Correlation of vitamin D and asymmetric dimethylarginine in children with bronchial asthma

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ABSTRACT

Bronchial Asthma is outlined as a chronic irritation of the airways in children. Vitamin D is a real immune system regulator which has a potential part in allergy. Asymmetric Dimethyl Arginine (ADMA) is an endogenous Nitric Oxide Synthase (NOS) inhibitor. This study is to determine if there is a role of vitamin D deficiency, ADMA in the pathogenesis of asthma in children, and whether the decreased arginine bioavailability and NOS suppression by ADMA contribute to respiratory tract blockage or not. We measured serum vitamin D, ADMA, nitric oxide and plasma L-arginine in 30 asthmatic and 10 healthy children. Serum 25-hydroxy vitamin D, plasma L-Arginine and serum Nitric Oxide were decreased significantly in asthmatic patients compared to healthy children. On the other hand, ADMA serum levels were increased significantly in asthmatic patients. In asthmatic children, there were positive correlations between serum vitamin D concentration and forced expiratory volume in the first, second FEV1 (% predicted). Furthermore, there were negative correlations between serum ADMA concentration and FEV1 (% predicted). In conclusion, marked reduction of vitamin D and elevated ADMA serum levels in asthmatic children has contributed to NOS-related pathophysiology, therefore ADMA and vitamin D could be considered reliable in managing oxidative stress in asthma.

1. Introduction

Bronchial asthma is considered one of the most popular chronic diseases influencing children, which is characterized by reversible airways obstruction (Boulet et al., 2015). Chronic respiratory tract infection may additionally set off airway remodeling, characterized by means of morphological modifications in the airway wall, which includes subepithelial fibrosis and elevated smooth muscle mass of the airway, progressive drop in lung function that could be a result of airway redesigning, and the occurrence of continual airway hyperresponsiveness (AHR) (Postma & Timens, 2006).

The prevalence of bronchial asthma in children may be brought on by several factors including environmental, gender, genetic, ethnic components, and socioeconomic standing (Yin et al., 2016). Recently, the complaint of low serum levels of vitamin D in cases of bronchial asthma increases nowadays all over the world (Babar et al., 2017; Parva et al., 2018). Even several studies have detected that there is a relation between the level of vitamin D and progressive wheezing (Einisman et al., 2015). Other studies returned the cause of increasing incidence of allergies epidemically to vitamin D deficiency due to its role in regulation of the immune system (Andújar-Espinosa et al., 2020).

Vitamin D helps in moderation of many immunological paths in various asthma endotypes, for instance, the regulation of the action of lymphocytes such as T cells, macrophages, Langerhans cells, mast cells, and B cells in order to reduce the different reactions of inflammation (Pfeffer & Hawrylowicz, 2018).

Vitamin D brought from solar exposure, nourishments, and supplements is biologically inactive and must go through two hydroxylations inside the body for activation. The primary hydroxylation, which takes place in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH) D], also recognized as ‘calcidiol.’ The secondary one takes place mainly in the kidney and forms the physiologically energetic 1, 25-dihydroxyvitamin D (1, 25(OH) 2D), which additionally acknowledged ‘calcitriol’ (Grant et al., 2018).

The lung is a predominant supply of asymmetric dimethylarginine (ADMA) (El-Alameey et al., 2017). Little information could be recognized about the role of (ADMA) in the diseases of airway inflammation. Increased ADMA levels have been validated in cystic fibrosis and in allergic asthma in both animal and human models (Carraro et al., 2013). ADMA is an L-arginine analogue that acts on inhibition of nitric oxide synthase enzyme (NOS). Previous research
revealed that higher levels of ADMA can additionally uncouple NOS activity, leading to a decrease in nitric oxide (NO) synthesis and accumulation of superoxide (O$_2^-$), which would result in higher levels of peroxynitrite (ONOO$^-$), a highly reactive nitrogen species that causes oxidative cell damage (Wells & Holian, 2007).

