

Serum vitamin D in Indian children with vernal keratoconjunctivitis

Priyanka Singh, Smriti Dabas, Anuj Mehta, L. Sarkar

Department of Ophthalmology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi 110029, India

Correspondence to: Priyanka Singh. Department of Ophthalmology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi 110029, India. doc.priyankasingh@gmail.com

Received: 2020-01-14 Accepted: 2021-12-09

Abstract

• **AIM:** To determine the vitamin D levels in vernal keratoconjunctivitis (VKC) patients in Indian population.

• **METHODS:** A total of 30 non-atopic healthy children and 30 children having VKC were included in the study. The serum vitamin D levels and the time spent outside was compared between the two groups ($P < 0.05$).

• **RESULTS:** The mean serum vitamin D level in the VKC group was significantly lower (mean 19.17 ± 10.76 ng/mL) compared to the control group (mean 31.19 ± 9.09 ng/mL) ($P = 0.0003$). The vitamin D levels were found to be deficient ($10-20$ ng/mL) in 43.33%, whereas severe deficiency (< 10 ng/mL) was found in 20% of the VKC patients. The deficiency of vitamin D correlated with the level of severity of VKC ($P < 0.02$). The time spent outside in the VKC group was 1.07 ± 0.76 h, whereas in the healthy subjects it was 2.08 ± 0.72 h ($P < 0.0001$), and it showed a significant correlation with the serum 25 (OH) D3 levels ($r = 0.478$, $P < 0.001$).

• **CONCLUSION:** The study shows that children with VKC had a significantly lower serum vitamin D levels as compared to the healthy children which correlated with time spent outside. The severity of VKC also correlated with the severity of vitamin D deficiency which suggests that vitamin D plays an important role in maintaining ocular surface health.

• **KEYWORDS:** vernal keratoconjunctivitis; vitamin D deficiency; children

DOI:10.18240/ier.2021.02.01

Citation: Singh P, Dabas S, Mehta A, Sarkar L. Serum vitamin D in Indian children with vernal keratoconjunctivitis. *Int Eye Res* 2021;2(2):63-68

INTRODUCTION

Vernal Keratoconjunctivitis (VKC) is a chronic, sight threatening, allergic disease of the eye, affecting mainly young males. It is characterized by a seasonally recurring, bilateral inflammation of the conjunctiva and the cornea^[1-5]. Vitamin D deficiency has been implicated in the pathophysiology of VKC along with endocrine, neurogenic, genetic, environmental and socioeconomic risk factors^[6-7].

There are 4 types of allergic conjunctivitis which have different types of pathological reactions: simple allergic conjunctivitis (seasonal, acute and perennial) is type I hypersensitivity reaction. Atopic and giant papillary conjunctivitis is combined type I and IV hypersensitivity reaction. Vernal Keratoconjunctivitis resembles Type I (Ig E dependent) and IV (Ig E independent) hypersensitivity reaction^[8]. It shows the presence of eosinophils, mast cells, fibroblasts, T and B cells^[9] which plays an active role in its pathogenesis.

VKC commonly affects young boys, but both sexes can be involved. Onset is usually around 5 years of age, around 95% of the cases show regression during or soon after puberty^[3]. Though the usual presentation is during spring and summer seasons, perennial cases are also seen. Symptoms consist of intense itching, blurred vision, lacrimation, photophobia, blepharospasm and mucoid discharge. The disease may be classified as tarsal (primarily involving the upper tarsal conjunctiva), limbal, or mixed phenotype. Tarsal disease presents with conjunctival hyperaemia and flat-topped macro-papillae (< 1 mm) known as cobblestone papillae, or giant papillae (> 1 mm) on the superior tarsal conjunctiva, whereas the limbal disease presents with gelatinous aggregates of epithelial cells and eosinophils (Horner-Trantas dots) at the limbus; corneal involvement may result in punctate epithelial keratitis, epithelial macro-erosions, shield ulcer and plaque formation^[6].

