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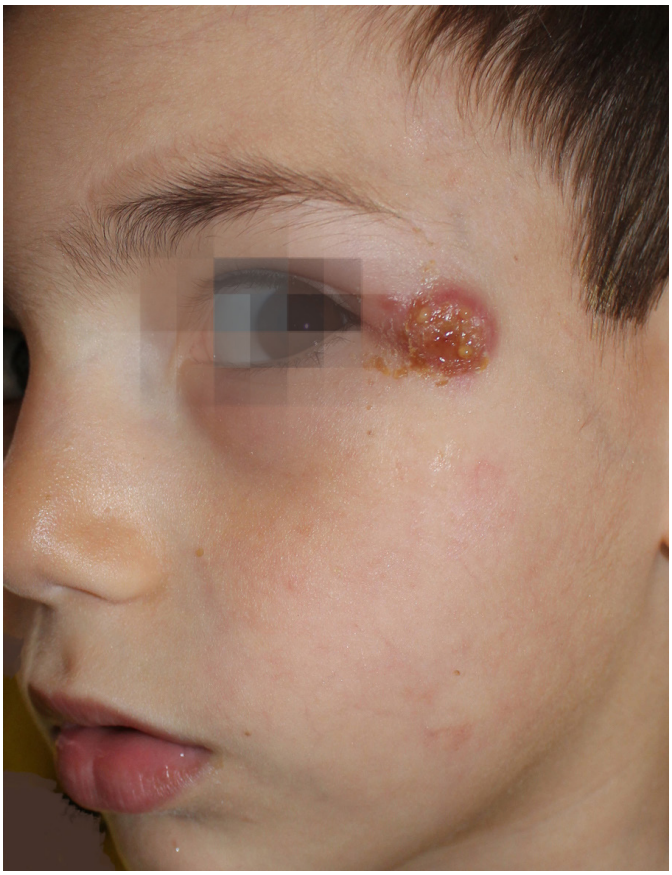

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On cover: Lymphomatoid papulosis type A

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Vitamin D3 supplementation reduced *Staphylococcus aureus* colonization in the skin of pediatric patients with atopic dermatitis.

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Summary

Atopic dermatitis (AD) is a common skin disorder resulting in malfunctioning of the skin barrier and immune system that facilitate the growth of various microorganisms. Several studies reported that there was a significant reduction of vitamin D and cathelicidin levels in AD patients. It was also reported that the administration of vitamin D in AD patients sharply increased cathelicidin level that can be advantageous as a skin defense against microbial infection. In this study the supplementation of vitamin D3 was administered to AD pediatric patients and the ability of Vitamin D3 to reduce infections in AD patients was measured by observing *Staphylococcus aureus* colonization. A double-blind randomized experimental study was carried out after appropriate sampling on 20 AD patients who came for treatment in Dermatopediatric Division of Dr. Soetomo General Hospital Surabaya and met the criteria for inclusion. A supplementation of vitamin D3 syrup was used in the experimental group, and a placebo syrup was used in the control group. Lesional skin swabs were taken before and after 28 days supplementation of vitamin D3. There was significant reduction of the *Staphylococcus aureus* colonization after vitamin D3 administration while there was no significant difference in the number of *Staphylococcus aureus* colonies after placebo administration. The statistic analysis of the two groups confirmed that there was a significant difference in the percentage of *Staphylococcus aureus* colonization in the vitamin D3 group compared to placebo. The supplementation of vitamin D3 decreased *Staphylococcus aureus* colonization in AD children, without significant side effects.

Keywords

Vitamin D3, *Staphylococcus aureus*, atopic dermatitis.

Atopic dermatitis (AD) is a chronic inflammatory skin disease that has an impact in the disruption of human skin barrier function and immune system (13). AD affects around 5-20% of children worldwide. Severe AD affects patient's quality of life, often causing patient morbidity as a result of associated skin infections (3). AD is characterized by dry, itchy skin, and immunological hyperresponsiveness to allergens. AD is frequently associated with other atopic diseases (such as allergic rhinitis, food allergy, and asthma), and is commonly the first manifestation of the atopic diathesis.

The impairment in skin barrier may facilitate the growth of bacteria, fungi, and viruses. Among the bacterial colonies *Staphylococcus aureus* is commonly found in 80% to 100% of AD patients is (21). The increased number of *Staphylococcus aureus* colonies is usually caused by various factors, including the defects in the skin barrier function, the reduction of antibacterial activity in the innate immunity system, and the imbalance

of the immune response. All of these factors play a shared and mutual inseparable role (2, 12).

