

Association of Serum Vitamin D Levels with Serum Pro-Inflammatory Mediators in Asthmatic Children

SAIMA PERVAIZ¹, MUHAMMAD BILAL FAROOQ², TAHIR SHAHEEN³, NIDA JAVED⁴, AFIFA JAVAID⁵, ASIFA AZHAR⁶

^{1,2}Senior Demonstrator, Pathology Department, Azra Naheed Medical College, Superior University Lahore.

³Senior Registrar, Endocrinology Department, Children's Hospital and The Institute of Child Health, Lahore.

⁴Senior Demonstrator Pathology, Al-Aleem Medical College Lahore.

⁵Forensic Medicine (continued), Demonstrator Forensic Medicine, CMH, Bahawalpur.

⁶WMO, Tehsil Headquarter, Sharaqpur Shareef.

Correspondence to Dr. Saima Pervaiz, H. 616, Shaheen Block, Bahria Town Lahore. Email, saimapz@hotmail.com, Cell: +92 331 4541646

ABSTRACT

Aim: To determine the frequency of vitamin D levels in asthmatic versus health controls and to determine the association of vitamin D levels with serum concentration of pro-inflammatory cytokines in asthma children.

Methods: In this case control study, a total number of 30 children presenting with asthma having age 5-14 years who were diagnosed of having asthma were included in this study. A similar number of healthy controls were also recruited. Venous blood samples were taken for each children and were analyzed for serum vitamin D levels and pro-inflammatory cytokines (IL-6 and IL-17) levels.

Results: There were 17(56.6%) children in asthma group who were having deficient vit. D levels, while there were only 01 (3.33%) children in control group having deficient vit. D levels (p-value <0.0001). Mean IL-17 levels were 18.7 ± 3.21 pg/ml in asthma patients versus 10.4 ± 3.3 in healthy controls (p-value 0.001). Mean IL-6 levels were 14.76 ± 4.03 pg/ml in asthma group versus 2.75 ± 0.98 pg/ml in control group (p-value <0.001). There was a significantly negative correlation between Vit. D levels and IL-17 in asthmatic patients ($r = -6.28$, p-value 0.001). We found a weak inverse correlation between the serum Vit. D and pro-inflammatory cytokines.

Conclusion: In our study, we found significant reduction of vitamin D levels in asthma patients. We also found significant correlation of vitamin D levels with inflammatory mediators in asthma patients.

Keywords: Asthma, vitamin D, pro-inflammatory cytokines.

INTRODUCTION

According to estimates, nearly 300 million people around the globe suffer from asthma, in recent decades, asthma has become more prevalent among adult population.¹ Out of 250 deaths one death is thought to be due to asthma, it is the 26th cause of death in South Asian population.^{1,2} The true prevalence of asthma is difficult to determine because of differences in evaluation and diagnosis of asthma in A total number of 30 children presenting with asthma having age 5-14 years who were diagnosed of having asthma according to the Global Initiative for Asthma (GINA) guidelines were included. The study was conducted in a Jinnah Hospital Lahore from Jan-2018 to April-2018. Severity of asthma was assessed before starting treatment. All children were taking routine steroids for asthma treatment. A similar number of healthy controls who presented in pediatric emergency having extra-respiratory symptoms were also included. Children suffering from any chronic disorders were excluded.

Determination of Vitamin D and Immune Cells: Venous blood samples were taken for each children and were analyzed for serum vit. D levels. Serum vitamin D levels were measured using radioimmunoassay kit. (Dia-Sorin, Stillwater, MN). Vitamin D levels were categorized into three categories; sufficient (≥ 30 ng/ml), insufficient (20-30 ng/ml) and deficient (≤ 20 ng/ml)¹².

Pro-inflammatory cytokines such as IL-6 and IL-17 levels were measured using ELIZA method.

Data Analysis: Data was analyzed using SPSS v23. Mann-Whitney u-test was used to compare study parameters between the asthmatic and non-asthmatic children. Chi-square test was used to compare vit. D

levels between the groups. Pearson correlation was used to determine the correlation of vitamin D levels with Pro-inflammatory cytokines.

