

Impact of Body Mass Index (BMI) on Serum Interleukin 4 level in Adult Bronchial Asthma Subjects

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ABSTRACT

Background: Overweight and obesity are in reported observational studies consistently associated with increased prevalence of asthma. Recently adipose tissue is considered as a source of inflammatory cytokines. Interleukin 4 (IL-4) mediates important pro-inflammatory functions in asthma, including induction of isotype rearrangement of IgE.

Objective: The aim of the work was to assess IL4 in relation to Body Mass Index (BMI) in allergic asthmatic patients.

Patients and Methods: 100 allergic asthmatic patients were enrolled in the study and classified according to BMI. All participants were subjected to routine laboratory investigations, serum IL4, skin prick test and pulmonary function tests.

Results: IL-4 was significantly higher in overweight, obese and massive obesity patients [560 (440-720) pg/ml, 560 (410 -730) pg/ml, 830 (445 -1095 pg/ml respectively] in comparison to non-obese asthmatic patients [160 (80-280) pg/ml] ($p < 0.001$). This difference in serum IL-4 level was strongly correlated to BMI ($r = 0.74$) and body weight ($r = 0.69$) ($p < 0.001$). Simple linear regression analysis revealed a strong relation between serum IL4 and BMI ($\beta = 0.705$, $p < 0.001$). **Conclusion:** It could be concluded that there is a strong association between the inflammatory cytokine IL4 and BMI among obese allergic asthmatic patients, this may suggest a state of ongoing subclinical inflammatory state in the obese-asthma phenotype.

Keywords: Interleukin 4, Obese, BMI, Allergic asthma

INTRODUCTION

Asthma is a worldwide major health problem, that affects all ages, genders, and ethnicities. Prevalence has been steadily increasing alongside that of allergy, as modern lifestyles are adopted and communities become more urbanized, a trend that is predicted to continue over the next two decades (1).

Overweight and obesity may affect the lungs through a mechanical effect on lung function or through inflammation that may predispose to asthma (2). It is likely that other factors associated with overweight and obesity such as dyslipidemia and increased mast cell activity, also implicated in the pathogenesis of asthma and decreased lung function (3, 4). This "obese asthma syndrome" was found to be complex and multifactorial.

The inflammatory response associated with obesity is originated in the adipose tissue cells (5). Adipose tissue consists of mature adipocytes and stromal-vascular cells, which play a role in obesity associated inflammation, including: leukocytes, macrophages, fibroblasts, endothelial cells, and pre-adipocytes (6).

Asthma is a complex genetic disorder that has been associated with IL-4 gene in promoter polymorphism and proteins involved in IL-4 (7). IL-4 mediates important pro-inflammatory functions in asthma, including induction of isotype rearrangement of IgE, expression of VCAM-1 molecules (vascular cell adhesion molecule 1), promoting eosinophilic transmigration through endothelium, mucus secretion

and T helper type 2 (Th2) leading to signaling cytokine release (8).

Airway hyperresponsiveness (AHR) is a hallmark of asthma. IL-4 and IL-13 induce hyper-responsiveness of the airway smooth muscle in isolated human small airways and was found to be glucocorticoid-insensitive (9). Many studies have reported that obesity-related asthma in children is more often non-atopic; yet some studies have reported that obesity in itself is associated with atopy (10, 11). To our knowledge, studies investigating obesity associated asthma in adults are few. So, this study was conducted to determine the relation between serum interleukin 4 (IL-4) level and body mass index (BMI) in adult atopic asthmatic patients.

PATIENTS AND METHODS

This comparative cross-sectional study included a total of 100 asthmatic adults, attending at Allergy Outpatient Clinics in Ain Shams University Hospitals.

The included subjects were divided into two groups; **Group 1 (non-obese asthmatics)** consisted of 50 asthmatic patients with BMI < 25 and **Group 2 (overweight/ obese /massive obesity asthmatics)** consisted of 50 asthmatic patients with BMI \geq 25.

Inclusion criteria: Allergic asthmatic patients according to **Global Initiative for Asthma (GINA)** criteria (12), Atopic patients were defined as having positive SPT or high specific IgE, or having a history of

other allergic diseases such as allergic rhinitis or atopic dermatitis.

Exclusion criteria: Other chronic illness e.g., DM, HTN, urticaria, COPD, severe asthma, age above 60 and below 20 years.

