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Dose-response association between vitamin D deficiency and atopic dermatitis in children, and effect modification by gender: A case-control study

Authors:

Amal Ahmed Mohamed, MD¹
Eman Mohamed Salah Ahmed, MD²
Youssef MK Farag, MD, PhD, MPH³
Nermeen Ibrahim Bedair, MD²
Nourelhuda Ahmed Nassar, MD⁴
Ayat Ibrahim Mohamed Ghanem, MD⁵

Affiliations:

¹Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, Egypt

Email: amalahmedhcp@yahoo.com

²Department of Dermatology, Andrology, Sexual Medicine and STDs, Faculty of Medicine, Helwan University, Cairo, Egypt

Email: salah.eman81@yahoo

Email: nermeen.bedair@med.helwan.edu.eg

³ Welch Center for Prevention, Epidemiology, and Clinical Research and Clinical Research and Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Email: yfarag@jhu.edu

⁴ Clinical Pathology Department, Elsayhel Teaching Hospital

⁵ Clinical Pathology Department, National Institute of Diabetics and Endocrinology, Egypt

Corresponding author:

Nermeen Ibrahim Bedair, MD

Department of Dermatology, Andrology, Sexual Medicine and STDs, Faculty of Medicine, Helwan University, Cairo, Egypt (nermeen.bedair@med.helwan.edu.eg)

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AA conceived the study

ES collected the data

AA, NA and AG ran sample assay

YMKF authored statistical analysis plan, performed data management and statistical analysis, generated raw and final tables and figures

NB and YMKF wrote the first draft of the manuscript

NB and YMKF reviewed manuscript and provided critical scientific input

All authors approved of final manuscript draft

NB had primary responsibility for the integrity of the original data collected and final content of the manuscript

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Abbreviations

NHTMRI: National Hepatology and Tropical Medicine Research Institute

AD: atopic dermatitis

25 (OH) D: 25-hydroxyvitamin D

PRs: prevalence ratios

UVB: ultraviolet B

VDR: vitamin D receptor

IFN- γ : interferon gamma

Key point:

Question: can atopic dermatitis be associated with vitamin D deficiency?

Finding: Serum 25(OH)D levels were significantly lower in cases with AD than in controls. Prevalence ratios for comparing male participants with intermediate and deficient vitamin D levels to those with optimal levels were 3.38 (1.21, 9.40) and 5.20 (1.91, 14.13), respectively, whereas in the female participants were 1.32 (0.96, 1.83) and 1.49 (1.04, 2.14), respectively (p-interaction <0.001).

Meaning: vitamin D deficiency is associated with AD in children, effect modification of this association by gender was also observed.

ABSTRACT**Background:**

Vitamin D is a regulatory factor for immunity and skin barrier functions. It is hypothesized to be linked to atopic dermatitis (AD) which is characterized by interaction between epidermal barrier dysfunction and dysregulation of skin immune functions.

Methods:

One hundred AD patients and one hundred and one normal controls were collected from outpatient clinic based on their clinical condition, both had measurement of 25-hydroxyvitamin D [25(OH)D]. We assessed the relationship between 25(OH)D deficiency and AD prevalence using adjusted Poisson regression model.

Results:

Serum 25(OH)D levels were significantly lower in cases than controls (mean 35.1 vs 22.6 ng/mL, $p < 0.001$). The unadjusted prevalence ratios (PRs) (95% CI) for AD for comparing

participants with intermediate and deficient vitamin D levels to those with optimal levels were 3.11 (1.91, 5.06) and 4.77 (2.99, 7.60), respectively. The association didn't materially change after adjusting for potential confounders. In the fully adjusted analysis stratified by gender, PRs for AD for comparing male participants with intermediate and deficient vitamin D levels to those with optimal levels were 3.38 (1.21, 9.40) and 5.20 (1.91, 14.13), respectively, whereas in the female participants were 1.32 (0.96, 1.83) and 1.49 (1.04, 2.14), respectively (p-interaction <0.001).