RESULTS

Baseline data of patients who presented with asthma is given in table 1. There were 70% male children who presented with asthma. Mean age of children was 9.7 years while it was 9.9 years in health controls. Severity of asthma was mild in 17(56.7%) children, and moderate in 12(40%) children.

There was significant difference in vit. D levels in asthma and control group. There were 17 (56.6%) children in asthma group who were having deficient vit. D levels, while there were only 01 (3.33%) children in control group having deficient vit. D levels (p-value <0.0001) (Table 2).

Table 1: Baseline data of asthma patients.

Total number	30
Male Gender	21 (70%)
Age	9.7 (range 5-15)
FVC (%)	87.02 (56-113)
FEV ₁ (%)	93.2 (54.122)
FEV ₁ /FVC	0.89 (0.78-1.0)
Severity of Asthma	
Mild Persistent	17 (56.7%)
Moderate Persistent	12 (40.0%)
Severe Persistent	01 (3.33%)

There were higher serum levels of IL-17 and IL-6 in asthma patients as compared to controls. Mean IL-17 levels were 18.7 ± 3.21 pg/ml in asthma patients versus 10.4 ± 3.3 in healthy controls (p-value 0.001). Mean IL-6 levels were

14.76±4.03 pg/ml in asthma group versus 2.75±0.98 pg/ml in control group (p-value <0.001) [Table 3].

On Pearson's correlation analysis, there was a significantly negative correlation between Vit. D levels and IL-17 in asthmatic patients ($r = -6.28$, p-value 0.001). We found a weak inverse correlation between the serum Vit. D and pro-inflammatory cytokines ($r = -0.076$, p-value 0.61).

Table 2: Comparison of Vitamin D levels between the groups.

	Asthma Pts	Control Group	P-value
Sufficient	06(20%)	23(76.67%)	<0.0001
Insufficient	07(23.3%)	06(20%)	
Deficient	17(56.6%)	03(3.33%)	

Table 3: Comparison of Cytokine Profiles in asthmatic and healthy children.

	Asthma Patients	Control Group	P-value
IL-17 (pg/ml)	18.7±3.21 (Range 9.65-22.46)	10.4±3.3 (Range 0.07-14.80)	0.001
IL-6 (pg/ml)	14.76±4.03 (Range 10.51-18.65)	2.75±0.98 (Range 0.02-8.44)	<0.001

DISCUSSION

In present study, we found significantly lower vit. D levels in asthma patients. Vit. D levels in asthma patients were significantly deficient as compared to control patients; 56.6% versus 3.33% respectively. The present study results are in accordance with the study of Chinellato et al. who found that vit. D deficiency is very common in asthma patients who are resident of Mediterranean countries and lower vit. D levels are associated with poor asthma control¹³. Brehm et al. also reported similar results, the authors reported higher frequency of vit. D deficiency in children having mild to moderate persistence asthma and is associated with higher risk of shifting to severe exacerbations in a 4 years follow-up¹⁴.

A recent study by Ojaimi et al. in an ex-vivo study, reported that vit. D has a significant role in innate immunity. And supplementation of vit. D can play a significant role in the management of various inflammatory disease¹⁵.

There are several studies who have found the correlation of vit. D levels with immune system malfunctioning^{16,17}. Several immune cells such as B & T lymphocytes dendritic cells and macrophages express vit. D receptors and their receptor expression is affected by deficiency in vit. D levels¹⁸.

It is hypothesized that variations in serum Vit. D levels may be responsible for inflammatory processes in asthma patients. Th1, ATh2, and Treg Acells have vit. D receptors (VDR), so these could be the main target of vit. D¹⁹. Moreover studies by Yu et al. and Maruotti et al. found that certain immune cells require expression of VDR receptors both intrinsically and extrinsically^{20,21}.

