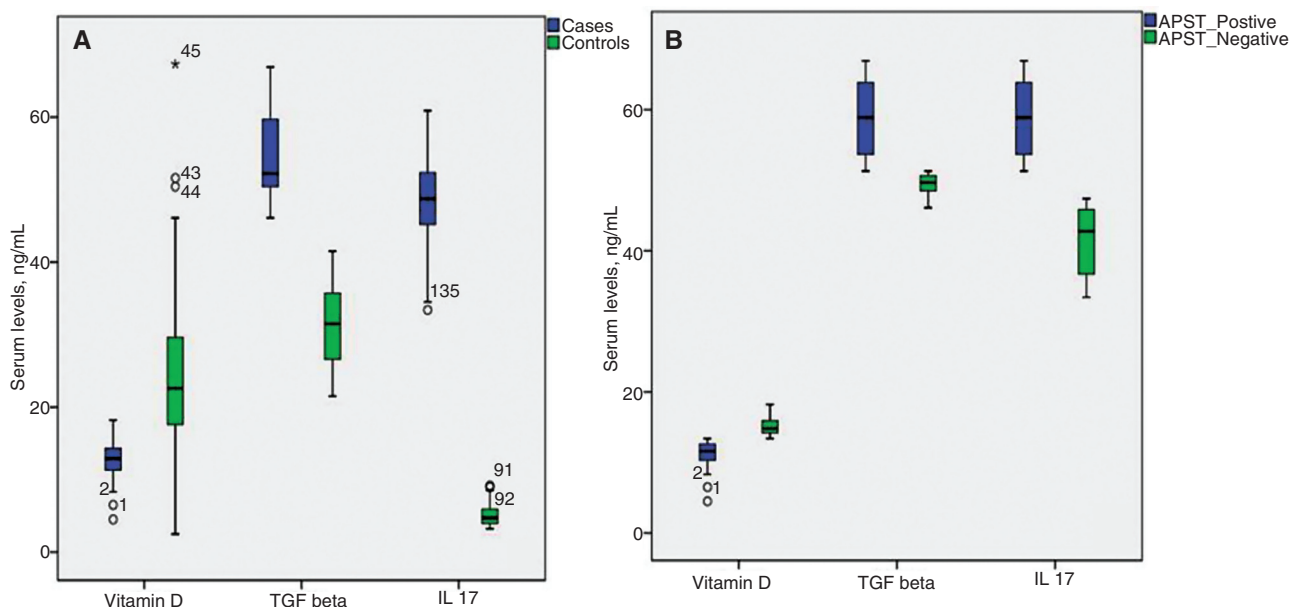


Figure 1 Box and Whisker plot comparing the serum levels of 25-hydroxy vitamin D, TGF- β 1 and IL-17 between patients with chronic urticaria and controls (A); between APST-positive and APST-negative patients with chronic urticaria (B).

