A randomized, double-blind, placebo-controlled clinical trial of the effects of vitamin D supplementation among diagnosed atopic dermatitis patients

Fatima DC. Jacinto-Calimag, MD[1]

ABSTRACT

Background: Atopic Dermatitis is an emerging public health concern. Recently, several studies have explored the role of Vitamin D in atopic dermatitis. To date, there is no local study using Vitamin D supplementation as an adjunct in the treatment of atopic dermatitis.

Objective: To determine the efficacy of Vitamin D supplementation in improvement of the disease severity in atopic dermatitis patients.

Methods: This is a Randomized, double blind, placebo-controlled clinical trial. The participants were newly diagnosed atopic dermatitis patients aged 19 to 50 years old. Participants were randomly assigned to take either 1 capsule of oral Vitamin D supplement (2200 IU/capsule) or a comparable placebo capsule, once daily for 60 days. Vitamin D level and disease severity using SCORAD index was evaluated at the start and end of the study.

Results: The mean value of serum Vitamin D levels at the start of treatment was deficient and comparable between the treatment and placebo group. The mean change in the serum Vitamin D levels of patients in the Treatment and Placebo group were 10.4 ng/mL ± 5.8 and -0.4 ng/mL ± 3.5, respectively. The mean change in the SCORAD index scores of patients in the Treatment and Placebo group were -20.2 ± 20.6 and 2.2 ± 6.8, respectively. Result of the two-sample independent t-test showed that the mean change in the SCORAD index scores significantly varied according to treatment group ($p<0.0001$).

Conclusion: The results from this study indicate that vitamin D supplementation may ameliorate clinical signs of the disease and can be considered as a safe and well-tolerated form of therapy.

Key words: Atopic dermatitis, Vitamin D, SCORAD index, t-test

INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, pruritic inflammatory condition of the skin which presents as having dry, itchy skin and immunological hyper-responsiveness to allergens [1]. This disease is greatly influenced by many factors, including genetics, environmental conditions, diet, infections and stress. The disease is highly prevalent and affects 10-20% of children and 1-3% adults worldwide [2]. Majority of the AD population are children comprising eighty-five percent, of which 30% continue to suffer in their adult years [3]. The yearly prevalence of AD in the Asia Pacific region was reported to be as high as 9% in Malaysia and Singapore, and as low as 0.9% in China for children aged 13-14 years old [4].

AD is an emerging public health concern, with its increasing prevalence and significant financial strain to the individual, family and the public healthcare system, particularly in industrialized countries including Southeast Asia. The estimated healthcare cost for an infant suffering from AD is estimated to be between

[1]Consultant, Department of Dermatology, East Avenue Medical Center

Corresponding author: Fatima DC. Jacinto-Calimag, MD,
East Avenue Medical Center,
Quezon City
fayejacinto@yahoo.com.ph

Source of funding: none
Conflict of interest: none

USD 199 and over USD 1,000 per year. The health costs of AD per patient per year ranged from USD 8 in the Philippines to USD 2,268 in South Korea [2].

In addition to the economic burden, quality of life is also affected especially in severe AD where skin infections are more common and the stigma associated with it, causing significant morbidity. Since majority of the AD population affected are children, another area of concern is the well-being of parents, families, and other caregivers of the patient [5]. Interestingly, it was found that it is more stressful taking care of a child with moderate-to-severe AD than a child with insulin-dependent diabetes [6]. Thus, AD represents a common disease that can negatively affect the QoL of both the patient and other family members.

The current treatment for AD include topical and oral corticosteroids, emollients, topical immunosuppressants (tacrolimus, pimecrolimus), oral antihistamines, refined-coal tar, and topical doxepin [7]. There is an emerging focus on complementary and adjuvant intervention given patients' hesitancies on conventional treatment. Diverse number of supplements have been proposed based on AD pathophysiology, including borage oil, docosahexaenoic acid (DHA), evening primrose oil, fish oil, gamma-linoleic acid, hempseed oil, probiotics, sea buckthorn oil, selenium, vitamins B6, C, D, and E, and zinc [8].

