Association of Serum Vitamin D Levels, IL-17a Levels with Disease Severity among 3- to 18-year-old Children with Asthma in the Philippine General Hospital

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ABSTRACT

Background. Previous studies show that Vitamin D has an inverse relationship with asthma severity, symptoms, exacerbations, medication usage, and a direct relationship with lung function. IL-17A was found to be increased in asthmatics, which was inhibited by Vitamin D. Associations found between vitamin D, IL-17A, and asthma may support the future role of vitamin D in the treatment of asthma in children.

Objective. To compare vitamin D and IL-17A levels between children with and without asthma and determine their association with asthma severity

Study Design. Cross-sectional study

Methods. There were 44 participants, aged 3 to 18 years: 22 with asthma (12 non-severe, 10 severe) and 22 without asthma. Participants with any disease-altering vitamin D metabolism, intake of vitamin D supplementation, and recent infection were excluded. Serum vitamin D and IL-17A levels were measured in all participants.

Results. There was no significant difference in mean vitamin D levels between participants with asthma (29.6 ± 12.6 ng/mL) and without asthma (27.6 ± 9.5 ng/mL) (p = 0.55) as well as between participants with non-severe asthma (29.8 ± 14.0 ng/mL) and severe asthma (29.4 ± 11.5 ng/mL) (p = 0.95). The overall prevalence of hypovitaminosis D (< 30ng/mL) is 61.4%; 59.1% among those with asthma and 63.6% without asthma. The prevalence of vitamin D insufficiency and/or deficiency did not significantly differ between those with and without asthma (all p-value > 0.05); prevalence ratios were: 1.05 for vitamin D insufficiency, 0.58 for vitamin deficiency, and 0.92 for vitamin D insufficiency and deficiency combined. There was also no significant difference in the prevalence of vitamin D insufficiency and/or deficiency between severe and non-severe asthma (all p-values > 0.05), with prevalence ratios: 0.74 for vitamin D insufficiency, 0.50 for vitamin D deficiency, and 0.75 for vitamin D insufficiency and deficiency combined. Serum IL-17A levels were below the minimum detectable levels in 96% of the participants using the MILLIPLEX Map Human TH17 Magnetic Band Panel; hence, could not be analyzed.

Conclusion. The mean serum vitamin D levels do not differ between children with asthma and healthy controls. There was no significant relationship between mean vitamin D levels and asthma severity. There was no association between the prevalence of vitamin D insufficiency and/or deficiency and asthma and its severity. The overall prevalence of hypovitaminosis D in this study is 61.4%. Serum IL-17A levels were undetectable in 96% of the study population.

Keywords: asthma, vitamin D, interleukin-17A, IL-17A

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways associated with airway hyperresponsiveness leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, usually at night or in the early morning.[1] According to the World Health Organization (WHO), asthma affected an estimated 262 million people in 2019.[2] It is the most common chronic disease in children. In the Philippines, the prevalence of asthma among Filipino children was reported between 9.2% to 27.4% based on at least four previous surveys.[3] In the Philippine General Hospital (PGH) Pediatric Asthma Clinic, there were 372 new cases of asthma diagnosed from 2007 to 2012.

Asthma prevalence has been increasing in both developed and developing countries. Although it is known that genetic predisposition, early allergen exposure, infections, diet, tobacco smoke exposure, and pollution are significant risk factors associated with the development and severity of asthma, recent evidence suggests that Vitamin D deficiency may be a factor predisposing to asthma.[4,5] Based on epidemiological evidence, there is a worldwide epidemic of vitamin D deficiency and that it has been linked to increased incidence and severity of asthma in children.[5]

Serum 25[OH]D is the best indicator of overall vitamin D status because this represents the total vitamin D from dietary intake, sun exposure, and the converted form from adipose stores in the liver. There is no known consensus as to the optimal level of serum 25[OH]D, but most experts use the following definition of serum 25[OH]D levels: (1) *vitamin D sufficiency* – more than or equal to 30 ng/mL (75 nmol/L) (2) *vitamin D insufficiency* – between 21-29 ng/mL (52.5-72.5 nmol/L); (3) *vitamin D deficiency* – less than or equal to 20 ng/mL (50 nmol/L). Risk factors for vitamin D deficiency/ insufficiency include lack of sunlight exposure (very little vitamin D is produced in areas beyond a latitude of 35 from October to March), non-white ethnicity (pigmented skin), concealed clothing, use of sunscreen, obesity, elderly, institutionalized individuals, multiple and short spaced pregnancies, liver disease, malabsorption, short bowel, and drugs (such as rifampicin, glucocorticoids, anticonvulsants).[4]

