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Pregnancy-associated plasma protein-A (PAPP-A) levels in patients with severe allergic asthma are reduced by omalizumab

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ABSTRACT

Background: Remodeling is a crucial feature of severe asthma and may be associated with activation of the allergic cascade by immunoglobulin E (IgE). Omalizumab, an anti-IgE monoclonal antibody, effectively targets the severe allergic asthma phenotype. Pregnancy-associated plasma protein-A (PAPP-A) is an insulin-like growth factor binding protein-4 (IGFBP-4) protease, increasing local insulin-like growth factor (IGF)-1 concentrations, which in turn initiating a cascade involved in the regulation of cell growth, differentiation, and proliferation in various tissues. In the present study, we evaluated the effects of omalizumab on serum PAPP-A, IGFBP-4, and IGF-1 levels in subjects with severe allergic asthma. **Methods:** We studied 36 asthmatic subjects and 36 healthy controls. An ultrasensitive enzyme-linked immunosorbent assay (ELISA) kit was used to measure serum PAPP-A levels, and routine commercial ELISA kits were employed to assess serum levels of IGF-1, IGFBP-4 in control subjects and asthmatic subjects before therapy (baseline) and after six months of omalizumab therapy in patients with severe asthma. **Results:** Compared to control subjects, serum PAPP-A and IGFB-4 levels were significantly higher in asthmatic subjects (both p values < 0.001). However, the serum IGF-I levels of asthmatic subjects were similar to those of control subjects ($p > 0.05$). In asthma subjects, 6-month omalizumab treatment significantly decreased the serum PAPP-A ($p < 0.001$), IGF-I ($p = 0.031$), and IGFB4 ($p = 0.025$) levels. **Conclusion:** PAPP-A level may be a useful biomarker for predicting airway remodeling in patients with severe asthma receiving omalizumab, and may also reflect the response to treatment.

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Introduction

Asthma is a chronic inflammatory airway disorder characterized by both inflammation and bronchoconstriction. Airway remodeling is critical in terms of asthma pathology, and the extent of remodeling correlates with disease severity (1). Severe asthma is a heterogeneous disease affecting 5–10% of all patients with asthma, and several different phenotypes have been described (2). Identifying biomarkers of the clinical response is crucial for successful management of severe asthma, and both periostin and galactin-3 levels have been suggested to be possible biomarkers (3,4). Additionally, we previously found that serum pregnancy-associated plasma protein-A (PAPP-A) levels were significantly higher in subjects with lung cancer (5), allergic rhinitis (6), and asthma (7) compared to those of healthy subjects.

Although its name suggests an association with pregnancy, PAPP-A is not a pregnancy-specific protein, but rather a growth-promoting enzyme that plays a crucial role in the insulin-like growth factor (IGF) pathway.

PAPP-A cleaves IGF binding protein-4 (IGFBP-4); this increases the local concentration of IGF-1, which initiates a cascade involved in cellular growth, differentiation, and proliferation in various tissues (8,9). PAPP-A has been suggested to increase local IGF-1 levels during vascular repair, wound-healing, and bone remodeling. Fibroblasts secrete PAPP-A and other growth factors that appear to be effective in thickening the basement membrane and encouraging lung fibroblast proliferation (10,11).

Airway remodeling is a crucial feature of severe asthma, and activation of the allergic cascade by immunoglobulin E (IgE) may be associated with such remodeling (12). Omalizumab is an anti-IgE monoclonal antibody that effectively targets the severe allergic asthma phenotype (13). In the present study, we evaluated the effect of omalizumab on serum PAPP-A, IGFBP-4, and IGF-1 levels, in patients with severe allergic asthma, to identify possible molecular mechanisms involved in the pathophysiology of the disease and to define biomarkers useful to evaluate the response to treatment.

Subjects and methods

Patient population

Overall, 36 patients with severe allergic asthma and 36 healthy subjects as a control group were enrolled to the study. Demographic characteristics, clinical findings, and spirometry results were determined before starting treatment with omalizumab. The exclusion criteria's were smoking, advanced liver or kidney failure, diabetes, heart failure, known hypertension, pregnancy, prerenal azotemia, known history of coronary artery disease, and history of major trauma or surgery within 6 months. All participants attended 12 visits, 4 weeks apart, in which they detailed about their symptoms and medications. Spirometric parameters and PAPP-A levels were reevaluated at sixth month in patients with treatment response at fourth month for omalizumab. Response to treatment was evaluated according to the physician's global evaluation of treatment effectiveness.

