Evaluation of IGF-1, TNF-α, and TGF-β Gene Expression after Oral Vitamin D Supplementation in School-Aged Children with Chronic Bronchial Asthma

Abeer Ramadan1*, Sara Sallam2, Rasha Yousef3, Mai Elsheikh4*, Asmaa Ali5, Yasmine Elhusseny6, Sally Ishak7

1Department of Molecular Genetics and Enzymology, Human Genetics and Genomic Research Institute, National Research Center, Giza, Egypt; 2Department of Child Health, Medical Research and Clinical Studies Institute, National Research Center, Giza, Egypt; 3Department of Clinical and Chemical Pathology, Medical Research and Clinical Studies Institute, National Research Center, Giza, Egypt; 4Department of Complementary Medicine, Medical Research and Clinical Studies Institute, National Research Center, Giza, Egypt; 5Department of Pulmonary Medicine, Abbassia Chest Hospital, Ministry of Health, Cairo, Egypt; 6Department of Biochemistry and Molecular Biology, New Giza University, Giza, Egypt; 7Department of Pediatric, Faculty of Medicine, Ain Shams University Hospital, Cairo, Egypt

Abstract

BACKGROUND: Airway remodeling in children with bronchial asthma is due to the effect of inflammatory mediators and growth factors on the bronchial epithelium. Vitamin D (VitD) has immunomodulatory effect in many inflammatory diseases as bronchial asthma. The anti-inflammatory and anti-fibrotic role of VitD could prevent or improve airway remodeling in asthmatic patients.

AIM: The study investigated the effect of VitD supplementation on the expression of transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), and insulin growth factor 1 (IGF-1) and to correlate them with asthma severity and level of control.

METHODS: The serum level of VitD and the mRNA expression of IGF-1, TGF-β, and TNF-α were estimated in 50 patients and 20 healthy controls using quantitative PCR in real-time. Asthmatic patients with VitD deficiency received VitD supplementation for 2 months followed by remeasurement of serum VitD and the genes expression of TGF-β, TNF-α, and IGF-1.

RESULT: Pre-intake of VitD and serum level of VitD were lower in all patients than control subjects (p = 0.005). VitD level was directly correlated with IGF-1 mRNA expression, which was indirectly correlated with TGF-β, r = 0.5 and −0.57; p = 0.0001 and 0.002, respectively. After VitD supplementation, the expression of the TGF-β mRNA gene was the only gene that decreased significantly (p = 0.04) together with improved asthma control and spirometric parameters.

CONCLUSIONS: VitD supplementation down regulated the gene expression of TGF-β and improved asthma control level, but it did not significantly affect the gene expression of TNF-α and IGF-1.

Introduction

Asthma is a chronic sustained inflammatory airway disease that ultimately leads to airway remodeling. Although the treatment guidelines help to improve symptoms of asthma, they did not affect airway remodeling, and treatment, now, is directed to be personalized for each patient according to individual biomarkers to prevent airway remodeling [1].

Transforming growth factor-beta (TGF-E) is involved in both the inflammatory and remodeling pathways of bronchial asthma [2]. It is considered a pro-inflammatory and pro-fibrotic mediator that are released from sensitized airways by allergens in response to the proliferation of T-helper 2 cells and the release of inflammatory cytokines and interleukins (IL-4, 5, and 13) [3]. TGF-E binds to its receptor complex that induces several pathways that end by gene induction, which finally leads to apoptosis of airway epithelial cells, and proliferation of goblet cells that aggravate asthma [4]. Furthermore, TGF-E induces fibrosis through activation of epithelial-mesenchymal transition in the airway epithelial cell [5]. TGF-E1 has a unique role in stimulating extracellular matrix accumulation. Therefore, TGF-E1 is suggested to be involved in progression of airway remodeling [6].

Tumor necrosis factor-alpha (TNF-D) is a pro-inflammatory cytokine that has a role in asthma and has been suggested as a therapeutic option for patients with steroid-resistant asthma [7]. However, the response to anti-TNF-D is controversial and the mechanism of action of TNF-D is still not well understood as it may be...
a direct effect on airway smooth muscles or through its effect on stimulating other cytokines and leukotrienes, in addition to its role as a chemoattractant for neutrophils and eosinophils and increasing the cytotoxicity of eosinophils on airway endothelial cells, leading to airway hyper-responsiveness [8].

