JMS 2022;5(1):11-19
UDC:616.248-085.356:577.161.2]-053.2
DOI:10.55302/JMS2251011m

VITAMIN D AND ASTHMA IN CHILDHOOD

Vesna Micevska[1], Tatjana Jakjovska Maretti[2], Ilija Kirovski[1], Olivera Jordanova[1]
[1] University Children's Hospital, Faculty of Medicine, Ss.Cyril and Methodius University in Skopje, R. North Macedonia
[2] Institute for Respiratory Diseases in Children–Kozle, Skopje, Faculty of Medicine, Ss.Cyril and Methodius University in Skopje, R. North Macedonia

Abstract

Asthma is the most common chronic disease in children and one of the most common chronic disease in adults. It is defined as a chronic inflammatory disease of the airways, caused by the influence of many different cells and cellular elements (eosinophiles, mast cells and T-lymphocytes).

The symptoms usually appear in childhood, and are associated with atopy and sometimes persist into adulthood. In the last decades, there has been an increased scientific interest in the research of vitamin D and its immunomodulatory effects, as well as the association of vitamin D deficiency and allergic disease development, especially asthma in childhood.

The study was prospective and was performed at the Pulmonology Department at the University Children's Hospital in Skopje on 120 oupatient children between the ages of 2-14 years. The examined group consisted of 60 children with asthma, and the control group consisted of 60 healthy children. Serum levels of vitamin D was measured in both groups.

The examined group was treated with inhaled corticosteroids, and the participants with vitamin D deficiency were also treated with oral vitamin D.

The results from the pulmonary function testing for bronchial hyperreactivity and the episodes of bronchoobstructions after 6 months follow-up were compared to the one at the beginning of the asthma therapy.

Mean serum vitamin D levels in the asthmatic children were 24.57 ± 10.0, and for the healthy children they were 28.74 ± 9.5, with a statistical significant difference of p=0,0021. 46,67% of the asthmatic children had vitamin D deficiency, versus 15% of the healthy children. In our study, vitamin D supplementation had no significant impact in asthma control efficacy.

Children with asthma have lower serum levels of vitamin D, versus healthy individuals. Although vitamin D supplementation in asthmatic children hasn't improved the asthma treatment efficacy, the data shows positive therapeutic effects in patients with more severe symptoms.

Keywords: asthma, vitamin D deficiency, children.

Introduction

Asthma is the most common chronic disease in children and one of the most common in adults (it is considered that about 300 million people have asthma worldwide) [1,2].

It is defined as a chronic inflammatory disease of the airways, caused by the influence of many different cells and cellular elements (eosinophiles, mast cells and T-lymphocytes). Asthma is manifested with repetitive wheezing episodes, cough, dyspnea and chest pain [3].

The symptoms usually appear in childhood, and are associated with atopy, and sometimes persist in adulthood. Asthma has a complex etiology. Genetic basis of the diseases can go from 35 to 95% [4]. Apart from genetics, different extrinsic factors can control its expression, such as: viral infections, early exposure to allergens, passive smoking, smoking during pregnancy, industrial and traffic airpollution [4, 5].

The development of inflammation plays the main role in the pathogenesis, which is presented by the accumulation of eosinophiles, mast cells and T-helper lymphocytes that release cytokines. Dendritic

cells are the main antigen presenting cells that activate T-lymphocytes and enhance IgE production and development of an allergic reaction [6].

Asthma diagnostic procedures in children covers: patient history, physical examination and investigations (serum IgE levels, allergen sensitization testing and pulmonary function tests) [3,5,7].

The therapeutic approach to asthma is based by the severity of the disease, the frequency of excacerbations and pulmonary function variability [3, 4].

The first line of therapy in asthmatic children is the administration of anti-inflammatory drugs, such as inhaled corticosteroids. They decrease the bronchial hyperresponsiveness, the need for treatment of acute excacerbation episodes and improve the quality of life of the patients [3,5,7].

Vitamin D (VD). In the last decades, there has been an increase in scientific interest in the research of VD and its immunomodulatory effects, as well as the association of VD deficiency and allergic disease development, especially childhood asthma. VD is a hormone with an impact in different immunological activities, apart from the main known role in calcium homeostasis [8,9].