Vitamin D is an important fat-soluble prohormone, having numerous functions in the body, including the regulation of calcium and phosphate metabolism^[10-11]. The effects are mediated via a receptor called as VDR. VDR has also been discovered in tissues and cells which are not involved in calcium homeostasis, such as on T cells, B cells, neutrophils, macrophages, and dendritic cells. It has been shown to have a role in the regulation of immunity, cells proliferation and

differentiation, angiogenesis and apoptosis^[12-16]. Vitamin D has been shown to suppress the allergic pathways by inhibiting the dendritic cells maturation, migration and reprogramming as tolerogenic phenotype, decreasing the interleukin-12 (IL-12), IL-23 and cytokine production, inducing IL-10 secreting regulatory T cells, and inhibiting Th1 and Th17 cells^[15-19].

Other effects such as prevention of cardiovascular, immune-related and infectious diseases have also been proposed^[20] and many studies have suggested a correlation between vitamin D levels and immuno-mediated and atopic diseases^[21-23].

Although many researchers evaluated the relationship between vitamin D and allergic disease providing conflicting results, its levels in patients with VKC have been assessed in only two studies: Zicari et al conducted a randomised prospective study on 110 children in Italy demonstrated that children affected by VKC have lower vitamin D levels when compared to healthy controls and highlights a significant correlation between its levels and disease severity.

Another study conducted in Turkey by Bozkurt et al on 62 non-atopic healthy children and 29 VKC children showed that children with VKC in the studied population had lower serum vitamin D levels compared with age and sex matched healthy children and half of these patients had severe vitamin D deficiency, which seems to develop secondary to decreased sun exposure^[6].

A recent study in Italy published in 2019 with 242 children with active VKC showed that ocular treatment with immunomodulator eye drops (cyclosporine or tacrolimus) could allow for improvement in serum vitamin D levels^[24]. Few other studies have mentioned vitamin D deficiency as risk for developing seasonal allergic conjunctivitis, childhood asthma and systemic allergies^[25-26].

In India, no such study has been done so far which correlates the association between VKC and serum vitamin D levels. The present study is intended to investigate the serum vitamin D levels in children affected by vernal keratoconjunctivitis.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Ethical Committee (Institute Ethics Committee, V.M.M.C. and Safdarjung hospital, New Delhi, India, 110029) and informed consent was taken from the parents.

A total of 30 VKC children and 30 healthy children were included in this case control study from April 2108 to November 2018. Newly diagnosed VKC patients with a VKC score ≥ 7 , in the age group of 5-15 years, willing to follow up were included in the study. Individuals having any prior ocular surgery, history of asthma or systemic allergy, any other ocular disease except refractive errors, history of rheumatoid arthritis, cystic fibrosis, sarcoidosis, thyroid dysfunction, obesity and on any drugs like barbiturates, bisphosphonate, sulphasalazine,

omega 3 fatty acids, calcium, and vitamin D, were excluded from the study. Control subjects were chosen from healthy children with no history of atopy or any ocular diseases other than refractive errors.

A meticulous history taking was done to rule out the exclusion criteria. Patients were asked about the symptoms like itching, photophobia, tearing, foreign body and burning sensation. Patients were also asked about how many hours they spend outdoors on an average per day. Clinical examination was done by evaluation of anterior segment using slit lamp examination to look for conjunctival hyperaemia, tarsal and/or limbal papillae, giant papillae, punctate epitheliopathy, or shield ulcer in patients with VKC. Best corrected visual acuity determination, refraction, fundus examination, corneal topography was also done for all the patients.

Determination of VKC score: 1) Conjunctival hyperaemia; 2) Tarsal and/or limbal papillae; 3) Giant papillae; 4) Itching; 5) Photophobia; 6) Tearing; 7) Foreign body sensation; 8) Burning sensation. Each variable was graded as follows: 0: absent, 1: mild, 2: moderate, 3: severe.

In the score other two parameters were included: Duration of symptoms (0 if <1 years; 1 if >1 years and 2 if >2 years); Corneal involvement (0: absent, 1: de-epithelialization, 2: keratitis, 3; ulcer). Children with a total score ≥ 7 were included in the study^[27].