Conclusion:

In this case-control study in children, we found a statistically significant dose-response association between vitamin D deficiency and AD. We also observed a statistically significant effect modification of this association by gender. Further research is recommended to study this association longitudinally, and to examine whether treating vitamin D deficiency may potentially improve AD.

Background

Atopic dermatitis (AD) is a chronic relapsing skin disease characterized by a defective skin barrier that affects mainly children, and less commonly adults with environmental, genetic and immunologic factors ^{(1) (2)}. Cell-mediated immunity is impaired in AD; where patients have both impaired skin barrier function as well as defective innate immunity of the skin.⁽³⁾ Recently, evidence has emerged showing that vitamin D, a recognized immunomodulator, may act as an important regulatory factor of the innate as well as adaptive immunity ⁽⁴⁾

7-dehydrocholesterol (provitamin D3) requires ultraviolet radiation in order to generate pre-vitamin D3 in the basal cell layer of the skin, which is then isomerized into vitamin D3 (cholecalciferol), the later then undergoes hydroxylation in the liver by 25-hydroxylase enzymes, forming 25-hydroxyvitamin D [25(OH)D]. Measurement of blood 25(OH)D, is used to determine vitamin D status. 25(OH)D then undergoes further hydroxylation in renal tubules by 1- α hydroxylase into 1,25-dihydroxyvitamin D [1,25(OH)2D], calcitriol, the biologically active form that binds nuclear vitamin D receptor (VDR) in target tissues, including skin. In human skin, calcitriol regulates several functions including formation of skin barrier ⁽⁵⁾

Cutaneous immune cells are capable of both synthesizing as well as responding to vitamin D ⁽⁶⁾. Vitamin D regulates the activity of monocytes, dendritic cells (DCs), T and B lymphocytes ⁽⁷⁾. Moreover, immune cells express vitamin D-activating enzymes allowing local activation of inactive vitamin into calcitriol leading to local paracrine as well as autocrine effects ⁽⁸⁾. Vitamin D role in regulating skin functions also includes inhibition of proliferation, stimulation of differentiation of keratinocytes as well as their formation of the permeability

barrier⁽⁵⁾. Vitamin D3 also increases innate immunity in skin and enables efficient antimicrobial defense at epithelial surfaces via its regulatory function of cathelicidin expression⁽⁹⁾. Since vitamin D is involved in regulation of both immune functions as well as skin barrier formation, it has been hypothesized that it may be involved in the pathogenesis of AD^{(10) (11)}.

Given the variability in the findings and the whole host of limitations from previous studies, we designed this case-control study was to investigate whether vitamin D status in children is associated with AD, and if there is an evidence of effect modification by age or gender.

Methods

Selection of cases and controls

We conducted an unmatched case-control study between November 2013 and October 2014. The participants were children who sought outpatient medical appointments in the Department of Dermatology, National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt. Study was approved by the NHTMRI research ethical committee. One hundred cases were identified by a licensed dermatologist and recruited to the study. Inclusion criteria included standard AD diagnostic criteria by Hanifin & Rajika⁽¹²⁾

(Supplementary Table 1). All patients were of skin types 3 and 4. We excluded children who reported prior diagnosis of systemic diseases other than bronchial asthma, including patients with diabetes, prior systemic treatment for AD (all kinds of phototherapy were considered as systemic treatment and were excluded), prior vitamin D supplementation and other concomitant dermatologic diseases (ichthyosis, psoriasis, vitiligo, etc). One hundred and one children were

identified and recruited as controls. Inclusion criteria were those without AD or any other dermatological disease and aged 1 to 14 years at the time of recruitment. None of the participants used any sunscreens and all of them used to go to their schools on foot which is common for the socioeconomic class of patients of the institute, participants Informed verbal consent was obtained from the parents of all cases and controls.