Children with asthma appear to be at increased risk of vitamin D deficiency or insufficiency. The complex role of vitamin D in the immune system has led to speculations on its potential role in asthma. Vitamin D is known to inhibit inflammation by maintaining regulatory T cells, and by direct induction of innate microbial mechanisms. It has also been associated with asthma based on its correlations with lung function, markers of inflammation, and modulation in response to steroids. Based on studies, low serum vitamin D levels in asthmatic children are associated with more asthma symptoms, exacerbations, reduced lung function, increased medication usage, and severe disease.[4]

T-helper2(Th2)-dependent allergic airway inflammation appears to be the main pathophysiologic pathway of asthma.[6] Th2-type cytokines, such as interleukin-4 (IL-4), IL-5, and IL-13, are thought to drive the disease pathology in patients.[7] The enhanced activity of Th2 cells in asthma stimulates IgE production and brings about eosinophilic airway inflammation and airway hyperresponsiveness.[4] Th2 asthma is generally easier to control since it usually responds to the standard asthma treatment.[6] Despite this, uncontrolled and severe asthma and high exacerbation rates persist even with the use of available asthma regimens. Recent evidence suggests that other T cells, such as T-helper17 (Th17) cells, may also be involved in asthma.[7] Th17 cells are proposed to play a role in asthma, including its severe and steroid-resistant forms.[8,9] IL-17A, a key pro-inflammatory cytokine of the Th17 pathway, may be involved in the neutrophilic inflammation and airway remodeling processes in severe asthma.[6]

In the study by Nanzer et al. (2013), it was found that patients with severe asthma exhibit increased levels of Th17 cytokines, which are not inhibited by steroids. When 1,25(OH)2D3 was placed in the culture, Th17 cytokine production was inhibited, irrespective of their clinical responsiveness to steroids. The role of vitamin D in inhibiting Th17 cytokines found in patients with asthma may demonstrate novel steroid-enhancing properties of vitamin D that may allow future advances in asthma management.[9]

With the emerging role of vitamin D and Th17 cytokines in asthma, it is important to evaluate the levels of vitamin D and IL-17A, and their relationship with disease severity in children with asthma to be able to assess the diagnostic and therapeutic implications of vitamin D in asthma management. If indeed, associations between vitamin D levels, IL-17A levels, and asthma severity are established, the possibility of vitamin D as a future treatment for asthma may be investigated.

The cause of asthma is complex. It involves interactions between genetic and environmental factors such as air pollution, tobacco smoke exposure, diet, allergen exposure, and infections. The impaired immunogenic tolerance and the relationship between cells and inflammatory mediators may further increase the airway obstruction in asthma.[10]

Vitamin D is believed to have a regulatory effect on the immune system; hence, having a potential role in allergic diseases. Vitamin D has been shown to have a role in the innate and adaptive immune systems that may help control the severity of asthma exacerbations. The airway epithelia have increased levels of the enzyme that converts 25-OH-vitamin D_3 to its active form, 1,25-OH-vitamin D_3. The active form of vitamin D decreases the inflammation brought about by the infections. The possible positive effect of Vitamin D on the adaptive immune system is through its effects on the Th1, Th2, and T-regulatory cells.[11] Other target cells of vitamin D in relation to asthma, include: Th17 cells, B cells, dendritic cells, lung bronchial smooth muscle cells, and mast cells. It specifically acts on Th17 cells by inhibiting the release of Th17 cytokines.[4] Gluco-

corticosteroids, the principal controller therapy for asthma, works by increasing the regulatory T cells and IL-10 synthesis and 1,25-dihydroxyvitamin D_3 can enhance their induction.[12]

