

Letter to the Editor

Vitamin D and antimicrobial peptide levels in patients with atopic dermatitis and atopic dermatitis complicated by eczema herpeticum: A pilot study

To the Editor:

Atopic dermatitis (AD) is a relapsing condition prone to infections such as herpes simplex virus, resulting in AD with eczema herpeticum (ADEH), a more severe clinical manifestation.¹ Recent medical research has implicated vitamin D (VD) in AD.^{2,3} It appears essential for skin barrier structure, increasing profilaggrin and lipid lamella production.⁴ Of interest is the effect of VD on the expression of antimicrobial peptide LL-37,³ which demonstrates significant antiviral activity against herpes simplex virus, and immune-modifying characteristics.⁵

Modern research has demonstrated low LL-37 levels in patients with AD and ADEH,⁶ increasing after VD supplementation.^{3,7} VD deficiency has been inversely correlated with AD severity.⁸ Furthermore, a few randomized controlled trials found AD improvement with VD supplementation.^{7,9,E1-E3} Despite these major advances, the extent of VD deficiency in AD is unknown.

We conducted a clinical service evaluation at the Sheffield Children's Hospital Dermatology Department to first determine the level of VD deficiency in children with AD and establish its association with disease severity. Second, we aimed to establish the effect of VD supplementation on AD, using LL-37 levels as a prognostic marker.

Following approval by the Sheffield Children's Hospital (CA309), children with AD were screened for VD deficiency during 3 summer months. 25(OH) VD levels were classified as follows: more than 75 nmol/L, sufficient; less than 75 nmol/L, insufficient (50-75 nmol/L, suboptimal; <50 nmol/L, deficient).^{E4} Children with AD with insufficient 25(OH) VD levels were then assessed clinically on a subsequent visit using SCORing Atopic Dermatitis (SCORAD) by a single dermatologist. Patient-Oriented Eczema Measure (POEM) scores were also determined. LL-37 levels were quantified from superficial samples of stratum corneum using a novel method described in this article's Online Repository at www.jacionline.org. This group was supplemented for 2 months depending on the level of deficiency and age (cholecalciferol 6000 IU daily in ages 1-12 years; 10,000 IU daily for ages 12-18 years) for 2 months, as recommended by the British National Formulary. Suboptimal levels were corrected with over-the-counter preparations containing 100% recommended daily allowance of VD. On the third visit, all levels were rechecked and clinical severity reassessed. Patients continued all other topical and oral medications. If in need of new oral treatment, the patient was not included in the final analysis.

Ninety children between the ages of 1 and 18 years (mean age, 9 years) attended the dermatology clinic during the period of this clinical audit and underwent screening for VD (see patients' demographic characteristics in Table E1 in this article's Online Repository at www.jacionline.org). Most patients were receiving topical immunotherapy; 11 were on oral immunosuppressants for more than 1 year before the study. Baseline

25(OH) VD levels revealed 57% patients with VD deficiency, and a further 26% with suboptimal levels, totaling 83% with insufficient VD levels.

Patients with ADEH comprised 51% of the sample population. Baseline 25(OH) VD levels were significantly lower in patients with ADEH (37 ± 20 nmol/L) than in patients with AD (61 ± 28 nmol/L; $P < .001$, 2-sample t test; Fig 1, A). Only 2 patients with ADEH had normal 25(OH) VD levels.

After screening, 18 patients were lost to follow-up (see Fig E1 in this article's Online Repository at www.jacionline.org); 10 patients were excluded because of commencement of oral therapy. Consequently, a total of 47 patients were analyzed: 12 with AD and 35 with ADEH. Patients with normal VD levels were not followed up as part of this audit.