There is a growing body of research evidence on the potential role of Vitamin D in AD. First, documentation of aggravation of AD especially in higher latitude countries during winter where serum 25(OH)D tends to be particularly low [9]. Second, many studies have shown improvement of AD symptoms with Vitamin D supplementation [10,11]. Third, genetic polymorphisms have been identified as contributors to AD development, including Vitamin D Receptor (VDR) and filaggrin gene mutation [12, 13]

Majority of the conducted studies on AD and Vitamin D are done in countries located at a higher latitude, where climate and season may have contributed to Vitamin D synthesis [14,15]. Moreover, bulk of the population of previous studies are children [10,11,16]. To date, there is no local study using oral Vitamin D supplementation as an adjunct in the treatment of atopic dermatitis. In line with this, this study aims to determine the effect of oral Vitamin D supplementation in the disease severity of diagnosed atopic dermatitis patients aged 19-50, in a Filipino population.

OBJECTIVES

Overall, the efficacy of oral vitamin D supplementation in improvement of the disease severity in diagnosed atopic dermatitis patients. Specifically, the following outcomes were determined in both groups: the serum Vitamin D levels of Atopic dermatitis patients at the start and end of the trial, disease severity measured by SCORAD (Scoring Atopic Dermatitis) index of Atopic dermatitis patients at the start and end of the trial, and if there is a significant difference in Vitamin D level and disease severity as measured by SCORAD index among atopic dermatitis patients given placebo and Vitamin D supplement.

METHODOLOGY

Patients and study design

This study was a randomized, double-blind, placebo-controlled clinical trial conducted at the the dermatology outpatient department of a tertiary dermatology referral center from September 2017 to July 2018. The hospital's review board approved the trial protocol before the study was started. Informed consent from all participants was likewise secured prior to treatment. This trial was carried out in accordance with the Declaration of Helsinki principles, Good Clinical Practice (GCP) guidelines, and local regulations. Prior to conduct, ethical review was obtained.

Participants of the study were new patients diagnosed with atopic dermatitis at the dermatology outpatient department. Inclusion criteria are as follows: (1) Age: 19-50 years old and (2) New and active cases of diagnosed Atopic Dermatitis (based on criteria of Hannifin and Rajka). Patients taking vitamin, mineral, fatty acid supplementation, oral contraceptive pills, steroid hormones (orally or injected), antiepileptic agents, and anticoagulants, using ultraviolet B or solar irradiation, elevated or decreased serum calcium and pregnant or nursing mothers were excluded from the study.

Materials

The Solgar Vitamin D3 tablets (clear gelatin capsules) were repackaged by the Thomas Aquinas Research Center Pharmacy laboratory of the University of Santo Tomas. The same laboratory prepared the cornstarch placebo into similar clear gelatin capsules. This was necessary to ensure that the physicians and the participants were blinded during the study. All

capsules were packaged in airtight containers (60 capsules per container).

Randomization, treatment allocation, and blinding

Randomization was done electronically. Treatment assignment and end-point assessment were done by independent physicians who were not aware of the treatment being administered.

Interventions

Patients were randomly assigned to 60 days of oral Vitamin D capsule (one capsule of 2200 IU daily) versus an identical-appearing placebo capsule. The participants were given a container filled with 60 capsules. The supplements and placebos were given to the patients in identical containers with label (from 1 to 26). Participants were allowed to continue previously prescribed AD therapies, including their own preferred emollients. Instructions were given not to take other medication or supplementation during the 60-day trial. Patient education on atopic dermatitis, vitamin D sources and basic skin care were performed on all patients.

Outcome measures

On initial visit, the participants were asked to fill out an information sheet with basic demographic questions. The severity of their eczema was evaluated based on SCORAD index by three evaluators (average of the three scores was obtained and used), and serum Vitamin D level determination was performed. Blood extraction for serum Vitamin D level was done after physical examination.

After 60 days of either placebo or Vitamin D, the participant was instructed to follow up for re-assessment. The severity of their eczema was re-evaluated based on SCORAD index by the three evaluators who made the initial evaluation (average of the three scores was obtained and used for data analysis), and serum Vitamin D level determination was performed. At the end of the trial, all participants who were still Vitamin D deficient were given appropriate treatment.

Checking of Compliance

Participants were given a Daily Monitoring Card (see Appendix E) where they can note if they have taken the capsule for the day, as well as side effects or other remarks (eg: vomiting). The numbers of remaining capsules were checked every 2 weeks. This was done thru tele-communication or follow-up as needed.

