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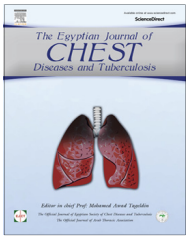

ORIGINAL ARTICLE

Association between anti-thyroid peroxidase and anti-cytokeratin 18 autoantibodies and bronchial asthma in women

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KEYWORDS

Allergic and non-allergic asthma;
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Thyroid function;
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Abstract *Background:* The mechanisms of intrinsic or non-allergic asthma remain uncertain as allergens have no obvious role in driving the inflammatory process in the airways. This study was designed to test the possible presence of an autoimmune pathogenesis of bronchial asthma and to investigate the similarities and differences between allergic and non-allergic asthma.

Design: Cross-sectional prospective cohort study.

Subjects and methods: 50 asthmatic women and 30 healthy control women were tested for thyroid function, anti-TPO, anti-CK18 autoantibodies, and total IgE measurements. Pulmonary function tests, skin-prick test and history of asthma risk factors were done for asthmatic women.

Results: Allergic asthma were found in half of the asthmatic patients and the other half (25/50) were non-allergic according to the results of skin-brick test and serum level of IgE. The thyroid function tests were not statistically different between asthmatic and control groups as well as between non-allergic and allergic asthma groups ($P > 0.05$). Serum anti-TPO autoantibodies and anti-CK18 autoantibodies' levels were significantly higher in asthmatic patients than the control group and also in non-allergic asthma patients than allergic asthma patients. In asthmatic patients serum anti-TPO autoantibodies showed negative correlation with FEV1 (pre- and post) and serum IgE.

Conclusion: Positive anti-TPO autoantibodies and anti-CK18 autoantibodies in asthmatic patients and their higher level in the non-allergic asthma group may strengthen the presence of a hidden autoimmune phenomenon in non-allergic asthma.

Abbreviations anti-TPO autoantibodies, anti-thyroid peroxidase antibodies; anti-CK18 autoantibodies, autoantibodies to cytokeratin 18; IgE, immunoglobulin E; Hb, hemoglobin; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; FEF, forced expiratory flow; CBC, complete blood picture; COPD, chronic obstructive pulmonary disease

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Introduction

Bronchial asthma is as a heterogeneous disease, usually characterized by chronic airways inflammation. It is defined by the history of respiratory symptoms such as wheezes, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation [1].

Asthma is a problem worldwide with an estimated 300 million affected individuals [2]. The global prevalence of asthma range from 1% to 18% of the population in different countries [3,4]. In Egypt, the bronchial asthma is a significant health problem among school children, and the prevalence was 7.7% [5]. Annual world deaths from asthma have been estimated at 250,000. Asthma is a major cause of absence from work in many countries [6–8].

Malfunction of the immune system is known in three main conditions: immunodeficiency, allergy, and autoimmunity. The appearance of autoimmunity and allergies has not been well appreciated. Recently, there are reports on the appearance of allergies concomitantly with autoimmunity, but the relationship is poorly understood. Allergy and autoimmunity are two potential outcomes of dysregulated immunity. Both are characterized by localized inflammation that leads to the injury and/or destruction of target tissues [9]. The allergic response to common environmental agents (allergens) has been regarded as an important mechanism in the development of airway inflammation of patients with asthma. However, allergic sensitization cannot be detected in a significant number of adult patients with asthma [10]. Non-allergic asthma usually begins at an older age and is clinically more severe than allergic asthma. Non-allergic asthma has been referred to as "intrinsic asthma" on the basis of a belief that there must be an etiologic agent in the patient's own body. However, the "intrinsic" etiology has not yet been defined [11].

One of the most important causes of thyroid diseases is autoimmunity in origin, and it seems that people with thyroid diseases present more signs of asthma. A little is known about the relation between thyroid disease and allergic diseases. The pathogenic mechanism for relationship between thyroid dysfunction and severity of asthma signs is not clearly understood. Knowledge of the presence of thyroid disease in patients with bronchial asthma is important. The reason is that hypothyroidism may coexist with allergic diseases such as bronchial asthma, while hyperthyroidism may be associated with a lower incidence of allergies. Some studies have shown that hypothyroidism ameliorates and hyperthyroidism exacerbates bronchial asthma [12].