Several studies demonstrated the relationship of vitamin D insufficiency or deficiency with asthma, and its inverse association with exacerbations, symptoms, and disease severity. In the study of Bener et al. (2012) in Qatar, serum vitamin D levels in children below 16 years of age were obtained from 483 children with asthma and 483 healthy controls. Results showed that children with asthma had significantly reduced serum vitamin D levels. Among all patients with asthma, 68.1% were noted to be vitamin D-deficient, while 36.1% of non-asthmatics were vitamin D-deficient.[5] In a cross-sectional study conducted by Al Zayadneh et al. (2020) in Al-afrak Governmental Hospital in Southern Jordan, it was found that out of 98 children with asthma, 25-OH vitamin D levels were deficient in 41.8% and insufficient in 34.7%. Only 23.5% had sufficient 25-OH vitamin D levels. There was a significant correlation found between the severity of asthma symptoms and 25-OH vitamin deficiency.[10] Brehm et al. (2010) examined the relationship between serum 25-hydroxy vitamin D3 and markers of allergy and asthma severity in a cross-sectional study on 616 Costa Rican children between 6 and 14 years of age. It was found that 28% of children with asthma had insufficient levels of vitamin D and that vitamin D levels were significantly and inversely associated with total IgE and eosinophil count. Results suggest that vitamin D insufficiency is relatively frequent in an equatorial population of children with asthma. Lower vitamin D levels were associated with increased markers of allergy and asthma severity.[13] In contrast to these findings, a study by Thuesen et al. (2015) on 4,999 Danish adults concluded that 25(OH)D levels showed no significant associations with atopy and asthma.[14]

The relationship between serum vitamin D levels, pulmonary function, and asthma control was investigated by Chinellato et al. (2011) among 75 Italian children. Results showed that 9.4% had normal vitamin D levels, 37.3% had insufficient vitamin D levels, and 53.3% were vitamin D-deficient. There was a positive association between vitamin D and asthma control. Lower vitamin D levels were associated with reduced asthma control and pulmonary function.[15] Similarly, in a cross-sectional study conducted by Alyasin et al. (2011), serum 25-hydroxyvitamin D3 level was obtained from 50 children with asthma and 50 healthy controls aged 6 to 18 years. It was found that serum 25-hydroxyvitamin D3 levels were inversely associated with asthma, and there was a direct and significant relationship between vitamin D levels and pulmonary function test results in asthmatic children.[16]

Searing et al. (2010) investigated the association between serum vitamin D levels and corticosteroid use in 100 children with asthma. It was found that 47% of subjects had insufficient vitamin D levels and 17% were vitamin D-deficient. FEV1 percent predicted and FEV1/FVC ratio showed a significant correlation with vitamin D levels. The use of inhaled steroids, oral steroids, and total steroid dose similarly showed significant inverse correlations with vitamin D levels. This shows that corticosteroid use and worsening airflow limitation are associated with lower vitamin D levels in asthmatic patients. Vitamin D enhances glucocorticoid action in the peripheral blood mononuclear cells (PBMCs) from asthmatic patients and enhances the immunosuppressive function of dexamethasone in vitro.[17] This may indicate the potential role of vitamin D in the severe forms of asthma, including steroid-resistant ones.

Asthma is mainly considered a Th2 cell-associated inflammatory disease. Although atopic asthma has an important Th2 cell component, the disease is extremely varied. Recent evidence suggests that other T cells, such as the subset of Th17 cells, may also contribute to the development of asthma.[7] Th17 cells is proposed to have a role in the severe and steroid-resistant forms of asthma.[8,9] Th17 cells were detected within the lung tissue of severe asthmatics and secrete IL-17A, IL-17F, IL-21, and IL-10, from which IL-17A is of major importance in mild to severe asthma. IL-17A, a key pro-inflammatory cytokine of the Th17 pathway, promotes inflammation by inducing proinflammatory cytokines and chemokines, recruiting neutrophils, enhancing antibody production, and activating T cells.[18] It may be involved in the neutrophilic inflammation and airway remodeling processes in severe asthma.[6]

In the study by Nanzer et al. (2013), asthmatic patients were found to synthesize much higher levels of IL-17A and IL-22 than non-asthmatic control subjects, with steroid-resistant asthma expressing the highest levels of IL-17A. This study included 10 healthy non-asthmatics and 28 patients with moderate to severe asthma who were treated for 6 months. Among the asthmatics, 18 were non-responsive, while 10 were responsive to steroids. It was found that glucocorticoids did not inhibit IL-17A cytokine expression in patients and enhanced production in cultures from control subjects. Treatment with 1,25(OH)2D3, however, with or without dexamethasone, significantly reduced both IL-17A and IL-22 levels. Patients with severe asthma showed increased levels of Th17 cytokines that were not inhibited by steroids but were inhibited by 1,25(OH)2D3 in all patients irrespective of their clinical response to steroids. This indicates that vitamin D might have novel steroid-enhancing properties that may be explored for the management of asthma in children.[9]

The roles of IL-17A and vitamin D in asthma should further be studied. Vitamin D may play an important part in asthma among children because of its known effect on the immune system, lung function, airway remodeling, asthma control, and steroid resistance. This study aimed to determine and relate vitamin D levels and IL-17A levels with asthma severity, among 3- to 18-year-old children with asthma.