Stopping guidelines

The study was stopped in patients who becomes pregnant during the trial period and who were advised to receive phototherapy. These patients were considered as withdrawals from the study. Those who did not comply to the once-a-day supplementation were also withdrawn from the study. Dropouts were defined as those who did not follow up within two weeks and whose outcome was unknown by the end of the study period.

Sample Size Computation

Using the sample size formula for t-test for two independent means, a minimum sample size of 24 patients (12 patients in the treatment group and 12 patients in the placebo group) was needed to achieve 80% power. Data encoding and editing was done using MS Excel for Mac 2009. For the profiling of patients, quantitative variables were described using the mean and standard deviation (S.D.). Meanwhile, responses to qualitative variables were summarized as frequency and percentage distributions. T-test for two independent means was used to compare the patients in the treatment and placebo groups in terms of the following: (a) Mean age; (b) Mean difference in serum Vitamin D levels at the start and end of the clinical trial; and (c) Mean difference in the SCORAD index scores at the start and end of the clinical trial.

Meanwhile, chi-square test of homogeneity was used to compare the gender distribution in the two treatment groups. A 5% level of significance was used in testing the hypotheses of this study.

Data processing and analysis

To determine if the groups were significantly different in their demographic characteristics, the T-test for two independent means was used to compare the patients in the treatment and placebo groups in terms of the mean age, mean difference in serum Vitamin D levels at the start and end of the clinical trial, and mean difference in the SCORAD index scores at the start and end of the clinical trial. Meanwhile, chi-square test of homogeneity was used to compare the gender distribution in the two treatment groups.

A 5% level of significance was used in testing the hypotheses of this study. Stata version 13 was utilized in producing the descriptive statistics and performing the analyses.

RESULTS

Study population

Twenty-five of 26 patients completed the 60-day trial, as shown in the flowchart (Figure 1). One subject was considered as dropout due to failure to follow up. No side effects were noted during the course of the trial. The two groups were comparable in age and gender. The mean age of the patients was 28.8 ± 10.9 years. Majority (21/25) of the patients are females. There were no significant differences between the two genders in serum Vitamin D level (male: 18.63 vs female: 18.19).

Clinical effects

At the beginning of the trial, the mean value of serum Vitamin D levels was deficient and comparable between the treatment and placebo group (19.9 ± 2.9 vs 16.5 ± 3.2). After 60 days of Vitamin D supplementation, it was found that in the treatment group, Vitamin D values were significantly higher compared to the starting levels (30.3 ± 3.3 vs 19.9 ± 2.9). There was no significant difference in the Vitamin D level of the placebo group (16.5 ±3.2 vs 16 ± 2). At the end of the trial, majority of the treatment group had sufficient vitamin D levels while the placebo group still had deficient Vitamin D level (Table 2).

For the treatment group, a reduction in the SCORAD index was observed (36.7 ± 9.1 at the initial visit vs 16.5 ± 4 at final visit). There was no significant difference in the SCORAD index for the placebo group. (37.2 ± 11.9 at the initial visit vs 39.3 ± 10.4 at final visit) (Table 3).

The mean change in the serum Vitamin D levels of patients in the Treatment and Placebo group were 10.4 ng/mL ± 5.8 and -0.4 ng/mL ± 3.5, respectively. Result of the two-sample independent t-test showed that the mean change in the serum Vitamin D levels was significantly varied according to treatment group ($p<0.0001$) (Table 4).

The mean change in the SCORAD index scores of patients in the Treatment and Placebo group were -20.2 ± 20.6 and 2.2 ± 6.8, respectively. Result of the two-sample independent t-test showed that the mean change in the SCORAD index scores significantly varied according to treatment group ($p<0.0001$) (Table 5).

Before supplementation, the degree of Atopic dermatitis severity of the treatment group was: mild = 5(38%), moderate = 5(38%) and severe = 3(24%). After 60 days of supplementation, there was a decrease in the disease severity 12(92%) were classified as mild AD and 1(8%) patient classified as moderate AD. On the other hand, for the placebo group, before supplementation, the degree of Atopic dermatitis severity was: mild = 5(42%), moderate = 5(42%) and severe = 2(16%). After 60 days of supplementation, there was no decrease in the disease severity: mild = 4(33%), moderate = 5(42%) and severe = 3(25%).