The idea of the possible involvement of an autoimmune mechanism in the pathogenesis of asthma has been proposed by previous studies that demonstrated high incidences of circulating autoantibodies to bronchial mucosa tissue (as anti-CK18 autoantibodies) in patients with asthma, especially in patients with non-allergic asthma [13–15].

This study was designed to investigate the frequency of autoimmune phenomena with and without thyroid diseases in women suffering from bronchial asthma by measuring serum level anti-TPO autoantibodies and anti-CK18 autoantibodies.

Subjects and methods

This study was conducted on 80 women over 18 years. Fifty of them had bronchial asthma whatever severity and thirty of them were healthy non-asthmatic. Women who attended to outpatient clinic at chest department and internal medicine department of El-Minia University Hospital were invited to participate in the study. The study was approved by the hospital's research ethics board.

Exclusion criteria

Women who had the diagnosis of COPD, connective tissue diseases, congestive heart failure, chronic renal failure or positive history of definite thyroid diseases or being under treatment were excluded. Pregnant and smoking women were also excluded.

The control group consisted of 30 women selected from people who participated in a field trial study. This group had no symptoms or signs of asthma disease in addition to all the above exclusion criteria.

The asthmatic patients were selected based on having characteristic respiratory symptoms such as wheezing, dyspnea, chest tightness or coughing and variable expiratory airflow limitation. Variable expiratory airflow limitation was confirmed by spirometry (ZAN 300, Germany). Reversibility refers to rapid improvement in FEV1 (12% and 200 ml) measured within minutes after inhalation of 400 μg of salbutamol [16]. Asthma severity was graded based on guidelines for diagnosis and treatment of asthma. Four groups; mild intermittent, mild persistent, moderate persistent and severe persistent were considered [17].

All asthmatic subjects underwent a skin-prick test with 12 common aeroallergens based on common aeroallergens in our region: mites, molds, pollens, animal dander, pigeon, house dust, feather, cats, strew and Candida. Histamine and saline were used as positive and negative controls. Patients with asthma were classified as having allergic asthma when the wheal diameter of any one allergen was greater than 3 mm over the negative control (normal saline) and there was a definite clinical history or objective evidence of asthmatic response induced by allergen exposure. Non-allergic asthma was defined as there being no positive skin reaction to any of the 12 common aeroallergens in the presence of a positive histamine control and serum total IgE concentration being within the normal range (less than 180 IU/ml). All patients with asthma had not received systemic steroid treatment for the 4 weeks before the study.

Five ml of venous blood was taken by sterile venipuncture after informed consent of all participants and divided as follows: one ml on EDTA for CBC, 4 ml on plasma tube was left to be collected and centrifuged to separate serum, and then serum was freezed at −70 °C for assay of anti-CK18 autoantibodies, total IgE, Anti-TPO, total T4 and TSH. All were measured by the EIA (Enzyme immune-assay) method using Huma reader, Germany.

Kits of Anti-TPO autoantibodies were supplied by Bios, USA, chemux Bioscience. Kits of autoantibodies to anti-CK autoantibodies were supplied by orgentec, diagnostic, Germany. Kits of total IgE were supplied by orgentec, diagnostic,

Germany. Kits of total T4 and TSH were supplied by Bios, USA, chemux Bioscience. CBC was determined by automated cell counter sysmex KX-2IN (TAO medical incorporation, Japan).

Anti-TPO autoantibodies >50 IU/ml were considered as positive. Hypothyroidism was based on TSH > 10 IU/ml and total T4 < 4.5 μg/dl 4. The normal level of total IgE was up to 180 IU/ml. The normal level of anti-CK autoantibodies was up to 13.4 pg/ml.

Principles of the anti-CK autoantibodies test: The microtiter plate provided in this kit has been pre-coated with an antibody specific to keratin, type 1 cytoskeletal 18. Standards or samples are then added to the microtiter plate wells with a biotin-conjugated poly clonal antibody preparation specific for keratin, type 1 cytoskeletal 18 and Avidin conjugated to Horseradish Peroxidase(HRP) is added to each microplate well and incubated. Then a TMB substrate is added to each well. Only those wells that contain keratin, type 1 cytoskeletal 18, biotin-conjugated antibody Avidin will exhibit a change in color. The enzyme substrate reaction is terminated by the addition of sulfuric acid solution and color change is measured specterophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of keratin, type 1 cytoskeletal 18 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis

Analysis of data was done by SPSS (statistical program for social science, version 17). The following statistical methods were used: Description of quantitative variables as means, SDs, and ranges; description of qualitative variables as numbers and percentages; chi-square test for comparisons of qualitative variables between groups; unpaired *t*-tests to compare two groups; Spearman correlation coefficient to rank different variables as either positive or inverse. The results were considered to be statistically significant at a P value < 0.05.