Insulin growth factor 1 (IGF-1) is produced by alveolar macrophages that are induced by IL-33 [9]; it increases in asthma and has a role in promoting inflammation, AHR, and inducing subepithelial fibrosis and smooth muscle hyperplasia. On the other hand, IGF-binding protein 3 inhibits the development of asthma by decreasing airway inflammation and AHR. Both IGF-1 and IGFBP3 are, now, considered therapeutic targets for asthma [10].

Vitamin D (VitD) is a dietary, immunomodulatory factor affecting the inflammatory process in asthmatic patients and although there was controversy regarding its role in improving asthma, its role in improving inflammation is well documented through decreasing many cytokines and interleukins, and also its effect on the cellular and humoral immune response [11].

The present study aims to find the change in the gene expression of TGF-£, TNF-£, and IGF-1 after 2 months of oral intake of VitD and to correlate them with asthma severity and level of control. intended for the quantitative determination of the total 25-OH vitamin D in human serum and plasma. The assay employs a vitamin D binding protein (VDBP) as capture protein, which binds to both 25-OH D3 and 25-OH D2 (Roche Diagnostics USA, n.d.).

Vitamin D deficiency was defined as 25(OH) D < 30 ng/mL and further categorized into three intensities: Mild, moderate, and severe vitamin D deficiencies; 25-OHD values of 20–30 ng/mL, 10–20 ng/mL, and <10 ng/mL, respectively [15], [16].

Measurement of the IGF-1, TGF-£, and TNF-D expression level: Total RNA from fresh venous blood samples was extracted using Qiagen QIAamp RNA Blood Mini Kit (Catalog number: 52304) according to the manufacturer’s instructions, followed by assuring RNA quality and purity using Nanodropper 2000 (ThermoScientific) [17].

cDNA synthesis: Total RNA was reverse transcribed into first-strand complementary DNA (cDNA) using a High Capacity cDNA Reverse Transcription Kit (Invitrogen, Life Sciences) using Bio-Rad T100TM Thermal Cycler.

Relative quantitative real-time PCR was performed by the LightCycler480 system using Maxima SYBER Green Q PCR Master Mix, 10 ng cDNA, and 200 nM of each forward and reverse primers according to manufacturer’s instructions in a final volume of 20 Pl. The PCR was performed through the following instructions: Initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for the 30 s, and extension at 72°C for 20 s. The values were normalized based on the expression level of the endogenous housekeeping gene beta-actin.

Primers were designed with Roche probe library (“Roche – Universal ProbeLibrary,” n.d.) 0 its sequences which were revised using primer blast (http://www.ncbi.nlm.nih.gov/BLAST/); 1-VDR primer sequence: Forward primer 5’ CATGCATTGGTCTTTTGTAATTGTCAC -3’, reverse primer 5’ AGGAGTCCCCGAAAAGG -3’ and 2- beta-actin primer sequence: Forward primer 5’: TGATGAAAGGCTTTTGTGTCAC -3’, Reverse primer 5’ CTGGCTCTCAAGTGCTAGTACAGGT -3’.

The second stage

It was an intervention study and included only 25 asthmatic subjects who agreed to continue, all patients with serum vitamin D level <20 ng/ml were asked to receive oral vitamin D3 (cholecalciferol) syrup (1000U/D) for 2 months duration [18], as an add-on therapy to their current antiasthma medications. The compliance of the patient was evaluated weekly. They were also regularly encouraged through mobile calls. The Pan African Clinical Trials Registry (PACTR.org); trial ID: PACTR201811682685226.
After 2 months of VitD supplementation, the VitD serum level and the gene expression of IGF-1, TGF-E, and TNF-D were reassessed.

### Statistical analysis

The accuracy of serum VitD, IGF-1, TGF-E, and TNF-D expression level was assessed with receiver operating curve analysis, assuming that the null hypothesis of AUROC was 0.5, at power 80%, CI 95%, and prevalence 23% of bronchial asthma [19], the sample size was calculated with a minimum total number of 40 using MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium). The best cutoff value of serum VitD serum level and the gene expression of IGF-1, TGF-E, and TNF-D expression level was selected with maximum sensitivity and specificity for prediction of asthma. The AUROC was also calculated, criteria to qualify for AUC were as follows: 0.90–1 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor, and 0.50–0.60 = fail. All tests were two-sided. p-value below 0.05 was considered significant. Another statistical analysis was performed using Minitab 17.1.0.0 for windows (Minitab Inc., 2013, Pennsylvania, USA). All tests were two-sided, p < 0.05 was considered significant. Data normality was checked for using the Shapiro–Wilk test. Independent t-test or Mann–Whitney U-tests were used for comparison between two groups of continuous data nature, and Chi-square test for comparison between two or more groups of categorical data. One-way (ANOVA) test or Kruskal–Wallis test was used to compare between more than two groups with multiple comparisons using Tukey methods. Person correlation coefficient test was used to examine the linear relationship between different continues data, (+) sign indicate positive correlation and (−) sign indicate a negative correlation. Paired t-test was applied to compare the parameters before and after treatment application.