There were no adverse effects noted in both groups.

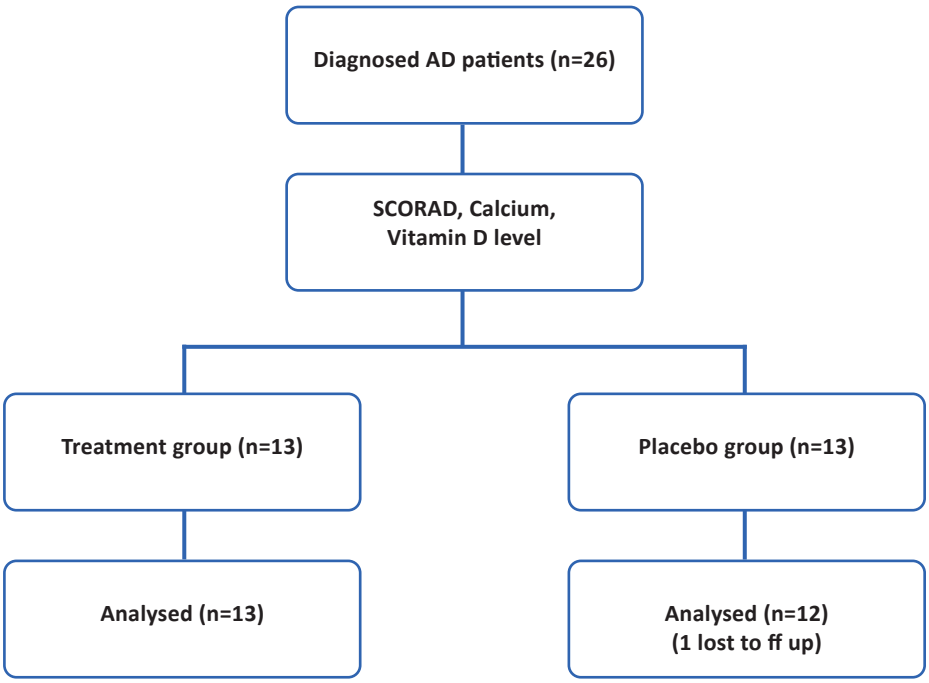


Figure 1. Screening, randomization, and analysis of patients in the study.

Table 1. Demographic profile of the patients according to their treatment group

Demographic Characteristic	Vitamin D Group (n=13)	Placebo Group (n=12)
	Mean (S.D.)	Mean (S.D.)
Age (in years)	28.8 (11.5)	29 (10.8)
	Frequency (Percent)	Frequency (Percent)
Gender Male Female	 2 (15.4%) 11 (84.6%)	 2 (16.7%) 10 (83.3%)

Table 2. Comparison of the improvement in the serum Vitamin D levels of patients in the treatment and placebo groups

Time Period	Mean	95% Confidence Interval
Baseline Vitamin D Group (n=13) Placebo Group (n=12)	 19.9 16.5	 17.0, 22.8 13.3, 19.7
End of study Vitamin D Group (n=13) Placebo Group (n=12)	 30.3 16.0	 27.0, 33.6 14.0, 18.1

Table 3. Comparison of the improvement in the SCORAD index scores of patients in the treatment and placebo groups

Time Period	Mean	95% Confidence Interval
Baseline Vitamin D Group (n=13) Placebo Group (n=12)	 36.7 37.2	 27.6, 45.8 25.3, 49.0
End of study Vitamin D Group (n=13) Placebo Group (n=12)	 16.5 39.3	 12.5, 20.4 28.9, 49.8

Table 4. Mean change in the serum Vitamin D levels (ng/mL) of the patients according to their treatment group

Treatment Group	Mean (S.D.)	p-value
Vitamin D Group (n=13)	10.4 (5.8)	<0.0001
Placebo Group (n=12)	-0.4 (3.5)	

Table 5. Mean change (i.e. end of study - baseline) in the SCORAD index scores of the patients according to their treatment group

Treatment Group	Mean (S.D.)	p-value
Vitamin D Group (n=13)	-20.2 (20.6)	<0.0001

Figure 2. AD Severity of treatment group (initial vs final visit)