Results

The present study included 80 subjects (50 patients with bronchial asthma and 30 control healthy subjects). Asthmatic patients were divided into; 25 (50%) patients had allergic and the others were non-allergic asthma, according to the results of skin-brick test and serum level of total IgE.

In the present study 42 (21/50)% of asthmatic women had allergic rhinitis, 36 (18/50)% had chronic sinusitis and 10 (5/50)% had urticaria. The women with non-allergic asthma had statistically significant lower history of allergic rhinitis, chronic sinusitis, urticaria and family history than those with allergic asthma. Within asthmatic women; 62% of lived in rural areas, 32% were bird breeders and 48% had history of dust exposure (Table 1).

Asthmatic patients had a significantly higher total IgE (P 0.001), anti-TPO (P 0.001) and anti-CK18 autoantibodies (P 0.03) than control subjects (Table 4). Non-allergic asthma patients had a significantly lower eosinophil count (P 0.002), higher pre- and post-bronchodilator FVC (P 0.04 for both), lower total IgE (P 0.001), and higher anti-TPO autoantibodies (P 0.03) and anti-CK18 autoantibodies (P 0.04) than those with allergic asthma (Table 5).

As regards thyroid function in the present study; there were three hypothyroid laboratory abnormalities in the asthma group (two in non-allergic asthma and one in allergic asthma) and none in the control group, and this difference was not statistically significant ($P > 0.05$) (Tables 2 and 3). There were not statistical differences between asthmatic and control groups as well as between non-allergic and allergic asthma groups as regards both total T4 and TSH and the mean values of both were within normal range (Tables 4 and 5).

A positive anti-TPO autoantibodies' serum level (>50 IU/ml) was found in 20% of women in the asthma group and 6.7% of control groups (Table 2), but this difference was statistically non-significant ($P > 0.05$). Also, positive anti-TPO autoantibodies were found in 28% of women in the non- allergic asthma group and 12% of patients in the allergic asthma group (Table 3) and this difference was statistically non-significant ($P > 0.05$).

A positive anti-CK18 autoantibodies' serum level (>13 IU/ml) was found in 16% of women in the bronchial asthma group and 10% of the control groups (Table 2), but this difference was statistically non-significant ($P > 0.05$). On the other hand, 40% of women in the non-allergic asthma group and 12% of patients in the allergic asthma group had positive anti-CK18 autoantibodies' levels (Table 3) and this difference was statistically significant (P 0.01).

There was a significantly negative correlation serum anti-TPO level and FEV1 (pre- and post-) Figure 1. There was also a significantly fair negative correlation serum anti-TPO level and total IgE (Figure 2). There was an insignificant correlation between anti-TPO and anti-CK18 autoantibodies and thyroid function (Table 6). There was an insignificant correlation between serum anti-CK18 autoantibodies' level and most of laboratory, and pulmonary function parameters in asthmatic patients except a positive correlation with post- bronchodilator PEF25–75.

There was a significant association between severity of bronchial asthma and anti-TPO autoantibodies' level (P 0.03), but there was no significant association between severity of bronchial asthma and anti-CK18 autoantibodies' level ($P = 0.6$) (Table 7).

Discussion

Among the fifty asthmatic patients, 50% patients were found to have allergic asthma and the other 50% had non-allergic asthma according to the results of both skin-brick test and serum level of IgE. The positivity of at least one skin prick test is usually used in epidemiology as a single diagnostic criterion for atopy. However, we chose two diagnostic criteria to obtain contrasted groups and to lower the risk of misclassification of patients.

In the present study we found that 50% (25 patients) of asthmatic patients were non-allergic. This proportion is high as the relative prevalence of non-allergic asthma is considered to reach 10%. This result is in agreement with Romanet–Manent et al., [18]. In similar studies the non-allergic reached up-to 30% [19]. The relatively high proportion of non-allergic asthmatics in our study is probably related to the hospital-based recruitment.