### Results

#### Stage I

The patients and control were matched as regarding age, sex, and BMI (p = 0.33, 0.31, and 0.2), respectively. Demographic and clinical criteria of the patients are summarized in Table 1. The median level of VitD in asthmatic patients was significantly lower compared to controls (p = 0.005) (Table 1), while, the qRT-PCR test showed that expression levels of IGF-1, TGF-E, and TNF-D mRNA were significantly increased in patients than the control group (p = 0.03, 0.002, and 0.009, respectively) (Table 1).

The diagnostic accuracy of serum VitD level, TGF-E, and TNF-D expression in prediction of bronchial asthma were good; AUC = 0.72, 0.74, and 0.7, respectively, and p = 0.004, 0.001, and 0.009, respectively, at cutoff value of VD < 11.52 (ng/mL), the sensitivity and specificity were 64% and 80%, respectively, the +LR = 3.2 and −LR = 0.45, while, in TGF-E and TNF-D expression the cutoff values >2.48E-04 and 6.9E-03, respectively, the sensitivity and specificity were 70 and 65% and 78 and 70%, respectively, the +LR = 2 and 2.6, respectively, and −LR = 0.46 and 0.31, respectively. Finally, the accuracy of IGF-1 expression was near to be fairly acceptable as the AUC was 0.67 and P = 0.02 (Table 2 and Figure 1).

#### Table 1: General characters of the studied groups

| Variables          | Asthmatic (N = 50) | Control (N = 20) | P     |
|--------------------|---------------------|------------------|-------|
| Age (years) (mean, SD) | 32 (6–11)          | 32.5 (6.1–17.9)  | 0.03  |
| Sex (male, %)      | 32 (64)             | 16 (80)          | 0.34† |
| BMI (Kg/m²) (mean, SD) | 15.6 (3.5)        | 16.5 (4.0)       | 0.2†  |
| VitD (ng/mL) (median, IQR) | 9.68 (6.36–14.31) | 13.94 (11.56–17.77) | 0.005† |
| IGF-1 mRNA (median, IQR) | 1.216E-04 (3.45E-05–6.65E-04) | 0.000341 (2.31E–05–1.17E-04) | 0.05† |
| Fold change         | 10.36               | 1.69             |       |
| TGF-E mRNA (median, IQR) | 1.42E-03 (1.21E-04–8.73E-03) | 1.81E-04 (7.86E–05–3.74E-04) | 0.002† |
| Fold change         | 33.91               | 2.22             |       |
| TNF-D mRNA (median, IQR) | 1.9E0-02 (7.5E-03–2.98E-02) | 4.76E-03 (2.32E–03–1.56E-02) | 0.009† |
| Fold change         | 1.9                 | 1.4              |       |

### Table 2: Variable tests predicted the bronchial asthma

| Parameters | VitD (ng/mL) | IGF-1 | TGF-E | TNF-D |
|------------|--------------|-------|-------|-------|
| AUC        | 72%          | 67%   | 74%   | 79%   |
| SE         | 0.06         | 0.06  | 0.06  | 0.06  |
| 95% CI     | 0.567–0.8383 | 0.5420–0.7960 | 0.6237–0.8543 | 0.5069–0.8351 |
| p          | 0.00         | 0.03  | 0.00  | 0.01  |
| Cutoff     | <11.52       | >6.96E-05 | >2.48E-04 | >6.96E-03 |
| Sensitivity | 64%          | 66%   | 70%   | 78%   |
| Specificity | 0.4919–0.7708 | 0.5123–0.7879 | 0.5539–0.8214 | 0.6040–0.8847 |
| 95% CI     | 0.0534–0.9427 | 0.4078–0.8461 | 0.4078–0.8461 | 0.4572–0.8811 |
| LR +       | 3.20         | 1.89  | 2.00  | 2.60  |
| LR −       | 0.45         | 0.52  | 0.46  | 0.31  |
| PV +       | 41%          | 29%   | 31%   | 36%   |
| PV −       | 91%          | 90%   | 91%   | 94%   |

*Number, SD: Standard deviation, BMI: Body mass index, VitD: Vitamin D, IGF-1: insulin growth factor 1, TGF-E: Transforming growth factor-beta, TNF-D: Tumor necrosis factor-alpha, IQR: Inter quartile range, continues data represented as mean and SD or median and IQR, categorical data represented as number and %; †: Independent t-test, ^: Mann Whitney test, #: Chi square test, P: considered significant <0.05.