Vitamin D status

Serum 1, 25(OH)D level was assessed using ELISA. Two milliliters of blood were withdrawn from patients and controls, allowed to clot, and then centrifuged at 3000 rpm for 15 minutes and serum separated for quantitative determination of 1,25-hydroxyvitamin D [25(OH)D] using commercial automated ELISA ,**DRG International Inc., USA according to manufacture instructions.**

For the primary analysis in this study, we categorized the serum 25(OH)D levels into three clinically relevant categories identified by the Endocrinology Society Clinical Practice Guidelines ⁽¹³⁾: deficient (<20 ng/mL), intermediate (20 to <30 ng/mL), and optimal ($\geq$ 30 ng/mL). For sensitivity analysis, we used the cutoff of 20 ng/mL identified by the National Academy of Medicine (NAM), formerly the Institute of Medicine, as adequate enough for health ⁽¹⁴⁾ to dichotomize the study participants into vitamin D deficient and non-deficient.

Atopic dermatitis

AD was diagnosed by a licensed dermatologist according to standard criteria⁽¹²⁾ (**Supplementary Table 1**). The severity of AD was assessed in all children by means of the

scoring atopic dermatitis (SCORAD) index. AD was classified as mild (SCORAD<25), moderate (SCORAD 25-40) and severe (SCORAD>40).

Other variables

In addition to the demographic information of the study participants, we also collected other laboratory data on the serum levels of fasting glucose, insulin, creatinine, triglycerides, cholesterol, alkaline phosphatase, and gamma-glutamyl transferase (GGT).

Statistical analysis

We presented the demographic, clinical and laboratory characteristics of the study population by the cases and controls; presence and absence of the AD. Continuous variables were presented using means and standard deviations, and we compared the cases and control groups using student's t-tests. Categorical variables were presented using frequencies and proportions, and we compared the cases and control groups using the chi-squared test.

To evaluate the association between vitamin D status and the prevalence of AD, we used Poisson regression models to estimate prevalence ratios (PRs) and 95% confidence intervals (CI) of AD comparing participants with deficient and intermediate vitamin D levels to those with optimal levels as the reference category.

In the sensitivity analyses, we modeled the exposure as 10 ng/mL decrease in 25(OH)D concentrations. To test for dose-response trend, the 3-group categorical variable of vitamin D level was entered as a continuous variable in the Poisson regression. Furthermore, we repeated

the Poisson regression analysis using the dichotomous vitamin D deficiency variable (<20 vs. ≥ 20 ng/mL). To further test for dose-response relationship, we used the box and whiskers plot to show the vitamin D levels by controls and AD severity and compared the median vitamin D levels across the four groups using the Kruskal-Wallis test. We also evaluated non-linear dose-response relationships using adjusted restricted cubic splines.

Our models were progressively adjusted for different sets of potential confounders. Model 1 was unadjusted; Model 2 was adjusted for age and gender; and Model 3 was further adjusted for metabolic risk factors (fasting serum glucose, serum insulin, serum creatinine, serum triglycerides, serum cholesterol, and serum GGT).

We used the fully adjusted Model 3 to test for effect modification by age or gender on the association between the 3-group categorical variable of vitamin D and AD. For this purpose, age was dichotomized by children (5-11 years) and adolescents (12-16 years).

Two-sided p-values <0.05 were considered statistically significant. The data were analyzed using Stata Software (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

Results:

The clinical and laboratory characteristics of the study participants are shown in **Table 1**. Serum 25(OH)D levels were significantly lower in cases than controls (mean 35.1 vs 22.6 ng/mL, $p<0.001$). Cases had more females ($p<0.001$), and slightly older children ($p<0.001$).

Vitamin D deficiency was present in 33% of cases vs 3% of controls ($p < 0.001$). Within the cases with AD, the prevalence of mild, moderate and severe AD was 8%, 17% and 25%, respectively. The median level of vitamin D was decreasing with increasing severity of the AD ($p < 0.001$) as shown in **Figure 1**.

The unadjusted PRs (95% CI) for AD for comparing participants with intermediate and deficient vitamin D levels to those with optimal levels were 3.11 (1.91, 5.06) and 4.77 (2.99, 7.60), respectively (**Table 2, Model 1**). As we progressively adjusted for potential confounders, the effect estimates were attenuated but maintained statistical significance and dose-response pattern ($p\text{-trend} < 0.001$) (**Figure 2**). In the fully adjusted model (**Table 2, Model 3**), the PR of AD for comparing participants with deficient 25(OH)D levels to those with optimal levels was 2.05 (1.44, 2.92). Additionally, there was a statistically significant 37% higher prevalence of AD for each 10 ng/mL decrease in vitamin D (**Table 2**).