The pathological process of asthma is mainly caused by inflammation and oxidation products of NO, which are vital inflammatory mediators in allergies (Fitzpatrick et al., 2009). However, the role of NO in bronchial asthma may be controversial. NO may have beneficial effects on airway function as a bronchodilator and it acts as a neurotransmitter of bronchodilator nerves in human airways (Dupont et al., 2014). On the other hand, NO may amplifyasthmatic inflammation by generation of toxic hydroxyl radicals and it also acts as a vasodilator, increasing plasma exudation and airflow obstruction (Tajti et al., 2018).

Currently, a growing body of literature supports an association between serum vitamin D levels and asthma. The aim of this study was to investigate vitamin D in children having bronchial asthma and assess its potential relationship with specific markers that indicates asthma severity (forced vital capacity (FVC) forced expiratory volume in the first second (FEV1), and peak expiratory flow (PEF)), and also to test the hypothesis of ADMA increased levels in asthma and ADMA inhibition to NOS contributing to airways obstruction.

Moreover, as the previous studies showed that the natural amino acid and plasma L-arginine augment NO levels and this can decrease asthmatic exacerbations, so in the current study the bioavailability of plasma L-arginine was also evaluated.

2 Patients and methods

A total of 40 children were registered in this study (30 asthmatic and 10 healthy) with an age range between 5 and 15 years. Patients with at least a 2-year record of allergic bronchial asthma had been selected from a pediatric outpatient clinic at Ain Shams University Hospital. Informed consents were taken from parents of all patients included in this study. This study meets the ethical principles of the Declaration of Helsinki in 1964 and the World Medical Association’s medical research ethics for human subjects in 2013.

2.1. Subjects’ selection

Patients selected those who were diagnosed as having mild or moderate persistent asthma. The diagnosis of bronchial asthma was performed by a pediatric respiratory doctor and based completely on traditional signs, symptoms (cough, shortness of breath, repetitive wheezing, and chest stiffness) and laboratory tests in accordance with the new Egyptian recommendation of examination and administration of bronchial asthma.

2.2. Subjects excluded

The following patients were excluded: patients with asthmatic crisis during the last month before conduction of the study, upper respiratory tract infections within the last three weeks, the presence of diseases linked to immune system disturbance, renal or hepatic diseases, and malabsorption syndrome.

2.3. Investigations performed

The following investigations were done:

- Routine biochemical tests: (liver function tests, renal function tests) the plasma levels of alanine transaminase (ALT) and aspartate transaminase (AST) were determined by automatic biochemical analyzer (Cobas 6000, Roche). Also, serum urea and creatinine were measured using Cobas Mira machine.
- The hemoglobin (Hb) parameters and total white blood cell (WBC) count were determined by Sysmex hematology analyzer, KX–21 (Sysmex Corporation, Japan). From the same sample, thin blood films were prepared for the assessment of differential count. Differential leukocyte count was done by examination of thin blood films stained with Wright stain under oil immersion objective. Absolute eosinophil count was measured using hemocytometer/Neubauer’s chamber.
- Measurement of serum vitamin D concentration (measured as 25-hydroxyvitamin D) was once carried out by the use of a radioimmunoassay strategy using the Diasorin RIA (DiasorinInc, Stillwater, MN) by means of Quest Laboratories that take an interest within the vitamin D outside Quality Appraisal Conspire (DEQAS)). Values have been categorized as adequate (>30 ng/mL), inadequate (20–30 ng/mL), and poor (<20 ng/mL) based on past suggestions (Searing et al., 2010).
- Spirometry for baseline functional respiratory tests: dynamic spirometry (Master screen Pneumo, Erich Jaeger GmbH, Germany) was performed to all patients and the healthy controls. The following data were obtained: FVC (liter), FEV1 (liter), FEV1%, and peak expiratory flow (PEF). For every parameter obtained, the actual and predicted values for age, sex, weight, and percentage (%) were calculated by converting the spirometer reading to a percentage of what would be predicted as normal based on several personal factors. The highest values of three forced expiratory maneuvers were estimated. The ratio FEV1/FVC is a measure of airflow obstruction. These measurements were performed according to the standards
of the European Respiratory Society and the American Thoracic Society (Miller et al., 2005).