After the diagnosis, patients were divided into four grades of VKC^[28] according to presenting symptoms and signs, *i.e.* mild, moderate, severe and blinding.

Fasting venous blood samples from the antecubital vein was obtained in the morning after overnight fasting and put into EDTA anticoagulated tubes. Serum vitamin D level was analysed by chemiluminescence assay in the department of pathology.

In this study serum 25 (OH) D3 levels were measured, as it has a significantly more stable hormone-receptor complex than 25 (OH) D2, and has been shown to provide levels of vitamin D in circulation more effectively. Detection limit for 25(OH) D3 is 5 ng/mL. The Endocrine Society Clinical Practice Guidelines suggested considering deficiency if 25 (OH) D is below 20 ng/mL (50 nmol/L), while levels <10 ng/mL are accepted as severe vitamin D deficiency^[29-30]. The children having vitamin D deficiency were referred to the department of paediatrics for treatment and vitamin D supplementation.

Statistical Analysis Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean $\pm$ SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non-parametric test was used.

Statistical tests were applied as follows: Quantitative variables were compared using Independent T test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups and ANOVA test for correlation of vitamin D level with severity. Qualitative variables were correlated using Chi-Square test.

Pearson correlation coefficient was used to assess the association of severity of VKC with vitamin D deficiency severity. A *P* value of <0.05 was considered statistically significant.

RESULTS

There were 30 patients in the VKC group (25 males, 83.33%; 5 females, 16.67%) and 30 patients in the control group (21 males, 70%; 9 females, 30%), with no statistical differences (*P*=0.22).

The mean ages of the VKC group was 8.27±2.79 years and the control group was 8.9±2.52 years, with no statistical difference (*P*=0.922).

The best corrected visual acuity (BCVA) ranged from 6/6-6/24, with 53.33% having 6/6, 40% having 6/9, 3.33% having 6/12 and 3.33% having 6/24 in VKC group. All the controls had a BCVA of 6/6.

The mean spherical refraction in the VKC group was -0.69±1.01, and in the control group was -0.68±1.02. The mean maximum (K1) and minimum (K2) topographic keratometric values in the VKC group were 43.83±0.78 D and 43.42±0.79 D respectively, and in the control group were 43.9±0.82 D (K1) and 43.57±0.87 D (K2). VKC was tarsal in 13 subjects (43.33%), limbal in 3 subjects (10%) and mixed in 14 subjects (46.67%). The severity of VKC was mild in 1 (3.33%), moderate in 19 (63.33%), severe in 9 (30.0%) and blinding in 1 (3.33%) patient.

The mean serum 25 (OH) D3 level in the VKC group was significantly lower (mean 19.17±10.76 ng/mL, median 17.05 ng/mL, range 5-53.4 ng/mL) as compared to the control group (mean 31.19± 9.09 ng/mL, median 32.15 ng/mL, range 12-49.2 ng/mL) (*P*=0.0003).

The vitamin D levels were found to be sufficient (>30 ng/mL) in 13.33%, in-sufficient (21-29 ng/mL) in 23.33%, deficient (10-20 ng/mL) in 43.33% and severe deficiency (<10 ng/mL) was found in 20% of the VKC patients. In the control group, the vitamin D levels were sufficient (>30 ng/mL) in 60%, in-sufficient (21-29 ng/mL) in 26.67%, deficient (10-20 ng/mL) in 10% and severe deficiency (<10 ng/mL) was found in 3.33% of controls (Figure 1).

According to severity of the disease, the mean vitamin D level in mild VKC was 30 ng/mL, moderate: 21.06±11.61 ng/mL, severe: 13.92±7.87 ng/mL and in blinding: 19.5 ng/mL (*P*=0.157) (Figure 2). The correlation of deficiency of vitamin D with the increased levels of severity of VKC was statistically significant (*P*<0.02).