Using SCORAD, patients were classified into the following groups: mild, less than 25; moderate, 25-50; and severe, more than 50,⁸ and showed a significant difference in 25(OH) VD levels (Fig 1, B; mean, 31 ± 17 , 40 ± 15 , and 57 ± 21 nmol/L, respectively; $P = .02$; 1-way ANOVA). Bonferroni post test showed a significant difference between mild and severe scores ($P = .01$). A significant inverse relationship was found between 25(OH) VD levels and SCORAD ($P = .01$; Pearson $r = -0.36$). LL-37 levels were also significantly different between the groups, with patients with the most severe AD displaying the lowest levels (Fig 1, C; $P = .018$; 1-way ANOVA). Bonferroni post test revealed a significant relationship between both mild and moderate ($P = .04$) and mild and severe ($P = .01$) groups. Children with ADEH had lower LL-37 levels than did children with AD ($n = 35$; mean score, 0.4 ± 0.5 $\mu\text{g/g}$; $n = 12$, mean score, 0.5 ± 0.6 $\mu\text{g/g}$, respectively; $P = .46$). Moreover 25(OH) VD levels were found to correlate with LL-37 levels (Fig 1, D; $r = 0.3$; $P = .02$).

Following a 2-month period of VD supplementation, SCORAD and POEM improved significantly, with a mean reduction of 42% and 47%, respectively ($P < 0.001$; Fig 2, A and B; paired t test). This improvement in severity was accompanied by a significant increase in LL-37 levels (lesional and nonlesional) by 4-fold (therapeutic or over the counter) ($P = .0004$; Fig 2, C-E; 2-sample t test). The severity of AD was significantly correlated with LL-37 levels ($r = -0.32$; $P = .01$), suggesting a causal relationship.

VD deficiency is now recognized as a worldwide problem. Recently, 35% to 40% of healthy UK^{E5} and US children^{E6} were found to be VD deficient. A study in Kuwait showed 57% of children with AD with VD levels of less than 50 nmol/L.^{E7} Our study shows VD levels significantly lower in children with moderate and severe AD than in children with mild AD, similar to recent studies.⁸ Children with ADEH also displayed significantly lower VD levels than did those with AD.

LL-37 levels are upregulated in wound injury to participate in reepithelialization.⁴ Previous studies have reported low LL-37 levels in patients with AD,³ with further reductions in patients with ADEH.^{6,E8} This was echoed here, but was not statistically significant, possibly due to the smaller sample size of patients with AD (AD = 12 vs ADEH = 35).

VD supplementation has previously been shown to increase lesional and nonlesional LL-37 levels in skin biopsies of patients