When we dichotomized vitamin D status (**Table 3**) the unadjusted PR for AD comparing those with deficient to those with non-deficient levels was 2.26 (1.83, 2.78) (**Table 3, Model 1**). This association was materially unchanged after adjusting for demographic and metabolic risk factors [1.41 (1.11, 1.78)] (**Table 3, Model 3**)

Using a fully adjusted model to test for effect modification (Table 4, Panel A) on the association between AD and vitamin D deficiency, there was no effect modification by age ($p\text{-interaction} = 0.12$), while there was independent dose-response pattern as presented earlier. However, we found evidence of statistically significant effect modification by gender on the

association between AD and vitamin D deficiency while maintaining the dose-response pattern. In the fully adjusted stratified analysis (**Table 4, Panel B**), PRs (95% CI) for AD for comparing male participants with intermediate and deficient vitamin D levels to those with optimal levels were 3.38 (1.21, 9.40) and 5.20 (1.91, 14.13), respectively, whereas in the female participants were 1.32 (0.96, 1.83) and 1.49 (1.04, 2.14), respectively (p-interaction <0.001).

Using restricted cubic splines to test the dose-response association between vitamin D and AD, we found an inverse association between AD and the serum vitamin D level (**Figure 3**).

Discussion:

The main findings of this study are the high prevalence of vitamin D deficiency among children with atopic dermatitis and the evidence of dose-response association between vitamin D levels and AD, as well as its severity using SCORAD index, in fully adjusted models. Another novel finding is the effect modification of the association between vitamin D deficiency and AD by gender, while maintaining dose-response association.

In this study conducted on children, 5-16 years of age, we observed a high prevalence of vitamin D deficiency among children with AD (33%) compared to healthy controls. Several cross-sectional studies examined vitamin D prevalence in Egyptian children in which it varied from 11-24%.^{(15) (16) (17)} Surprisingly, Cairo weather is sunny during most of the year, and the threshold value of 18 mJ/cm² (that is minimal ultraviolet B (UVB) radiation level that produces a significant increase of serum vitamin D3) is generally reached even during spring, fall and winter seasons^{(18) (19)}. However, several cultural and social factors have been suggested to influence

vitamin D levels in children, in addition to insufficient dietary calcium intake⁽¹⁵⁾. On the other hand, Thyssen (20) and his colleagues contested this proposal when they performed a pooled meta-analysis of over 9950 European individuals of the association between filaggrin gene (*FLG*) mutations, vitamin D deficiency and AD. *FLG* mutation carriers possess high serum vitamin D levels(21). They found that higher prevalence of AD is not associated with vitamin D insufficiency because of reduced solar UVB exposure, even after adjusting for potential confounders, and testing for effect modification by age or gender. However, the results of the Thyssen study may be only generalizable to the Europeans and their descendants. On the other hand, VDR gene has been shown to have pleiotropic effects that modulates autoimmunity, inflammation and several allergic conditions. VDR polymorphism may have the potential to explain the association of the disease with lower vitamin D levels, as data showed in a study in AD in adults⁽²²⁾. Another recent meta-analysis concluded that VDR polymorphism contribute to asthma susceptibility (23) in both adults and children.

Our results showed effect modification by gender on the association between vitamin D deficiency and AD, this can be explained by gender variation of cytokine response to 1,25-(OH)2D3; as Correale and coworkers showed that 1,25-(OH)2D3 reduced the number of IFN- γ -producing cells significantly more in females than in males in both patients of multiple sclerosis and normal controls⁽²⁴⁾, and patients with AD are known to have reduced INF- γ levels⁽²⁵⁾. This is a unique finding that wasn't robustly tested before in children with AD.