The patients were classified according to Global Initiative for Asthma Guideline disease severity criteria. Spirometric parameters (forced expiratory volume in 1 sec (FEV1) and forced vital capacity (FVC)) were measured according to European Respiratory Society guidelines. Three comparable FVC maneuvers were completed with the best of three attempts recorded. FEV1 values were required to be within 100 mL of each attempt to ensure reproducibility. Reversibility testing was conducted by remeasuring FEV1 20 minutes after inhalation of 400 mg of salbutamol. Positive reversibility was considered at 12% or greater increase in postbronchodilator FEV1 and spirometry results are expressed in percentage of predicted values according to the European reference equations (13).

Uncontrolled severe asthma was defined as asthma that could not be controlled despite treatment with 2,000 mg/d or greater of beclomethasone dipropionate or equivalent and a second controller medication. Skin prick tests were performed using a common panel including cat dander, dog dander, house dust mite, *Aspergillus fumigatus*, tree mix, grass pollen, and latex (ALK, Abello Denmark). A wheal size 3 mm larger than the negative control represented positive sensitization (atopy).

Asthma control was assessed by the asthma control test (ACT), consists of five questions regarding signs, symptoms, and rescue medication use in the last 4 weeks (14,15). In the present study, the following ACT scores were used for defining the level of asthma control: controlled asthma (CA), 25 points; partially controlled asthma (PCA), 20–24 points; and uncontrolled asthma, < 19 points (14,15).

The relevance for omalizumab use in asthmatic patients (Xolair, Novartis, Pharma AG, Basel, Switzerland) has been determined by the Ministry of Health on a basis of the following criteria: (1) clinical and functional asthma diagnosis; (2) patients with severe asthma who are not well controlled with ICS + LABA and/or leukotriene receptor antagonist (LTRA), long-acting theophylline; (3) serum IgE level: between 30 and 700 IU/mL; (4) perennial allergen sensitivity is demonstrated by skin test and/or specific IgE measurement; (5) age > 12 years; and (6) body weight < 150 kg (16,17).

The study was conducted at the Sureyyapasa Chest Disease and Chest Surgery Educational and Research Hospital and Acibadem Labmed Clinical Laboratories, Istanbul, Turkey. The study protocol was approved by the Ethics Committee of Acibadem University. Prior to study entry, all subjects provided written informed consent.

Biochemical assays

Compared to nonpregnant women and men, the serum PAPP-A level is very high in pregnant women (18). Indeed, the serum PAPP-A levels in nonpregnant women and men are so low as to render quantification by conventional enzyme-linked immunosorbent assay (ELISA) impossible. Thus, we used an ultrasensitive ELISA kit (DRG Instruments, Marburg, Germany) to measure serum PAPP-A levels. We used routine ELISA kits to measure the serum levels of IGF-1 (Mediagnost, Reutlingen, Germany) and IGFBP-4 (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturers' instructions (19–21).

Statistical analysis

We used SPSS software version 20 (Inc., Chicago, Illinois, USA) for statistical analyses. Power analysis was performed using G * Power (v3.1.9) program to determine the number of samples. According to the effect size coefficients defined by Jacob Cohen (22), assuming that the evaluations to be made between any two independent groups would have a large effect size ($d = 0.8$), it was calculated that at least 26 people should be in the group when calculated. Similarly, assuming that the evaluations to be made between two time periods in the patient group are assumed to have a large effect size ($d = 0.8$), it is estimated that at least 15 people should be calculated. Descriptive statistics was used to summarize the data. Kolmogorov–Smirnov test was employed to evaluate variables' adherence to a normal distribution. Data were presented as median (first quartile, third quartile), or mean $\pm$ standard deviation, as appropriate. Independent

Table 1. Clinical and demographic characteristics of the participants.