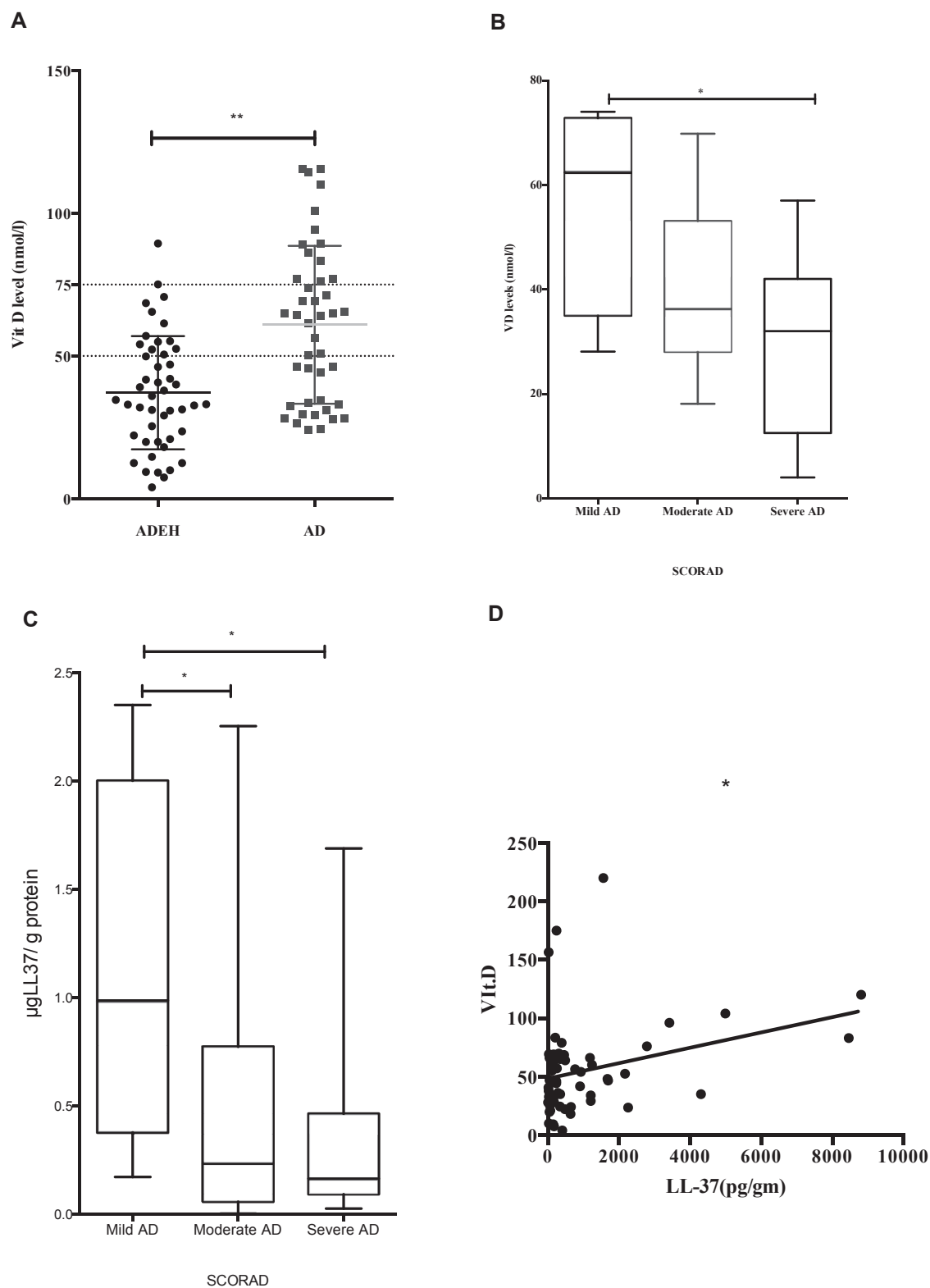


FIG 1. Presupplementation analysis. **A**, Baseline VD level comparison. Children with ADEH (black dots, $n = 45$) with lower VD levels compared with children with AD (gray squares, $n = 45$). Levels: 50-75 nmol/L (dotted lines), suboptimal; less than 50 nmol/L, deficient. **B**, Patients with AD (47) classified into severe AD ($n = 13$), moderate AD ($n = 30$), and mild AD ($n = 4$) showed significantly different VD levels. **C**, Baseline LL-37 levels stratified according to SCORAD were significantly different. **D**, VD and LL-37 correlation ($P = .01$). * $P < .05$ and ** $P < .01$.