Prior studies showed controversial results regarding association between AD severity and vitamin D levels. We have found that lower vitamin D levels are inversely associated with AD

severity in a dose-response pattern while other studies didn't^{(26) (27) (28) (29)}. Our results are in accordance with some studies^{(30) (31) (32) (33)}. Specifically, a recent systematic review and meta-analysis of clinical trials involved vitamin D supplementation on the mean difference in the AD severity symptoms using the SCORAD index⁽³⁴⁾. The pooled mean difference of -5.81 (95% CI: -9.03, -2.59) comparing those who received vitamin D supplementation to those who didn't. However, some of the studies included had serious limitations including very small sample sizes, non-blinding of participants, investigators or the outcome assessors, and selective reporting.

This study provides further insight in the association between vitamin D and AD, and the role of gender in modifying this association. Several clinical and public health implication arise from these findings, not only increasing awareness to consume vitamin-D-fortified food and increase exposure to sunlight, but also the availability of over-the-counter vitamin D supplementation that maybe use to treat and improve the symptoms of AD. A recent case report⁽³⁵⁾, albeit not enough, has shown successful treatment of AD in a young girl with daily oral calcitriol and cholecalciferol for 2 months. Large randomized double-blind placebo-controlled clinical trials, long enough to show efficacy and maintenance of treatment effect and powered to detect small improvements in SCORAD and other AD indices can only answer our research question, with an overarching goal of overcoming the limitations of previously conducted clinical trials⁽³⁶⁾ that were exploratory in nature.

Our study has several methodologic and scientific strengths. We used our case-control study to estimate prevalence of vitamin D deficiency in AD patients. We performed detailed and robust statistical analysis to improve shortcomings in the design. This includes adjustment for

potential confounders, including age and gender, given that we didn't match on them at the recruitment stage. In addition, we explored linear and non-linear dose-response association and effect modification by age and gender, which is not frequently done in dermatologic research. Although we didn't perform *a priori* formal sample size and power calculation, closer look at the narrow confidence intervals reveals that our study was powered enough.

It is important to consider the important findings of our study with some limitations in mind. First, this is a case-control study; thus, both reverse causation and residual confounding are of potential concern. Second, there is lack of information regarding sun exposure, obesity, dietary habits and medication use among cases and controls. In order to partially overcome this limitation, we excluded participants who reported to take systemic steroids, since oral corticosteroids are known to affect vitamin D levels⁽³⁷⁾, while topical steroids do not⁽³⁸⁾. Third, we were only able to assemble a small sample size which might affect statistical evaluation and conclusions.

In conclusion, our study suggests that vitamin D deficiency is associated with the prevalence as well as severity of AD, with significant effect modification by gender. We advocate further studies evaluating the potential use of over the counter vitamin D supplements as an adjuvant therapy to improve AD.

Author Contributions: Drs Mohamed and Ahmed , had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: , (Mohamed , ahmed, Farag, Bedair, Nassar, Ghanem). Acquisition, analysis, and interpretation of data: , , Farag, Bedair. Drafting of the manuscript: Bedair, Farag. Critical revision of the

manuscript for important intellectual content: Farag. Statistical analysis: Farag. Obtained funding: None.

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Table 1: Baseline demographic, clinical and laboratory characteristics of cases and controls