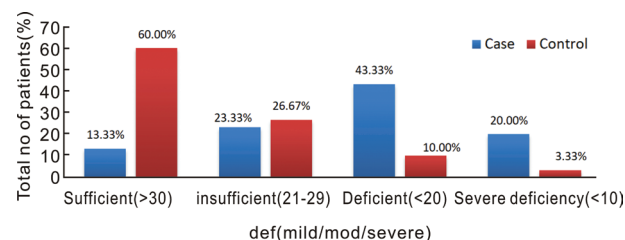


Figure 1 Chart depicting the distribution of serum vitamin D levels in cases and controls.

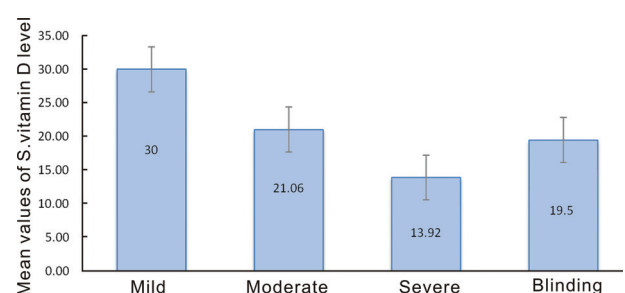


Figure 2 Chart depicting the mean vitamin D levels according to severity of VKC.

The VKC score and the serum vitamin D levels did not show a statistically significant correlation (correlation coefficient -0.342, *P*=0.0646) but was clinically significant.

The time spent outside in the VKC group was 1.07±0.76h, with a median of 1h and range 0-3h, whereas in the healthy subjects it was 2.08±0.72h, median 2h and range 1-4h, which was statistically significant (*P*<0.0001).

The time spent outside correlated with the vitamin D levels, with higher Vitamin D levels in children who spent more time outside (*r*=0.478, *P*<0.001).

DISCUSSION

Vernal keratoconjunctivitis is one of the disorders in the vast spectrum of allergic eye diseases. Various studies have been done evaluating the risk factors for seasonal allergic conjunctivitis. It has been suggested that Vitamin D deficiency may play a role in the onset of allergic diseases^[31]. In this study, a total of 60 subjects were taken, 30 in the VKC group and 30 in the control group (non-VKC group). There was no statistical difference in the age and sex distribution between the two groups.

Of the 30 patients, majority had mixed VKC (46.47%), followed by tarsal (43.33%) and limbal (10%). 19 patients (63.3%) had a moderate (intermittent) disease and 9 (30%) had a severe disease. One patient had a mild disease and one had blinding disease. The child with mild disease was included in the study due to the severity of his presenting symptoms, which resulted in his severity score being 7. We encountered 2 patients with chronic moderate VKC but as they were already on topical medications, they were not included in the study. The mean VKC score was 10.33±2.62 (median 10,

range 7-15). Each patient was treated based on the severity of VKC with mild patients receiving topical lubricants, mast cell stabilizers/ antihistamines, moderate severity patients receiving low dose steroids in addition, severe patients receiving potent topical steroids with tacrolimus/cyclosporine and those with blinding disease receiving management of shield ulcer, in addition to the above^[28]. In a study by Bozkurt *et al*^[6], 51.7% patients had tarsal VKC and 48.3% had mixed VKC.

The best corrected visual acuity ranged from 6/6-6/24 (median: 6/6). The visual acuity of 6/24 was found in the child having blinding disease, which was attributed to the corneal ulceration that was present. The patients having BCVA 6/9 (40%) and 6/12 (3.33%) had varying degrees of superficial punctate erosions and macro-erosions of the cornea, along with intense photophobia. All the children in the control group had a BCVA of 6/6. The mean spherical refraction in the VKC group was -0.69 ± 1.01 , and in the control group was 0.68 ± 1.02 .