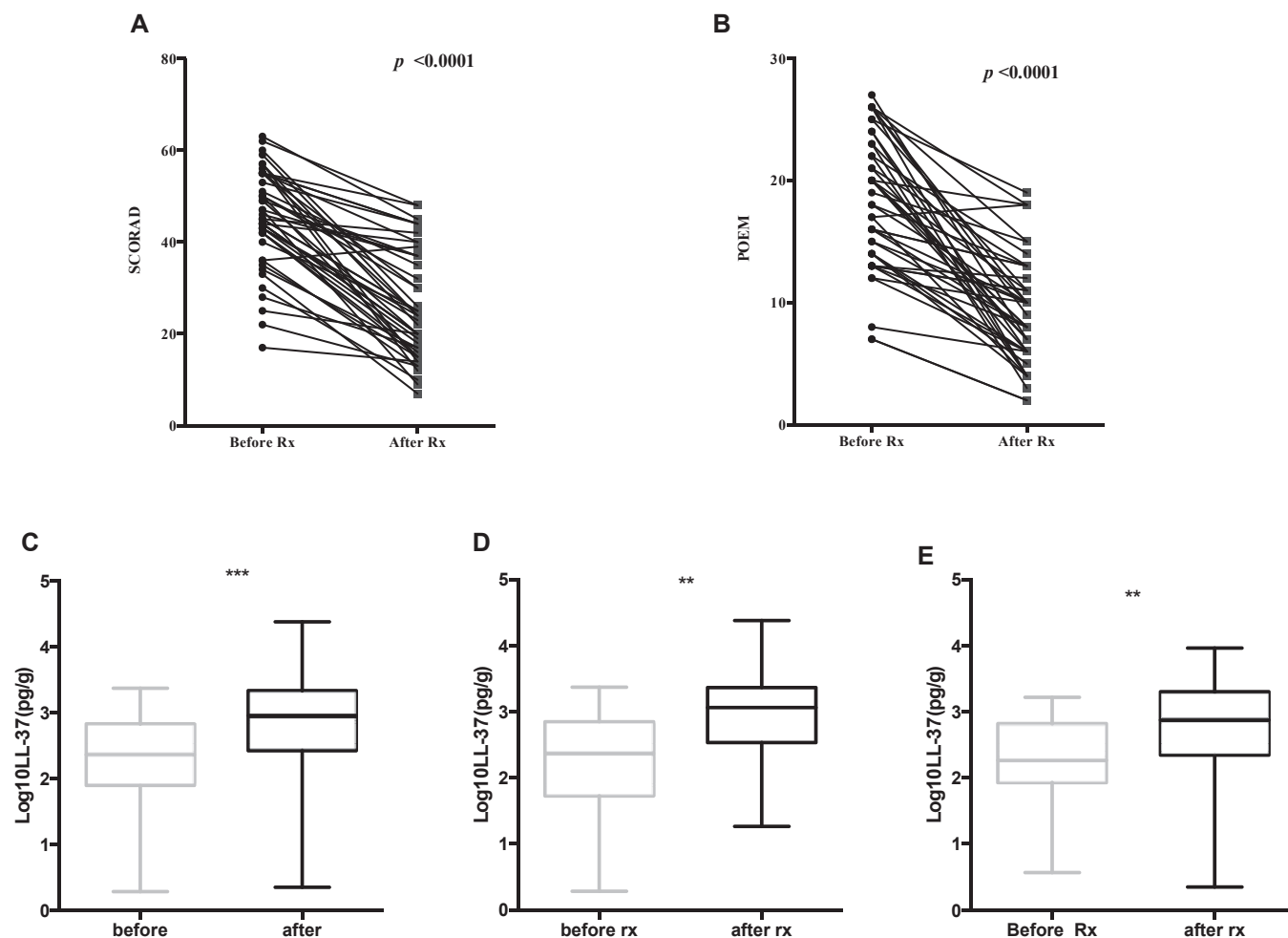


FIG 2. Postsupplementation analysis (n = 47). **A**, SCORAD reduced 42.3% postsupplementation. **B**, POEM showed significant reduction (46.6%). **C**, LL-37 levels increased significantly from 2 ± 0.7 Log10(LL-37 pg/g) to 2.8 ± 0.8 Log10(LL-37 pg/g). **D**, Lesional LL-37 levels increased from 2.3 ± 0.7 Log10LL-37 pg/g to 3 ± 0.7 Log10(LL-37 pg/g). **E**, Nonlesional LL-37 levels increased from 2.3 ± 0.6 Log10(LL-37 pg/g) to 2.7 ± 0.8 Log10(LL-37 pg/g). Rx, Treatment. ** $P < .01$ and *** $P < .001$.

with AD.^{2,3} Moreover, randomized controlled trials have reported reduced AD severity with VD supplementation.^{7,9,E1-E3} In our study, 2-month VD supplementation significantly improved AD and ADEH severity; LL-37 levels also increased significantly within the stratum corneum. Therefore, VD deficiency could lead to a decrease in LL-37 levels, resulting in reduced antimicrobial defense and increased disease severity with secondary infections.