Ethical Consideration:

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Written informed consent of all the participants was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patients were subjected to detailed history & clinical examination, measuring BMI, laboratory investigations (including CBC, liver function tests, kidney function tests, serum total IgE level, serum IL4 level), pulmonary function tests and Skin prick test using a panel of common allergens (prepared at the allergen extract unit, Faculty of Medicine).

Body mass index: is calculated as body weight divided by the square of the body height and is universally expressed in units of kg/m^2 . Body weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively, by using standardized equipment and procedures. Patients were classified as non-obese ($\text{BMI} < 25$), overweight ($\text{BMI} 25\text{-}29.9$), obese ($\text{BMI} 30\text{-}40$), massive obesity range ($\text{BMI} \geq 40$)^(13, 14).

Serum Total IgE Level: It was performed by the enzyme-linked immunoassay (ELISA) technique. Total serum IgE concentrations were reported in international units (IU/ml). Reference total serum IgE is < 100 IU/ml⁽¹⁸⁾.

Serum IL-4 by ELISA: Detection of serum IL4 was performed by (ELISA) method by using R&D Systems kits (R&D Systems, Wiesbaden, Germany). A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn up through the points on the graph. These calculations were best performed with computer-based curve-fitting software and the best fit line was determined by regression analysis.

Pulmonary Function Test (PFT): The test was performed in the standing posture according to ACOEM recommendations.⁽¹⁵⁾ The patient's age, height, and weight (wearing light clothes without shoes) were recorded. The age was expressed in years. Height and weight were expressed in meters and kilograms respectively. The appropriate technique was explained to all patients before the test⁽¹⁶⁾. Forced expired volume in one second (FEV1), forced vital capacity (FVC) and FEV1/VC ratio were measured by a spirometer (Microspiro HI-601 Spiro Analyzers, Chest MI Inc., Japan). Calculation of FEV1/FVC ratio to differentiate

obstructive from restrictive ventilatory defects. The FEV1 was expressed as a percentage of the predictive value and classified according to severity of the impairment. A low FEV1/FVC signifies an obstructive defect. A reduced FEV1 more than reduced FEV1/FVC is considered obstructive defect⁽¹⁷⁾

Statistical analysis

Data were fed to the computer and analyzed using IBM Statistical Package of Social Science (SPSS) software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Spearman correlation and simple linear regression analysis were performed. Significance of the obtained results was judged at the 5% level. P value < 0.05 was considered significant.

RESULTS:

This study was conducted on 100 asthmatics patients (4 patients were missed as they didn't complete their workup). Patients were recruited from Allergy and Immunology Outpatient Clinics. They were 47 males and 49 females. The participants were divided into 2 groups, according to BMI. 48 patients were "normal weight", and 48 patients were "overweight, obese and massive obesity". Their age ranged from 20 years old to 60 years old.

Comparison between the two groups was done regarding CBC parameters, pulmonary function tests (FEV1% predicted, FVC % predicted and FEV1/FVC ratio), skin test, and IL4 serum level (Table 1).

Comparison between all studied groups as regards IL-4 level showed a highly significant difference ($p\text{-value} < 0.001$), being higher in overweight, obese and massive obesity asthmatics [560 (440-720) pg/ml, 560 (410 -730) pg/ml, 830 (445 -1095) pg/ml respectively] more than average weight asthmatics [160 (80-280) pg/ml] (Table 1 & Figure1).

Also, there was a highly significant difference between the studied participants as regard total IgE level. Otherwise, there were no statistically significant differences regarding the CBC parameters or the skin prick test. Interestingly, there was no statistically significant difference between patients as regard the pulmonary function tests (Table 1).

Correlation between IL4 level and the other tested parameters among the participants showed a highly significant positive correlation with BMI, body weight and total IgE (Table 2 & Figure 2). We applied further statistical measures (simple linear regression analysis) to understand the relative contribution of BMI measures to serum IL4 level, and we found a highly significant relation (Table 3 & Figure 3).