There are two main sources of VD in the human body: ultraviolet sunlight induced skin production and several dietary supplements [8, 10-13]. VD action in human tissues is made through vitamin D receptors (VDR). They are found in almost all human cells and tissues [14, 15].

VD is well known for its immunomodulatory effects, its VDR are very common in T-lymphocytes, macrophages, and especially in immature thymic immune cells and mature T-lymphocites [16, 17].

Many epidemiologic studies suggest that VD deficiency is associated with an increased risk for wheezing, respiratory tract infections and asthma development [20-22]. VD controls activities of macrophages, dendritic cells and many TLR (toll-like receptors) mediated processes in neutrophilic cells, decreases the dendritic cells activities by slowing down and inhibition of their maturation, antigen presentation and cytokine production (interleukins IL-12, IL-23) [18, 19, 23, 26].

The main mechanism of the allergic response to asthma symptoms is determined by the Th2 immune response activation, that causes secretion of IgE and inflammatory cytokines, which leads to inflammation and bronchial hyperreactivity (BHR) of the airways [24, 26]. Moreover, VD affects airway remodelling [25].

Literature data proves the benefit of VD supplementation in the treatment of allergic diseases. Therapeutic doses of VD are recommended for people with VD deficiency (VD serum level < 20ng/ml). Optimal levels of VD are considered from 30 to 50 ng/ml. The maximum recommended doses for VD oral intake range from 1000 to 4000 I.U., depending on the age and VD serum levels [27].

Aim

To measure and compare VD serum levels in healthy and asthmatic children, to present the distribution of common allergens in the examined group, and determine the role of VD supplementation in children with VD deficiency by comparing the results to the rest of the patients after 6 months asthma therapy.

Methods

The study was prospective and was performed at the Pulmonology Department at the University Children's Hospital in Skopje on 120 oupatient children between the age of 2-14 in a 3-year period. The examined group (EG) consisted of 60 children with asthma, and the control group (CG) consisted of 60 healthy children. Both groups were additionally divided into two age subgroups, due to the diagnostic protocols for asthma in childhood [7].

Serum levels of VD were measured in both groups. The results from both groups were compared. The examined group had passed the diagnostic procedures from standardized asthma protocol from GINA (Global INitiative for Asthma). For our study, we analyzed the VD serum levels, results from allergic sensitization tests and the pulmonary function testing for BHR (bronchodilatatory testing–BDT), which was performed for the subgroup of asthmatic children at the age of 6 and above.

All asthmatic children were treated with inhaled corticosteroids.

Additionally, patients with VD deficiency underwent treatment with oral VD in the recommended doses. All patients were followed up after 6 months, for episode frequency of acute bronchoobstuctions (BO), and control testing of pulmonary function (BDT) in the older subgroup.

The results from the control examination of the patients with VD treatment, and the other without, were compared.

Statistical analysis was prepared by SPSS 23.0, using parametric and non-parametric tests and correlation testing. The statistical significance was defined at level p<0,05.

Results

Our analisys was performed on 120 children divided into two groups (EG and CG), each consisted of 60 children aged between 2-14 years.We presented the sex distribution and VD levels for both groups of children. Table 1. shows that although there was a male predominance in both groups, it still made no statistical significant difference in sex distribution.

Table 1. Sex distribution of the participants. X^2 (Pearson Chi-square)

Sex	Groups			**p-level**
	n	**EG** n(%)	**CG** n(%)	
Female	48	22 (36.67)	26 (43.33)	X^2=0.56 p=0.46 ns
Male	72	38 (63.33)	34 (56.67)	

Table 2. and Image 2. show the mean VD serum levels in both groups.

The difference of 4,17 ng/ml was statistically significant (p=0,021). That proves that children with asthma have significantly lower serum levels of VD, compared to the healthy children (non-asthmatic children).