The importance of positive family history in classifying patients to allergic and non-allergic asthma is outlined in

Table 1 Descriptive data of asthmatic patients and comparison between allergic and non-allergic asthma groups.

		All asthmatic (*n* 50)	Allergic asthma (*n* 25)	Non-allergic asthma (*n* 25)	*P* value
Age		38.7 ± 11.7[∂]	39.72 ± 11.7	37.6 ± 11.7	0.5
Residence	Rural	31 (62%)[∂∂]	17 (68%)	14 (56%)	0.5
	Urban	19 (38%)	8 (32%)	11 (44%)	
Risk factors	Bird breeding	16 (32%)	10 (40%)	6 (24%)	0.3
	Dust exposure	24 (48%)	14 (56%)	10 (40%)	0.3
Clinical data	Cough	38 (76%)	21 (84%)	17 (68%)	0.3
	Dyspnea	50 (100%)	25 (100%)	25 (100%)	0.6
	Wheeze	48 (96%)	25 (100%)	23 (92%)	0.5
	Allergic rhinitis	21(42%)	17 (68%)	4 (16%)	0.001*
	Sinusitis	18 (36%)	14 (56%)	4 (16%)	0.001*
	Urticaria	6 (12%)	6 (24%)	0	0.02*
	Nocturnal attack	45 (90%)	25 (100%)	20 (80%)	0.5
	Family history	14 (28%)	11 (44%)	3 (12%)	0.03*
	Disease duration (yrs.)	7.53 ± 7.13[∂]	7.79 ± 7.21	7.28 ± 7.19	0.8
Medication	Regular use	11 (22%)	8 (32%)	3 (12%)	0.1
	ICS	24 (48%)	17 (68%)	7 (28%)	0.01*
	SABA	40 (80%)	23 (57.5%)	17 (68%)	0.05*
	LABA	31 (62%)	19 (76%)	12 (48%)	0.009*
	Systemic steroids	4 (8%)	3 (12%)	1 (4%)	0.6
	Antihistaminic	3 (6%)	3 (12%)	0	0.2
	Leukotriene modifiers	3 (6%)	3 (12%)	0	0.2
	Theophyllines	15 (30%)	11 (44%)	4 (16%)	0.01*

[∂] Data presented as mean ± SD.
[∂∂] Data presented as number (percentage).
* Significant $P < 0.05$.

Table 2 Comparison of frequency of anti-TPO and anti-CK18 autoantibodies and hypothyroid abnormalities in asthma and control groups.

	Control group (*n* 25)	Asthma group (*n* 25)	*P* value
Anti-TPO autoantibodies > 50 IU/ml	2 (6.7%)	10 (20%)	0.1
Anti-CK18 autoantibodies > 13 pg./ml	3 (10%)	13 (16%)	0.08
Hypothyroid	0 (0%)	3 (6%)	0.2

Table 3 Comparison of frequency of anti-TPO and anti-CK18 autoantibodies and hypothyroid abnormalities abnormality in allergic and non-allergic asthma groups.

	Allergic asthma (*n* 25)	Non-allergic asthma (*n* 25)	*P* value
Anti-TPO > 50 IU/ml	3 (12%)	7 (28%)	0.07
Anti-CK18 > 13 pg/ml	3 (12%)	10 (40%)	0.01*
Hypothyroid	2 (8%)	1 (4%)	0.95

* Significant $P < 0.05$.

Table 4 Comparison between asthmatic patients and control as regards serum level of different laboratory tests.

	Asthma group (*n* 25)	Control group (*n* 25)	*P* value
Total IgE IU/ml	280.8 ± 313.3	45.1 ± 38.3	0.001*
Anti-TPO autoantibodies IU/ml	44.7 ± 31.9	23.0 ± 20.7	0.001*
Anti-CK18 autoantibodies pg/ml	22.1 ± 30.8	9.7 ± 5.1	0.03*
Total T4 µg/dl	8.9 ± 2.3	7.8 ± 2.1	0.1
TSH µg/dl	1.89 ± 1.69	1.81 ± 0.52	0.2

* Significant $P < 0.05$.

adults. Classification of non-atopic asthma by Rackemann proposed stronger familial association asthma than in non-atopic asthma in adults [20]. Strong genetic predisposition to atopy and allergy indicates that atopic asthma in adults is more a family associated phenotype. In our study, positive family history was found in 44% of allergic asthma and 12% of non-allergic asthma and this difference was statistically significant ($P = 0.03$).