MATERIALS AND METHODS

Subjects

This study was performed among 3-to-18-year-old children of the University of the Philippines - Philippine General Hospital (UP-PGH) Department of Pediatrics, who consulted at the outpatient clinics from October to November 2013. A total of 44 children were included, 22 with asthma and 22 without asthma.

Patients with asthma were those with physician-diagnosed asthma based on the Global Initiative for Asthma (GINA) guidelines, who were not in acute asthma exacerbation and with good compliance to appropriate medication. Excluded from the study were patients with asthma with a history of (1) intake of vitamin D supplement or any drugs that modulate the serum vitamin D level (such as rifampicin, systemic glucocorticosteroids, anticonvulsants), (2) malabsorption or short bowel syndrome, (3) liver disease, (4) other chronic diseases, (5) obesity, and (6) other chronic pulmonary conditions. Patients with an acute respiratory infection or any systemic infection were also excluded.

The control group consisted of healthy controls without asthma with no history of (1) allergic disorders, (2) atopy in the first-degree relatives, (3) previous asthma diagnosis, (4) usage of any inhaler or asthmatic medications, (5) liver disease, (6) intake of vitamin D supplements or any drugs that modulate vitamin D levels, (7) malabsorption or short bowel syndrome, (8) other chronic diseases, and (9) obesity. Those with an acute respiratory infection or any systemic infection were also excluded.

Sample Size

A sample of 19 patients with asthma and 19 without asthma would satisfy the minimum requirement based on sample size estimation using the mean differences of vitamin D among those with and without asthma. A mean vitamin D level of 26.8 ng/mL among patients without asthma, and a mean vitamin D level of 17.2 among those with asthma were used based on the study by Bener et al. (2012).[4] This would have 80% power ($\beta = 0.20$) to detect the estimated differences in mean vitamin D levels between those with and without asthma. The sample size of 3 per group for the severe versus non-severe asthma groups was computed using the mean vitamin D level of 17.0 ng/mL in non-severe cases and mean vitamin D level of 11.2 ng/mL in severe cases, based on the study by Gupta et al.[19] Ideally, the asthmatic sample should contain similar numbers of severe and non-severe asthmatics. To achieve this, the sample size was increased to 20 with asthma and 20 without asthma, divided into 10 non-severe and 10 severe asthma. This would have more than adequate power at acceptable reliability levels to detect mean differences in vitamin D levels between severe and non-severe asthma.

Data Collection

After a thorough explanation of the objectives, procedure, risks, and benefits of the study by the principal investigator, and upon signing of the informed or assent consent form by the participant or his/her guardian, eligible participants were classified into three groups based on the severity of disease: (1) control (healthy, non-asthmatics), (2) non-severe asthma (intermittent or mild persistent asthma classification based on GINA guidelines), and (3) severe asthma (moderate or severe persistent asthma classification based on GINA guidelines).

All asthmatic patients initially underwent a short interview regarding their asthma history (such as a family history of atopy, history of allergy, use of anti-inflammatory drugs, hospitalization in the previous year, and unscheduled visits in the previous year). Then, an asthma control test was completed by the patients or by the parents or guardians (for younger patients). For patients 4-11 years old, the 7-item childhood asthma control test was used. The first 4 questions were answered by the child using pictures to score how he/she feels about his/her asthma and asthma symptoms. The last 3 questions were answered by the parents/ guardian regarding the asthma symptoms of the child. For patients >12 years old, the asthma control test, consisting of 5 questions, was used. Questions on asthma symptoms, activity limitation, and asthma medication use for the past four weeks were included.

Each participant underwent a single measurement of serum 25-hydroxyvitamin D and serum IL-17A. Following the standard procedure for blood extraction, a total of 5-6 mL of peripheral blood was obtained, centrifuged, and divided into aliquots. Serum samples were frozen to a minimum of -30-degree Celsius storage. The serum vitamin D levels were processed at the St. Luke's Medical Center Extension Clinic Laboratory by a one-step polyclonal chemiluminescent microparticle immunoassay using the Abbott Architect 25-OH Vitamin D Assay Reagent Kit, while the serum IL-17A levels were processed at the Unilab Research Laboratory using the MILLIPLEX Map Human Th17 Magnetic Band Panel.