- Serum nitric oxide (as nitrite) was evaluated using Nitric Oxide Assay kit (Colorimetric) (ab65328), based completely on colorimetric determination of NO at 540 nm (Miranda et al., 2001).
- Plasma L-arginine level by high-performance liquid chromatography (HPLC): serum concentration of arginine was determined by liquid chromatography–tandem mass spectrometry of the butyl esters on an Applied Biosystems 3200 Q-Trap instrument (Applied Biosystems, Scoresby, Victoria).

2.4. Statistical analysis

The measurements were presented as mean ±SD. The t-test was utilized to distinguish between two groups, and P < 0.05 was estimated to be as statistically significant. A one-way analysis of variance (ANOVA test) was utilized to distinguish between multiple groups by the usage of F-ratio. Pearson and Spearman’s relationship tests were used to correlate between each parameter and within the same group to distinguish between positive and negative relationships and to locate significant differences.

3. Results

Patients and controls were age and sex matched with nonsignificant weight difference. Regarding pulmonic parameters function (FEV1, FVC, and PEF), there were significant differences among the two groups. According to the hematological parameters, hemoglobin and total leucocyte counts show nonsignificant difference between two groups; however, absolute and relative counts of eosinophils were significantly high in asthmatic patients (Table 1). Similarly, for the liver and renal functions, there were nonsignificant differences between the two groups.

Children with asthma showed decreased levels of serum 25-OH(D), serum NO, and plasma L-arginine as compared to healthy children. The mean serum ADMA was significantly higher in asthmatic patients group compared to control group Table 2.

Figures (1) and & Figure (2) show the relationships between serum 25-OH(D), ADMA, and FEV1 (% predicted) in asthmatic children. There were positive correlations between serum vitamin D concentration and FEV1 (% predicted). Furthermore, there were negative correlations between serum ADMA concentration and FEV1 (% predicted).

Figure (3) shows negative correlations between serum ADMA concentration and serum NO.

4. Discussion

The results of this study showed that vitamin D levels in the asthmatic children were significantly less than levels in healthy children (P < 0.001), as shown in Table 2. Deficiency of vitamin D level eventually increases the incidence of asthma as it decreases the inhibitory influence, which causes over production of Th2-type cytokines and disproportion of Th1/Th2 cytokines (Tamašauskiene et al., 2015).

In addition, optimal levels of vitamin D can diminish the proliferation and division of the cells of the smooth muscles, which lead to diminished flow of air and obstruction of small airways (Liu et al., 2019). Hence, it is hypothesized that the deficiency of vitamin D levels can increase children’s susceptibility to airway infection, resulting in upper respiratory tract infection and encourage attacks of asthma (Bener et al., 2014).

The results of this study were in agreement with the studies done by Kang et al. (2018), who showed that vitamin D levels in asthmatic children were essentially less than those in ordinary children, their study suggested that vitamin D supplementation is an essential one in all of the adjuvant therapy to avoid and treat children’s asthma. On the other hand, Ganji et al. (2020) found that serum 25(OH)D and the frequency of asthma are not related to each other in both multivariate-adjusted and unadjusted models. Additionally, a recent study done by Von Mutius & Martinez (2020) stated that the incidence of children’s asthma and their persistent wheeze are not affected by vitamin D supplementation to their mothers during pregnancy.

Table 1. Anthropometric measures, pulmonary function parameters, and hematological parameters in asthmatic and control groups.

|                         | Asthmatic (n = 20) | Control (n = 10) | P  |
|-------------------------|--------------------|-----------------|----|
| Age (years)             | 9 ± 3.6            | 9 ± 2           | NS |
| Sex (m/f)               | 13/7               | 6/4             | NS |
| Wt (kg)                 | 37.5 ± 15.6        | 35.9 ± 14.5     | NS |
| FEV1 (% predicted)      | 72.96 ± 2.78       | 106.4 ± 9.7     | <0.01|
| FVC (% predicted)       | 75.4 ± 3.8         | 99.6 ± 11.5     | <0.01|
| PEF (% predicted)       | 73.3 ± 2.9         | 101.7 ± 10.4    | <0.01|
| Total leucocyte count   | 9.36 ± 2.51        | 8.02 ± 1.30     | NS |
| (10^9/ L)               |                    |                 |    |
| Eosinophils             | 0.76 ± 0.27        | 0.42 ± 0.15     | <0.01|
| Hemoglobin (g/dL)       | 10.46 ± 3.14       | 11.65 ± 3.18    | NS |
| Eosinophils (%)         | 9.5 ± 3.4          | 2.7 ± 0.3       | <0.01|