Wang et Al. (22) carried out a study by measuring vitamin D levels (serum 25 hydroxyvitamin D) in 498 DA patients and 328 non-DA patients in Hong Kong. The Authors found a lower vitamin D levels in AD patients compared to non-AD patients. Moreover, Darwish et Al. (5) also showed a lower levels of vitamin D and cathelicidin in AD patients. The administration of vitamin D in AD patients proved to be effective in increasing cathelicidin level (8). Vitamin D increased the expression of cathelicidin, that can be used by the skin as a defense factor against bacterial infection including *Staphylococcus aureus* (13).

Due to the very limited research on the effect of vitamin D (11) and its correlation with the reduction of *Staphylococcus aureus* colonization in AD patients, we carried out a study aimed at proving the effect of vitamin D3 on the reduction of *Staphylococcus aureus* colonization in the skin of AD pediatric patients.

Methods

The actual research is a randomized, double-blind, placebo-controlled experimental study, applying to all pediatric AD patients who came to receive treatment at the Dermatopediatric Division of Dr. Soetomo General Hospital Surabaya from March to August 2018. The patients were then selected using consecutive sampling and met the inclusion criteria of the study.

The inclusion criteria were as follows: children aged 2-12 years with a history of AD, who were not in an exacerbation condition, known to have a colonization of *Staphylococcus aureus* before intervention (i.e. supplementation of vitamin D3 or placebo), who were willing to enter the study and whose tutor signed an informed consent.

Exclusion criteria applied to patients using corticosteroids and oral or topical antibiotics in the past week, suffering from other diseases responsible for immune suppression that could increase the risk of bacterial infections such as diabetes, malignancy, liver and kidney function disorders. Other exclusion criteria were as follows: patients who were taking antiepileptic drugs (phenytoin, carbamazepine, oxcarbazepine, phenobarbital), theophylline, antituberculosis, antiretroviral, and antifungal (ketoconazole) drugs, suffering from skin cancer, acne, psoriasis, rosacea, vitiligo, scleroderma, pemphigus vulgaris, bullous pemphigoid, systemic lupus erythematosus, and taking other vitamins.

The study was approved by the Ethics Committee of Dr. Soetomo General Hospital Surabaya and the methods were performed in accordance with approved guidelines. All the subjects signed an informed consent (child patients being represented by parents or guardians). The eligible research subjects who met the inclusion criteria underwent lesional skin swab to measure the colonization of *Staphylococcus aureus* before the administration of vitamin D3 and placebo. Study subjects and the intervention were then randomized. The randomization was carried out and recorded by a pharmacist who kept information about who took vitamin D3 and placebo secret. Vitamin D3 syrup and placebo produced by Apotek UBAYA (pharmacy of Universitas Surabaya) were given to the patients for 28 days. At the end of this period all subjects again underwent lesional skin swab using sterile stick cotton to measure the colonization of *Staphylococcus aureus*. Measurement of *Staphylococcus aureus* colonization was carried out at Surabaya Health Laboratory.

Vitamin D3 syrup and placebo were produced (17) by grinding of 0.004 g vitamin D3, wetting by 0.5 ml propylene glycol 10%, stirring together with simple syrup, 0.025 ml lime flavor and additional simple syrup to provide the final volume of 5 ml (each dose). The given dose of vitamin D3 was 400 IU in 5 ml syrup daily; the placebo syrup was made by similar composition of all ingredients except for vitamin D3. The colonization of *Staphylococcus aureus* before and after 28 days of Vit D3 and placebo administration were then analyzed using Wilcoxon Signed Ranks Test. During administration of vitamin D3 and placebo, an evaluation of the side effects was recorded.

Material and results

Of 20 pediatric AD subjects, 12 patients (60%) were male and 8 patients (40%) were female. Ten

subjects received vitamin D3 syrup and other 10 subjects received a placebo syrup. Six female (60%) and four male (40%) patients received Vitamin D3 syrup, and eight male (80%) and 2 female (20%) patients received placebo syrup. Statistically gender differences did not significantly affect the S. aureus colonization (p value = 0.170). It was also found that age difference did

TABLE 1: Baseline data characteristic of research subjects.