The correlation between IGF mRNA expression and the level of VD in patients was significantly positive (r = 0.5; p = 0.001), while TGFβ mRNA showed significant negative correlation with it (r = −0.57; p = 0.002) (Figure 2).
Ramadan et al. IGF-1, TNF-α, and TGF-β Gene Expression after Vitamin D Supplementation in Children with Bronchial Asthma

and

Ozyilmaz

reported a significant (2019) proved that IGF-1 is elevated in recent research of our group that showed a significant pulmonary function test (PFT) outcome had a direct and correlated with asthma severity and that VitD levels and found that serum VitD levels were proven to be inversely corroborated by Alyasin discussed controversially [20], [21], and [22]. This was on numerous gene expression biomarkers in asthma was

Discussion

The role of serum level of VitD and its implication on numerous gene expression biomarkers in asthma was discussed controversially [20], [21], and [22]. This was corroborated by Alyasin et al., and Whiting et al., who found that serum VitD levels were proven to be inversely correlated with asthma severity and that VitD levels and pulmonary function test (PFT) outcome had a direct and significant association [23], [24]. This is in line with a recent research of our group that showed a significant reduction of asthma symptoms and increase spirometry parameters when VitD was added to the asthma treatment [25]. However, these findings were limited by investigating solely vitamin D receptor expression.

Before administration of VitD, TGF-E, IGF-1, and TNF-D gene expression were higher in asthmatic patients compared to the control subjects. The studies of various international groups, reported (TNF-alpha) genes, are already identified and the known locus of it might influence the inflammatory activity. Consequently, latest studies reported that TNF-alpha is one of the genes that play a leading role in the disease development [26]

The study by Chen and Xu (2018) was against our observation, which found that TNF-D was higher, while TGF-E was lower in asthmatics than control [27], and surprisingly, a recent study done by Han et al. (2021) showed that IGF-1 has a beneficial role in asthmatic adults and is associated with better pulmonary functions [28]. However, the study of Mu et al. (2019) proved that IGF-1 is elevated in the lungs and BAL of asthmatic mice and it inhibits phagocytosis and development of apoptotic cells after lung injury, and after using blocking antibodies to IGF-1, phagocytosis, and apoptosis improved that ultimately lead to a decrease inflammatory cells infiltration and improvement of asthma [9].

Regarding TGF-E, Manuyakorn et al. found significantly higher serum TGF-E1 in pediatric patients with atopic asthma compared to controls, but they did not find a correlation between it and asthma duration, treatment, or pulmonary functions [29]. Moreover, Redington et al. (1997) found a significant increase of TGF-E1 in bronchoalveolar lavage (BAL) of asthmatic patients that increased more on allergen exposure, suggesting the role of TGF-E1 on airway remodeling [30]. Hereby, the expression of TGF-b1 is likely to be inducible and transient in nature, and that up-regulation from baseline level requires an antigen challenge [31].

Furthermore, Joseph et al. and Ozyilmaz et al. reported that TGF-E1 was significantly higher in asthmatic cases versus the control group [32], [33]. Furthermore, Ivanovna et al. showed a highly significant increase in the serum TGF-E1 in both asthmatic groups with mild and severe grades versus control group [34]. Interestingly, El-Sayed et al. reported a significant increase in the serum TGF-E1 level in the mild bronchial asthma group and a significant decrease in the serum TGF-E1 level in severe asthma [35].

In our study, TGF-E gene expression correlated negatively with VitD levels and its level of gene expression was decreased after VitD levels increased due to the identified suppression of TGF-E1 gene expression. This agrees with the study of Isik et al. who suggested the role of TGF-E1 in inducing fibrosis in patients with VitD deficiency [36]. This suggested that the level of TGF-E1 expression might correlate with severity and exacerbation.
Many studies such as Al-Alawi et al., [3] Makinde et al., [4], and Howell et al. [37] suggested the potential therapeutic role of decreasing TGF-E in preventing remodeling in asthmatic patients and that it can be used as individualized therapy by biological therapy as steroids did not decrease its level in the study done by Chakir et al. [38]. Thus, sensitive monitoring of TGF-E gene expression might assas asthma severity.