Table 2. Level distribution of serum VD

Vitamin D (ng/ml) Statistic parameter	Groups	
	EG	**CG**
mean±SD	24.57 ± 10.0	28.74 ± 9.5
min – max	3.7 – 48.4	9.2 – 60.4
p-level	t=2.34*p=0.021 sig	

t(Student t-test); *p<0.05

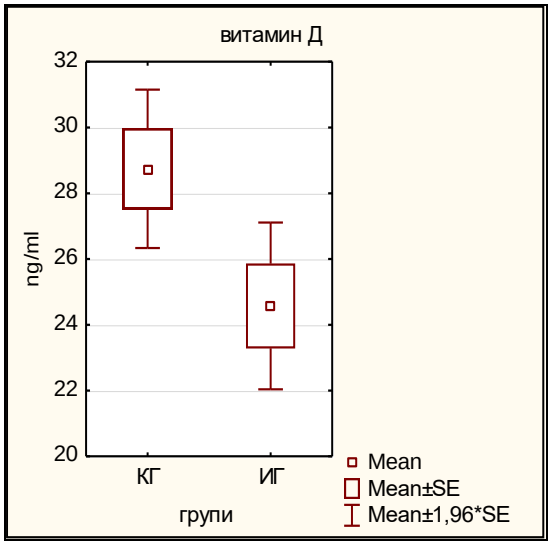

Image 2. Level distribution of serum VD.

Moreover, we divided the groups due to the serum concentration of VD in children with: deficient(<20 ng/ml), insufficient (20-30ng/ml) and sufficient(>30 ng/ml) VD levels.

We found a statistically significant difference in EG and CG regarding the distribution of all three levels (p=0.00028) (Table 3.).

Table 3. Distribution of VD serum levels.

Vitamin D ng/ml	Groups			**p-level**
	n	**EG** n(%)	**CG** n(%)	
20-30	40	12 (20)	28 (46.67)	X^2=16.4 ***p=0.00028 sig
<20	37	28 (46.67)	9 (15)	
>30	43	20 (33.33)	23 (38.33)	

X^2 (Pearson Chi-square); ***p<0.0001

Table 4. Presents the distribution of most common allergens in the EG.

EG	
Variable	**n(%)**
Grass pollens	
positive	12 (20)
negative	48 (80)
Tree pollens	
positive	6 (10)
negative	54 (90)
Dermatophagoides	
positive	29 (48.33)
negative	31 (51.67)
Mold	
positive	7 (11.67)
negative	53(88.33)
Cow's milk	
positive	3 (5)
negative	57 (95)
Eggs	
positive	4 (6.67)
negative	56 (93.33)

After 6 months of therapy, all 60 participants from the EG were followed up. We found that only 1 of the participants with normal VD levels, had an acute episode of bronchoobstruction (BO). It showed that no statistically significant difference appeared between the children with VD supplementation (VD deficient asthmatic children–VDD) and the ones without (VDN). Table 5.

Table 5. Number of episodes of bronchoobstruction.

BO episodes	**Vitamin D (**ng/ml)			**p-level**
	n	**VDN n(%)**	**VDD n(%)**	
none	27	26 (96.3)	1 (100)	**Fisher exact p=1.0 ns**
1 – 2 /month	1	1 (3.7)	0	

In the subgroup of asthmatic children aged 6-14 years (VDD2, VDN2), only one had positive bronchodilatatry testing (BDT) after 6 months of therapy. It was the one with normal VD serum levels. It shows no statistically significant difference in the results from BDT, due to VD serum levels of the participants. (Table 6).

Table 6. Results from control BDT.

BDT	**Vitamin D (**ng/ml)			**p-level**
	n	**VDN2 n(%)**	**VDD2 n(%)**	
Positive	1	1 (6.67)	0	Fisher exact p=0.23 ns
Negative	15	14 (93.33)	1 (100)	

Discussion

Due to the results in our study, we found that significantly more of the asthmatic children (participants from the EG) had serum VD deficiency, compared with the healthy children (children without asthma), 46,7% and 33,3% accordingly.

Our study proved that asthmatic children have significantly lower levels of serum VD than healthy children. Literature data from different and various studies, shows that asthmatic people (children and adults) have lower levels of serum VD no matter of their age, ethnic, cultural or geographic differences [28-37].

Our study showed that 75% of our asthmatic patients had a positive sensitization test for at least one of the analyzed specific allergens, and additionally 40% of them had a positive sensitization for more than one specific allergen.

