VITAMIN- D, INTERLEUKINS, AND ASTHMA IN OBESE AND NON-OBESE PATIENTS

Kamel Abd Elgafar Hassan[1]; Atef W. Elrifai[1*]; Saad Eldeen Mohamed Elsheref [2]; Mahmoud Saad Berengy[2], Ahmed Salama Al-Adl[2]; Osama Abdelhamid Alian[3]; Alsaid A. Aboulmagd[4]; Hany Awadallah[5]; Sayed Abd-Elsabour Kinawy [6]; Eman M. Mahmoud[7]

Abstract

Background: Obesity is associated with bronchial asthma, and asthma greatly restricts the patient's activity, which predispose to obesity. However, the possible pathogenic mechanisms had not been fully understood. Vitamin-D deficiency is associated with obesity and asthma. However, its values in obesity-associated asthma did not fully explored. The current work designed to estimate the serum levels of vitamin-D, some interleukins in obese and non-obese subjects with and without asthma.

Methodology: Four groups of subjects (each included 65 subjects) were included. The first for healthy controls, the second for obese asthmatics (OA), the third for asthmatic non-obese (ANO) and the fourth for obese non-asthmatic (ONA). All were assessed clinically, then vitamin D and interleukins (IL-1β, IL-18, IL-17A, IL-6, IL-5, IL-4, IL-9 and IL-10) were measured in blood. Bronchoalveolar lavage (BAL) cellular count was determined and pulmonary function tests were completed. Then asthma was categorized according to its severity.

Results: The severe form of asthma was higher among OA than NOA (67.7% vs 30.8%). There was significant increase of cellular count in BAL of asthmatics (obese and non-obese) when compared to control group. Vitamin D was significantly reduced in OA, ANO and ONA than the control group (14.5±3.1, 18.0±3.7 and 19.89±2.9 compared to 21.8±3.0 ng/dl). IL-10 was significantly reduced, but other interleukins were increased in OA, ANO and ONA when compared to controls. BMI and asthma grade had significant proportionately correlation with asthma grade, cellular count in BAL and all interleukins expect IL-10 and respiratory functions (that showed inverse correlation). However, vitamin-D had negative correlation with cells in BAL, all interleukins except IL-10 who had positive correlations.

Conclusion: Bronchial asthma and obesity shares the low grade-inflammatory process and low vitamin D seems to play a pathogenic role in both conditions, and normal levels could protect against asthma and obesity.

Keywords: Cytokines; Interleukins; Vitamin-D; Body mass index; Respiratory Function Tests.

[1]Department of Chest Diseases, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.
[2]Department of Internal Medicine, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.
[3]Department of Internal Medicine, Assiut Faculty of Medicine, Al-Azhar University, Assiut, Egypt.
[4]Department of Internal Medicine, Mansoura Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[5]Department of Gastroenterology and Hepatology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.
[6]Department of Chest diseases, Faculty of Medicine, Aswan university, Egypt - Najran General Hospital, KSA.
[7]Department of Chest diseases, Faculty of Medicine, Port-Said University, Port-Said, Egypt.

*** Corresponding Author:** Atef W. Elrifai
***** Email: atefchest@gmail.com

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INTRODUCTION

Asthma is a common disease. It is characterized by airflow limitations and clinically presented by different symptoms. It is associated with a marked health burden all over the world **(1).**

Obesity increased worldwide and reached an epidemic. About 650 million adults or more are obese**.** Increased obesity presses on the healthcare resources with increased morbidity and mortality. Obesity had a harmful impact on the different pulmonary diseases **(2).**

Asthma associated with obesity is a significant challenge. It is usually associated with low response to steroids and other medications, poor control, frequent hospitalizations, and poor quality of life **(3-6).** In addition, obesity specific comorbid conditions (e.g., obstructive sleep apnea syndrome) is associated with worse outcome of asthma **(7).**

The effect of obesity on respiratory diseases is multifactorial. Obesity mechanically impairs wall dynamics, impair ventilation and increased airflow limitations. These are reflected by reduced FEV1 with airway narrowing **(8).** In addition, airway inflammation may play a role in asthma pathogenesis **(9,10)**. However, it had not been studied sufficiently.

Vitamin D deficiency relationship with asthma had been reported in previous studies. Its pathophysiological mechanisms include anti-inflammatory effects on chronic lung inflammation. In addition, lower levels of vitamin-D are linked to obesity. Thus, vitamin D deficiency may play a role in asthma and obesity **(11).**

Here, the current work aimed to estimate the values of serum vitamin D levels and different interleukins and its relation to the severity of asthma in obese and non-obese subjects.