Concerning the reported positive correlation between the increased level of IGF-1 and VitD, Bereket et al. [39] and Soliman et al. [40] confirmed the same conclusion. This was explained by the well-established experimental data that IGF-1 stimulates the synthesis of 1, 25(OH)2D in the kidney, causing the increase of the blood levels of both VitD and IGF-1 reciprocally, but the mechanism that interplay between VitD and IGF-1 is complicated [41].

On the other hand, we did not find a significant change in the gene expression of TNF-D or that of IGF-1 after VitD supplementation. Furthermore, Sinha-Hikim et al. (2015) in their study on inflammatory markers in pre-diabetic patients did not find a significant effect of VitD on TNF-D or IGF-1 levels [42]. Other studies on the effect of Vit D on IGF-1 showed different results. Some go with our results as Trummer et al. in their study on patients with arterial hypertension [43], and Kamycheva et al. a study on obese patients [44]. Furthermore, the meta-analysis done by Kord-Varkaneha et al. (2020) showed no significant increase in IGF-1 after VitD supplementation in eight different studies [45]. In contrast, Hyppönen et al., in their study on patients with metabolic syndrome, found that IGF-1 increased after VitD supplementation to 75-85 nmol/l but not more [46].

Bogazzi et al. reported positive correlation between VitD and IGF-1 especially in patients with severe VitD deficiency (<20 ng/ml) [47]. In addition, Ameri et al. concluded that VitD increased the levels of IGF-1 in adults with growth hormone deficiency after 12 weeks supplementation of 7000 IU/week vitamin D3 [48].

Regarding TNF-D, Chandler et al. did not find a significant correlation between VitD levels and soluble tumor necrosis factor-alpha receptor type 2 (sTNF-R2) in African-Americans [49]. Furthermore, the results of Dadaei et al. (2015) showed that although the level of TNF-D decreased in Iranian patients with inflammatory bowel diseases after VitD supplementation, the decrease was not statistically significant [50]. However, Haddad et al. (2018) showed that VitD caused downregulation in the gene expression of TNF-D in diabetic hemodialysis patients [51]. Interestingly, Kuo et al. (2010) found that Vit D3 decreases TNF-D and T-helper 1 related chemokines in asthmatic patients at lower doses, but higher doses and excessive use of VitD supplementations enhance T-helper 2 related chemokines and worsen asthma, autoimmune diseases, and other T-helper 2 related allergic [52].

## Conclusions

VitD intake is of beneficial value in asthma control. The mRNA gene expression of TGF-E, TNF-D, and IGF-1 could be potential candidate genes in the pathogenesis and assessment level of bronchial asthma control.

## References

1. Zhang J, Dong L. Status and prospects: Personalized treatment and biomarker for airway remodeling in asthma. J Thorac Dis. 2020;12(10):6090-101. https://doi.org/10.21037/jtd-20-1024
PMid:33209441

2. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: A key for understanding inflammation and remodeling. Allergy. 2012;67(10):1193-202. https://doi.org/10.1111/j.1398-9995.2012.02880.x
PMid:22913656

3. Al-Alawi M, Hassan T, Chotirmall SH. Transforming growth factor-E and severe asthma: A perfect storm. Respir Med. 2014;108(10):1409-23. https://doi.org/10.1016/j.rmed.2014.08.008
PMid:25240764

4. Makinde T, Murphy RF, Agrawal DK. The regulatory role of TGF-beta in airway remodeling in asthma. Immunol Cell Biol. 2007;85(5):348-56. https://doi.org/10.1038/sj.icb.7100044
PMid:17325694

5. Gong JH, Cho IH, Shin D, Han SY, Park SH, Kang YH. Inhibition of airway epithelial-to-mesenchymal transition and fibrosis by kaempferol in endotoxin-induced epithelial cells and ovalbumin-sensitized mice. Lab Invest. 2014;94(3):297-308. https://doi.org/10.1038/labinvest.2013.137
PMid:24378645

6. Miramani Mirzamani MS, Nourani MR, Fooladi AA, Zare S, Ebrahimi M, Yazdani S, et al. Increased expression of transforming growth factor-E and receptors in primary human airway fibroblast from chemical inhalation patients. Iran J Allergy Asthma Immunol. 2013;12(2):144-52.
PMid:23754353

7. Babu KS, Davies DE, Holgate ST. Role of tumor necrosis factor alpha in asthma. Immunol Allergy Clin North Am. 2004;24(4):583-97, v-vi. https://doi.org/10.1016/j.iac.2004.06.010
PMid:15474860