Table 2. The laboratory findings in asthmatic patients and control groups.

|                     | Asthmatic (n = 30) | Control (n = 10) | P  |
|---------------------|--------------------|-----------------|----|
| 25-OH(D) (ng/ml)    | 15.26 ± 12.03      | 29.07 ± 20.67   | <0.001|
| ADMA (Umol/L)       | 2.28 ± 0.35        | 0.52 ± 0.5      | 0.006 |
| NO (Umol/L)         | 28.34 ± 3.75       | 49.87 ± 8.44    | <0.001|
| Plasma L-arginine   | 53.7 ± 9.8         | 72.2 ± 12.3     | <0.001|
| Creatinine(mg/dL)   | 0.66 ± 0.24        | 0.45 ± 0.25     | NS  |
| Urea (mg/dL)        | 28 ± 6             | 26 ± 7          | NS  |
| ALT (IU/L)          | 18 ± 4.5           | 19 ± 3.5        | NS  |
| AST (IU/L)          | 29 ± 5.6           | 28 ± 6.1        | NS  |
Additionally, the results of the current study showed that there was a direct correlation between vitamin D levels and pulmonary functions of the lung, which come in agreement with the study done by Sakr et al. (2020) who concluded that there was significant association between vitamin D levels and pulmonary function tests.

This study revealed that there is statistically significant increase of serum ADMA ($P = 0.006$), which was reported previously by Carraro et al. (2013), who evaluated the levels of ADMA in both serum and exhaled breath condensation, and proved its role in different pathological processes of bronchial asthma such as (airway irritation, oxidative stress, bronchial hyperresponsiveness, and collagen deposition). El-Alameey et al. in (2017) found that serum concentrations of oxidizing agent markers such as ADMA and malondialdehyde were increased in patients complaining of asthma, while an antioxidant marker such as paraoxonase was mainly reduced in comparison with controls. Moreover, Ito et al. (2018) found that irrespective to weight, the concentrations of ADMA

**Figure 1.** There was a positive correlation between serum levels of 25-OH(D) and forced expiratory volume in the first and the second (FEV1) (% predicted) in the whole data set ($n = 30$).

**Figure 2.** There was a negative correlation between forced expiratory volume in the first and the second (FEV1) (% predicted) and asymmetric dimethylarginine (ADMA) serum levels in the whole data set ($n = 30$).
are more prominent in both asthmatic grown-ups and children in comparison with their respective controls, and plasma L-arginine levels are lower in healthy obese subjects. Therefore, it is not surprising that the convergence of these two chronic diseases lowers the L-arginine/ADMA balance, uncoupling airway epithelial NOS (Winnica et al., 2017).

Inflammation has an effective role in the pathogenesis of asthma. Abundant inflammation can be ascribed to helper T cell type 2 cytokine actuation, and its levels are connected to the seriousness of the illness (Chung & Barnes, 1999). One of the mediators of inflammation that has a significant consideration in bronchial asthma is NO, which is created by NOS from arginine. NO could be a basic vasodilator of the bronchi, with both bronchodilatory and anti-inflammatory effects (Morris et al., 2004). The NO can be a messenger particle that is vital for the upkeep of healthy lung and normal airway function. Enzymatic transformation of L-arginine to L-citrulline is catalyzed by (NOS) producing NO.

Increased expression of inducible NOS in airway inflammation is assumed to raise the breathed out NO levels that can be found in asthmatic patients (Scott et al., 2013). Until now, few studies have assessed levels of plasma arginase or arginine, the substrate for NO formation in patients with asthma (Benson et al., 2011), as asthma seemed to be a condition of decreased NO bioavailability, rather than its formation as a result of irritation (Morris et al., 2004).