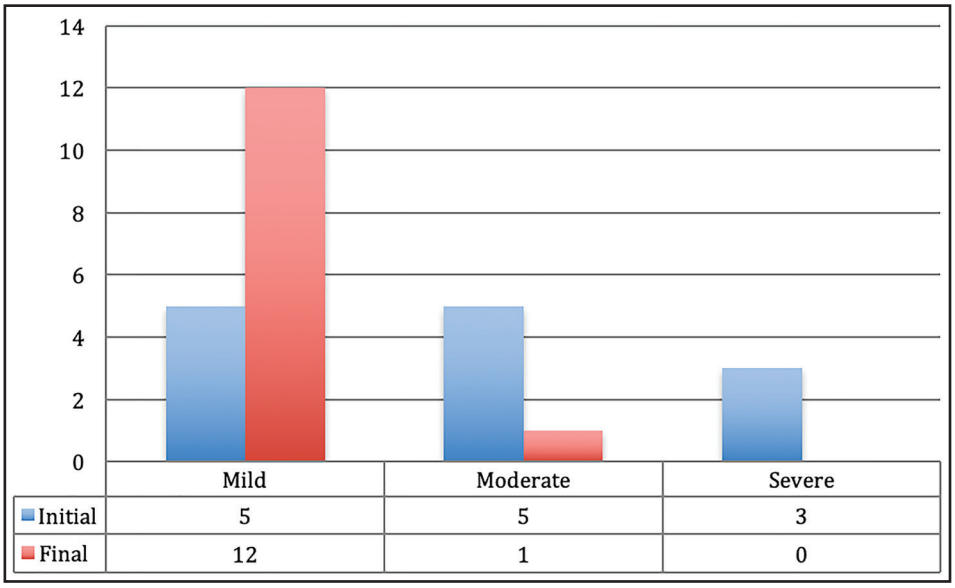
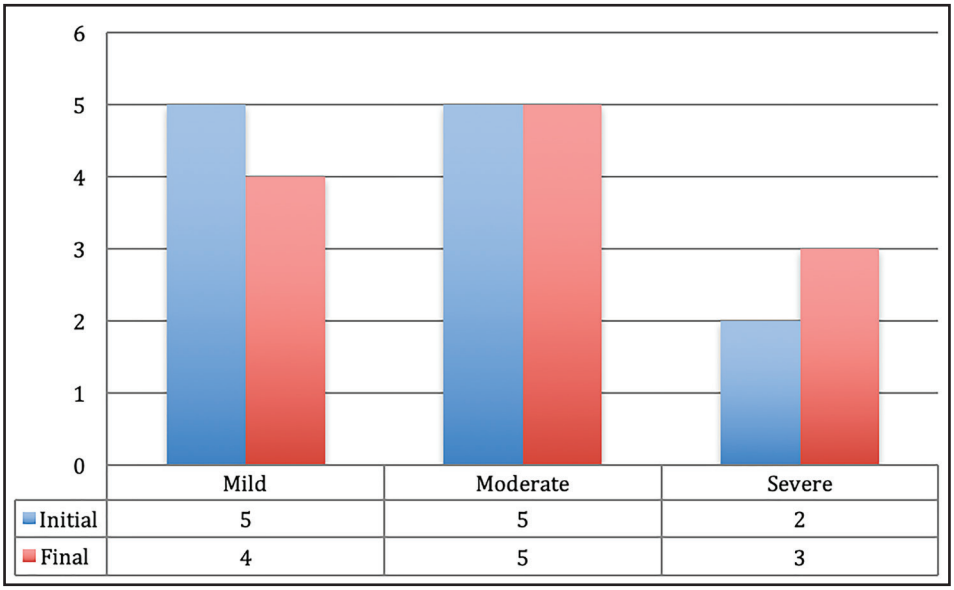


Figure 3. AD Severity of Placebo group (initial and final visit)



	Mild	Moderate	Severe
Initial	5	5	2
Final	4	5	3

DISCUSSION

The results of this research showed considerable improvement in AD patients taking oral vitamin D supplementation. Furthermore, the researchers found a significant negative correlation between the SCORAD change and vitamin D change in the subgroup of patients in which the supplementation was able to increase vitamin D levels. This negative association between SCORAD and the serum Vitamin D level could imply its influence in improvement and potential as an adjunct therapy. Despite the small size of the sample, it was interesting to note that without supplementation, there was no significant change in the placebo group between the initial and final visit in the following: (1) Vitamin D levels (16.5 ± 3.2 vs 16 ± 2) and (2) SCORAD (37.2 ± 11.9 vs 39.3 ± 10.4). In contrast to the participants who took Vitamin D supplement where AD severity, SCORAD and Vitamin D levels were markedly improved.