As per the Endocrine Society Clinical Practice Guidelines, vitamin D deficiency is considered if serum 25 (OH) D is below 20 ng/mL (50 nmol/L), while levels <10 ng/mL are accepted as severe vitamin D deficiency^[29-30]. Insufficiency is considered at levels 21-29 ng/mL. In our study there was a significant difference in the mean serum 25 (OH) D3 levels between the two groups, with the VKC group having a mean level of 19.17 ± 10.76 ng/mL and controls having a mean of 31.19 ± 9.09 ng/mL. The vitamin D levels were found to be deficient (43.33%) in majority of the VKC patients. The vitamin D deficiency (sufficient, insufficient, deficiency and severe deficiency) correlated with the severity of VKC (mild, moderate, severe and blinding), with patients having increased severity of VKC having lower vitamin D levels ($P=0.0216$, correlation coefficient: 0.418).

At least two studies have reported an association between VKC and vitamin D levels^[6,32]. In a study by Zicari *et al*^[32], lower levels of vitamin D were found in the VKC group when compared to controls (20.4 ± 6.7 ng/mL vs 25.2 ± 6.5 ng/mL). They also found an increase in vitamin D levels after therapy with cyclosporine eye drops 1% although this increase was lower than that of healthy controls.

Another study done by Bozkurt *et al*^[6], mean 25 (OH) D3 level in the VKC group was 11.02 ± 5.16 ng/mL, and in the controls was 15.99 ± 7.36 ng/mL which was significantly lower. They found severe deficiency (<10 ng/mL) in 48.3% and levels <20 ng/mL in 93.1% of the VKC children. This could be attributed to the fact the patients with VKC have intense photophobia and most often avoid direct exposure to sunlight, wear sunglasses or hats to decrease the exposure. Also, people having increased pigmentation, such as those belonging to India, Middle East or Africa, are more predisposed to deficiency of vitamin D.

In our study, there was a significant reduction in the time spent

outside in the VKC group (1.07 ± 0.76 h, with a median of 1h) as compared to the controls (2.08 ± 0.72 h, with a median of 2h). The time spent outside correlated with the vitamin D levels, with higher vitamin D levels in children who spent more time outside ($r=0.478$, $P<0.001$), but it did not show a significant correlation with the VKC score (correlation coefficient -0.047 , $P=0.8038$). Bozkurt *et al*^[6] also found a significant difference in the time spent outdoors between the VKC group (229.5 ± 101.2 min) and the controls (160 ± 65.9 min), and they found a significant correlation between 25 (OH) D3 levels and the time spent outside.

The most important source of vitamin D is the sun, UV B radiation from the sunlight helps in converting the naturally occurring 7-Dehydrocholesterol (present in the skin) to cholecalciferol (Vit D3). Dietary products like fish and meat are also minor sources of vitamin D3. Cholecalciferol is a biologically inactive form, and is converted to the biologically active 1, 25 dihydroxycholecalciferol by two reactions, firstly 25-hydroxylation in the liver, followed by 1 alpha hydroxylation in the kidneys. The synthesis of Vit D3 in the skin depends upon the amount of UV B radiation absorbed, which further depends upon the geographical latitude, season, type of clothing worn, use of sunscreen and the amount of skin pigmentation^[33-35].

Several recent studies have found the presence of vitamin D target cells and vitamin D hydroxylase activity (*i.e.* the ability to convert 25 hydroxycholecalciferol to 1, 25 dihydroxycholecalciferol) in numerous ocular cells, which suggests that the ocular tissue may have a role in activation and regulation of Vitamin D metabolism^[36-39]. In the eye, administration of vitamin D has been found to decrease retinal inflammation and neovascularization, decrease the macrophage number and dose dependent inhibition of capillary endothelial cell morphogenesis^[40-41].

Vitamin D has also been shown to inhibit the expression of IgE on B cells and its deficiency is associated with eosinophilia, increased serum IgE levels and sensitization to allergen^[42-45]. In a few studies on asthma patients, low vitamin D levels correlated with the elevated serum IgE levels, severity of asthma and the number of eosinophils, and supplementation with Vitamin D was shown to increase the steroid responsiveness in those who were steroid resistant^[43-50]. In our study the mean VKC score was 10.33 ± 2.62 (median 10, range: 7-15) and it showed significant correlation with severity of VKC ($P=0.001$). The VKC score and the serum vitamin D levels did not show a statistically significant correlation (correlation coefficient -0.342 , $P=0.0646$).