In the present study, there was a significant increase in the prevalence of allergic symptoms as allergic rhinitis ($P = 0.001$), allergic sinusitis ($P = 0.001$) and urticaria ($P = 0.02$) in allergic asthma than non-allergic asthma. In a study from an Iowa population, 78% of allergic asthmatics were rhinitic [21]. Pedersen et al. found a prevalence of 28% rhinitic patients in an asthmatic population [22]. In study of Romanet et al. [18], and Leynaert et al. [23], the prevalence of rhinitic symptoms was similar in both groups, irrespective

Table 5 Comparison between mean levels of different laboratory parameters of allergic and non-allergic asthma groups.

	Allergic asthma (*n* 25)	Non-allergic asthma (*n* 25)	*P* value
Hb%	12.2 ± 1.1	11.9 ± 0.9	0.5
WBCs × 10^3	7.3 ± 3.1	6.8 ± 2.4	0.4
Eosinophils%	1.3 ± 0.5	1.0 ± 0.1	0.002*
Pre-bronchodilator FVC%	79.6 ± 8.7	84.3 ± 7.4	0.04*
Post-bronchodilator FVC%	88 ± 6.2	91.7 ± 6.4	0.04*
Pre-bronchodilator FEV1%	59 ± 11.7	61.24 ± 11.4	0.5
Post-bronchodilator FEV1%	74.3 ± 11.3	75.8 ± 10.3	0.6
Pre-bronchodilator PEF25–75%	47.1 ± 15.1	56.2 ± 19.1	0.06
Post -bronchodilator PEF25–75%	64.1 ± 15.1	71.2 ± 14.9	0.1
Total IgE IU/ml	503.1 ± 310.1	58.5 ± 35.9	0.001*
Anti-TPO autoantibodies IU/ml	34.8 ± 23.6	54.6 ± 36.3	0.03*
Anti-CK18 autoantibodies pg/ml	13.6 ± 9.9	30.7 ± 4.1	0.04*
Total T4 µg/dl	8.8 ± 2.9	8.9 ± 3.7	0.8
TSH µg/dl	1.81 ± 1.48	1.97 ± 1.91	0.7

* Significant $P < 0.05$.

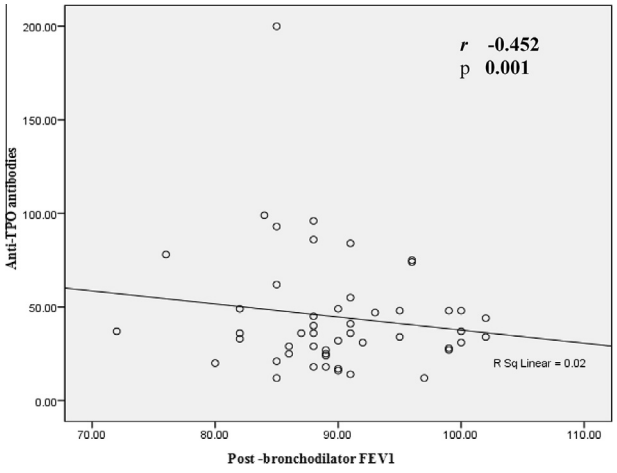

Figure 1 Correlation between anti-TPO and post-bronchodilator FEV1.

of allergic status. The authors found that the probability of displaying asthma among non-allergic subjects was strongly associated with perennial rhinitis, and also are probably due to sinusal pathology. This is suggested by the higher prevalence of nasal polyposis and sinusitis in this group.

In the present study, there was a significant statistical elevation of total IgE level in patients with bronchial asthma than in healthy subjects and in patients with allergic asthma than those with non-allergic asthma ($P < 0.001$ for both). These results agree with those of Takeoka and colleagues and Abd El-Aziz et al. [24,25].