These results are comparable to recent studies done by Armendariz et al [14] and Lara-Corrales et al [15] where SCORAD Index scores decreased after Vitamin D supplementation. Armendariz et al. [14] supplemented participants aged 2–54 with 5000 IU Vitamin D daily for 3 months, while Lara-Corrales et al [15] included patients between the ages of 0 and 18 years and administered 2000 IU Vitamin D for 3 months. In addition, Armendariz et al [14] and Lara-Corrales et al [15] showed favorable improvement in the SCORAD Index score (Mean difference: − 13.3 compared to the placebo group: − 1.27, $p < 0.05$; SCORAD change: − -11.92 compared to placebo group: − 6.32, respectively).

Of note, in a study conducted by Galli et al. [16] including children (48 boys) with a median age of 68 months (range 6–195 months), underwent 3-month consecutive supplementation with 2000 IU Vitamin D daily failed to show a statistical correlation between the serum levels of vitamin D and eczema severity. The variety of dosing, duration and population may be the culprit of these conflicting results. Likewise, a study done by Huang et al [5] has suggested that Vitamin D supplementation may not be advantageous for all children and an increased intake of Vitamin D during infancy was correlated with a higher risk of AD at age 6. Therefore, the age at which supplementation is initiated should be considered carefully.

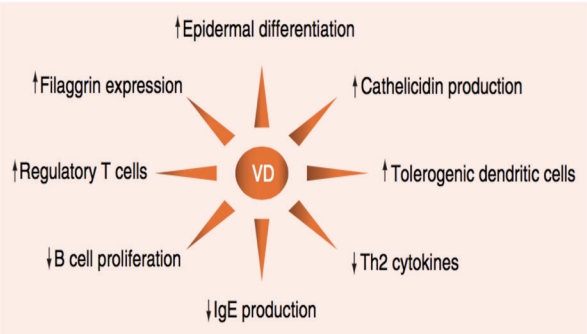
Vitamin D is a secosteroid with an endocrine mechanism of action that can be derived from dietary intake or synthesized from ultraviolet radiation exposure of the skin. It is primarily considered a significant regulator of calcium and phosphate homeostasis. However, current evidence suggests that Vitamin D may also contribute in the prevention and control of cardiovascular disease, autoimmune disease, and cancer. Recent studies found a negative association between serum Vitamin D levels and AD severity. Additionally, Vitamin D supplementation was proposed as an efficacious and safe treatment for AD.

The observed AD improvement from vitamin D supplementation has strong biological plausibility [5].

Vitamin D promotes skin barrier integrity and increases antimicrobial peptides (AMPs) which is responsible for the prevention of skin infections, thus suppressing inflammatory response. Vitamin D may exert its role in epidermal barrier function by modulating structural proteins of the cornified dermis layer, regulating the glycoseramides essential for the hydrating protective lipid barrier which keeps the skin moisturized and intact.

Innate immunity is improved by modulation of AMPs and cytokine response leading to reduction of skin infection risk. Amon et al. [17] further elucidated on the immunomodulatory function of Vitamin D including inhibition of monocyte production (via Toll-like receptors) and inhibition of dendritic cell activity and increased mast cell release of IL10. In addition, Amon et al. [17] also noted that vitamin D may have an inhibitory effect on IgE release by reducing B cell function which reduces the release of proinflammatory cytokines from Th1 cells. Therefore, from an antimicrobial viewpoint, vitamin D can reduce the infection susceptibility and regulate both local immune and inflammatory response in AD patients [1].

The results of this study also showed that all participants had serum concentrations of 25(OH)D3, below 30 ng/mL at the start of the trial. This finding concurs with a study done by Camargo et al who found similarly low vitamin D levels in patients with AD. In the two largest studies to date on Vitamin D supplement in AD, both consisting of 306 participants, a significantly higher serum Vitamin D level was found in patients with milder forms of AD [5]. Thus, vitamin D deficiency appears to contribute to several of the hallmark characteristics of AD: altered barrier function, immune dysregulation, and inadequate bacterial defense (Figure 4).