8. Brightling C, Berry M, Amrani Y. Targeting TNF-alpha: A novel therapeutic approach for asthma. J Allergy Clin Immunol. 2008;121(1):5-12. https://doi.org/10.1016/j.jaci.2007.10.028
PMid:18036647

9. Mu M, Wu F, He J, Tang X, Ma H, Guo S, et al. Insulin-like growth factor 1 inhibits phagocytosis of alveolar epithelial cells in asthmatic mice. Mol Med Rep. 2019;19(3):2381-8. https://doi.org/10.3892/mmr.2019.10496
PMid:31322198.
10. Lee H, Kim SR, Oh Y, Cho SH, Schleimer RP, Lee YC. Targeting insulin-like growth factor-I and insulin-like growth factor-binding protein-3 signaling pathways: A novel therapeutic approach for asthma. Am J Respir Cell Mol Biol. 2014;50(4):667-77. https://doi.org/10.1165/rcmb.2013-0397TR
PMid:24219511

11. Hall SC, Agrawal DK. Vitamin D and bronchial asthma: An overview of data from the past 5 years. Clin Ther. 2017;39(5):917-29. https://doi.org/10.1016/j.clinthera.2017.04.002
PMid:28449868

12. Bateman ED, Hurd SS, Barnes PJ, Boussenet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J. 2008;31(1):143-78. https://doi.org/10.1183/09031936.00138707
PMid:18166595

13. Celli BR. ATS standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. Eur Respir Rev. 1996;6(39):276-81.

14. Talaei A, Yadegari N, Rafee M, Rezvanfar MR, Moini A. Prevalence and cut-off point of vitamin D deficiency among secondary students of Arak, Iran in 2010. Indian J Endocrinol Metab. 2012;16(5):786. https://doi.org/10.4103/2230-8210.100676
PMid:23087865

15. Saad K, Abd Aziz NH, El-Hakim IZ, El-Kerdani TA, Ghanem HM. Serum vitamin D concentrations in children with bronchiolitis: A randomized, double-blind, placebo-controlled study. Pediatr Allergy Immunol Pulmonol. 2015;28(2):102-6.

16. Wang E, Miller LD, Ohmacht GA, Liu ET, Marincola FM. High-fidelity mRNA amplification for gene profiling. Nat Biotechnol. 2000;18(4):457-9. https://doi.org/10.1038/74546
PMid:10748532

17. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: Review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-417. https://doi.org/10.1542/peds.2007-1894
PMid:18676559

18. Huh SY, Gordon CM. Vitamin D deficiency in children and adolescents: Epidemiology, impact and treatment. Rev Endocr Metab Disord. 2008;9(2):161-70. https://doi.org/10.1007/s11154-007-9072-y
PMid:18157220

19. Zedan M, Settine A, Farag M, Ezz-Elgmal M, Osman E, Fouda A. Prevalence of bronchial asthma among Egyptian school children. Egypt J Bronchol. 2009;3(2):124-30.

20. Holmlund-Suila E, Koskiivirta P, Metso T, Andersson S, Mäkkitie O, Vilkajainen HT. Vitamin D deficiency in children with a chronic illness—seasonal and age-related changes in serum 25-hydroxy vitamin D concentrations. PLoS One. 2013;8(4):e60856. https://doi.org/10.1371/journal.pone.0060856
PMid:23585857

21. Kolokotroni O, Papadopoulou A, Middleton N, Kouta C, Raftopoulos V, Nicolaidou P, et al. Vitamin D levels and status amongst asthmatic and non-asthmatic adolescents in Cyprus: A comparative cross-sectional study. BMC Public Health. 2015;15:48. https://doi.org/10.1186/s12889-015-1385-2
PMid:26583166

22. Hatami G, Ghasemi K, Motamed N, Firoozbakht S, Movahed A, Farrokhii S. Relationship between Vitamin D and childhood asthma: A case–control study. Iran J Pediatr. 2014;24(6):710-4.
PMid:26019776

23. Alyasin S, Momen T, Kashef S, Alipour A, Amin R. The relationship between serum 25 hydroxy Vitamin D levels and asthma in children. Allergy Asthma Immunol Res. 2011;3(4):251. https://doi.org/10.4168/aair.2011.3.4.251
PMid:21966605

24. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: An examination in children and adults with and without supplement use. Am J Clin Nutr. 2011;94(1):128-35. https://doi.org/10.3945/ajcn.111.013268
PMid:21595303