The results from the allergen distribution showed predominance of sensitization for mite allergens (Dermatophagoides) over the distribution of grass and tree polens, mold, cow's milk and eggs.

Data from other studies show differences in distribution of specific allergens in children from different countries and nations. Most of them had the same predomination as in our study [38-39, 42-44], and some of them present predominance of grass polens over mite allergens [40].

In the group of our asthmatic participants, we also followed the effect of VD supplementation in patients with VD deficiency, comparing the results we found between them and the group of asthmatic children that had no VD deficiency.

According to our results, VD supplementation showed no significant difference in the asthma treatment efficacy. Data from literature differs due to the role of VD supplementation in asthmatic children and adults as well [45-54].

Conclusions

Asthmatic children have significantly lower levels of serum VD, compared to healthy individuals. The most common allergens in asthmatic children in our country are mite allergens (dermatophagoides) and grass pollens, and appear to be the same as for most of the regions worldwide.

Although vitamin D supplementation in asthmatic children hasn't improved asthma treatment efficacy, data shows positive therapeutic effects in patients with more severe symptoms.

References

1. Most Recent Asthma Data, CDC Centre for Disease Control and Prevention,February 2017.
2. Global Burden of disease due to asthma,The Global Asthma Report 2014.
3. Global Initiative for Asthma (GINA). 2021 GINA Global strategy for asthma. management and prevention 2021 update. 2021 GINA Main Report - Global Initiative for Asthma - GINA (ginasthma.org).
4. Ernst E, Fabio M. Paediatric Respiratory Medicine, 2013 (first edition), published by ERS;
5. Проф.д-р Дејан Докиќ и соработници. "Алергологија". 2017; Скопје
6. O'Byrne P, Persson CG, Church MK. Cellular and mediator mechanisms of the early phase response. Allergy. 2001;325-36.

7. Papadopoulos NG, et al. International consensus on (ICON) pediatric asthma. Allergy.2012; 67:976-997 doi: 10.1111/j.1398-9995.2012.02865.x.
8. Hoxha M, Zoto M, Deda L, VyshkaG.Vitamin D and Its Role as a Protective Factor in Allergy. International Scholarly Research Notices. 2014; Article ID 951946:7 pages
9. Giustina AD, Landi M, Bellini F, Bosoni M, Ferrante G, Onorari M, Travaglini A, Pingitore G, PassalacquaG.Vitamin D, allergies and asthma: focus on pediatric patients.World Allergy Organization Journal. 2014; 7:27.
10. Stelmach I, Jerzyńska J, Podlecka D. Immunomodulatory Effect of Vitamin D in Children with Allergic Diseases. 2017 Apr; DOI: 10.5772/65072
11. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004 Dec;80(6 Suppl):1689–96.
12. Kanis JA. Vitamin D metabolism and its clinical application. J Bone Joint Surg Br. 1982;64(5):542–560.
13. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014 Mar 20;21(3):319–329. doi: 10.1016/j.chembiol.2013.12.016. Epub 2014 Feb 13.
14. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev. 1998 Oct;78(4):1193–1231.
15. Kimmel-Jehan C, Darwish HM, Strugnell SA, Jehan F, Wiefling B, DeLuca HF. DNA bending is induced by binding of vitamin D receptor-retinoid X receptor heterodimers to vitamin D response elements. J Cell Biochem. 1999 Aug 1;74(2):220–228.
16. Von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat Immunol. 2010 Apr;11(4):344–349.
17. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J. 2001 Dec;15(14):2579–85.
18. Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 years of age. Am J Clin Nutr. 2007 Mar;85(3):788–795.
19. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S, Helms PJ, Seaton A, Weiss ST. Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr. 2007 Mar;85(3):853–859.
20. Jerzynska J, Stelmach W, Rychlik B, Lechańska J, Podlecka D, Stelmach I. The clinical effect of vitamin D supplementation combined with grass-specific sublingual immunotherapy in children with allergic rhinitis. Allergy Asthma Proc. 2016 Mar;37(2):105–114.
21. Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polańska K, Gromadzińska J, Wąsowicz W, Hanke W. Cord serum 25-hydroxyvitamin D correlates with early childhood viral-induced wheezing. Respir Med. 2015 Jan;109(1):38–43.
22. Majak P, Jerzyńska J, Smejda K, Stelmach I, Timler D, Stelmach W. Correlation of vitamin D with Foxp3 induction and steroid-sparing effect of immunotherapy in asthmatic children. Ann Allergy Asthma Immunol. 2012 Nov;109(5):329–335.
23. Esposito S, Lelii M. Vitamin D and respiratory tract infections in childhood. BMC Infect Dis. 2015 Oct 28;15:487.
24. Najada AS, Habashneh MS, Khader M. The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. J Trop Pediatr. 2004 Dec;50(6):364–368.
25. Principi N, Bianchini S, Baggi E, Esposito S. Implications of maternal vitamin D deficiency for the fetus, the neonate and the young infant. Eur J Nutr. 2013 Apr;52(3):859–867.
26. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Hudson TJ. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. Am J Respir Crit Care Med. 2004 Nov 1;170(9):967–973.
27. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010.