	VITAMIN D3 n = 10 (%)	**PLACEBO n = 10 (%)**	**P-VALUE**
GENDER			
Male	4 (40%)	8 (80%)	p = 0.170
Female	6 (60%)	2 (20%)	
AGE			
2 - 5 age	4 (40%)	2 (20%)	p = 0.170
6 -12 age	6 (60%)	8 (80%)	
FAMILY HISTORY			
Allergic rhinitis	4 (40%)	2 (20%)	p = 0.628
Asthma	5 (50%)	4 (40%)	p = 1.00
Atopic dermatitis	7 (70%)	5 (50%)	p = 0.65
BODY MASS INDEX			
Underweight	0	0	p = 1.00
Normal	7 (70%)	6 (60%)	
Overweight	3 (30%)	4 (40%)	
LOCATION			
Surabaya	10 (100%)	10 (100%)	p = 1.00
FITZPATRICK SKIN TYPE			
Type IV	6 (60%)	6 (60%)	p = 1.00
Type V	4 (40%)	4 (40%)	
SUN EXPOSURE (HOUR)			
Mean ± SD	2.5054 ± 0.636	2.5310 ± 0.802	p = 0.938
SUN PROTECTIVE			
Protective 1	6 (60%)	5 (50%)	p = 1.00
Protective 2	4 (40%)	5 (50%)	
FOODS			
Containing vitamin D	6 (60%)	8 (80%)	p = 0.628
Milk consumption (ml) (mean ± SD)	304 ± 241.8	331 ± 192.1	p = 0.569
USE OF MOISTURIZERS			
Yes	1 (10%)	1 (10%)	p = 1.00
No	9 (90%)	9 (90%)	

TABLE 2: *Staphylococcus aureus* colonization before and after administration of vitamin D3.

	***S. aureus* colonization**	**p-value**
Pre-vitamin D3 (mean ± SD)	998.1 ± 360.9	p = 0.0001
Post-vitamin D3 (mean ± SD)	206.8 ± 227.1	
Pre-Placebo (median) (minimum – maximum)	404.5 (12-1250)	p = 0.386
Post-Placebo (median) (minimum – maximum)	22.5 (0-958)	

TABLE 3: Comparison test initial colonization *Staphylococcus aureus* vitamin D3 and placebo.

	Vitamin D3	**Placebo**	**p-value**
Initial colonization *Staphylococcus aureus*	998.1 ± 360.9	461.1 ± 434.5	p = 0.008
Mean ± SD			
Delta (Δ) % ± DS	-791.3 ± 428.5	-161.5 ± 787.1	p = 0.043

not significantly affect the effect of Vitamin D3 in the *S. aureus* colonization (p value = 0.170).

The effect of Vitamin D3 is influenced by several factors, for example geographical location, body mass index, pigmentation, sun exposure, protection against sunlight, foods containing vitamin D, and consumption of milk. The baseline data characteristic of research subjects can be seen in table 1. The twenty research subjects were residing in Surabaya, so that there were no geographical differences in the vitamin D3 and placebo groups. There were no significant differences in body mass indexes (p value = 1.00). Body mass index was adjusted according to the anthropometric standard of the Health Ministry of the Republic of Indonesia. There was no significant difference (p value = 1.00) of Fitzpatrick skin type in the vitamin D3 groups and placebo groups. The average duration of sun exposure in vitamin D3 study group was 2.5054 hours and in placebo study group was 2.5310 hours (p value = 0.938). There was no significant difference in the value of protection against sun exposure (p value = 1.00) and consumption of foods containing vitamin D (p value = 0.628). The average milk consumption in the vitamin D3 group was 304 ml and in the placebo group was 331 ml (p value = 0.569).

Wilcoxon Signed Ranks Test comparing median scores was used to determine the differences in *Staphylococcus aureus* colonization before and after placebo. There were no significant differences (table 2) in colonization before and after placebo (p value = 0.386). Paired T-test comparing the mean before and after vitamin D3 was used in vitamin D3 group. The results of the analysis showed that there were significant differences in colonization before and after administration of vitamin D3 with p value of 0.0001 (table 2).