Source: Borzutzky, A. and Camargo, C. (2013). Role of vitamin D in the pathogenesis and treatment of atopic dermatitis. Expert Review of Clinical Immunology, 9(8), pp.751-760.

Figure 4. Possible mechanism of action of Vitamin D in atopic dermatitis.

A notable weakness in this study was the relatively small sample size. Thus, larger prospective randomized controlled trials are needed to delineate the benefits of vitamin D treatment for AD. Ideally, studies utilizing varied Vitamin D doses and durations should be performed, to establish the most effective supplementation regimen for AD. Additionally, clinical factors such as subjects' physical activity, duration of outdoor and thus sunlight exposure, and detailed dietary intakes that regulate vitamin D homeostasis should also be considered. Prospective cohorts are needed to accurately address the influences of these confounders on vitamin D deficiency. Another limitation of this study is that the data included is composed mainly of mild and moderate AD with only few severe cases (5/25). The specific reduction in SCORAD seen, and difference in 25(OH)D between AD patients may differ in children from that found in this study which focused on adult patients with AD. Finally, our results are based on Vitamin D supplementation of 2200 IU per day for 60 days and SCORAD reductions observed seen are likely to differ with higher or lower doses and longer or shorter duration.

In conclusion, among adult patients (19-50 years old) diagnosed with atopic dermatitis, vitamin D supplementation of 2200 IU/day for 60 days may ameliorate clinical signs of the disease and can be considered as a safe and well-tolerated form of therapy.

Acknowledgment

The author would like to thank Dr. Adrian Patrick Calimag for his technical editing and proofreading.

REFERENCE

1. Kalshetti VT, Bhate VM, Haswani N, Bothikar ST. Staphylococcus aureus: a major causative agent of community - acquired pyoderma. Int.J.Curr. Microbiol.App.Sci (2014) 3(9) 94-97

2. Bowen AC, Mahe A, Hay RJ, Andrews RM, Steer AC, Tong ST, et al. The global epidemiology of impetigo: a systematic review of the population prevalence of impetigo and pyoderma. PLoS One 2015;10(8):e0136789.

3. Philippine Dermatological Society Health Information System. Internet. Available from http://192.168.2.140/pdshis/index.php

4. Gandhi S, Ojha SK, Ranjan KP, and Neelima. Clinical and Bacteriological Aspects of Pyoderma. N Am J Med Sci. 2012; 4(10): 492–495.

5. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. The Canadian Journal of Infectious Diseases & Medical Microbiology. 2008;19(2):173-184.

6. Templer J, Maximo SB. Bacterial Skin and Soft Tissue Infections. Curr Opin Infect Dis. 2009; 45(3): 9-16, 26

7. Tong, SYC, Davis, J, Eichenberger, E., Holland, T, Fowler VG. Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbio Rev 2015: 603–661

8. Chen, C.J, Huang, Y.C, New epidemiology of Staphylococcus aureus infection in Asia. Clinical Microbiology and Infection 2014: 20(17): 605-2

9. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbio Mol Biol Rev 2010; 74(3): 417–433

10. Hetem, D.J., Bonten, M.J. Clinical relevance of mupirocin resistance in Staphylococcus aureus. J Hosp Infect. 2013; 85(4): 249-56

11. Gisby J, Bryant J. Efficacy of a New Cream Formulation of Mupirocin: Comparison with Oral and Topical Agents in Experimental Skin Infections. Antimicrobial Agents and Chemotherapy 2000; 44(2): 255-260.

12. Eells L.D., Mertz P.M., Piovanetti Y., Pekoe G.M., Eaglstein W.H. Topical antibiotic treatment for impetigo with mupirocin. Arch Dermatol 1986; 122: 1273-6.

13. Breneman D.L. Use of mupirocin ointment in the treatment of secondarily infected dermatoses. J AM Acad Dermatol 1990; 22: 886-92.