28. El-Abd Ahmed A, Hassan MH, Toghan R, Rashwan NI. Analysis of 25-hydroxy cholecalciferol, immunoglobulin E, and vitamin D receptor single nucleotide polymorphisms (Apa1, Taq1, and Bsm1), among sample of Egyptian children with bronchial asthma: A case-control study. Pediatric Pulmonology, 2020 April; https://doi.org/10.1002/ppul.24785.
29. Hollams EM, Hart PH, Holt BJ, Serralha M, Parsons F, de Klerk NH, Zhang G,Sly PD, Holt PG. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. Eur Respir J. 2011; 38:1320–27.
30. Comberiati P. Tsabouri S, Piacentini GL, Moser S, Minniti F, Peroni DG, Rossi GB, Piazzale LA. Is vitamin D deficiency correlated with childhood wheezing and asthma ?. Frontiers in Bioscience. 2014 Jan 6; 31-39.
31. Sathyamurthy R, Manney S, Wood A, Perkins G, Webster C, Krishna MT, MansurAH.
    a. Does Vitamin D axis have an effect on the severity of asthma?. Thorax.2010;Volume 65, Issue Suppl 4. doi:10.1136/thx.2010.150961.14.
32. JoshiVB, Dyke B, Rahman T, Townshend C, Patil V, Kurukullaratchy R.Vitamin D deficiency in the difficult asthma population; findings from a difficult asthma clinic. Thorax 2012; 67: A67 doi: 10.1136/thoraxjnl-2012-202678.149.
33. Chinellato I, Piazza M, Sandri M, et al. Vitamin D serum levels and markers of asthma control in Italian children. J Pediatr. 2011; 158:437–441.
34. Poon AH, Mahboub B, Hamid Q.Vitamin D deficiency and severe asthma. Pharmacol Ther. 2013; 140(2): 148-155.
35. Brehm JM, Celedón JC, Soto-Quiros M E, Avila L, Hunninghake G M, 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. American Journal of Respiratory and Critical Care Medicine. Volume 179, Issue 9: http://dx.doi.org/10.1164/rccm.200808-1361OC.
36. Turkeli A, Ayaz O, UncuA,Ozhan B, Bas VN, Tufan AK,Yylmaz O,Yuksel H. Effects of vitamin D levels on asthma control and severity in pre-school children. European Review for Medical and Pharmacological Sciences. 2016;20:26-36.
37. Bener A, Ehlayel MS,Tulic MK, Hamid Q.Vitamin D Deficiency as a Strong Predictor of Asthma in Children. Int Arch Allergy Immunol. 2012;157:168–175 DOI: 10.1159/000323941.
38. Segovia-Ortí R, Bennasar BA, De Sotto-Esteban D, Sanchís Cortés P, Pons J. Association between vitamin D status and allergen sensitization in pediatric subjects in the Balearic Islands. Pediatr Allergy Immunol. 2021 Aug;32(6):1183-1189. doi: 10.1111/pai.13513. Epub 2021 Apr 18. PMID: 33811785.
39. Chih-Yung Ch et al. Longitudinal vitamin D deficiency is inversely related to mite sensitization in early childhood. Pediatric Allergy and immunology. 2017 Dec; https://doi.org/10.1111/pai.12846.
40. Baghdasaryan A et al. Sensitisation pattern to inhalant allergens in Armenian children. Clinical and Translational Allergy. 2014; 4(1):P56, DOI:http://www.ctajournal.com/content/4/S1/P56.
41. Zheng YW, Chen S, Lai XX, Gjesing B, Zhong NS, Spangfort MD. Indoor mite allergen levels, specific IgE prevalence and IgE cross-inhibition pattern among asthmatic children in Haikou, southern China. Chin Med J (Engl). 2012 Sep;125(17):3059-63. PMID: 22932181.
42. Huang HW, Lue KH, Wong RH, Sun HL, Sheu JN, Lu KH. Distribution of allergens in children with different atopic disorders in central Taiwan. Acta Paediatr Taiwan. 2006 May-Jun;47(3):127-34. PMID: 17078465.
43. Montealegre F, Quiñones C, Michelen V, Bayona M, Fernández-Caldas E, Vazques O, Colón F, Chardón D, García M. Prevalence of skin reactions to aeroallergens in asthmatics of Puerto Rico PR. Health Sci J. 1997 Dec;16(4):359-67. PMID: 9580406.
44. Li J, Sun B, Huang Y, Lin X, Zhao D, Tan G, Wu J, Zhao H, Cao L, Zhong N. China Alliance of Research on Respiratory Allergic Disease-A multicentre study assessing the prevalence of sensitizations in patients with asthma and/or rhinitis in China. Allergy. 2009 Jul;64(7):1083-92. doi: 10.1111/j.1398-9995.2009.01967.x. Epub 2009 Feb 11. PMID: 19210346.