Table (1): showing Comparison between groups regarding lab investigations, pulmonary function, and skin prick tests

	BMI		t*	P value
	Normal weight (n=48)	Overweight/obese/massive obesity (n=48)		
WBC (10 ³ /UL) (Mean ±SD)	7.60± 1.41	7.39 ± 1.14	0.44	0.66
Hemoglobin (g/dL)	11.95 ± 1.50	11.88 ± 1.22	0.25	0.81
Eosinophils (10 ³ /UL) (Mean ±SD)	0.23 ± 0.09	0.17 ± 0.02	1.03	0.31
Platelets (10 ³ /UL)	264.50 ± 8.79	272.77 ± 9.66	0.45	0.65
Total IgE (IU/L) Median (IQR)	43.35 (20.85-40.35)	127.00 (66.50-80.50)	4.36**	<0.001
IL-4 level (pg/ml) Median (IQR)	160 (80-100)	560 (410-500)	7.57**	<0.001
FEV1 % predicted	82.04 ± 20.24	80.74 ± 15.16	0.36	0.72
FVC % predicted	89.13 ± 18.59	90.36 ± 12.50	0.38	0.71
FEV1/FVC	0.91 ± 0.08	0.89 ± 0.11	0.94	0.35
Skin prick test [n (%)]	Negative	21 (43.8%)	0.70***	0.40
	Positive	27 (56.3%)		

*Student t test **Mann Whitney U test *** Chi square test
 P > 0.05: Non-significant; P < 0.05: Significant; P < 0.01: Highly significant

Table (2): Correlation between IL4 level and the other tested parameters among the participants

	IL4 level	
	R	p-value
Age (years)	- 0.083	0.447
Weight (kg)	0.69**	0.000
Height (cm)	- 0.077	0.481
BMI (kg/m ²)	0.74**	<0.001
Hemoglobin (g/dL)	- 0.017	0.873
WBCs (mcL)	- 0.014	0.889
Esinophils (10 ³ /UL)	- 0.155	0.13
Platelets (mcL)	- 0.068	0.513
Total IgE (IU/L)	0.343	0.001
FEV1 % (predicted)	-0.027	0.791
FVC % (predicted)	0.015	0.881
FEV1/FVC ratio	0.074	0.471

Spearman correlation coefficients

Table (3): Simple Linear regression analysis for effect of BMI on IL4 level

	Unstandardized Coefficients		Standardized Coefficients	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
(Constant)	-494.823	95.110		<0.001	-683.667	-305.979
BMI	33.806	3.505	0.705	<0.001	26.847	40.764