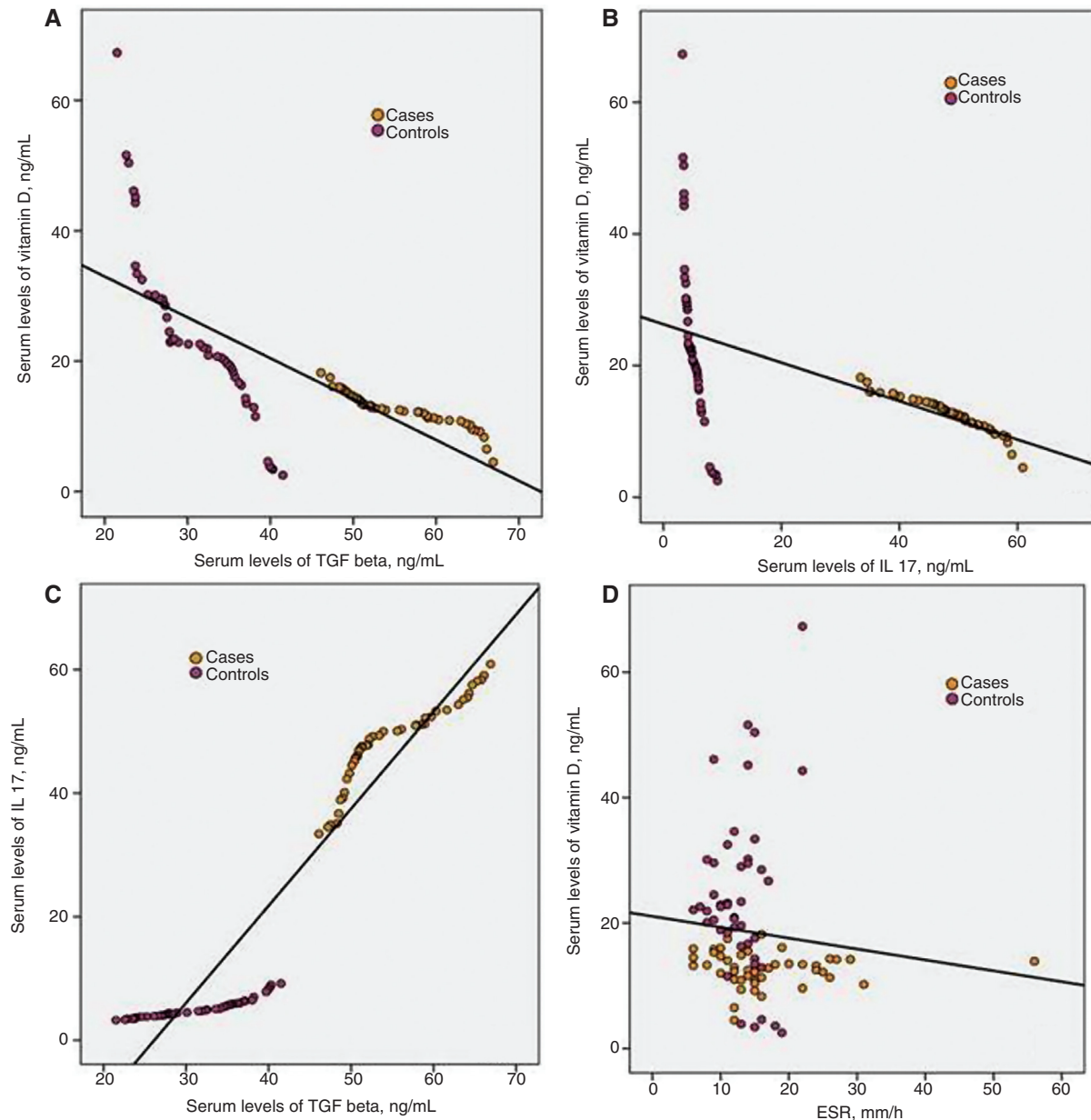


Figure 2 Scatter plot demonstrating the correlation between 25-hydroxy vitamin D and TGF- β (A); between 25-hydroxyvitamin D and IL-17 (B); between IL-17 and TGF- β (C), and 25-hydroxy vitamin D and ESR (D).

by Thorp et al. [7], study subjects with CU showed significantly reduced 25-hydroxy vitamin D levels compared with controls and the 25-hydroxy vitamin D levels did not correlate with duration or severity of disease, autoimmunity or thyroid autoantibody testing. In contrast, we were able to demonstrate significant lowering of 25-hydroxy vitamin D in cases of CU, especially in the auto-reactive type as estimated by APST and correlating with the disease severity. Atwa et al. [2] reported serum concentrations of IL-17, IL-23 and TNF- α to be significantly higher in CU patients as compared with the healthy controls, positively

correlating with disease severity [2]. However, in *Anisakis simplex*-associated urticaria, IL-17 levels were reported to be lowered in CU and there was no difference in TGF- β 1 levels between cases of CU and controls [8]. We were also able to demonstrate that serum IL-17 and TGF- β 1 were significantly elevated in cases of CU, more so in auto reactive cases. In addition, we also have demonstrated a negative correlation of 25-hydroxy vitamin D with IL-17 and TGF- β 1, both of which are elevated in CU.

IL-17 is a pleiotropic cytokine, which mediates tissue inflammation by inducing many pro-inflammatory

cytokines and chemokines. Whereas IL-6 induces Th17 differentiation together with TGF- β , IL-6 inhibits TGF- β -induced Treg differentiation. Lowered 25-hydroxy vitamin D may be the consequence of systemic inflammation seen in CU, which was reported by us in a prior paper [9]. This is also demonstrated by the significant negative correlation of 25-hydroxyvitamin D with ESR in the present work. Moreover, there is widespread prevalence of varying degrees (50%–90%) of vitamin D deficiency with low dietary calcium intake in Indian population [10]. Hence, it is difficult to attribute lowered 25-hydroxy vitamin D levels to be a cause of CU.

We need to perform this study in a larger sample size and measurements of 25-hydroxy vitamin D levels post-urticaria resolution also need to be done. The question

whether supplementation with 25-hydroxy vitamin D would lower the systemic inflammation seen in CU needs to be addressed in a clinical trial.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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