Methodology

Study settings and duration:

The current work included four groups of patients (each 65 patients). The first included healthy subjects as a control group. The second included obese patients (BMI above 30 kg/m^2) with asthma (OA). The third included asthmatic non obese patients (ANO) and the fourth included obese non-asthmatic subjects (ONA).

The following criteria were used to include asthmatic patients: confirmed diagnosis of asthma through clinical history and either bronchodilator responsiveness (>12% and 200 mL improvement in forced expiratory volume in 1 s [FEV1] after 180 µg salbutamol metered-dose inhaler) or airway hyperresponsiveness (AHR) (PC_{20} methacholine < 8 mg/mL).

Current smokers or those who had received systemic corticosteroids (CS) for one month or longer before evaluation were excluded from the study.

Sampling:

Blood samples were collected by peripheral venipuncture from the antecubital or suitable vein. All samples collected on capillary tubes. Samples were centrifuged at 5000g/min for 10 minutes after coagulation. Serum was collected in Eppendorf tubes and stored at -80 ^{o}C till the time of analysis.

Estimation of serum levels of vitamin D3

The measurement of serum levels of vitamin D levels was performed by ELISA according to the manufacturer's regulations (Cusabio Biotech Co, Wuhan, China). Values were determined at the 450 nm by micro-reader.

Interleukins measurement

Amounts of different interleukins (IL-1β, IL-18, IL-17A, IL-6, IL-5, IL-4, IL-9 and IL-10) were measured by ELISA using R&D Systems (USA) for IL-1β and IL-18. However, cytometric bead array was used to determine other interleukins by a FACScalibur flow cytometer. All were performed according to manufacturer instructions.

Total and differential cell counts in BAL

The BAL was collected and saved as pellets. This pellet was resuspended by 200 mL of phosphate buffer solution (PBS). Then 50µl of cell suspension was used to count cells by a hemocytometer. Another 50 ml of suspension was further subjected to cytospin at 450 rpm for 5 min, followed by Diff-Quick staining to detect the inflammatory cells. A total of 300 cells were counted under microscopic examination. Cells were counted on the basis of morphological criteria and staining characteristics. These were eosinophils, neutrophils, macrophages, or lymphocytes.

Forced spirometry was performed according to American Thoracic Society/European Respiratory Society (ERS/ATS) standards **(12).**

Statistical analysis:

The collected data was coded and fed to a personal computer, running Microsoft Windows 10®. The statistical measurements and tests of comparison

were performed by the statistical package for social sciences, for windows, version 18 (IBM®SPSS®, USA). The quantitative data were presented by their mean and standard deviations as measurements of central tendency and dispersion, respectively. Otherwise, qualitative data were summarized by their frequency and percentages. Groups were compared by one-way analysis of variance, with post Hoc Least significant differences (LSD) for comparison between groups if data were quantitative and by Chi square if data were qualitative. Pearson's correlation coefficient (*r*) was calculated to determine bivariate correlations. P value < 0.05 was set as the indicator of significant differences.

RESULTS

In the current work, study groups were comparable regarding patient age and gender. The age ranged between 24 to 61 years, with female sex predominance among all groups. However, body mass index (BMI) was significantly higher among Obese asthmatic and obese non-asthmatic groups when compared to control and asthmatic non-obese groups. The severe asthma was significantly higher among obese asthmatic than non-obese asthmatic subjects (67.7% vs 30.8%, respectively). Finally, respiratory functions were significantly reduced in obese asthmatic than non-obese asthmatics, controls and obese-non asthmatic groups. In addition, a similar situation was observed when asthmatic non-obese patients were compared to obese non-asthmatic and control groups. Obese non-asthmatic group also showed significant reduction of respiratory function and significant increase of cellular count in BAL when compared to control group (Table 1).

Serum levels of vitamin D were significantly lower in obese asthmatic, non-obese asthmatic, and asthmatic non-obese groups than the control group. Values were much reduced in obese asthmatic than asthmatic non-obese and obese non-asthmatic groups. A similar situation was reported for interleukin10, where it was significantly reduced in OA than ANO, ONA and control groups. However, other interleukins (IL-1β, IL-18, IL-4, IL-5, IL-6, IL-9 and IL-17A) were significantly higher among OA than ANO, ONA and control groups (Table 2).