25. Ramadan A, Sallam SF, Elsheikh MS, Ishakd SR, Abdelsayed MG, Salah M, et al. VDR gene expression in asthmatic children patients in relation of vitamin D Status and supplementation. Gene Rep. 2019;15:100387. https://doi.org/10.1016/j.genrep.2019.100387

26. Siezen CL, Bont L, Hodemaekers HM, Ermers MJ, Doornbos G, Van’t Slot R, et al. Genetic susceptibility to respiratory syncytial virus bronchiolitis in preterm children is associated with airway remodeling genes and innate immune genes. Pediatr Infect Dis J. 2009;28(4):333-5. https://doi.org/10.1097/INF.0b013e318181e2aa9
PMid:19258923

27. Chen Y, Xu T. Association of vitamin D receptor expression with inflammatory changes and prognosis of asthma. Exp Ther Med. 2018;16(6):5096-102. https://doi.org/10.3892/etm.2018.8687
PMid:30542464

28. Han YY, Yan Q, Chen W, Forno E, Celemón JC. Serum insulin-like growth factor-1, asthma, and lung function among British adults. Ann Allergy Asthma Immunol. 2021;126(3):284-91.e2. https://doi.org/10.1016/j.anai.2020.12.005
PMid:33316372

29. Manuyakov W, Kamchaisawan W, Atamasiriik K, Sasisakulporn C, Direkwattanachai C, Benjaponpitak S. Serum TGF-beta1 in atopic asthma. Asian Pac J Allergy Immunol. 2008;26(4):185-9.
PMid:19317336.

30. Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, et al. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am J Respir Crit Care Med. 1997;156(2 Pt 1):642-7. https://doi.org/10.1164/ajcc.156.2.9605065
PMid:9279252

31. Halwani R, Al-Muhsen S, Hamdan Al-Jahdali H, Hamid Q. Role of transforming growth factor–b in airway remodeling in asthma. Am J Respir Cell Mol Biol. 2011;44:127-33.

32. Joseph J, Benedict S, Badrinath P, Wassef S, Joseph M, McLaurin K. Effect of asthma exacerbations on health care costs among asthmatic patients with moderate and severe persistent asthma. J Allergy Clin Immunol. 2012;129(5):1229-35. https://doi.org/10.1016/j.jaci.2012.01.039
PMid:23364231

33. Ozylmez E, Canbakan S, Capan N, Erturk A, Guhan M. Correlation of plasma transforming growth factor beta 1 with asthma control test. Allergy Asthma Proc. 2009;30(1):35-40. https://doi.org/10.2500/aap.2009.30.3192
PMid:19331718

34. Ivanova JI, Bergman R, Birnbaum HG, Colice GL, Silverman RA, McLaurin K. Effect of asthma exacerbations on health care costs among asthmatic patients with moderate and severe persistent asthma. J Allergy Clin Immunol. 2012;129(5):1229-35. https://doi.org/10.1016/j.jaci.2012.01.039
PMid:23236484

35. El-Sayed ZA, El-Hakim IM, El-Kerdani TA, Ghanem HM. Serum transforming growth factor-beta 1 in asthmatic children. Egypt J Pediatr Allergy Immunol. 2004;2(1):46-51.

36. Isik S, Ozuguz U, Tutuncu YA, Erden G, Berkner D, Acar K, et al. Serum transforming growth factor-beta levels in patients with vitamin D deficiency. Eur J Intern Med. 2012;23(1):93-7. https://doi.org/10.1016/j.ejim.2011.09.017
37. Howell JE, McNulty RJ. TGF-beta: Its role in asthma and therapeutic potential. Curr Drug Targets. 2006;7(5):547-65. https://doi.org/10.2174/138945006778818692
PMid:16719766

38. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma: Effect of steroids on TGF-beta, IL-11, IL-17, and Type I and Type III collagen expression. J Allergy Clin Immunol. 2003;111(6):1293-8. https://doi.org/10.1067/mai.2003.1557
PMid:12789932

39. Bereket A, Cesur Y, Özkan B, Adal E, Turan S, Onan SH, et al. Circulating insulin-like growth factor binding protein-4 (IGFBP-4) is not regulated by parathyroid hormone and vitamin D in vivo: Evidence from children with rickets. J Clin Res Pediatr Endocrinol. 2010;2(1):17-20. https://doi.org/10.4274/jcrpe.v2i1.17
PMid:21274331