As regards the pulmonary function tests in this study, there was non-significant difference in FEV1 in non-allergic

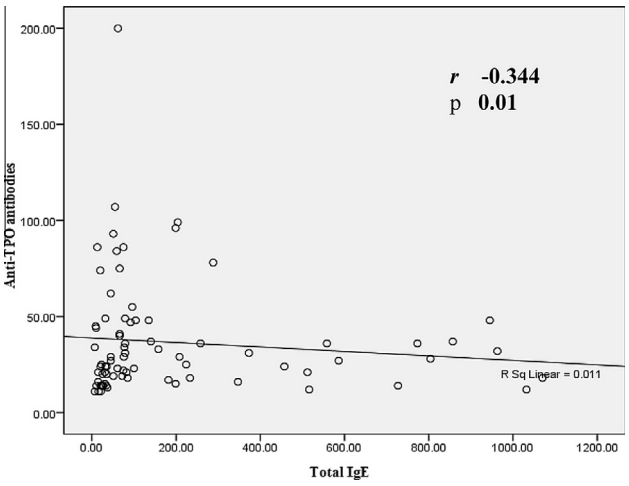

Figure 2 Correlation between anti-TPO and IgE.

asthmatic patients compared to the allergic asthma allergic asthmatic (*P* 0.5). This result is in agreement with Maria study [26]. Inouye et al. compared asthma severity in two groups of asthmatics with negative and positive skin tests to aeroallergens. They found greater exercise limitation, more frequent asthma-related symptoms, and a greater use of oral steroids in the negative skin test group, suggesting more severe asthma. Nevertheless, these differences disappeared after adjusting for age and asthma duration [27]. Furthermore, bronchial obstruction assessed by FEV1 was not significantly different between both groups. Accordingly, Cline et al. [28] did not find positivity of skin tests to be related to FEV1 nor to asthma severity.

In the present study, anti-TPO autoantibodies were statistically higher in bronchial asthma patients than in healthy subjects ($P < 0.001$). These results agree with previous studies [25,29,30]. The explanation was that the Th2 response-enhanced antibodies (as in asthma and other allergic diseases), and IL4, IL5, and IL13 (involved in allergic diseases) stimulate B cells to secrete thyroid antibodies, which in turn decrease thyroid hormone synthesis and secretion [31].

In our study, anti-TPO autoantibodies' level had significant negative correlation with total IgE in bronchial asthma patients ($r = -0.344$, *P* 0.01) and these results confirm that the changes in serum levels of antibodies can be used as an indicator of allergic and non-allergic asthma. These results are similar to those of Abd El-Aziz, et al. ($r = -0.56$, $P < 0.05$) [25].

As regards thyroid function, there were no statistical differences between asthmatic and control groups as well as between non-allergic and allergic asthma groups as regards both total T4 and TSH suggesting that asthma was not associated with changes in thyroid function. Abd El-Aziz et al. reported similar results [25]. Biscaldi and colleagues reported that T3 and T4 levels were higher in a control group than in asthmatic patients; but still, the asthmatic patients' thyroid hormone levels were within the reference range, suggesting that asthma was not associated with changes in thyroid function [32]. Landyshev and colleagues reported that thyroid function undergoes biphasic changes in bronchial asthma patients [33]. They also reported that as patients with mild bronchial asthma progressed to paroxysmal exacerbation of their

Table 6 Correlation between anti-TPO autoantibodies, anti-CK18 autoantibodies and total IgE and other parameters in all asthmatic patients.

	Anti-TPO autoantibodies		Anti-CK18 autoantibodies		Total IgE	
	r	*P*	*r*	*P*	*r*	*P*
Eosinophils%	0.123	0.4	−0.127	0.3	0.398	0.004*
Pre-bronchodilator FVC%	−0.219	0.1	0.232	0.1	−0.156	0.3
Post-bronchodilator FVC%	−0.142	0.3	0.183	0.2	−0.069	0.6
Pre-bronchodilator FEV1%	−0.372	0.008*	0.103	0.5	−0.044	0.7
Post-bronchodilator FEV1%	−0.452	0.001*	0.074	0.6	−0.045	0.8
Pre-bronchodilator PEF25–75%	−0.157	0.3	0.185	0.2	−0.223	0.1
Post-bronchodilator PEF25–75%	−0.184	0.2	0.315	0.02*	0.234	0.1
Total IgE IU/ml	−0.344	0.01*	−0.160	0.3	1.00	
Anti-TPO autoantibodies IU/ml	1.00		0.259	0.05*	−0.344	0.01*
Anti-CK18 autoantibodies pg/ml	0.259	0.05	1.00		−0.160	0.3
Total T4 μg/dl	0.109	0.4	−0.001	0.9	−0.044	0.8
TSH μg/dl	0.131	0.2	−0.032	0.7	0.022	0.8

* Significant $P < 0.05$.