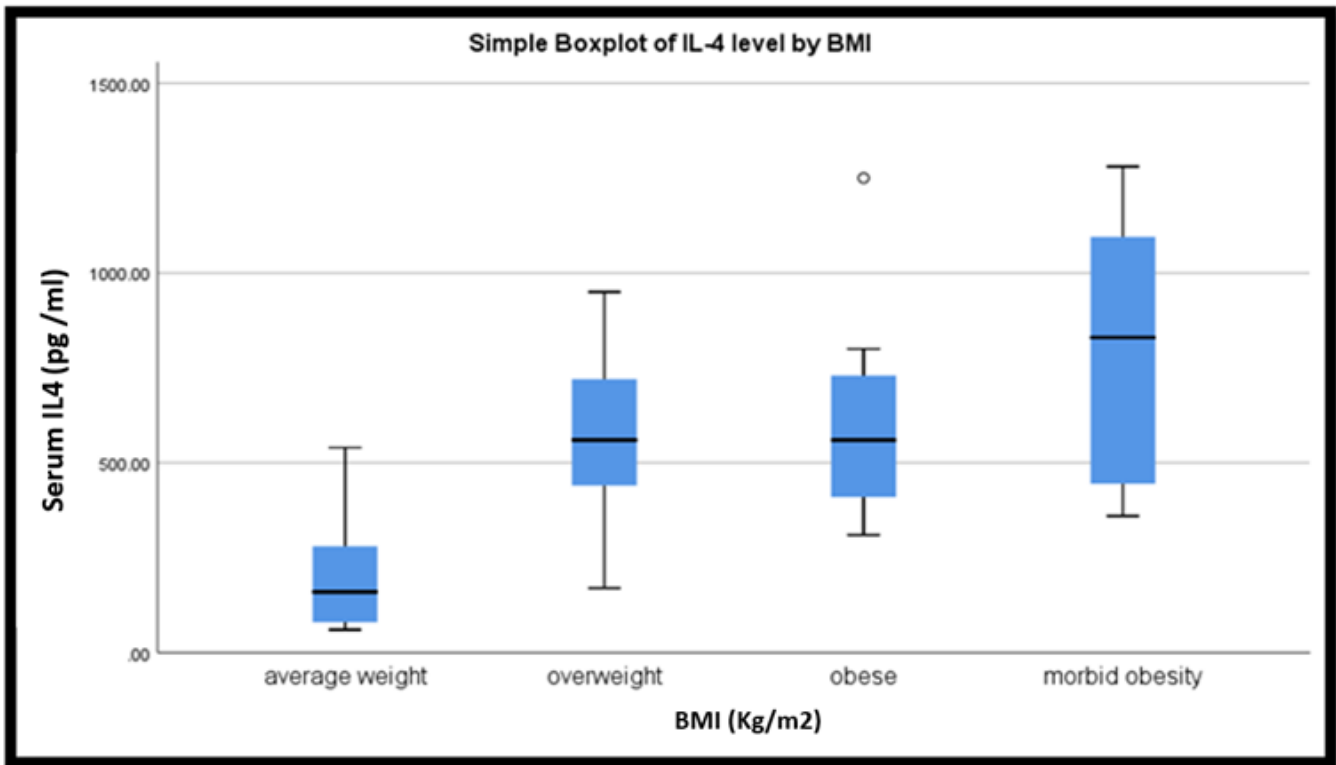


Figure (1): Comparison of IL-4 level between all studied groups (as subdivided according to BMI).

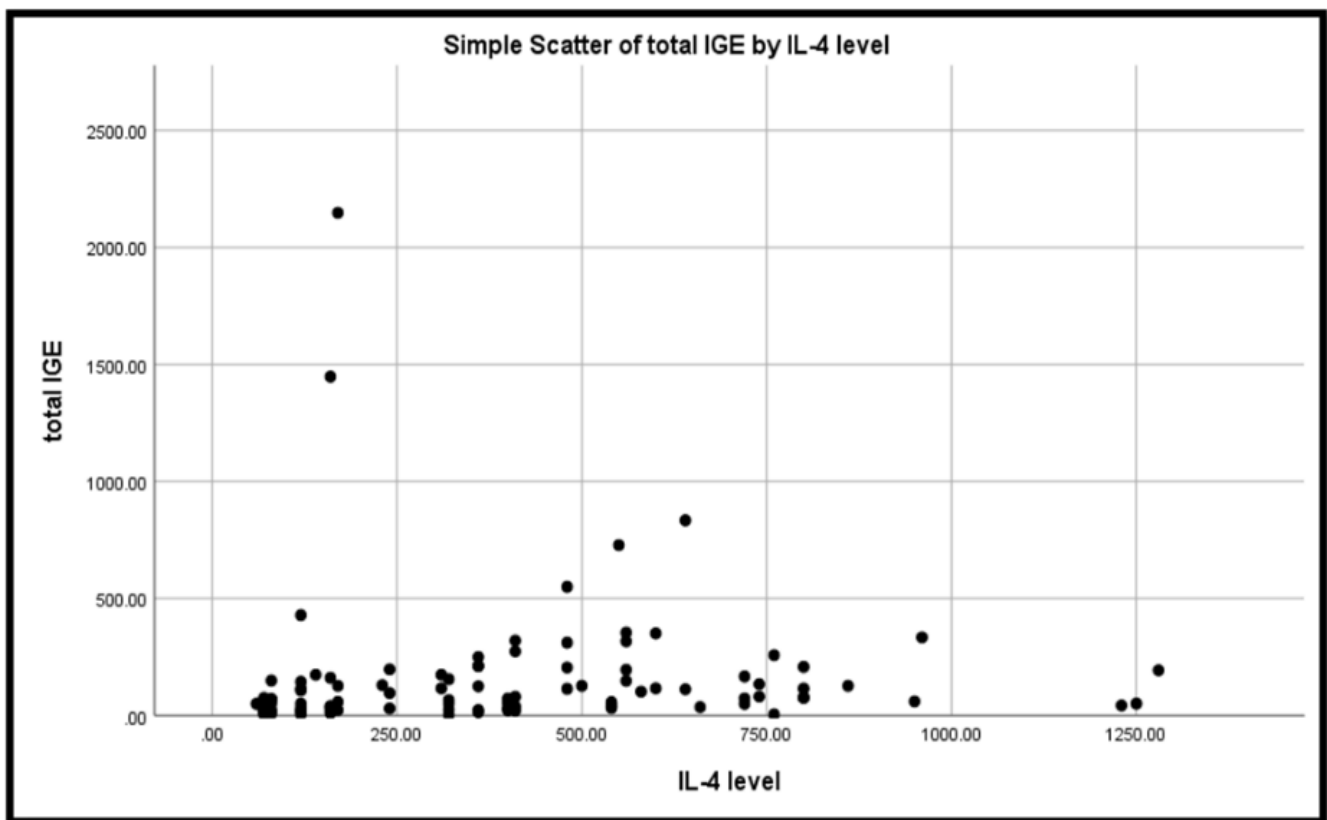


Figure (2): Simple scatter graph showing the relation between IL4 and total IgE.