In Table (2), there is a critical decrease in plasma L-arginine levels ($P < 0.001$) in asthmatic children, with the reduction of arginine substrate, which may be explained by the increased need of NO in asthmatic patients to keep the dilatation of the bronchi at its basal bronchial tone, compensating the increased demand of NO in the course of oxidative stress. Our findings were in agreement with the explanation of Holguin et al. (2019), who stated that in order to have typical levels of nitric oxide, it is pivotal to preserve an adjustment between arginine and ADMA. For people with adult-onset asthma and asthma with overweight, having lower levels of arginine and higher levels of ADMA essentially bring down their NO levels, which sequentially causes more wheezing, shortness of breath, and other different asthma symptoms. This finding is promising because it proposes that expanding arginine might establish NO levels. This would have an effective positive impact on aviation profession and asthma symptoms (Holguin et al., 2019). Regarding respiratory function parameters, there were significant differences between the two groups of children ($<0.01$) as shown in Table (1); in addition, there was a negative correlation between serum ADMA levels and the severity of airway obstruction as indicated by respiratory function tests shown in Figure (2). Lau et al. (2013) stated that high ADMA serum levels have been found to be in close relation with the deterioration of FEV1% predicted, FVC% predicted, and with the seriousness of symptoms in asthma and obesity.

5. Conclusion
Vitamin D deficiency is found more often in children with bronchial asthma, and because of the limited

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Figure 3. There was a negative correlation between asymmetric dimethylarginine (ADMA) serum levels and nitric oxide (NO) serum levels in the whole data set ($n = 30$).
data, an ideal cutoff dose for 25-di-hydroxyvitamin D in bronchial asthma has not been established. In a view of ADMA, it suppresses the formation of NO in asthma airways and subsequently it has a physiological importance to improve the airways hyper-responsiveness. Therefore, ADMA might be a good target for novel plans sketched out to treat oxidative stress in asthma. Elevated ADMA concentration in asthma competes with substrate for NOS action, thus increasing L-arginine bioavailability for NOS and could improve pulmonary functions in asthma patients.

A lot of comprehensive randomized medical trials with extensive follow-up are required to substantiate the outcomes. Repetitive assessment of vitamin D in asthmatic children is encouraged; meanwhile, more studies are required to assess the effect of vitamin-D supplementation on bronchial asthma in children.

Disclosure statement
No potential conflict of interest was reported by the author(s).

References
Andújar-Espinosa, R., Salinero-González, L., Illán-Gómez, F., Castilla-Martínez, M., Hu-Yang, C., & Ruiz-López, F. J. (2020). Effect of vitamin D supplementation on asthma control in patients with vitamin D deficiency: The ACVID randomised clinical trial. Thorax Published Online First: 05 November 2020. https://doi.org/10.1136/thoraxjnl-2019-213936

Babar, M. Z. M., Hussain, M., & Majeed, S. A. (2017). Vitamin D supplementation improves FEV1 in patients of Bronchial Asthma. Pakistan Journal of Medical Sciences, 33, 1144–1147. https://doi.org/10.12669/pjms.335.12990

Benner, A., Ehlayel, M. S., Bener, H. Z., & Hamid, Q. (2014). The impact of Vitamin D deficiency on asthma, allergic rhinitis and wheezing in children: An emerging public health problem. Journal of Family & Community Medicine, 21, 154–161. https://doi.org/10.4103/2230-8229.142967

Benson, R. C., Hardy, K. A., & Morris, C. R. (2011). Arginase and arginine dysregulation in Asthma. Journal of Allergy, 2011, 12. https://doi.org/10.1155/2011/736319

Boulet, L. P., FitzGerald, J. M., & Reddel, H. K. (2015). The revised 2014 GINA strategy report: Opportunities for change. Current Opinion in Pulmonary Medicine, 21(1), 1–7.