In the studied population, children with VKC had a significantly lower serum vitamin D levels as compared to the healthy children, which correlated with time spent outside.

Positive correlation was found between the increasing severity of disease and the severity of vitamin D deficiency. Majority of the patients had severe vitamin D deficiency which seems to develop secondary to decreased sunlight exposure.

The possibility of vitamin D deficiency should be kept in mind, and appropriately managed in VKC patients, as it may contribute to the pathophysiology of the disease. However, further studies are warranted to establish vitamin D deficiency as a risk factor in VKC and understand the course of remission of the disease after giving vitamin D supplementation.

ACKNOWLEDGEMENTS

Conflicts of Interest: Singh P, None; Dabas S, None; Mehta A, None; Sarkar L, None.

Peer Review File Available at: http://ier.ijo.cn/gjykier/ch/reader/download_attache_file.aspx?seq_id=20210625162007001&flag=1&journal_id=gjykier&year_id=2021&issue=2

REFERENCES

- Vichyanond P, Pacharn P, Pleyer U, Leonardi A. Vernal keratoconjunctivitis: a severe allergic eye disease with remodeling changes. *Pediatr Allergy Immunol* 2014;25(4):314-322.
- Leonardi A, Busca F, Motterle L, Cavarzeran F, Fregona IA, Plebani M, Secchi AG. Case series of 406 vernal keratoconjunctivitis patients: a demographic and epidemiological study. *Acta Ophthalmol Scand* 2006;84(3):406-410.
- Bonini S, Bonini S, Lambiase A, Marchi S, Pasqualetti P, Zuccaro O, Rama P, Magrini L, Juhas T, Bucci MG. Vernal keratoconjunctivitis revisited: a case series of 195 patients with long-term followup. *Ophthalmology* 2000;107(6):1157-1163.
- Pucci N, Novembre E, Lombardi E, Cianferoni A, Bernardini R, Massai C, Caputo R, Campa L, Vierucci A. Atopy and serum eosinophil cationic protein in 110 white children with vernal keratoconjunctivitis: differences between tarsal and limbal forms. *Clin Exp Allergy* 2003;33(3):325-330.
- Lambiase A, Minichiotti S, Leonardi A, Secchi AG, Rolando M, Calabria G, Orsoni J, Zola E, Ferreri G, Aragona P, Reibaldi A, Chisari G, Bonini S. Prospective, multicenter demographic and epidemiological study on vernal keratoconjunctivitis: a glimpse of ocular surface in Italian population. *Ophthalmic Epidemiol* 2009;16(1):38-41.
- Bozkurt B, Artac H, Ozdemir H, Ünü A, Bozkurt MK, Irkeç M. Serum vitamin D levels in children with vernal keratoconjunctivitis. *Ocul Immunol Inflamm* 2018;26(3):435-439.
- Spadavecchia L, Fanelli P, Tesse R, Brunetti L, Cardinale F, Bellizzi M, Rizzo G, Procoli U, Bellizzi G, Armenio L. Efficacy of 1.25% and 1% topical cyclosporine in the treatment of severe vernal keratoconjunctivitis in childhood. *Pediatr Allergy Immunol* 2006;17(7):527-532.
