

Pharmacological effects of oral vitamin D intake on pulmonary function Test and Interleukin-17 in adult asthmatic patients in Karachi

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Abstract: Vitamin D is an anti-inflammatory and immuno-modulatory secosteroid. Previous studies showed strong link between childhood and adult onset asthma with vitamin D. Interleukin 17 is an inflammatory cytokine and plays a major role in the worsening of asthma. The aim of this study is to compare the effects of serum vitamin D on serum IL 17 and pulmonary function test (FVC, FEV₁, FEV₁/FVC) before and after oral vitamin D supplementation. Fifty severe asthmatic patients were selected from out patient department of Chest Medicine Ward, Jinnah post graduate medical center, Karachi. Spirometry was performed by vitalograph compact. Baseline values were as follows: serum vitamin D=13.19±2.37ng/ml, IL-17=20.70±2.13ng/ml, FVC=2.31±0.40L, FEV₁=1.40±0.28L, FEV₁/FVC=60.15±4.61%. Subjects were given 1000 IU of oral vitamin D capsule per day for six weeks. After this trial all values were found as serum vitamin D=19.03±1.26ng/ml (p<0.001), IL 17=15.40ng/ml (p<0.001), FVC=2.90±0.60 L (p<0.001), FEV₁=2.01±0.10L (p<0.001), FEV₁/FVC=63.79% ±1.45 (p<0.001). It may be concluded that improvement in serum vitamin D levels improves the status of lung functions, decreases the airway inflammation and hence may decrease the asthma severity.

Keywords: Inflammation, asthma, spirometry, PFTs, vitamin D.

INTRODUCTION

Asthma is an inflammatory condition in which bronchoconstriction causes dyspnea, dry or productive cough and wheeze like symptoms (Hall *et al.*, 2016). Worldwide, 334 million people are affected by this disease. In Pakistan, due to urbanization and lack of health facilities, 20% of children are affected by asthma (Sabar *et al.*, 2018). Globally in adults, prevalence of childhood asthma is increased upto 8.6%, while in adult asthmatics upto 9.7% (Ferrante *et al.*, 2018). For early diagnosis, FEV₁ is the best parameter to show the status of lung function. PFTs should be performed not only for diagnosis but to check the prognosis of patients after the commencement of treatment.

Vitamin D has a great influence on immune system of our body. This immune system plays an important role in the different inflammatory and autoimmune disorders. Previous studies showed that deficiency of Vitamin D is correlated with childhood and adult onset asthma. Besides its main function in the maintenance of calcium homeostasis, vitamin D has its immunomodulatory effects on T and B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, antibodies, dendritic cells and cytokines (Barragan *et al.*, 2015; Berraies *et al.*, 2014; (Mirzakhani *et al.*, 2015). Vitamin D has its anti inflammatory effect on Th 17 cells resulting in inhibition of IL17 secretion which has a major role in the development of severe adult types of asthma (Hatta and fellows (2017).

Due to these widespread beneficial effects of vitamin D on different immune cells we design an interventional study to observe the therapeutic effects of vitamin D in asthmatic adults.

MATERIALS AND METHODS

This interventional study was conducted in the Department of Physiology, University of Karachi from 2016-2019. Diagnosed asthmatic subjects were selected from Chest Medicine OPD of JPMC, Karachi. Study was approved by Board of Ethical Review of JPMC, Karachi, for the selection of adult asthmatic patients. Age of all asthmatic patients were from 20 to 60 years, including both males and females and identified according to GINA guideline (2019). Subjects with chronic pulmonary disorders, or taking drugs which may alter serum vitamin D levels (anti epileptic, systemic steroids) or pregnant women were excluded from study. Baseline serum vitamin D and IL 17 were checked. Likewise, baseline spirometry was performed to check pulmonary function test for the assessment of asthma severity. With the regular treatment of asthma, all patients were given 1000 IU/day of oral vitamin D capsule for six weeks. After that, serum levels of vitamin D, IL17 and pulmonary function test were reassessed.