45. Lei S, Guo-tao G. The clinical effect and mechanism of vitamin D in the treatment of bronchial asthma in preschool children. Current Immunology, 2021; 41(4): 325-30.
46. Adcock I, Amrani Y,Role of Vitamin D Deficiency in Allergic Diseases: Is this Due to an Impaired Patients' Response to Corticosteroid Therapy?. Soj Immunology.2014February 17.
47. Gupta A, Bush A, Hawrylowicz C, Saglani S.Vitamin D and asthma in children. Paediatric Respiratory Reviews. 2012; 13(4): 236-243.
48. Tahiri KR, Najmuddin F, Avasthi Kh. Effect of vitamin d therapy on the pulmonary functions in pediatric asthma and allergic rhinitis. European Respiratory Journal. 2016; 48: PA1244; DOI: 10.1183/13993003.congress-2016.PA1244
49. Menon B, Nima G, Dogra V, Mittal A, Kaur Ch, Mittal U. Evaluation of vitamin D in bronchial asthma and the effect of vitamin D supplementation on asthma severity and control: A randomised control trial. European Respiratory Journal. 2014; 44: P4049.
50. Sannette C, Agrawal HDK. Vitamin D and Bronchial Asthma: An Overview of Data From the Past 5 Years. DOI: https://doi.org/10.1016/j.clinthera.2017.04.002
51. Augusto A. LitonjuaMD, Vitamin D Deficiency as a risk factor for childhood allergic disease and asthma. Curr Opin Allergy Clin Immunol. 2012 April ; 12(2): 179–185. doi:10.1097/ACI.0b013e3283507927
52. Martineau AR, Cates CJ, Urashima M, Jensen M, Griffiths AP, Nurmatov U, Sheikh A, Griffiths CJ.Vitamin D for the management of asthma. Cochrane Airways Group. 2016 Sept 6; DOI: 10.1002/14651858.CD011511.pub2
53. Pfeffer PE , Mann EH, Hornsby E, Chambers ES, Chen Y-H, Rice L, Hawrylowicz CM. Vitamin D Influences Asthmatic Pathology through Its Action on Diverse Immunological Pathways. AnnalsATS. 201405;204AW. PubMed: 25525739https://doi.org/10.1513
54. Bokobza I, El Hadi N, Bush A, Makrinioti H. Can vitamin D3 supplementation reduce the time to severe asthma exacerbations in children with asthma? Breathe (Sheff). 2021 Sep;17(3):210071. doi: 10.1183/20734735.0071-2021. PMID: 35035547; PMCID: PMC8753645.