Carraro, S., Giordano, G., Piacentini, G., Kantar, A., Moser, S., Cesca, L., Beradi, M., Gangi,L., Baraldi,E. (2013). Asymmetric dimethylarginine in exhaled breath condensate and serum of children with asthma. Chest, 144(2), 405–410. https://doi.org/10.1378/chest.12-2379

Chung, K. F., & Barnes, P. J. (1999). Cytokines in asthma. Thorax, 54,825–857. http://dx.doi.org/10.1136/thx.54.9.825

Dupont, L. L., Gynlos, C., Bracke, K. R., Brouckaert, P., & Brusselle, G. G. (2014). Role of the nitric oxide–soluble guanylyl cyclase pathway in obstructive airway diseases. Pulm Pharmacol Ther, 29(1), 1–6. https://doi.org/10.1016/j.pupt.2014.07.004

Einisman, H., Reyes, M. L., Angulo, J., Cerda, J., López-Larra, M., & Castro-Rodriguez, J. A. (2015). Vitamin D levels and vitamin D receptor gene polymorphisms in asthmatic children: A case-control study. Pediatric Allergy and Immunology : Official Publication of the European Society of Pediatric Allergy and Immunology, 26, 545–550. https://doi.org/10.1111/pai.12409

El-Alameey, I. R., Fathy, G., Shady, M. A., Ali, A., Fathy, H., Younis, E., & Nasr, S. (2017). Relationship of oxidant and antioxidant markers to asthma severity in Egyptian asthmatic children. Open Access Macedonian Journal Medical Science, 5 (5), 645–650.

Fitzpatrick, A. M., Brown, L. S., Holguin, F., & Teague, W. G. (2009). National institute of health/National heart, lung, and blood institute severe asthma research program. Levels of nitric oxide oxidation products are increased in the epithelial lining fluid of children with persistent asthma. The Journal of Allergy and Clinical Immunology, 124, 990–996. https://doi.org/10.1016/j.jaci.2009.08.039

Ganji, V., Al-obahi, A., Yusuf, S., Dookhy, Z., & Shi, Z. (2020). Serum vitamin D is associated with improved lung function markers but not with prevalence of asthma, emphysema, and chronic bronchitis. Scientific Reports, 10, 67967. https://doi.org/10.1038/s41598-020-67967-7

Grant, W. B., Boucher, B. J., Bhattoa, H. P., & Lahore, H. (2018). Why vitamin D clinical trials should be based on 25-hydroxyvitamin D concentrations. The Journal of Steroid Biochemistry and Molecular Biology, 177, 266–269. https://doi.org/10.1016/j.jsbmb.2017.08.009

Holguin, F., Grasemann, H., Sharma, S., Winnica, D., Wasil, K., Smith, V., Cruse, M. H., Perez, N., Coleman, E., Siclla, T. J., & Que, L. G. (2019). L-Citrulline increases nitric oxide and improves control in obese asthmatics. JCI Insights, 4(24), e131733. https://doi.org/10.1111/jacrjcmconference.2019.199.1_MeetingAbstracts.A7088

Ito, T., Kubo, M., Nagaoka, K., Funakubo, N., Setiawan, H., Takemoto, K., Eguchi, E., Fujikura, Y., & Ogino, K. (2018). Early obesity leads to increases in hepatic arginase I and related systemic changes in nitric oxide and L-arginine metabolism in mice. Journal of Physiology and Biochemistry, 74(1), 9–16.

Kang, Q., Zhang, X., Liu, S., & Huang, F. (2018). Correlation between the vitamin D levels and asthma attacks in children: Evaluation of the effects of combination therapy of atomization inhalation of budesonide, albuterol and vitamin D supplementation on asthmatic patients. EXPERIMENTAL AND THERAPEUTIC MEDICINE, 15, 727–732. https://doi.org/10.3892/etm.2017.7543

Lau, E. M. T., Morgan, P. E., Belousova, E. G., Toelle, B. G., Ayer, J. G., Celermajer, D. S., & Marks, G. B. (2013). Asymmetric dimethylarginine and asthma: Results from the Childhood Asthma Prevention Study. European Respiratory Journal, 41, 1234–1237. https://doi.org/10.1183/09031936.00162212

Liu, J., Dong, Y., Yin, J., Yao, J., Shen, J., Cheng, G., Li, K., Lv, H., Fang, X., & Wu, W. (2019). Meta-analysis of vitamin D and lung function in patients with asthma. Respiratory Research, 20,161. https://doi.org/10.1186/s12931-019-1072-4

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., & Coates, A. (2005). Standardisation of spirometry. European Respiratory Journal, 26, 319–338. https://doi.org/10.1183/09031936.05.00034805