	Controls N=101	Cases N=100	p-value
Gender			
Males	63 (62.4%)	39 (39.0%)	<0.001
Females	38 (37.6%)	61 (61.0%)	
Age (years)	9.2±3.3	11.0±2.7	<0.001
Age categories			
Children	78 (77.2%)	53 (53.0%)	<0.001
Adolescents	23 (22.8%)	47 (47.0%)	
Vitamin D (ng/mL)	35.1±15.5	22.6±8.7	<0.001
Vitamin D Deficiency (<20 ng/mL)			
Yes	3 (3.0%)	33 (33.0%)	<0.001
Vitamin D Deficiency - 3 Categories			
>=30	63 (62.4%)	15 (15.0%)	<0.001
20-29	35 (34.7%)	52 (52.0%)	
<20	3 (3.0%)	33 (33.0%)	
Fasting Glucose (mg/dL)	100.8±16.0	143.2±30.3	<0.001
Diabetes			
Prediabetes	32 (31.7%)	25 (25.0%)	<0.001
Diabetes	6 (5.9%)	69 (69.0%)	
Insulin (mIU/L)	4.6±3.0	11.3±4.4	<0.001
Serum Creatinine (mg/dL)	1.0±0.2	0.8±0.4	<0.001
Serum Creatinine Categories			<0.001
Elevated	67 (66.3%)	42 (42.0%)	
Serum Triglycerides (mg/dL)	156.2±31.6	194.1±38.1	<0.001
Triglycerides categories			
Borderline high	32 (31.7%)	58 (58.0%)	<0.001
High	18 (17.8%)	37 (37.0%)	
Cholesterol (mg/L)	163.7±27.7	198.4±27.7	<0.001
Cholesterol categories			
Borderline high	2 (2.0%)	21 (21.0%)	<0.001
High	3 (3.0%)	13 (13.0%)	
Alkaline Phosphatase (U/L)	189.4±46.8	229.4±89.8	<0.001
Alkaline Phosphatase categories			0.67
Elevated	14 (13.9%)	16 (16.0%)	
GGT (IU/L)	34.3±9.5	54.7±19.9	<0.001
GGT Categories			<0.001
Elevated	47 (46.5%)	83 (83.0%)	

Table 2: Prevalence ratios (PR) of atopic dermatitis and 95% confidence intervals (95% CI) by vitamin D categories

Vitamin D Deficiency	≥30 ng/mL	20-30 ng/mL	<20 ng/mL	per 10 ng/mL decrease in Vitamin D	p-trend
Model 1	1.00	3.11 (1.91, 5.06)	4.77 (2.99, 7.60)	1.88 (1.44, 2.45)	<0.001
Model 2	1.00	2.79 (1.71, 4.52)	4.14 (2.59, 6.63)	1.73 (1.34, 2.23)	<0.001
Model 3	1.00	1.66 (1.18, 2.32)	2.05 (1.44, 2.92)	1.37 (1.13, 1.66)	<0.001

Model 1 is unadjusted

Model 2 is adjusted for age and gender

Model 3 is further adjusted for metabolic biomarkers (glucose, insulin, serum creatinine, triglycerides, cholesterol, and GGT)

Table 3: Prevalence ratios (PR) of atopic dermatitis and 95% confidence intervals (95% CI) by vitamin D categories

Vitamin D Categories	≥20 ng/mL	<20 ng/mL
Model 1	1.00	2.26 (1.83, 2.78)
Model 2	1.00	2.05 (1.65, 2.55)
Model 3	1.00	1.41 (1.11, 1.78)

Model 1 is unadjusted

Model 2 is adjusted for age and gender

Model 3 is further adjusted for metabolic biomarkers (glucose, insulin, serum creatinine, triglycerides, cholesterol, and GGT)

Table 4: Effect modification by age (panel A) and gender (panel B) on the association between vitamin D and atopic dermatitis showing prevalence ratios (PR) and 95% confidence intervals (95% CI)

(A)

Vitamin D Categories	≥30 ng/mL	20-30 ng/mL	<20 ng/mL	p-interaction
Children (5-11 years)	1.00	1.77 (1.04, 3.01)	2.55 (1.47, 4.41)	0.12
Adolescents (12-16 years)	1.00	1.54 (1.07, 2.22)	1.57 (1.04, 2.39)	

(B)

Males	1.00	3.38 (1.21, 9.40)	5.20 (1.91, 14.13)	<0.001
Females	1.00	1.32 (0.96, 1.83)	1.49 (1.04, 2.14)	

The model is adjusted for age, gender and metabolic biomarkers (glucose, insulin, serum creatinine, triglycerides, cholesterol, and GGT)

Figure 1: Boxplot showing the distribution of vitamin D levels in the controls and different severity stages of AD