As VD itself has been reported to influence lipid lamellae formation,⁴ it could have contributed to improved AD in our cohort by improving permeability barrier function. The discovery of increased VDR polymorphisms in patients with AD in comparison to healthy controls suggests an important role of VD in the pathogenesis of AD.^{E9}

This was a practice evaluation study designed to emulate regular clinical practice not a randomized controlled trial. All medications continued with no constraints. Another limitation of this study is the lack of clinical scores in patients with AD with normal 25(OH) VD levels, to allow for unknown factors that could contribute to clinical improvement. In addition, our study could be underpowered to detect differences due to the sample

size. Nevertheless, the significant results of VD supplementation in this study renders that possibility unlikely.

In conclusion, VD deficiency is common, and could lead to decreased LL-37 levels and increased severity of AD and ADEH. We developed a novel, noninvasive method for quantifying LL-37 that simplifies collection, permitting analysis of larger and younger populations. Monitoring of this peptide may be a useful prognostic clinical marker. Further research and larger samples are necessary to fully examine the relationship between VD and LL-37.

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REFERENCES

1. Beck LA, Boguniewicz M, Hata T, Schneider LC, Hanifin J, Gallo R, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. *J Allergy Clin Immunol* 2009;124:260-9.
2. Hata TR, Kotol P, Jackson M, Nguyen M, Paik A, Udall D, et al. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J Allergy Clin Immunol* 2008;122:829-31.
3. Hata TR, Jackson M, Nguyen M, Udall D, Park A, Kotol P, et al. Rescue of diminished cathelicidin antimicrobial peptide expression in subjects with atopic dermatitis by administration of oral vitamin D. *J Invest Dermatol* 2008;128:368.
4. Bikle D. Vitamin D and the skin: physiology and pathophysiology. *Rev Endocr Metab Disord* 2012;13:3-19.
5. Schaubert J, Gallo RL. Antimicrobial peptides and the skin immune defense system. *J Allergy Clin Immunol* 2008;122:261-6.
6. Hata TR, Kotol P, Boguniewicz M, Taylor P, Paik A, Jackson M, et al. History of eczema herpeticum is associated with the inability to induce human beta-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. *Br J Dermatol* 2010;163:659-61.
7. Hata TR, Audish D, Kotol P, Coda A, Kabigting F, Miller J, et al. A randomized controlled double-blind investigation of the effects of vitamin D dietary supplementation in subjects with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2014;28:781-9.
8. Peroni DG, Piacentini GL, Cametti E, Chinellato I, Boner AL. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol* 2011;164:1078-82.
9. Javanbakht MH, Keshavarz SA, Djalali M, Siassi F, Eshraghian MR, Firooz A, et al. Randomized controlled trial using vitamins E and D supplementation in atopic dermatitis. *J Dermat Treatment* 2011;22:144-50.

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METHODS

Sample collection and quantification of LL-37

Skin cells were collected using noninvasive cytology brushes (Cytotak brushes, Medical Wire Co, Corsham, United Kingdom) from lesional and nonlesional sites of VD-deficient patients. Three brushes were gently brushed against the skin surface, placed in a 1.5-mL tube, and frozen at -80°C . The samples were extracted in 800 μL buffer (10 mM disodium phosphate, pH 7.4, 0.2% sodium dodecyl sulphate, 0.5% propylene glycol) containing protease inhibitors (Complete mini, Roche, Mannheim, Germany) for 30 minutes with sonication (Ultrawave Ltd, Cardiff, United Kingdom). The LL-37 levels were determined using the Human LL-37 ELISA kit (Hycult, Uden, The Netherlands), and expressed as a proportion of total protein, determined by Bicinchoninic Acid analysis (Sigma-Aldrich, Dorset, United Kingdom).