40. Soliman AT, Al Khalaf F, AlHemaidi N, Al Ali M, Al Zyoud M, Yakoot K. Linear growth in relation to the circulating concentrations of insulin-like growth factor I, parathyroid hormone, and 25-hydroxy vitamin D in children with nutritional rickets before and after treatment: Endocrine adaptation to Vitamin D deficiency. Metabolism. 2008;57(2):298-305. https://doi.org/10.1016/j.metabol.2007.08.011
PMid:18003755

41. Ameri P, Giusti A, Boschetti M, Murialdo G, Minuto F, Ferone D. Interactions between vitamin D and IGF-I: From physiology to clinical practice. Clin Endocrinol. 2013;79(4):457-63. https://doi.org/10.1111/cen.12268
PMid:23789983

42. Sinha-Hikim I, Duran P, Shen R, Lee M, Friedman TC, Davidson TC, et al. Effect of long term vitamin D supplementation on biomarkers of inflammation in Latino and African-American subjects with pre-diabetes and hypovitaminosis D. Horm Metab Res. 2015;47(4):280-3. https://doi.org/10.1055/s-0034-1383652
PMid:25011019

43. Trummer C, Schweiz V, Pandis M, Grübler MR, Verheyen N, Gaksch M, et al. Effects of Vitamin D supplementation on IGF-1 and calcitriol: A randomized-controlled trial. Nutrients. 2017;9(6):623. https://doi.org/10.3390/nu9060623
PMid:28629132

44. Kamrycheva E, Berg V, Jorde R. Insulin-like growth factor I, growth hormone, and insulin sensitivity: The effects of a one-year cholecalciferol supplementation in middle-aged overweight and obese subjects. Endocrine. 2013;43:412-18. https://doi.org/10.1007/s12020-012-9825-6
PMid:23109222

45. Kord-Varkaneh H, Rinaldi G, Hekmatdoost A, Fatahi S, Tan SC, Shadmoush M, et al. The influence of vitamin D supplementation on IGF-1 levels in humans: A systematic review and meta-analysis. Ageing Res Rev. 2020;57:100996. https://doi.org/10.1016/j.arr.2019.100996
PMid:31816443

46. Hyppönen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: A cross-sectional study in the 1958 British Birth Cohort. Diabetes. 2008;57(2):298-305. https://doi.org/10.2337/db07-1122
PMid:18003755

47. Bogazzi F, Rossi G, Lombardi M, Tomisti L, Sardella C, Manetti L, et al. Vitamin D status may contribute to serum insulin-like growth factor I concentrations in healthy subjects. J Endocrinol Invest. 2011;34(8):e200-3. https://doi.org/10.3275/7228
PMid:20671418

48. Ameri P, Giusti A, Boschetti M, Bovio M, Teti C, Leoncini G, et al. Vitamin D increases circulating IGF1 in adults: Potential implication for the treatment of GH deficiency. Eur J Endocrinol. 2013;169(6):767-72. https://doi.org/10.1530/EJE-13-0510
PMid:24005315

49. Chandler PD, Scott JB, Drake BF, Ng K, Manson JE, Rifai N, et al. Impact of vitamin D supplementation on inflammatory markers in African Americans: Results of a four-arm, randomized, placebo-controlled trial. Cancer Prev Res (Phila). 2014;7(2):218-25. doi:10.1158/1940-6207.CAPR-13-0338-T
PMid:24327720

50. Dadaei T, Safapoor MH, Asadzadeh Aghdaei H, Balaii H, Pourhoseingholi MA, Naderi N, et al. Effect of vitamin D3 supplementation on TNF-D serum level and disease activity index in Iranian IBD patients. Gastroenterol Hepatol Bed Bench. 2015;8(1):49-55. https://doi.org/10.2336/gbhb.2015.0021
PMid:25854176

51. Haddad Kashani H, Seyed Hosseini E, Nikzad H, Soleimani A, Soleimani M, Tamadon MR, et al. The effects of Vitamin D supplementation on signaling pathway of inflammation and oxidative stress in diabetic hemodialysis: A randomized, double-blind, placebo-controlled trial. Front Pharmacol. 2018;9:50. doi:10.3389/fphar.2018.00050

52. Kuo YT, Kuo CH, Lam KP, Chu YT, Wang WL, Huang CH, et al. Effects of vitamin D3 on expression of tumor necrosis factor-alpha and chemokines by monocytes. J Food Sci. 2010;75(6):H200-4. https://doi.org/10.1111/j.1750-3841.2010.01704.x
PMid:20722932