BMI showed proportionately significant correlation with asthma grade, different cells in BAL and all interleukins except IL-10, where there was a significant inverse correlation between BMI and IL-10. In addition, BMI was inversely correlated with respiratory function tests and vitamin-D concentrations. However, the correlation with age was non-significant. Asthma grade had similar correlations with other variables like BMI. However, the correlation with FVC % of predicted did not reach statistical significance. Otherwise, vitamin-D had negative, significant correlation with cellular count in BAL, all interleukins except IL-10 and respiratory functions (The correlations were positive). However, no significant correlation between vitamin D and patient age (Table 3).

Table (1): Comparison between study groups regarding patient demographics, respiratory functions and cellular count in bronchoalveolar lavage (BAL).

Variables	Measures	Control (n=65)	OA (n=65)	ANO (n=65)	ONA (n=65)	test	P
Age (years)	Mean±SD	50.9±7.5	51.7±6.1	52.3±5.2	51.4±5.8	0.58	0.63
	Min.-Max.	24-61	33-62	33-62	33-59		
Gender (n,%)	Male	22 (33.8%)	20 (30.8%)	19 (29.2%)	23 (35.4%)	0.70	0.87
	Female	43(66.2%)	45(69.2%)	46 (70.8%)	42(64.6%)		
BMI (kg/m^2)	Mean±SD	23.6±1.0	32.3±0.9	23.8±0.8	31.1±0.8	**1699**	**<0.001***
Asthma grade (n,%)	Mild	-	3(4.6%)	17(26.2%)	-	**20.97**	**<0.001***
	Moderate	-	18(27.7%)	28(43.1%)	-		
	Severe	-	44(67.7%)	20(30.8%)	-		
FVC% Predicted	Mean±SD	126.5±3.0	106.0±3.4	105.9±3.5	117.8±5.4	**414.9**	**<0.001***
FEV1 % predicted	Mean±SD	95.7±1.9	50.1±9.1	60.0±9.7	94.9±2.9	**768.3**	**<0.001***
FEV1/FVC	Mean±SD	75.7±2.3	47.3±8.5	56.7±9.3	80.7±4.1	**354.4**	**<0.001***
BAL Total Cells (x 10^4)	Mean±SD	10.0±2.3	**37.3±6.6**	*30.3±8.2*	18.0±2.2	**320.5**	**<0.001***
BAL Eosinophil %	Mean±SD	5.0±1.2	**18.0±2.9**	*14.7±3.8*	9.2±1.3	**331.4**	**<0.001***
BAL Neutrophil %	Mean±SD	11.1±2.4	**23.4±4.0**	*20.7±4.8*	13.6±1.9	**179.8**	**<0.001***

OA: Obese asthmatic; ANO: Asthmatic non-obese; ONA: Obese non-asthmatic

Table (2): Serum levels of vitamin D and different interleukins among study groups

Variables	Control (n=65)	OA (n=65)	ANO (n=65)	ONA (n=65)	test	P
Vitamin D(ng/ml)	21.8±3.0	14.5±3.1	18.0±3.7	19.89±2.9	62.49	<0.001*
IL-1β (ng/ml)	12.6±2.8	**89.9±22.7**	72.1±27.6	36.7±8.1	232.9	<0.001*
IL-18 (ng/ml)	41.6±9.3	**71.6±12.1**	57.3±17.8	56.7±7.0	64.57	<0.001*
IL-4 (ng/ml)	21.0±2.4	**60.6±14.6**	47.6±15.2	44.4±11.2	122.7	<0.001*
IL-5 (ng/ml)	115.0±12.6	**287.2±44.3**	281.9±64.3	163.9±12.4	301.51	<0.001*
IL-6(pg/ml)	30.8±4.8	**265.9±31.7**	153.4±73.3	158.4±27.5	335.5	<0.001*
IL-9(ng/ml)	3.6±1.0	**11.8±3.8**	6.2±4.1	3.9±0.9	112.3	<0.001*
IL-10 (pg/ml)	120.8±12.6	31.4±8.8	41.4±11.8	115.1±10.6	1186.0	<0.001*
IL-17A(pg/ml)	12.1±1.7	**47.2±7.7**	37.1±11.5	36.8±8.2	221.2	<0.001*

NB: Data are presented by mean ±Standard deviation (SD).