Operational Definitions

- Non-severe Asthma: intermittent and mild persistent asthma based on GINA guidelines.
- Severe Asthma: moderate persistent and severe persistent asthma based on the GINA guidelines.
- Vitamin D status: based on serum levels of 25-hydroxyvitamin D.
- Vitamin D Sufficiency: serum 25-hydroxyvitamin D levels more than or equal to 30 ng/mL (75 nmol/L).
- Vitamin D Insufficiency: serum 25-hydroxyvitamin D levels between 21 to 29 ng/mL (52.5 to 72.5 nmol/L).
- Vitamin D deficiency: serum 25-hydroxyvitamin D levels less than or equal to 20 ng/mL (50 nmol/L).
- Hypovitaminosis D: Vitamin D level below 30 ng/mL (Vitamin D insufficiency and deficiency combined).

Statistical Analysis

Measures of central tendency and variation were used to describe quantitative variables, and frequency distributions were used for categorical variables. Independent t-test was used to compare the mean vitamin D levels between participants (1) with asthma versus without asthma, and (2) with non-severe vs. severe asthma. Prevalence ratios were computed to describe the relationship between (1) vitamin D deficiency and asthma and (2) vitamin D deficiency and asthma severity; Fisher's exact test was used to determine the significance of the prevalence ratios. Statistical analyses utilized STATA Version 6.0 Software. All inferential analyses were performed at a 5% level of significance ($\alpha = 0.5$). The relationship between vitamin D and IL-17A levels and the relationship between IL-17A levels and asthma severity could not be determined statistically since almost all the IL-17A levels obtained were undetectable.

RESULTS

Clinico-demographic Profile

Forty-four participants were included in the study, 22 with asthma and 22 without asthma. Among the participants with asthma, 12 had non-severe asthma and 10 had severe asthma. Table 1 presents the demographic profile of these participants. None of the patients were obese.

The clinical profile of the participants with asthma is presented in Table 2. All of those with non-severe asthma had a family history of asthma, compared to only 60% of those with severe asthma. Among all the asthmatics, 82% (18 out of 22) had a family history of asthma. The majority (66%) of those with non-severe asthma was controlled versus only 40% of those with severe asthma. Mean daytime symptoms per week were numerically close for both groups (0.6 ± 2.0 for non-severe asthma and 0.5 ± 1.0 for severe asthma). On the other hand, the number of nighttime symptoms was numerically higher among severe asthma (1.6 ± 2.9) compared to non-severe asthma (0.3 ± 0.6). The number of hospital admissions due to asthma is also numerically higher among the group with severe asthma (1.0 ± 1.15) compared to non-severe asthma (0.3 ± 0.5). The same is true with the number of attacks in the previous year (5.9 ± 5.9 for severe asthma vs. 3.3 ± 3.8 for non-severe asthma) and the use of systemic steroids in the previous year (1.1 ± 1.7 for severe asthma vs. 0.2 ± 0.4 for non-severe asthma).

Comparison of Vitamin D Levels

The mean vitamin D levels among participants with asthma was 29.6 ± 12.6 ng/mL with a range of 4.8 – 56.6 ng/mL. For those without asthma, it was 27.6 ± 9.5 ng/mL, ranging from 8.4 - 45.5 ng/mL. There is no significant difference in mean vitamin D levels between participants with and without asthma ($p = 0.546$).

Comparing Vitamin D levels in terms of severity of asthma, participants with non-severe asthma had levels of 29.8 ± 14.0 ng/mL, ranging from 9.2–56.6 ng/mL, while those with severe asthma had levels of 29.4 ± 11.5 ng/mL with a range of 4.8–45.8 ng/mL. There was no significant difference between the two groups ($p = 0.947$).