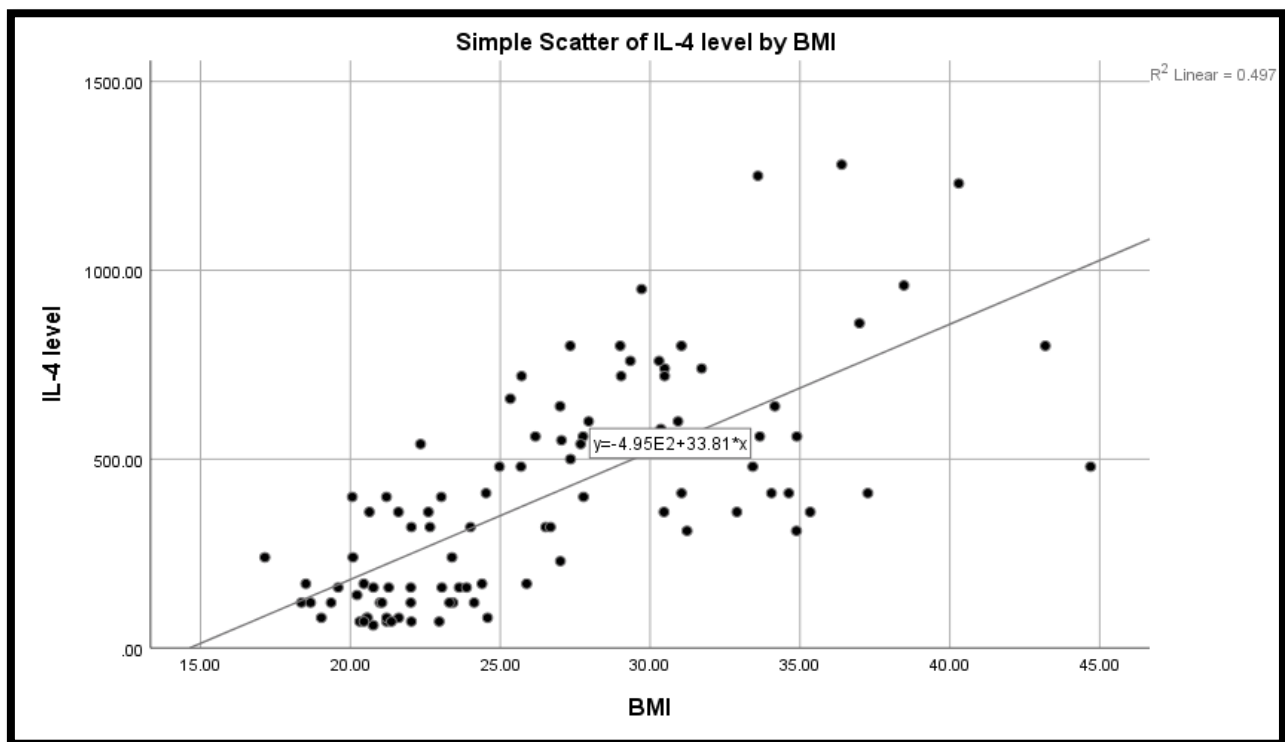


Figure (3): Simple scatter graph showing the correlation between IL4 and BMI.

DISCUSSION

Obesity is a risk factor for both incident and prevalent asthma. The interrelationship between obesity and asthma derives from a complex interplay of biologic, physiologic, and environmental factors⁽¹⁹⁾.

Interleukin 4 is a cytokine that directs the differentiation of naive helper T cells (Th0 cells) to Th2 cells which subsequently produce more IL-4 in a positive feedback mechanism. IL-4 sources are mast cells, Th2 cells, eosinophils and basophiles⁽⁸⁾.

This study was aimed to determine serum level of IL-4 in asthmatic patients in relation to BMI. this study was conducted on 96 participants divided into two groups that healthy weight group and obese group which included (overweight, obese and massive obesity asthmatics) with BMI equal or higher than 25 kg/m² and normal weight asthmatics with BMI from 18 to 24.9 kg/m² in an age group equal or higher than 20 years old and equal or less than 60 years old. All participants subjected to detailed history and physical examination, skin prick test, pulmonary function tests, measurement of eosinophils, total IgE and serum IL-4 level.