Collection of blood sample and storage

Following all aseptic precautions, three ml of venous blood was collected from all subjects. Blood was taken into a plain tube and allowed to clot at 37⁰ centigrade for ten minutes. The clotted sample was centrifuged at 4000

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rpm for ten minutes. The serum was taken and kept in freezer at -70° centigrade.

Determination of serum vitamin D

Determination of 25 OH D in serum was done by ELISA kit. By incubating the 15ul of serum with chemical 1 and 2 first incubation was done by which the attached 25- OH D was free from VDBP. Specimen was again nursed with ruthenium label with VDBP by formation of compound with 25- OH D. micro particles which were dusted with Streptavidin and biotin labeled vitamin D were added, empty area of VDBP were engaged and making a complex of VDBP and biotin bound vitamin D. This compound was taken into cells of measurements. Here particles were caught by magnetic force on electrode. Final results were calculated by a calibration curve, specific to the machine.

Reference range

- Vitamin D deficient levels <10ng/ml
- Vitamin D insufficient levels (10 – 29ng/ml)
- Vitamin D sufficient levels \geq 30ng/ml

Spirometry

Pulmonary functions were performed by spirometer. First, flowhead was connected to the Vitalograph compact. Vitalograph was ON by connecting the plug to socket. A small button was ON, located near the lower part of the instrument. Data was taken by clicking the new subject icon appeared on screen. All the required information was filled according to the instructions appeared on screen. FVC button was pressed to perform a Single Breath Test. Deep inhalation was done as possible. Nose clip was applied and mouthpiece was inserted between teeth. lips were approximated to prevent air escaping. Air exhalation was completed as much as possible. The values of FVC and FEV1 were appeared on screen.

Determination of serum IL-17

IL-17 levels in serum was determined by Human IL-17 ELISA kit. Anti IL-17 antibody against IL 17 was treated to the wells. Antigen antibody reaction was taken place between anti IL 17 antibody and antigen present in serum. There was a particular time to incubate. Then, the amount of bounded antigen antibody was detached from unbound antigen through washing. By the addition of one more antibody having horseradish peroxidase enzyme, reaction was occurred and HRP-Antigen- Biotin complex was formed on wells. Again, there was a washing step to remove extra enzyme. Chromogen substrate was used to create blue color. The color was changed from blue to yellow by adding acid. The strength of color is related to the concentration of IL 17, which was determined by spectrophotometer. By the help of standard solutions a dose response curve was achieved.

Reference range 0.7-20 pg/mL

STATISTICAL ANALYSIS

Data was entered in SPSS 21. Mean values and SD were determined for continuous parameters. Frequency and percentages were determined for categorical parameters. Categorical parameters were analyzed by Chi square while continuous parameters by Independent sample T test. P value less than 0.05 was taken as to assess the significance.

RESULTS

Table 1 shows the descriptive information about asthmatic subjects. Age of these patients were 43.20 ± 10.94 , including 44% females and 56% males. 34% patients having positive family history of asthma and 58% had history of smoking. Out of fifty, 12% were vitamin D deficient and 88% were related to vitamin D insufficient category.

Table 2 describes the comparison of pulmonary functions before and after oral vitamin D supplementation. These lung functions included FVC, FEV₁ and FEV₁/FVC ratio. Values of all these variables were increased and showed statistically significant differences after intake of vitamin D ($p < 0.001$).

Table 3 shows comparison of serum vitamin D and IL 17 in asthmatic subjects before and after vitamin D intake. Mean vitamin D were 13.19 ± 2.37 ng/ml and 19.03 ± 1.26 ng/ml. Likewise mean IL-17 were 20.70 ± 2.13 pg/ml and 15.40 ± 0.06 pg/ml. Differences of both these serum parameters were statistically significant ($p < 0.001$).

DISCUSSION

The aim of this study is to find out the effects of oral vitamin D supplements on lung functions as well as on interleukin -17(IL-17) in diagnosed asthmatic patients aged between 20-60 years of either gender. We gave vitamin D capsule along with the standard treatment of asthma.