In the colonization measurement before the treatment, the mean value in vitamin D3 group was significantly higher than the placebo group (mean value ± SD vitamin D3 group was 998.1 ± 360.9 and mean placebo group was 461.1 ± 434.5 with p value = 0.008. Thus, the proportion was adjusted using percentage of reduction (Δ%) of the final measurement of colonization compared to the initial value of colonization. In vitamin D3 group there was a decrease in percentage of 791.3% compared to the placebo group with a decrease of 161.5%. The results were then analyzed with statistical tests using paired T-test, obtaining

p value of 0.043. There was a significant difference in the percentage of *Staphylococcus aureus* colonization in the vitamin D3 group compared to placebo (table 3). There were no side effects in the vitamin D3 and placebo groups.

Discussion

Aimed at proving the effect of vitamin D3 on the reduction of *Staphylococcus aureus* colonization, we carried out a study in 20 pediatric patients with AD in Dr. Soetomo Hospital Surabaya. We found a similar result in the percentage of subject gender with the study performed by Shi et. Al. employing 1008 AD patients in China – percentage of male patients more than women with a ratio of 1.63:1 – (19). In the actual study 20 research subjects showed more male patients than female with a ratio of 1.5:1. A greater incidence of *Staphylococcus aureus* colonization in male (70.8%) than in female (61.1%) was also confirmed by Alenizi et Al. in Saudi Arabia (1). In a different research carried out by Sollid et Al. the male patients had a higher risk for nasal carriage of *Staphylococcus aureus* than females (20).

With regard to the family history of atopy, we found that patients with a family history of atopic dermatitis were at highest risk of suffering from AD (60%), followed by asthma (45%). and allergic rhinitis (30%). These results were in accordance with the results of Gomes et Al. According to these Authors the colonization of *Staphylococcus aureus* was higher (65%) in AD patients with atopic dermatitis history in their families (7).

Geographical position also affects the production of vitamin D by the skin. In the area above 37° north latitude the number of UVB photons reaching the Earth's atmosphere decreases 80% to 100% in winter; as a consequence, a little amount of vitamin D3 is produced in the skin (10). Surabaya's geographical location, which all patients of the actual study shared, is close to the equator allowing a huge production of vitamin D3 on the skin all over the year.

Body mass index affects vitamin D levels. A study carried out by Hata et. Al. (2013) regarding vitamin D administration in AD patients (9) found an inverse correlation between 25 (OH) D levels and body mass index ($r = -0.38$. with p value = 0.04). There was no significant difference in body mass index in the control group. Thirteen study subjects taken together were in a normal condition of body mass index, suggesting that they did not have excessive sequestration of vitamin D in the subcutaneous fat tissue.

Skin pigmentation determines the duration of sun exposure needed to reach a certain concentration of vitamin D. UVB exposure is not evaluated through a fixed amount of energy (UVB light) but is expressed as a minimum erythematous dose (MED). More exposure to UVB rays is needed to produce MED in dark-skinned people; therefore, people with dark skin need longer exposure to the sun than light-skinned people for the same response. This is related to melanin as a competitive inhibitor for vitamin D receptors in the skin. Melanin will absorb UVB light, thereby reducing absorption of UVB rays by vitamin D receptors on the skin (15, 17). The research data showed that the subjects in both the vitamin D3 and placebo groups had Fitzpatrick skin types between 4 and 5. However, there were no significant differences for Fitzpatrick's skin type in the two groups (p value = 1).

Vitamin D is a lipid soluble vitamin produced effectively when ultraviolet B (UVB) radiation in a wavelength of 255-330 nm reaches the epidermis. Outdoor activities also affect the formation of vitamin D in the skin related to the intensity of UVB exposure to the skin (9, 15). Moy carried out a cross-sectional study using a questionnaire on 380 people in Malaysia (16) and found a positive correlation between 25 (OH) D levels and sun exposure scores ($r = 0.27$. $p < 0.001$). In the actual study there was no difference in the value of sun exposure in the vitamin D3 (mean: 2.5054 hours) and placebo (2.5310 hours) groups (p value = 0.938).

Less than ten percent of vitamin D is obtained from food supplementation. It is a minor amount as compared to the production of vitamin D by UVB light exposure. Food supplementation provides two different form of Vitamin D, namely vitamin D3 and vitamin D2. Vitamin D3 (cholecalciferol) is produced from animals and vitamin D2 (ergocholecalciferol) from plants. Animal

sources of vitamin D3 are salmon, cod liver oil, egg yolks, mackerel and tuna. Plant sources are orange juice, cereals and mushrooms (14). Vitamin D2 and vitamin D3 have the same activity potential. However, most recent studies suggested that vitamin D3 has a potential that is 3 times higher than that of vitamin D2. In the actual study there were no significant differences in the amount of milk consumption in subjects given vitamin D3 and placebo (p value = 0.569). Furthermore, there was no significant difference (p value = 0.628) in the two group regarding the fortification of foods with vitamin D.