Table 7 Relationship between severity of allergic asthma and anti-TPO and anti-CK18 autoantibodies' serum levels.

	Total	Mild intermittent ($N = 0$)	Mild persistent ($N = 1$)	Moderate persistent ($N = 27$)	Severe persistent ($N = 22$)	*P* value
Negative anti-TPO autoantibodies ⩽ 50 IU/ml	40	0 (0%)	1 (100%)	25 (93.6%)	14 (63.6%)	0.03*
Positive anti-TPO autoantibodies > 50 IU/ml	10	0 (0%)	0 (0%)	2 (6.4%)	8 (36.4%)	
Positive anti-CK18 autoantibodies < 13.4 pg/ml	37	0 (0%)	1 (100%)	21 (77.8%)	15 (68.2%)	0.6
Negative anti-CK18 autoantibodies > 13.4 pg/ml	13	0 (0%)	0 (0%)	6 (22.2%)	7 (31.8%)	

* Significant $P < 0.05$.

asthma, hypo-function of the patients' thyroid glands developed.

Also, we found the frequency of hypothyroidism was not statistically-different between the asthmatic group and control groups. Mitra and colleagues reported similar results [34]. In contrast, in the study of Jerez et al. on 49 asthmatics the prevalence of hypothyroidism was higher in the test group [35]. This may be due to lower sample size in the present study.

In our study, anti-CK18 autoantibodies' level in bronchial asthma patients was statistically higher than in healthy subjects ($P < 0.03$). Anti-CK18 autoantibodies' level was statistically higher than in non-allergic asthma than in allergic asthma ($P < 0.04$). Also, positive anti-CK18 autoantibodies' level (>13) was present in 40% of non-allergic asthma patients and 12% of allergic asthma patients which was statistically significant (P 0.01). Dong, et al. [36] reported similar results as they reported that circulating IgG autoantibodies to the anti-CK18 were detected in 43% of patients with non-allergic asthma, 11% of patients with allergic asthma and 9% of age-matched healthy volunteers ($P < 0.005$).

Another study reported that circulating autoantibodies against bronchial epithelial cells was detected in 9.1% (2 of 22) of non-atopic asthma (which is relatively low) and in none of 22 atopic asthma patients and of 22 healthy control subjects [37].

The identification of anti-CK18 autoantibodies as a bronchial epithelial auto-antigen associated with non-allergic asthma might provide a clue to exploring the autoimmune hypothesis in the pathogenesis of non-allergic asthma or it could be just a reflection of epithelial damage in patients with asthma [38].

Increased concentrations of circulating IgG antibodies against bovine cytokeratin 18 antigen have been reported in patients with idiopathic pulmonary fibrosis and autoimmune hepatitis [39,40], and increased concentrations of circulating IgA antibodies to bovine cytokeratin 18 antigen have also been reported in patients with rheumatoid arthritis [41]. These findings suggest that the anti-CK18 antigens may not be specific to non-allergic asthma.

The present study found association between severity of bronchial asthma and anti-TPO autoantibodies' level (Tables 6 and 7), but there was no association between severity of bronchial asthma and anti-CK18 level. Mitra et al. [34] found no association between severity of bronchial asthma and anti-TPO autoantibodies' level ($P = 0.54$). This difference may be related to the small number of our studied patients.

IgG antibodies from patients with non-allergic (intrinsic) asthma have recently been shown to have cytotoxic effects on epithelial cells, whereas these antibodies are not found in extrinsic asthma. It is possible that these autoantibodies damage airway epithelial cells so that super-antigen producing organisms are able to infect the airway surface more easily [42].

Conclusions

Asthma might just be a syndrome including various heterogeneous diseases regarding etiology, natural history, and sever-

ity. Our study suggests the possible existence of a subgroup of patients with asthma characterized by the presence of autoantibodies to bronchial epithelial antigens. We identified anti-CK18 autoantibodies (as an antibody response to bronchial epithelial autoantigens) and anti-TPO autoantibodies (as thyroid autoantibody) associated with non-allergic asthma. Further studies are needed to determine the significance of autoimmunity in non-allergic asthma.

Limitations of the study include performing the study on females, small sample size. Also, some other factors which could affect the anti-TPO and anti-CK18 autoantibodies are not included in the study.

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