14. Upton, A., Lang, S., Heffernan, H. Mupirocin and Staphylococcus aureus: a recent paradigm of emerging antibiotic resistance. Journal of Antimicrobial Chemotherapy. 2003;51:613-617

15. A.R. Caffrey, B.J. Quilliam, K.L. LaPlante, Risk factors associated with mupirocin resistance in meticillin-resistant Staphylococcus aureus, J Hosp Infect 2010; 76(3): 206-210

16. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin Resistance in Staphylococcus aureus Causing Recurrent Skin and Soft Tissue Infections in Children. Antimicrob Agents and Chemother. 2011;55(5):2431-2433

17. Lagda D, Sutantoyo CJ, Galang MC. In vitro evaluation of Makabuhay stem extract as an Antimicrobial Agent against Staphylococcus aureus Steptococcus pyogenes and Pseudomonas aeruginosa. 2015, Unpublished manuscript

18. Ahmad W, Jantan I, Bukhari SNA. Tinospora crispa (L.) Hook. f. & Thomson: A Review of Its Ethnobotanical, Phytochemical, and Pharmacological Aspects. Front Pharmacol. 2016;7:59

19. Tan RS, Bajo. LM. Modulation of Tinospora rumphii and zinc salt on DNA damage in quinoline-induced genotoxicity and hepatotoxicity in male albino mice. Advances in Toxicology. 2014

20. Castillo, A, Osi, MO, Ramos, JD, De Francia JL, Dujunco MU, Quilala, PF. Efficacy and safety of Tinospora cordifolia lotion in Sarcoptes scabiei var hominis-infected pediatric patients: A single blind, randomized controlled trial. J Pharmacol Pharmacother. 2013; 4(1):39-46

21. Dela Torre, LT, Ponsaran G, Ponsaran, MG, De Guzman, KD, De Guzman, LD, Manalo, AM, et al. Safety, efficacy, and physicochemical characterization of Tinospora crispa ointment: a community-based formulation against Pediculus humanus capitis. The Korean Journal of Parasitology. 2017; 55(4): 409-416

22. Hipol, R.L., Cariaga, M.F.N.M, and Hipol, R.M. Antiinflammatory activities of the aqueous extract of the stem of Tinospora crispa (Family Menispermaceae). Journal of Nature Sudies. 2012; 11(1&2): 88-95

23. Galia, MLD., Galia, J.D. Angiogenetic activity of Tinospora rumphii Boerl (Makabuhay) Leaf and Stem Extracts. International Research Journal of Biological Sciences. 2016; 5(1): 54-59

24. Choudhary N, Siddiqui MB, Azmat S, Khatoon S. Tinospora cordifolia: Ethnobotany, Phytopharmacology and Phytochemistry Aspects. IJPSR, 2013; 4(3): 891-899.

25. Choudhary, MI, Ismail M, Ali, Z, Shaari K, Lajis NH, Atta-ur-Rhaman. Alkaloidal Constituents of Tinospora crispa. Nat Prod Commun. 2010;5(11):1747-50

26. Mohammed, AI. Antimicrobial activity of Tinospora crispa root extracts. International Journal of Research in Ayurveda and Pharmacy. May-June 2012 3(3):417-419

27. Ragasa, C, Cruz, MC. Antimicrobial Diterpenes from Tineaspora rumphii. Journal of Research Science, Computing and Engineering, 2005;2(1)

28. Sutantoyo CJ, Lagda D, , Dayrit JF, Gabriel, MT. An Evaluation of Rabbit Skin Irritation from Makabuhay Cream. 2016. Unpublished manuscript

29. Galang MC, Chia, CLN, , Gabriel, MT, Dayrit, JF, Teodosio, GB. Use of skin patch testing in determining safely of Tinospora rumphii 25%, 50%, 90% cream on normal skin. 2016. Unpublished manuscript

30. Panis L, Cejar R, Gabriel MT, Dayrit JF, A randomized, double-blind, controlled study on the safety and efficacy of 10% bee propolis ointment versus 2% superficial pyodermas caused by Staphylococcus aureus. 2014. Unpublished manuscript

31. Adasa, G, A Randomized, Double-blind, Controlled Study on the Safety and Efficacy of 2.5% Moringa oleifera Lam (Moringaceae) Leaf Extract Ointment versus 2% Mupirocin Ointment on Superficial Pyodermas caused by Staphylococcus aureus . Unpublished manuscript

32. Yusoff, M, Hamid, H, Houghton, P. Anticholinesterase inhibitory activity of quaternary alkaloids from Tinospora crispa. Molecules 2014; 19:1201-1211