Table (3): Correlation between BMI, Asthma severity and vitamin-D with other variables

		BMI		Asthma grade		Vitamin D	
		r	p	r	p	r	p
Asthma grade		0.408**	<0.001*				
BAL	Total cells x10^4	0.391**	<0.001*	0.851**	<0.001*	-0.739**	<0.001*
	Eosinophils%	0.409**	<0.001*	0.840**	<0.001*	-0.731**	<0.001*
	Neutrophils%	0.287**	<0.001*	0.814**	<0.001*	-0.706**	<0.001*
IL-1β (pg/ml)		0.370**	<0.001*	0.869**	<0.001*	-0.775**	<0.001*
IL-10 (pg/ml)		-0.179**	0.004*	-0.960**	<0.001*	0.649**	<0.001*
IL-6 (pg/ml)		0.681**	<0.001*	0.845**	<0.001*	-0.734**	<0.001*
IL-4 (pg/ml)		0.539**	<0.001*	0.902**	<0.001*	-0.733**	<0.001*
IL-17A (pg/ml)		0.607**	<0.001*	0.928**	<0.001*	-0.705**	<0.001*
IL-9 (ng/ml)		0.406**	<0.001*	0.887**	<0.001*	-0.769**	<0.001*
IL-5 (ng/ml)		0.234**	<0.001*	0.835**	<0.001*	-0.734**	<0.001*
IL-18 (ng/ml)		0.496**	<0.001*	0.864**	<0.001*	-0.727**	<0.001*
Age (years)		-0.004	0.944	0.046	0.602	-0.059	0.347
FVC% predicted		-0.275**	<0.001*	-0.073	0.407	0.528**	<0.001*
FEV1% predicted		-0.208**	0.001*	-0.973**	<0.001*	0.722**	<0.001*
FEV1/FVC		-0.162**	0.009*	-0.957**	<0.001*	0.731**	<0.001*
Vitamin D (ng/ml)		-0.366**	<0.001*	-0.874**	<0.001*	-	-

DISCUSSION

There are many reports indicating association between asthma and obesity. However, the possible mechanisms linking asthma and obesity remain largely unknown. Obesity increases the incidence of asthma, aggravate symptoms and reduce the response to asthma medications. Thus, obesity associated asthma was recognized as a new phenotype of asthma with specific features. One association between obesity and asthma is the low-grade chronic inflammatory state of adipose and lung tissues. However, the possible mechanisms remain unknown. It is expected that; discovery of such mechanism will improve the treatment modalities **(11).**

The current study is a trial to elucidate the possible association between asthma and obesity. The current work showed an association between obesity from one side and severity of asthma from the second side. Vitamin D deficiency was an association with both conditions (obesity and asthma). However, the deficiency was marked in obese asthmatics. Similarly, there was significant association between studied interleukins. Again, the association was more marked in obese asthmatics. This association was confirmed through significant correlation between the body mass index, severity of asthma and vitamin D with other interleukins. These results indicate that all studied factors could play a role in the pathogenesis of obesity and asthma. The relationship between both conditions seems to be reciprocal. The pathogenesis seems to be achieved through a chronic inflammatory process (the hallmark of both obesity and asthma). Clinically, it is expected that weight reduction will improve asthma. Micronutrient supplementation (vitamin D) could improve both conditions.

Results of the current work come in agreement with previous studies indicated that, vitamin D has a marked effect on the immune function. It inhibits $CD4^+$ T cell proliferation. It also reduced the production of IL-17 and interferon-gamma **(13).**

In asthma, evidence from experimental studies suggested beneficial effects of vitamin-D, and its insufficiency had been associated with higher morbidity. For example, **Han YY, *et al.* (14)** showed that, vitamin D insufficiency was linked to the development of asthma and wheezy chest in children as well as in adults. Other studies suggested that, vitamin D is a protector agent against asthma exacerbations. For example, **Kreindler *et al.* (15)** showed that vitamin D improved immunity reaction in patients with asthma. **Schedel *et al.* (16)** showed that, supplementation by vitamin D, prevents the conversion of IL-4 to IL-13 producers. Interestingly, lower levels of vitamin D correlates with the high adiposity **(17).** Obesity itself reduces the levels of vitamin D, due to reduced bioavailability of vitamin D3 due to its deposition in body fats **(18, 19).**

Most recently, **Zajac and Wojciechowski (20)** summarized the role of different vitamins in asthma, including vitamin D. They reported that, vitamin-D had an important property as it is able to inhibit inflammation by regulation of the proliferation and migration of different immune cells (e.g., mast cells, neutrophils, and eosinophils). It also inhibits cytokine release from these cells, and reduce production of different pro-inflammatory cytokines including IL-1β, IL-6, and tumor necrosis factor-alpha (TNFα), and an increase in the secretion of the anti-inflammatory IL-10. This was confirmed in the current work.