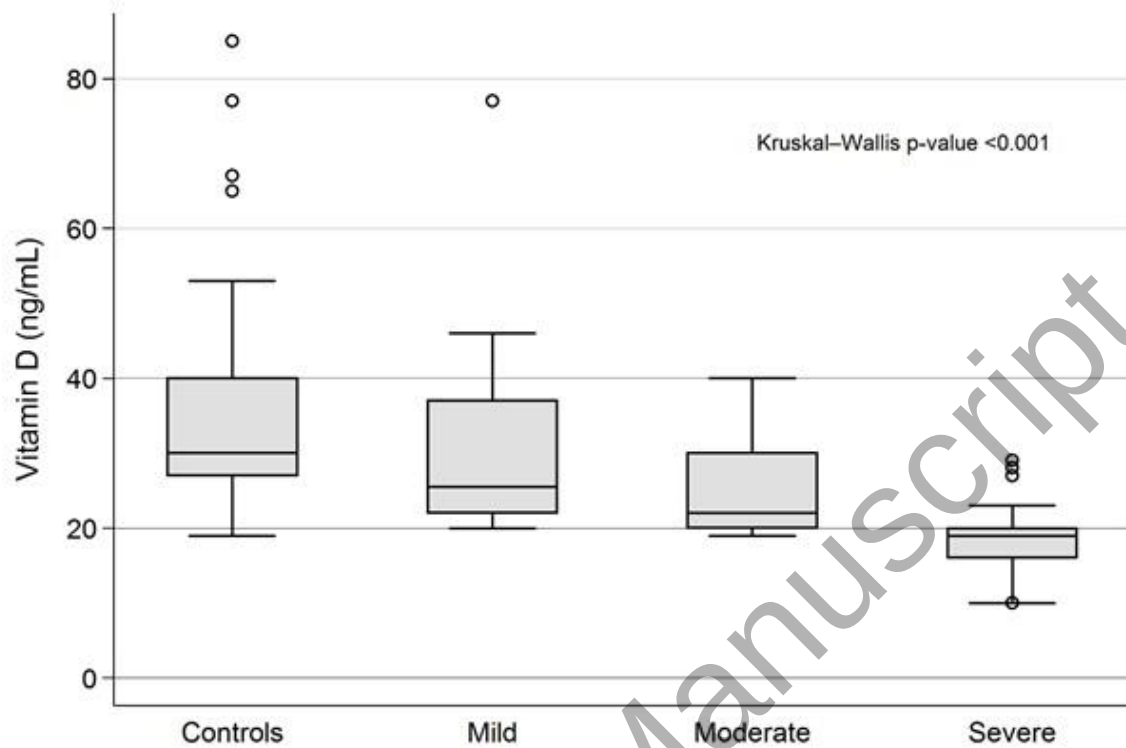


Figure 1: Boxplot showing the distribution of vitamin D levels in the controls and different severity stages of AD

Figure 2: Prevalence ratios for atopic dermatitis comparing vitamin D deficient children to those who are not