The correlation between vit. D and pro-inflammatory cytokines also raises the question regarding the origin of vit. D deficiency in asthma patients. There is a need to conduct more studies to clarify the pathophysiological mechanisms by which vit. D regulates immune system and to explore genetic factors influencing vit. D production

CONCLUSION

We found significant reduction of Vit D levels in asthma patients. We also found significant correlation of vit. D levels with inflammatory mediators in asthma patients.

REFERENCES

- Masoli M, Fabian D, Holt S, Beasley R, Program GfA. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004;59(5):469-78.
- Burney P, Jarvis D, Perez-Padilla R. The global burden of chronic respiratory disease in adults. *Int J Tuberc Lung Dis*. 2015;19(1):10-20.
- Sá-Sousa A, Jacinto T, Azevedo LF, Morais-Almeida M, Robalo-Cordeiro C, Bugalho-Almeida A, et al. Operational definitions of asthma in recent epidemiological studies are inconsistent. *Clin Trans Allergy*. 2014;4(1):24.
- Song W-J, Kang M-G, Chang Y-S, Cho S-H. Epidemiology of adult asthma in Asia: toward a better understanding. *Asia Pacific Allergy*. 2014;4(2):75-85.
- Razzaq S, Nafees AA, Rabbani U, Irfan M, Naeem S, Khan MA, et al. Epidemiology of asthma and associated factors in an urban Pakistani population: adult asthma study-Karachi. *BMC Pulm Med*. 2018;18(1):184-.
- Li F, Peng M, Jiang L, Sun Q, Zhang K, Lian F, et al. Vitamin D deficiency is associated with decreased lung function in Chinese adults with asthma. *Respiration*. 2011;81(6):469-75.
- Chinellato I, Piazza M, Sandri M, Peroni DG et al. Serum vitamin D levels and exercise-induced bronchoconstriction in children with asthma. *Eur Respir J*. 2011;37(6):1366-70.
- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-81.
- Hewison M. Vitamin D and the intracrinology of innate immunity. *Mol Cell Endocrinol*. 2010;321(2):103-11.
- Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. *Curr Allergy Asthma Rep*. 2011;11(1):29
- Hamzaoui A, Maalmi H, Berraïes A, Abid H. Transcriptional characteristics of CD4+ T cells in young asthmatic children: RORC and FOXP3 axis. *J Inflamm Res* 2011;4:139.
- Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006;84(1):18-28.
- Chinellato I, Piazza M, Sandri M, Peroni D, Piacentini G, Boner AL. Vitamin D serum levels and markers of asthma control in Italian children. *J Pediatr*. 2011;158(3):437-41.
- Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*. 2010;126(1):52-8. e5.
- Ojaimi S, Skinner NA, Strauss BJ, Sundararajan V, Woolley I, Visvanathan K. Vitamin D deficiency impacts on expression of toll-like receptor-2 and cytokine profile: a pilot study. *J Trans Med*. 2013;11(1):176.
- Zosky GR, Berry LJ, Elliot JG. Vitamin D deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med*. 2011;183(10):1336-43.
- Cannell J, Hollis B, Zasloff M, Heaney R. Diagnosis and treatment of vitamin D deficiency. *Expert Opin Pharmacother*. 2008;9(1):107-18.
- Maalmi H, Berraïes A, Tangour E, Ammar J et al. The impact of vitamin D deficiency on immune T cells in asthmatic children: a case-control study. *J Asthma Allergy*. 2012;5:11.
- Baeke F, Korf H, Overbergh L, van Etten E, Verstuyf A, Gysemans C, et al. Human T lymphocytes are direct targets of 1, 25-dihydroxyvitamin D3 in the immune system. *J Steroid Biochem Mol Biol*. 2010;121(1-2):221-7.
- Yu S, Cantorna MT. The vitamin D receptor is required for iNKT cell development. *Proceedings of the National Academy of Sciences*. 2008;105(13):5207-12.
- Aranow C. Vitamin D and the immune system. *J Investig Med*. 2011;59(6):881-6.