In the past the application of emollients on the skin surface was focused on improving skin barrier function and inhibiting transepidermal water loss. Nowadays, several theories reveal that the application of emollients can change skin microbiomes and pH, and increases antimicrobial peptides and immune expression on the skin barrier, therefore indirectly influencing bacterial colonization. Czarnowicki et. Al. showed through skin biopsies an emollient-induced increase in antimicrobial peptides and innate immune genes. Therefore, an increase in antimicrobial peptide is expected to reduce *Staphylococcus aureus* colonization (4).

Emollients were proven to be useful in preventing AD development in high-risk newborns. Glatz et. Al. discovered that emollients applied to 3 weeks old pediatric patients with family history of atopy for 6 months were able to modify skin microbiota. The Authors showed that the emollient group had a lower skin pH than the control group. The number of bacterial taxa in the emollient group was higher than in the control group at all sites. Particularly, the proportion of *Streptococcus salivarius* was higher in emollient versus control group in all locations. Proportion of *S. salivarius* appeared higher in infants without AD than in infants with AD (6). In the actual study there were no significant differences in the use of emollients in both research subjects (p value = 1).

S. aureus colonization in AD is commonly associated with the induction of a malfunction of the immune system which results in toxicity to keratinocytes and lymphocytic apoptosis. It is also associated with the induction of AD chronicity through lymphocyte stimulation to produce IFN. The products secreted by *S. aureus* are linked to T cell receptors and stimulate their polyclonal activation. They also activate macrophages and antigen-presenting cells inducing specific IgE production against a superantigen that functions as an allergen. This process culminates in the activation of basophils and IgE-mediated inflammation. Moreover, it was postulated that this process could induce erythema and a burning sensation in the AD lesions (18).

The actual study proves that the supplementation of vitamin D3 syrups for 28 days reduced *Staphylococcus aureus* colonization in AD pediatric patients without significant side effects. Considering the purported immunologic mechanisms that contribute to AD, it is possible that vitamin D may influence this disorder through its immunomodulatory properties. This hypothesis is strengthen by the knowledge that the active form of vitamin D enhanced expression of antibacterial peptides and prevented skin infections. In addition, vitamin D stimulates protein synthesis that is necessary for building stratum corneum barrier.

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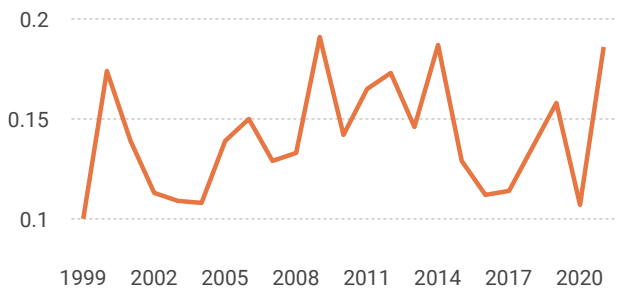

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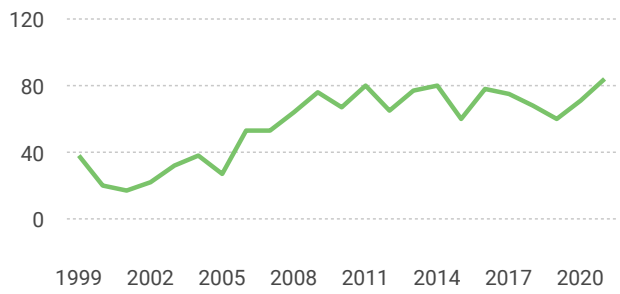

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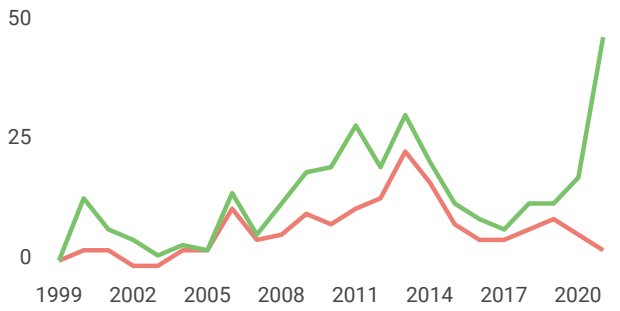