Statistical analysis

GraphPad Prism (La Jolla, Calif) was used for statistical analysis. AD and ADEH groups were analyzed by 2-sided 2-sample t test. Scatter plot of AD versus ADEH shows mean and SD. One-way ANOVA was used to compare VD levels and LL-37 levels stratified by clinical severity. *Post hoc* comparisons were analyzed with Bonferroni multiple comparisons test. Boxplot graphs show midline to represent median; boxes represent the 25th and 75th quartile, and whiskers represent the minimum to maximum values. Pearson analysis was used for correlations between LL-37 levels, VD levels, and SCORAD. SCORAD and POEM measurements collected before and after VD supplementation were analyzed by paired t test. LL-37 levels collected before and after were analyzed by 2-sample t test. Level of significance was set at .05. All values were expressed as mean $\pm$ SD.

REFERENCES

- E1. Sidbury R, Sullivan AF, Thadhani RI, Camargo CA. Randomized controlled trial of vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. *Br J Dermatol* 2008;159:245-7.
- E2. Amestajani M, Salehi BS, Vasigh M, Sobhkhiz A, Karami M, Alinia H, et al. Vitamin D supplementation in the treatment of atopic dermatitis: a clinical trial study. *J Drugs Dermatol* 2012;11:327-30.
- E3. Camargo CA Jr, Ganmaa D, Sidbury R, Erdenedelger K, Radnaakhand N, Khandsuren B. Randomized trial of vitamin D supplementation for winter-related atopic dermatitis in children. *J Allergy Clin Immunol* 2014;134:831-5.e1.
- E4. Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt P, Fuleihan GEH, et al. IOF position statement: vitamin D recommendations for older adults. *Osteopor Int* 2010;21:1151-4.
- E5. Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One* 2011;6:6.
- E6. Gordon C, Feldman HA, Sinclair L. Prevalence of vitamin D deficiency among healthy infants and toddlers. *Arch Pediatr Adolesc Med* 2008;162:505-12.
- E7. Al-Mutairi N, Issa BI, Nair V. Photoprotection and vitamin D status: a study on awareness, knowledge and attitude towards sun protection in general population from Kuwait, and its relation with vitamin D levels. *Ind J Dermatol Venereol Leprol* 2012;78:342-9.
- E8. Howell MD, Wollenberg A, Gallo RL, Flaig M, Streib JE, Wong C, et al. Cathelicidin deficiency predisposes to eczema herpeticum. *J Allergy Clin Immunol* 2006;117:836-41.
- E9. Heine G, Hoefer N, Franke A, Nothling U, Schumann RR, Hamann L, et al. Association of vitamin D receptor gene polymorphisms with severe atopic dermatitis in adults. *Br J Dermatol* 2013;168:855-8.