- Zicari AM, Zicari A, Nebbioso M, Mari E, Celani C, Lollobrigida V, Merzelli AC, Occasi F, Duse M. High-mobility group box-1 (HMGB-1) and serum soluble receptor for advanced glycation end products (sRAGE) in children affected by vernal keratoconjunctivitis. *Pediatr Allergy Immunol* 2014;25(1):57-63.
- Baah S, Kinzer EE. Allergic conjunctivitis. *StatPearls. Treasure Island (FL)* 2019;1.
- Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004;80(6):1678S-1688S.
- Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, Lichtenstein A, Patel K, Raman G, Tatsioni A, Terasawa T, Trikalinos TA. Vitamin D and calcium: a systematic review of health outcomes. *Evid Rep Technol Assess (Full Rep)* 2009(183):1-420.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357(3):266-281.
- Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol* 2010;10(4):482-496.
- Hewison M. Vitamin D and innate and adaptive immunity. *Vitam Horm* 2011;86:23-62.
- Bikle DD. Vitamin D and immune function: understanding common pathways. *Curr Osteoporos Rep* 2009;7(2):58-63.
- May E, Asadullah K, Zügel U. Immunoregulation through 1, 25-dihydroxyvitamin D3 and its analogs. *Curr Drug Targets Inflamm Allergy* 2004;3(4):377-393.
- Bantz SK, Zhu Z, Zheng T. The role of vitamin D in pediatric asthma. *Ann Pediatr Child Health* 2015;3(1):1032.
- Hamzaoui A, Berraies A, Hamdi B, Kaabachi W, Ammar J, Hamzaoui K. Vitamin D reduces the differentiation and expansion of Th17 cells in young asthmatic children. *Immunobiology* 2014;219(11):873-879.
- Nanzer AM, Chambers ES, Ryanna K, Richards DF, Black C, Timms PM, Martineau AR, Griffiths CJ, Corrigan CJ, Hawrylowicz CM. Enhanced production of IL-17A in patients with severe asthma is inhibited by 1 α , 25-dihydroxyvitamin D3 in a glucocorticoid-independent fashion. *J Allergy Clin Immunol* 2013;132(15):297-304.
- Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. *Atherosclerosis* 2009;205(1): 255-260.
- Christakos S, DeLuca HF. Minireview: Vitamin D: is there a role in extraskeletal health? *Endocrinology* 2011;152(8):2930-2936.
- Dogru M, Kirmizibekmez H, Yesiltepe Mutlu RG, Aktas A, Ozturkmen S. Clinical effects of vitamin D in children with asthma. *Int Arch Allergy Immunol* 2014;164(4):319-325.
- McFadden JP, Thyssen JP, Basketter DA, Puangpet P, Kimber I. T helper cell 2 immune skewing in pregnancy/early life: chemical exposure and the development of atopic disease and allergy. *Br J Dermatol* 2015;172(3):584-591.
- Ghiglioni DG, Bruschi G, Gandini S, Osnaghi S, Peroni D, Marchisio P. Vitamin D serum levels in children with vernal keratoconjunctivitis and disease control. *Int J Immunopathol Pharmacol* 2019;33: 2058738419833468.