Miranda, K. M., Espey, M. G., & Wink, D. A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide - Biology and Chemistry, 5, 62–71. https://doi.org/10.1006/niox.2000.0319

Morris, C. R., Poljakovic, M., Lavrishia, L, Machado, L, Kuyers, F. A., & Morris, S. M., Jr. (2004). Decreased arginine bioavailability and
increased serum arginase activity in asthma. *American Journal of Respiratory and Critical Care Medicine*, 152(2), 148–153. https://doi.org/10.1164/rccm.200309-1304OC

Parva, N. R., Tadeipalli, S., Singh, P., Qian, A., Joshi, R., Kandala, H., Nookala, V. K., & Cheriyath, P. (2018). Prevalence of vitamin D deficiency and associated risk factors in the US Population (2011–2012). *Cureus*, 10(6), e2741. https://doi.org/10.7759/cureus.2741

Pfeffer, P. E., & Hawrylowicz, C. M. (2018). Vitamin D in Asthma: Mechanisms of action and considerations for clinical trials. *Chest*, 153(5), 1229–1239. https://doi.org/10.1016/j.chest.2017.09.005

Postma, D. S. A., & Timens, W. (2006). Remodeling in asthma and chronic Obstructive pulmonary disease. *Proceedings of the American Thoracic Society*, 3, 434–439. https://doi.org/10.1513/pats.200601-006AW

Sakr, M., Elsamnod, Y. M., Elrifai, A. W., Abd-Al-Samee, H. S., & Saad, A. A. (2020). Correlation between Vitamin-D level and pulmonary function tests in children with bronchial asthma. *International Journal of Medical Arts*, 2(1), 308–312. https://doi.org/10.21608/ijma.2020.14621.1018

Scott, J. A., & Grasemann, H. (2013). Asymmetric dimethylarginine: A disease marker for asthma? *Chest*, 144(2), 367–368. https://doi.org/10.1378/chest.13-0480

Searing, D. A., Zhang, Y., Murphy, J. R., Hauk, P. J., Goleva, E., & Leung, D. Y. (2010). Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *Journal of Allergy and Clinical Immunology*, 125(5), 995–1000. https://doi.org/10.1016/j.jaci.2010.03.008

Tajti, G., Papp, C., Kardos, L., Keki, S., Pak, K., Szilasi, M., Gesztelyi, R., Mikaczo, A., Fodor, A., Szilasi, M., & Zsuga, J. (2018). Positive correlation of airway resistance and serum asymmetric dimethylarginine (ADMA) in bronchial asthma patients lacking evidence for systemic inflammation. *Allergy, Asthma & Clinical Immunology*, 14(2), 2–12. https://doi.org/10.1186/s13223-017-0226-5

Tamašauskienė, L., Gasiūnienė, E., Lavinskiene, S., Sakalauskas, R., & Šitkauskienė, B. (2015). Evaluation of vitamin D levels in allergic and non-allergic asthma. *Medicina (Kaunas)*, 51, 321–327. https://doi.org/10.1016/j.medici.2015.11.003

Von Mutius, E., & Martinez, F. D. (2020). Vitamin D supplementation during pregnancy and the prevention of childhood asthma. *N. Engl. J. Med.*, 382, 574–575. https://doi.org/10.1056/NEJMe1915082

Wells, S. M., & Holian, A. (2007). Asymmetric dimethylarginine induces oxidative and nitrosative stress in murine lung epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, 36(5), 520–528. https://doi.org/10.1165/rcmb.2006-0302SM

Winnica, D., Holguin, F., Wenzel, S. E., Freeman, B., Holguin, F., Wenzel, S. E., Freeman, B., & Holguin, F. (2017). l-citrulline prevents asymmetric dimethylarginine-mediated reductions in nitric oxide and nitrosative stress in primary human airway epithelial cells. *Clinical Experimental Allergy*, 47, 190–199. https://doi.org/10.1111/cea.12802

Yin, G. Q., Jiang, W. H., Wu, P. Q., He, C. H., Chen, R. S., & Deng, L. (2016). Clinical evaluation of sublingual administration of dust mite drops in the treatment of allergic asthma and allergic rhinitis of children. *European Review for Medical and Pharmacological Sciences*, 20, 4348–4353.