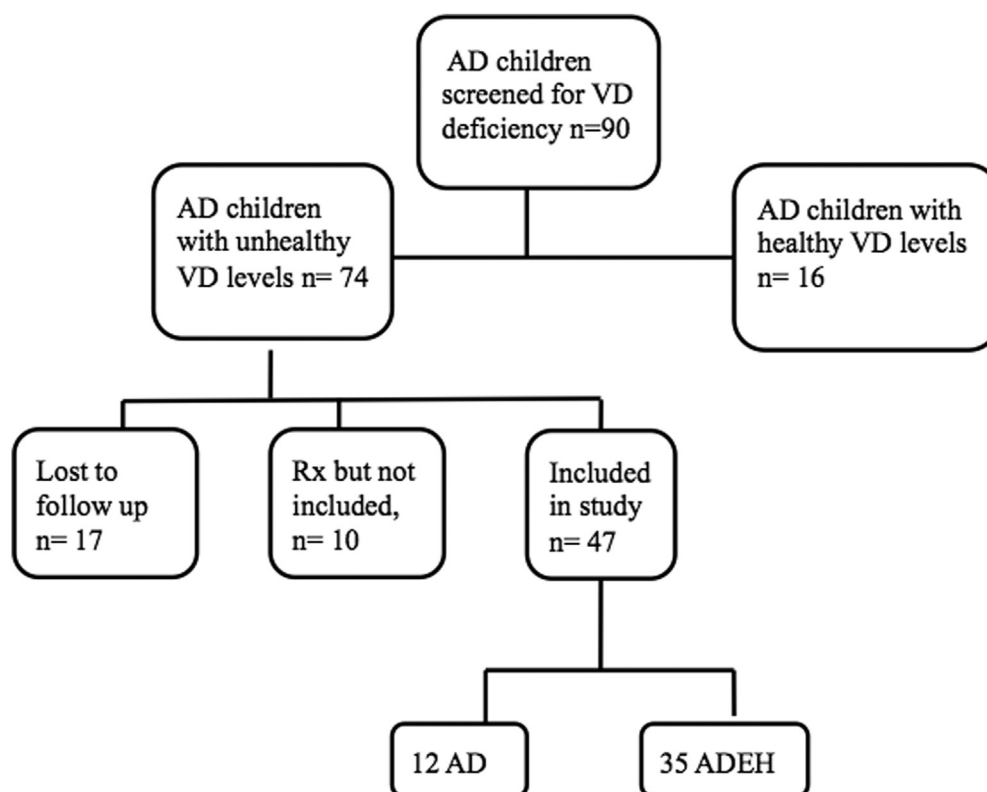


FIG E1. Flowchart of study. Ninety children with AD screened for VD deficiency and classified into AD and ADEH groups on initial consultation. Of these 90, 15 were found to have normal VD levels and 75 had low VD levels. Of the 75 children with AD, 18 were lost to follow-up and 10 were excluded. Therefore, 47 children were included in the practice evaluation study. These 47 children were clinically assessed and supplemented.

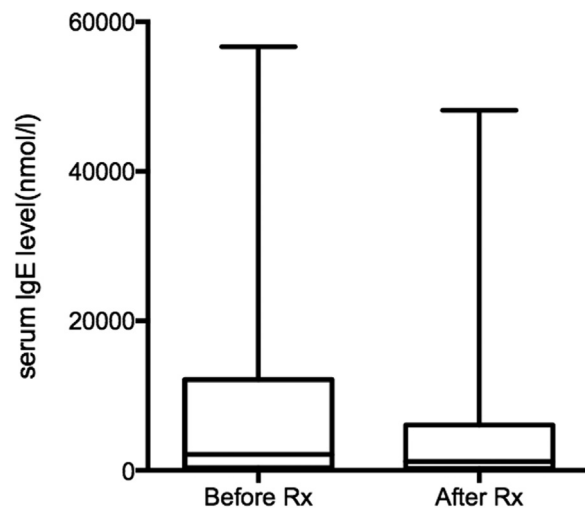


FIG E2. The IgE level did not show a significant change from mean score 7010 ± 2370 nmol/L to 7824 ± 3221 nmol/L ($P = .93$; unpaired t test). Rx, Treatment.

TABLE E1. Demographic characteristics of children with AD

Characteristics	Initial children with AD screened (n = 90)	Children with AD included in study (n = 47)
Age (y), mean (range)	9 (1-18)	11 (1-18)
Sex		
Female	47 (52)	21 (45)
Male	43 (48)	26 (55)
Ethnicity		
White	57 (63)	25 (53)
Asian	24 (27)	14 (30)
Chinese	3 (4)	3 (6)
African	2 (2)	1 (2)
Mixed	4 (4)	4 (9)
Clinical classification		
AD	45 (50)	12 (26)
ADEH	45 (50)	35 (74)
Healthy 25(OH) VD levels	15 (17)	
Unhealthy 25(OH) VD levels	75 (83)	47
Suboptimal 25(OH) VD (50-75 nmol/L)	23 (26)	12 (25)
Deficient 25(OH) VD (<50 nmol/L)	35 (39)	23 (50)
Severely deficient VD (<25 nmol/L)	17 (18)	12 (25)

n = 90 the total number of children screened initially. n = 47 the number of children entered into the practice evaluation study, supplemented, and clinically evaluated. Values represent n (%) unless otherwise indicated.