- 25 Doğan Ü, Ağca S. Investigation of possible risk factors in the development of seasonal allergic conjunctivitis. *Int J Ophthalmol* 2018;11(9):1508-1513.
- 26 Litonjua AA. Vitamin D deficiency as a risk factor for childhood allergic disease and asthma. *Curr Opin Allergy Clin Immunol* 2012;12(2):179-185.
- 27 Bonini S, Sacchetti M, Mantelli F, Lambiase A. Clinical grading of vernal keratoconjunctivitis. *Curr Opin Allergy Clin Immunol* 2007;7(5):436-441.
- 28 Gokhale NS. Systematic approach to managing vernal keratoconjunctivitis in clinical practice: Severity grading system and a treatment algorithm. *Indian J Ophthalmol* 2016;64(2):145-148.
- 29 Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911-1930.
- 30 Braegger C, Campoy C, Colomb V, Decsi T, Domellof M, Fewtrell M, Hojsak I, Mihatsch W, Molgaard C, Shamir R, Turck D, van Goudoever J, ESPGHAN Committee on Nutrition. Vitamin D in the healthy paediatric population. *J Pediatr Gastroenterol Nutr* 2013;56(6):692-701.
- 31 Frieri M, Valluri A. Vitamin D deficiency as a risk factor for allergic disorders and immune mechanisms. *Allergy Asthma Proc* 2011;32(6):438-444.
- 32 Zicari AM, Cafarotti A, Occasi F, Lollobrigida V, Nebbioso M, Pecorella I, De Castro G, Spalice A, Loffredo L, Maria PV, Duse M. Vitamin D levels in children affected by vernal keratoconjunctivitis. *Curr Med Res Opin* 2017;33(2): 269-274.
- 33 Gilchrist BA. Sun exposure and vitamin D sufficiency. *Am J Clin Nutr* 2008;88(2):570S-577S.
- 34 Engelsen O, Brustad M, Aksnes L, Lund E. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochem Photobiol* 2005;81(6):1287-1290.
- 35 Basit S. Vitamin D in health and disease: a literature review. *Br J Biomed Sci* 2013;70(4):161-172.
- 36 Verstappen A, Parmentier M, Chirnoaga M, Lawson DEM, Pasteels JL, Pochet R. Vitamin D-dependent calcium binding protein immunoreactivity in human retina. *Ophthalmic Res* 1986;18(4):209-214.
- 37 Alsalem JA, Patel D, Susarla R, Coca-Prados M, Bland R, Walker EA, Rauz S, Wallace GR. Characterization of vitamin D production by human ocular barrier cells. *Invest Ophthalmol Vis Sci* 2014;55(4):2140-2147.
- 38 Yin ZH, Pintea V, Lin YP, Himmock BD, Watsky MA. Vitamin D enhances corneal epithelial barrier function. *Invest Ophthalmol Vis Sci* 2011;52(10):7359-7364.
- 39 Reins RY, McDermott AM. Vitamin D: Implications for ocular disease and therapeutic potential. *Exp Eye Res* 2015;134:101-110.
- 40 Dickson IR, Maher PM. The influence of vitamin D metabolites on collagen synthesis by chick cartilage in organ culture. *J Endocrinol* 1985;105(1):79-85.
- 41 Albert DM, Scheef EA, Wang SJ, Mehraein F, Darjatmoko SR, Sorenson CM, Sheibani N. Calcitriol is a potent inhibitor of retinal neovascularization. *Invest Ophthalmol Vis Sci* 2007;48(5):2327-2334.
- 42 Vasilidou JE, Lui S, Walker SA, Chohan V, Xystrakis E, Bush A, Hawrylowicz CM, Saglani S, Lloyd CM. Vitamin D deficiency induces Th2 skewing and eosinophilia in neonatal allergic airways disease. *Allergy* 2014;69(10):1380-1389.
- 43 Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, Laskey D, Sylvia JS, Hollis BW, Weiss ST, Litonjua AA. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179(9):765-771.
- 44 Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D deficiency as a strong predictor of asthma in children. *Int Arch Allergy Immunol* 2012;157(2):168-175.
- 45 Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the national health and nutrition examination survey 2005-2006. *J Allergy Clin Immunol* 2011;127(5):1195-1202.
- 46 Goleva E, Searing DA, Jackson LP, Richers BN, Leung DYM. Steroid requirements and immune associations with vitamin D are stronger in children than adults with asthma. *J Allergy Clin Immunol* 2012;129(5):1243-1251.
- 47 Brehm JM, Acosta-Pérez E, Klei L, Roeder K, Barmada M, Boutaoui N, Forno E, Kelly R, Paul K, Sylvia J, Litonjua AA, Cabana M, Alvarez M, Colón-Semidey A, Canino G, Celedón JC. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2012;186(2):140-146.
- 48 Beigelman A, Zeiger RS, Mauger D, Strunk RC, Jackson DJ, Martinez FD, Morgan WJ, Covar R, Szeffler SJ, Taussig LM, Bacharier LB. The association between vitamin D status and the rate of exacerbations requiring oral corticosteroids in preschool children with recurrent wheezing. *J Allergy Clin Immunol* 2014;133(5):1489-1492.e3.
- 49 Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010;125(5):995-1000.
- 50 Majak P, Rychlik B, Stelmach I. The effect of oral steroids with and without vitamin D3 on early efficacy of immunotherapy in asthmatic children. *Clin Exp Allergy* 2009;39(12):1830-1841.