Comparison of Prevalence of Vitamin D Insufficiency/Deficiency

The prevalence ratio is a measure of association in cross-sectional studies. This measures how the prevalence of asthma differs in those with vitamin D insufficiency and/or deficiency as compared to those whose vitamin D levels are sufficient. A prevalence ratio greater than 1 in the context of

Table 1. Demographic profile of 3- to 18-year-old patients with and without asthma at the UP-PGH

Characteristics	Number (%) or value		
	Without asthma (n = 22)	Non-severe asthma (n = 12)	Severe asthma (n = 10)
Age (years)			
Mean ± SD[1]	11.7 ± 4.1	9.7 ± 3.7	11.0 ± 4.1
Range	4 – 18	4 – 15	4 – 18
Gender			
Female	15 (68)	9 (75)	6 (60)
Male	7 (32)	3 (25)	4 (40)
Body Mass Index (kg/m^2)			
Mean ± SD	16.3 ± 2.0	16.1 ± 3.4	18.0 ± 6.0
Range	13.8 – 20.3	11.7 – 22.4	12.7 – 28.4

Table 2. Clinical profile of 3–18-year-old patients with severe and non-severe asthma seen at the UP-PGH

Characteristics	Group	
	Non-severe asthmatics (n = 12)	Severe asthmatics (n = 10)
Family history of asthma		
Positive	12 (100)	6 (60)
Negative	0	4 (40)
Asthma control		
Controlled	8 (66)	4 (40)
Partially controlled	2 (17)	4 (40)
Uncontrolled	2 (17)	2 (20)
Number of daytime symptoms per week		
Mean ± SD	0.6 ± 2.0	0.5 ± 1.0
Range	0 – 7	0 – 3
Number of night-time symptoms per week		
Mean ± SD	0.3 ± 0.6	1.6 ± 2.9
Range	0 – 2	0 – 7
Number of hospital admissions		
Mean ± SD	0.3 ± 0.5	1.0 ± 1.15
Range	0 – 1	0 – 4
Number of attacks in the previous year		
Mean ± SD	3.3 ± 3.8	5.9 ± 5.9
Range	0 – 12	1 – 19
Use of systemic steroids in the previous year		
Mean ± SD	0.2 ± 0.4	1.1 ± 1.7
Range	0 – 1	0 – 5

Table 3. Comparison of prevalence of vitamin D insufficiency/deficiency among 3- to 18-year-olds with and without asthma seen at the UP-PGH

Vitamin D status	Group		Prevalence ratio	p-value*
	With asthma	Without Asthma		
Sufficient† (≥30ng/mL)	9	8		
Insufficient (21–29 ng/mL)	10	8	1.05	0.88
Deficient (≤20 ng/mL)	3	6	0.58	0.43††
"Not-sufficient" (<30 ng/mL)	13	14	0.92	0.76

**Chi-square test used, except otherwise stated; †Used as comparison group; ††Fisher's exact test used*

Table 4. Comparison of prevalence of vitamin D insufficiency/deficiency among 3- to 18-year-old patients seen at the UP-PGH based on the severity of asthma

Vitamin D status	Group		Prevalence ratio	p-value*
	Non-severe asthmatics	Severe asthmatics		
Sufficient† (≥30 ng/mL)	4	5		
Insufficient (21–29 ng/mL)	6	4	0.74	0.66
Deficient (≤20 ng/mL)	2	1	0.50	1.00
Not sufficient (<30 ng/mL)	8	5	0.75	0.67

**Fisher's exact test used; †Used as a comparison group*

this study, means that the prevalence of asthma is greater in those with vitamin D insufficiency and/or deficiency, while a prevalence ratio less than 1 means that the prevalence of asthma is greater in those whose vitamin D levels are sufficient.

The prevalence of vitamin D insufficiency and/or deficiency among asthmatics compared to non-asthmatics is presented in Table 3 using participants with sufficient vitamin D as the comparison group. The prevalence ratio is 1.05 for vitamin D insufficiency, 0.58 for vitamin D deficiency, and 0.92 for vitamin D insufficiency and deficiency combined. There is no significant difference in the prevalence of vitamin D insufficiency and/or deficiency between groups with and without asthma (all p-values > 0.05). When looking at the prevalence ratios, the only group that had a substantial risk for asthma is those with Vitamin D insufficiency (level of Vitamin D at 21–29 ng/mL). However, even this association is not statistically significant.

The prevalence of vitamin D insufficiency and/or deficiency between those with non-severe asthma compared to severe asthma is presented in Table 4 using participants with sufficient vitamin D as the comparison group. The prevalence ratio is 0.74 for vitamin D insufficiency, 0.50 for vitamin D deficiency, and 0.75 for vitamin D insufficiency and deficiency combined. The prevalence ratios are all less than 1, however, these ratios are not statistically significant. There is no significant difference in the prevalence of vitamin D insufficiency and/or deficiency between severe and non-severe asthma.