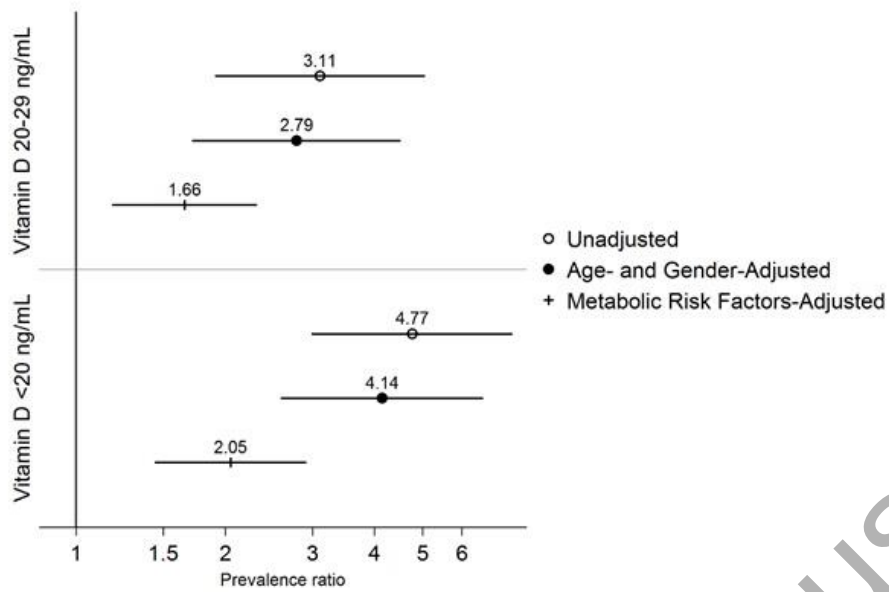


Figure 2: Prevalence ratios for atopic dermatitis comparing vitamin D deficient children to those who are not

Figure 3: Restricted cubic spline of 25(OH)D and adjusted prevalence ratios of AD

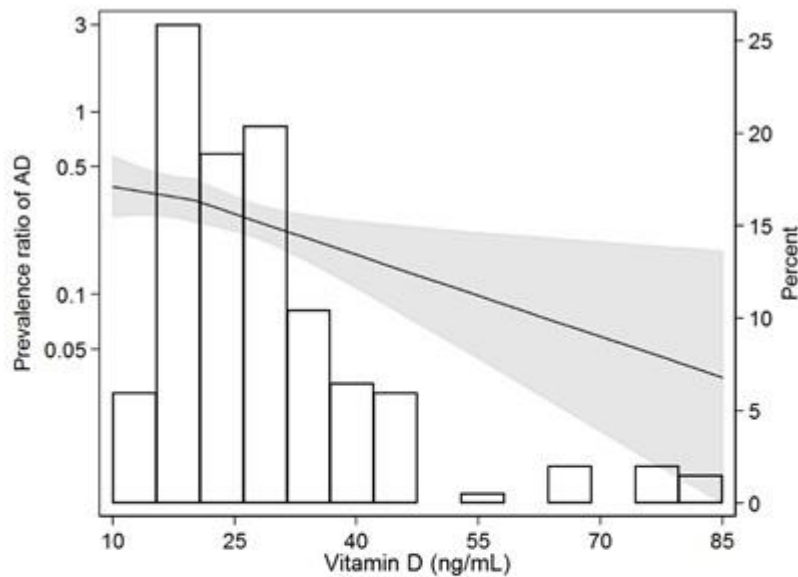


**Figure 3: Restricted cubic spline of 25(OH)D and
adjusted prevalence ratios of AD**

Curve represent adjusted prevalence ratios (solid line) and the 95% confidence intervals (dashed lines) based on restricted cubic splines for 25(OH)D level with knots at 10, 20, 30, 40 ng/ml. The reference values were set at 20 ng/ml. Model is adjusted for age, gender, glucose, insulin, serum creatinine, triglycerides, cholesterol, and GGT.

Supplementary Table 1: Diagnostic Standard of Hanifin & Rajika

1. Must have three or more basic features described below
(1) Pruritus (2) Typical morphology and distribution - Flexural lichenification in adults - Facial and extensor eruptions in infants and children (3) Chronic or chronically relapsing dermatitis (4) Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)
2. Must have three or more following minor features:
(1) Xerosis (2) Ichthyosis/palmar hyperlinearity, keratosis pilaris (3) Immediate (type I) skin test reaction (4) Elevated serum IgE (5) Early age of onset (6) Tendency toward cutaneous infections (7) Tendency toward non-specific hand or foot dermatitis (8) Nipple eczema (9) Cheilitis (10) Recurrent conjunctivitis (11) Dennie-Morgan infraorbital fold (12) Keratoconus (13) Anterior subcapsular cataracts (14) Orbital darkening (15) Facial pallor, facial erythema (16) Pityriasis alba (17) Anterior neck folds (18) Itch when sweating (19) Intolerance to wool and lipid solvents (20) Periofollicular accentuation (21) Food intolerance (22) Course influenced by environmental and emotional factors (23) White dermographism, delayed blanch

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