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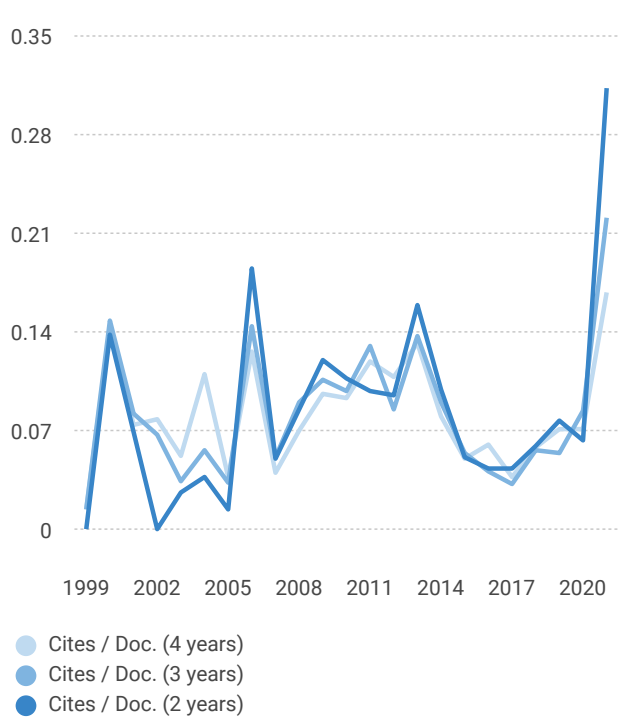

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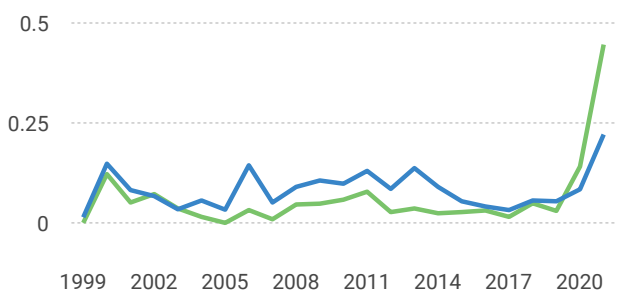

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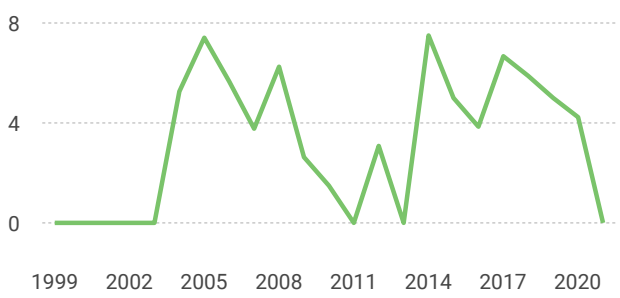

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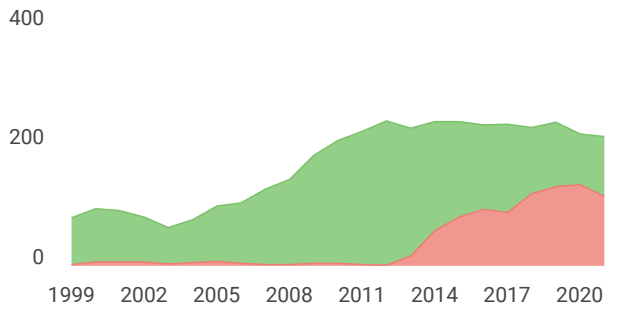

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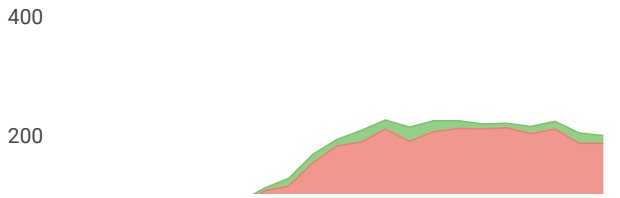

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European Journal of Pediatric Dermatology

Scopus coverage years: from 1994 to 2021

Publisher: Dermatologia Pediatrica

ISSN: 1122-7672

Subject area: Medicine: Pediatrics, Perinatology and Child Health | Medicine: Dermatology

Source type: Journal

CiteScore 2021
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Category	Rank	Percentile
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