The prevalence of "sufficient" or "not-sufficient" vitamin D status among participants with and without asthma is presented in Table 5. Although numerically, the prevalence of the not-sufficient status (insufficiency/deficiency) is higher than the sufficient status in both groups (59.1% vs. 40.9% among those with asthma; 63.6% vs. 36.4% among those without asthma), the 95% confidence intervals (CI) of these prevalence measures overlap in both groups. Hence, it could not be concluded with 95% certainty that the prevalence of the non-sufficient status is higher than that of the sufficient status. Moreover, the 95% CIs are quite wide suggesting that a bigger sample may help define if there are actual differences in the said prevalence values.

In this study, the overall prevalence of hypovitaminosis D (vitamin D insufficiency and deficiency) among all participants is 61.4%; 59.1% among participants with asthma, and 63.6% without asthma.

IL-17A Levels

Most of the participants (96%) had undetectable levels of IL-17A (Table 6). These were in two separate runs – one with a minimum detectable IL-17A level of 6.77 pg/mL (73%) and the other with a minimum detectable IL-17A level of 7.37 pg/mL. Only 2 participants (4%) had detectable IL-17A, one with a level of 7.09 pg/mL from the first run. This participant had severe asthma with a sufficient vitamin D level of 44.6 ng/mL. The other had an IL-17A level of 16.91 pg/mL from the second run. This participant had no asthma and with a sufficient vitamin D level of 40.4 ng/mL. Due to the inadequate quantitative data (only two have actual values) correlation analysis was not feasible between vitamin D levels and IL-17A levels. Similarly, independent t-tests to compare mean IL-17A levels among participants with severe and non-severe asthma were not possible. The only way the IL-17A data could be categorized was as <7.37 pg/mL (98%) vs. 16.91 pg/mL (2%), which is not advisable to analyze inferentially due to the minimal variability.

Table 5. Prevalence (95% CI) of vitamin D status among 3- to 18-year-old patients with or without asthma seen at the UP-PGH

Vitamin D status	With Asthma		Asthmatics	
	Frequency	Prevalence % (95% CI*)	Frequency	Prevalence % (95% CI*)
Sufficient[3] (≥30 ng/mL)	9	40.9 (20.7, 63.6)	8	36.4 (17.2, 59.3)
Not sufficient (<30 ng/mL)	13	59.1 (36.4, 79.3)	14	63.6 (40.7, 82.8)
Total	22		22	

**CI, Confidence Interval*

Table 6. IL-17 levels of 3- to 18-year-old patients with and without asthma seen at the UP-PGH

IL-17 level (pg/mL)	Frequency (%)	Remarks
Undetectable		Those with < 6.77 and < 7.37 belong to different test runs.
<6.77	32 (73)	The machine determines the minimum detectable amounts
<7.37	10 (23)	in the runs as 6.77 and 7.37 pg/mL, respectively.
Detectable		Severe asthma with sufficient vitamin D (44.6 ng/mL)
7.09	1 (2)	Non-asthma with sufficient vitamin D (40.4 ng/mL)
16.91	1 (2)	

DISCUSSION

The mean serum vitamin D levels between participants with and without asthma, and between non-severe and severe asthma showed no significant difference. This is not consistent with most of the previously reviewed studies, except for the study by Thuessen et al. (2015) among adult patients, which similarly showed no significant association between 24(OH)D levels and asthma.[14]

The prevalence of vitamin D insufficiency and/or deficiency did not differ in patients with asthma compared to those without asthma, as well as between non-severe and severe asthma. The single vitamin D measurement obtained in the study may influence such results, since it may have not been sufficient to establish the long-term vitamin D status in the studied population.[20] Follow-up studies may be needed to consider changes in the vitamin D status, in relation to changes in lifestyle and behavioral practices, such as clothing, indoor or outdoor activities, use of sunscreen, and diet, within a longer interval. Genetic polymorphisms of vitamin D receptors and the enzymes for the metabolism of vitamin D precursors may also be considered in explaining the inconsistent results of this study with the previous studies. Differences in ethnicity, race, or culture among the different populations involved in the previous studies may also influence the differences in the vitamin D levels obtained.