In our study, comparison between the groups as regard serum IL-4 level showed that there was a statistically significant difference, being higher in overweight, obese and massive obesity asthmatics than average weight asthmatics (p value <0.001).

Few previous observational studies have noticed the relation between obesity and elevated IL-4 levels. **Schmidt and colleagues**⁽²⁰⁾, in a cross-sectional study aimed to characterize the profile of a broad range of pro- and anti-inflammatory cytokines in patients with general obesity, central obesity, and non-obese subjects and impact of physical activity on them, found that in

participants with general obesity, level of IL-4 was significantly elevated in generally obese participants with low physical activity. Also, **EL-Wakkad et al.**⁽²¹⁾ in a study conducted on 86 female obese adolescence girls found that there was a significant increase in serum IL-4 level in centrally obese female than non-centrally obese at (p value <0.0005) (p value <0.0001) respectively, this study was conducted on obese persons only and didn't include non-obese controls.

On the other hand, **Al-Ayed and coworkers**⁽²²⁾ in a study conducted on schoolchildren to elucidate the possible role of interleukin IL-4, in linking obesity with childhood asthma, they found that there was no significant differences between obese and non-obese asthmatic children as regard serum IL-4 level, this may be due to different age group between this study and our studies and subsequent different body fat content.

We suggest that our results can be supported by the increasing evidence that obese patients should be regarded in a state of persistent low-grade systemic inflammation. IL-4 also plays a crucial role in the regulation of IgE synthesis and induces the expression of the low-affinity IgE receptor on macrophages⁽²³⁾. And this may explain the higher levels of IL-4 in overweight and obese asthmatics rather than average weight asthmatics.

In our study there was a positive correlation between IL-4 in asthmatic patients and BMI (p=0.000) and this may indicate role of IL-4 in pathogenesis linking obesity with asthma. In accordance with our study, **EL-Wakkad et al.**⁽²¹⁾ found that there was high positive correlation between central obesity and IL-4 at (p<0.001). On the contrary, **Schmidt et al.**⁽²⁰⁾ found that there was no correlation between IL-4 and BMI

($p=0.156$), but this study was conducted on non-asthmatic patients.

Interestingly, Comparison between studied groups as regard spirometry parameters (FEV₁, FVC % of predicted and FEV₁/FVC) showed that there was no statistically significant difference between the groups. This is contrary to a recent study by **Huang and coworkers** ⁽²⁴⁾ who found that adults who had both abnormal BMI (overweight and obesity) had substantially decreased pulmonary function tests in asthmatic and non-asthmatic patients. Other previous studies have reported slightly lower FEV₁/FVC in adults with asthma ⁽²⁵⁾. We suggest that our result can be explained by the narrow range of studied parameters as we excluded the severe asthmatics from the study. Surprisingly, this goes in agreement with a Meta-analysis done by **Forno and colleagues** ⁽²⁶⁾ who concluded that the effect sizes for FEV₁ and FVC were much larger among subjects without asthma than among those with asthma, and they speculate that obesity may have larger effects on FEV₁ and FVC in healthy subjects.

In our study there was no correlation between IL-4 level and FEV₁% Pred, FVC Pred and FEV₁/FVC ratio and this may exclude effect of IL-4 level on pulmonary function parameters. **Huang et al.** ⁽²⁷⁾ in a study aimed to correlate IL-4 level with pulmonary function in patients with asthma, acute exacerbation of chronic obstructive pulmonary disease (COPD) and asthma –COPD overlap syndrome found that level of IL-4 was positively correlated with FEV₁% Pred and FEV₁/FVC ($p=0.018$) ($p< 0.001$) respectively, this may be due to different study population as we excluded COPD and asthma –COPD overlap patients.

In the present study, we found a significant correlation between serum IL4 and total IgE among obese and non-obese asthmatics. This goes in agreement with early study by **Punnonen and coworkers** ⁽²⁸⁾ who detected a strong correlation between IL4 and level of IgE synthesis.

We would also like to report potential weaknesses of the present study, as using direct measure of body fat such as CT or body composition densitometry instead of the indirect measures of body fat would have been more accurate, but this was not applicable mainly for logistical reasons due to the large number of study participants.

CONCLUSION

It could be concluded that IL-4 may play a pathogenic role in obese-asthma phenotype. Indicating a pro-inflammatory state, thus targeting IL-4 therapy should be considered in this subgroup of patients. Also, lifestyle modification and weight reduction may be important for modulating the biochemical structure of those obese asthmatic patients.

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