It was observed that most of the patients were smokers. Jones and colleagues (2016) in their study related the harmful effects of smoking in asthma. Smoking results in airway inflammation which causes resistance to corticosteroid therapy in these patients. Tamasauskiene and fellows (2015) noticed direct correlation between serum levels of vitamin D and FEV₁/FVC in smokers asthmatic patients.

Comparison of family history of asthma showed that most of the patients had positive history. Comberati and co workers (2017) noticed that among all risk factors, family history was the most important factor and it may help to identify individuals who were at high risk to develop asthma.

Table 1: Descriptive data of study participants

Variable		Asthmatic patient n =50 mean±SD
Age (years)		43.20±10.94
Gender		N (%)
	Female	22(44)
	male	28(56)
Family history of asthma	Yes	17(34)
	No	33(66)
Smoking	Yes	29(58)
	No	21(42)
Vitamin D deficient level (< 10ng/ml)		6(12)
Vitamin D Insufficient level(10-29ng/ml)		44(88)

Table 2: Comparison of pulmonary function test before and after vitamin D supplementation

Variables	Before vitamin D intake Mean ± SD	After vitamin D intake Mean ± SD	P -value
FVC(L)	2.31±0.40	2.90±0.60	<0.001
FEV1(L)	1.40±0.28	1.85±0.10	<0.001
FEV1/FVC (%)	60.60±4.61	63.79±1.45	<0.001

Table 3: Comparison of serum vitamin D and IL 17 before and after vitamin D supplementation

Variables	Before vitamin D intake Mean ± SD	After vitamin D intake Mean ± SD	P- value
Vitamin D Level (ng/ml)	13.19±2.37	19.03±1.26	<0.001
IL-17 (pg/ml)	20.70±2.13	15.40±0.06	<0.001

In this study first the baseline measurements of vitamin D, IL-17 and pulmonary function test including FEV1, FVC and FEV1/FVC% were performed. Then 1000 IU of vitamin D capsules once per day for six weeks was prescribed to all participants. All parameters were rechecked after six weeks of trial.

It was noticed that no subject in this study had sufficient levels of vitamin D. Riaz and his colleagues (2016) also found that vitamin D deficiency was common in our population in all ages and both in males and females. Less exposure to sunlight and lack of adequate amount of vitamin D intake are the main causes of vitamin D deficiency worldwide. It was also observed that there was a significant difference in serum levels of vitamin D before and after supplementation. Menon and colleagues (2014), also noticed the same result. Lack of Vitamin D results in aggravation of inflammation and upon exposure to any allergen causes release of inflammatory mediators and their recruitment towards site of inflammation (Tian and Cheng, 2017).

After six weeks of therapeutic intervention with vitamin D, decrease levels of IL-17 were estimated as compared to the levels before supplementation and this difference is statistically significant. Hatta and fellows (2017) showed in their study that Interleukin 17 had a strong correlation with asthma.

Our results were also consistent with Kupaev and Nurdina (2018). They also found inverse relationship between vitamin D and IL 17 in their asthmatics. Chronic history of asthma causes adverse structural changes in the airways. This is caused by continued inflammation which was followed by healing process of the airways (Fehrenbach *et al.*, 2017). Vitamin D affects on smooth muscles of airways and inhibits the production of factors causing remodelling and structural changes in bronchial smooth muscles. In this way improves lung functions (Berraies *et al.*, 2014).

Regarding pulmonary function test before and after vitamin D intake, these parameters were significantly higher after six weeks. These parameters were reflecting the functional status of lungs and their lower values shows air flow limitations and improper exhalation. Another study conducted in Turkey showed decrease FEV1 in asthmatic patients with low vitamin D (Beyhan-Sagmen *et al.*, 2017). They showed that decrease serum level of vitamin D were associated with uncontrolled asthma and poor lung function.

CONCLUSION

It may be concluded that improvement in serum vitamin D levels by giving supplements may improve the status of lung functions, decreases the airway inflammation by its anti-inflammatory property and hence may decrease the asthma severity.

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