The overall prevalence of vitamin D insufficiency and deficiency among participants is 61.4%, with a prevalence of 59.1% among participants with asthma and 63.6% among those without asthma. The decreased vitamin D levels among participants may be influenced by factors such as skin pigmentation, behavioral practices (sunscreen use, use of concealed clothing, decreased time outdoors), or low vitamin D in the diet. In addition, since most of the participants lived within or near the National Capital Region, urban lifestyle practices such as prolonged indoor stay and use of protective gears, may have influenced the vitamin D results in this study. In a review done by Arabi et al. (2010) on the hypovitaminosis D status of developing countries, the prevalence of hypovitaminosis D in children in East Asia and Pacific countries were: 50% among pre-school and 32% among 9-to-11-year-old children in Mongolia; 3–29% among 12-24 months age group and 7–45% among 12- to 14-year old children in China.[21] The overall prevalence of hypovitaminosis D of 61.4% among the participants in this study is considerably higher compared to the prevalence reported in children from nearby developing countries, though this finding may not be generalizable to the study population due to the large and overlapping 95% CIs of the prevalence measured. A population-based study with a larger sample size may be recommended for further assessment of hypovitaminosis D among Filipino children.

Serum IL-17A levels using the MILLIPLEX Map Human Th17 Magnetic Band Panel were not able to detect blood concentrations of participants below its threshold level. The inability to detect IL-17A under 6.77 pg/mL or under 7.37 pg/mL is a machine-limitation. Future studies may explore other methods that can analyze lesser quantities of IL-17A. It is not unlikely that IL-17A levels under these values are present among those with and without asthma. For example, in a study in Saudi Arabia consisting of 100 asthma patients and 102 ethnically-matched controls, the mean IL-17A levels obtained were 2.242 pg/mL among controls and 2.752 pg/mL among those with asthma (p = 60.096), measured in plasma using the enzyme-linked immunoabsorbent assay.[22]

We can infer that the IL-17A levels of the participants had higher than minimum detectable concentrations depending on the asthma status of the patient at the time of testing. If the population examined was in acute exacerbation, was not maintained on any asthma medications

(such as inhaled corticosteroids), or was documented to be steroid-resistant, IL-17A levels could be more detectable. Differences in asthma phenotypes of the patients compared to other study populations may also influence the response of IL-17A. Since 82% (18 out of the 22) of the patients with asthma in this study had a family history of asthma, a Th2 asthma phenotype was most likely involved, and thus, may explain the low (undetectable) IL-17A levels. The steroid responsiveness of the participants may have also influenced the results.

There was no relationship between vitamin D and IL-17A levels between participants with and without asthma. Based on the findings of this study, vitamin D may not be a significant factor in the development of asthma and its severity among Filipino children with asthma. Further investigation regarding IL-17A levels among asthma belonging to specific phenotypes is recommended.

CONCLUSION

The mean serum vitamin D levels do not differ between patients with asthma and healthy controls. There was no significant relationship between mean vitamin D levels and severity of asthma disease. The prevalence of vitamin D insufficiency and/or deficiency among those with and without asthma and between non-severe and severe asthma was not significantly different. There was no correlation found between vitamin D insufficiency and/ or deficiency and asthma and its severity. The prevalence of hypovitaminosis D among all participants is 61.4%. Serum IL-17A levels were undetectable in 96% of the study population using the MILLIPLEX Map Human TH17 Magnetic Band Panel.

Recommendations

In future studies, a long-term vitamin D status instead of a single vitamin D measurement is suggested to be able to incorporate the changes in lifestyle and behavioral practices of the participants that may influence vitamin D levels.

A genetic study on vitamin D receptors and enzymes responsible for the metabolism of vitamin D precursors may also be done to determine possible differences in vitamin D response.

Levels of vitamin D in association with IL-17A may be examined in patients with asthma with ongoing airway inflammation such as those who are in acute exacerbation, those who are not maintained on any asthma medications, or those who are unresponsive to steroids.

IL-17A levels may also be obtained in a subset of patients with asthma who belong to the non-Th2 asthma phenotype or the steroid-resistant patients. Allergy tests may be done to properly evaluate the specific asthma phenotype of the patients.

Aside from the MILLIPLEX Map Human Th17 Magnetic Band Panel, other methods of cytokine analysis may also be explored that may have higher sensitivities in detecting IL-17A levels in children. Use of other specimens, aside from serum, may also be studied. Other novel cytokines that may be associated with asthma in children may further be investigated, which may be helpful in future therapeutic advancements, as well.

A population-based study with a larger sample size is recommended to obtain the hypovitaminosis status of the pediatric population in the Philippines.

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Statement of Authorship

Both authors contributed in the conceptualization of work, acquisition and analysis of data, drafting and revising and approved the final version submitted.

Author Disclosure

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