Migliaccio *et al.* (19) summarized the available evidence regarding the relation between vitamin D insufficiency and obesity. They noticed the potential role of obesity leading to reduction of vitamin D uptake and utilization and the reciprocal role of vitamin D deficiency in development of obesity. Low vitamin-D in obese subjects was observed in the current study regardless presence or absence of asthma.

Results of the current work are in line with the study of **Bantula *et al.* (21)** who reported that obese asthma patients had reduced forced capacity and FEV1 than healthy controls. In addition, FEV1/FVC ratio was significantly reduced in non-obese and obese asthma subjects when compared to obese non-asthmatic and healthy controls. They added, eosinophils were higher in non-obese asthmatics in comparison to healthy controls and obese non-asthmatic subjects.

In line with the current work, previous researchers reported elevated levels of serum IL-18 in obese subjects and interestingly, these values are reduced with weight loss **(22).**

In addition, patients with uncontrolled asthma had significantly higher levels of IL-18 **(2)** and asthma exacerbations **(24).** A recent study reported higher expression of IL-18 receptor in the lung of patients with severe asthma, indicating that IL-18 signaling may play a significant and pivotal role in the pathogenesis of asthma, especially severe forms **(25).**

IL-9 levels and their role in inflammatory conditions is controversial. It was reported that IL-9 promotes t-helper cells differentiation and increased production of IL-17 and IL-6 **(26).** However, IL-9 on the other side, promotes the suppressive functions of regulatory t-cells **(27).**

Patients with asthma have an increased number of t-helper cells in their peripheral blood, with increased levels of interleukin-9 (IL-9) in their serum and BAL **(28, 29).** IL-9 itself increases the production of other interleukins (IL-5 and IL-13) and stimulates different immune cells (e.g., mast cells and eosinophils) **(30).**

Bantula *et al.* (21) reported significantly higher levels of serum IL-9 in obese asthmatics than healthy and obese-non asthmatic subjects. These results are in line with the current work and suggest that, asthma and obesity may increase the production of IL-19 as a result of chronic inflammatory state. However, weight reduction did not lead to significant reduction of IL-9 and they attributed this effect to higher females in their study, where sexual dimorphism could affect the production of IL-9 (female gender had a protective effect against increased production of IL-9).

Overall, we could say that, both asthma and obesity had an effect on each condition on the other. This could be explained by low-grade inflammatory state in both conditions. In addition, vitamin D plays a critical role in the pathogenesis of both conditions, due to its anti-inflammatory properties exerted on different immune cells and stimulate secretion of different cytokines, mainly interleukins.

Papamichael *et al.* (31) studied the role of vitamin-D deficiency on the pulmonary function in obese and non-obese children with mild asthma. They reported that, suboptimal vitamin D was significantly higher among asthmatic children regardless of their BMI. However, on the contrary, lung function was significantly associated with

BMI and vitamin D. When they adjusted their results for patient age, the association between obesity, low vitamin D and mild asthma was more evident and higher BMI was linked to both asthma and low vitamin D. They, then stated that, the interplay between the three conditions is more complex and needs further studies. The authors themselves explains the non-association between vitamin D, adiposity and asthma they found first to the small sample size of obese subjects.

Lautenbacher *et al*. (32) documented lower respiratory function indices among asthma patients with obesity and vitamin D-deficiency than sufficient peers. The importance of maintaining lean body weight and optimal levels of vitamin D status was confirmed to improve the lung function indices in healthy and subjects with asthma **(33). Willeboordse *et al*., 2016).**

Conclusion:

The chronic inflammatory state associated with obesity and asthma could explain the association between the two conditions. The excessive synthesis of proinflammatory interleukins produced by fatty cells (IL-1β and IL-6) may contribute to the pathogenesis of asthma. However, higher expression of interleukins keeping lean state in asthma (e.g., IL-4 and IL-5) makes the interpretation of the complex association between asthma and obesity difficult. Thus, other possible pathogenic players could be included in this association, and this warrants further studies.

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