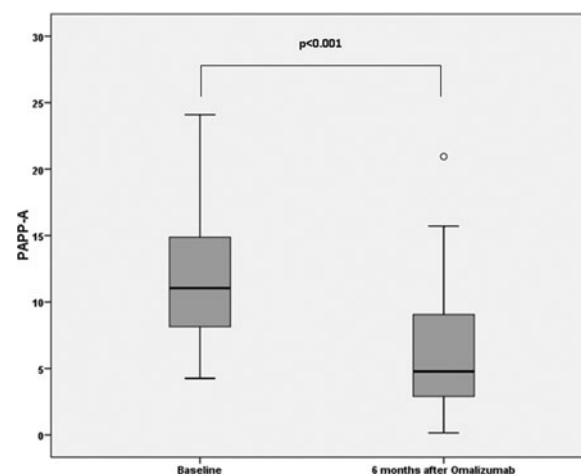
	Asthma patients (n = 36)	Control subjects (n = 36)	p
Age, years	41.69 ± 10.53	33.36 ± 6.94	<0.001** ^a
Gender, F/M	22 (61.1)/14 (38.9)	18 (50)/18 (50)	0.343 ^b
Disease duration, years	17.14 ± 9.21	—	—
Disease severity, n (%)	—	—	—
FEV1 ≥ 80%	4 (11.1)	—	—
FEV1 50–80%	12 (33.3)	—	—
FEV1 30–79%	17 (47.2)	—	—
FEV1 < 30%	3 (8.3)	—	—
Omalizumab use time, months	—	—	—
Mean ± SD	20.61 ± 7.09	—	—
Range	14–28	—	—
Omalizumab dosage, mg	—	—	—
Mean ± SD	233.33 ± 118.62	—	—
Range	150–600	—	—

^aIndependent samples t test. ^bPearson chi-square test. **p < 0.01.

samples t test was used to compare age between groups. Pearson chi-square test was used to compare gender between groups. Three separate analysis of covariance (ANCOVA) were conducted to analyze the effect of group on serum PAPP-A, IGF-I, and IGFB-4 levels, where age was introduced as covariate. We used paired t test (normal data) and Wilcoxon signed-ranks test for comparison of serum biomarker level in baseline and 6 months follow-up. Pearson correlation coefficient was used to evaluate association between quantitative variables. A *p* value of < 0.05 was considered as statistically significant

Results

Their demographic and clinical characteristics, omalizumab dosages, pulmonary function test (PFT) results, and extent of airway obstruction are shown in Table 1. Age was statistically significantly different between asthma and control groups (*p* < 0.001). Three separate ANCOVA were conducted to analyze the effect of group on serum PAPP-A, IGF-I, and IGFB-4 levels, where

**Figure 1.** PAPP-A levels of asthmatic patients before and after omalizumab.

age was introduced as covariate. Analysis revealed that PAPP-A and IGFB-4 levels were significantly higher in patients with asthma (*p* < 0.001, *p* = 0.013, respectively). On the other hand, serum IGF-I levels of asthmatic patients were similar to that of control subjects (Table 2) (*p* > 0.05). In asthma patients, the 6-months treatment of omalizumab significantly decreased serum PAPP-A, IGF-I, and IGFB-4 levels (*p* < 0.001) (Figure 1, 2, 3).

In addition to PAPP-A, the treatment of omalizumab for 6 months significantly increased the PFT values, including FVC, FEV1, FEV1/FVC, and FEF 25–75 (Table 3, all *p* < 0.01). Additionally, a statistically significant decrease in asthmatic attack frequency in a month or a year and hospitalization in a year were observed (Table 4, all *p* < 0.01). And also, asthma control test (ACT) score after omalizumab therapy was significantly higher (Table 4, *p* < 0.001).

There was no statistically significant correlation between the delta changes spirometry values and the delta changes with serum PAPP-A in patients with allergic asthma during initial and sixth month after

Table 2. The medians and ranges of PAPP-A, IGF-I, and IGFBP-4 of severe allergic asthma and control subjects.

		Beta (95% CI)	p	Partial eta squared	Observed power
PAPP-A	Corrected model	—	<0.001 ^b	0.503	1.000
	Intercept	1.26 (−3.03, 5.54)	0.560	0.006	0.089
	Age	0.05 (−0.06, 0.17)	0.355	0.014	0.151
	Group	8.00 (5.66, 19.34)	<0.001 ^b	0.429	1.000
IGF-I	Corrected model	—	0.913	0.003	0.063
	Intercept	163.01 (107.60, 218.41)	<0.001 ^b	0.362	1.000
	Age	−0.12 (−1.64, 1.41)	0.876	0.001	0.053
	Group	6.36 (−23.37, 36.10)	0.670	0.003	0.071
IGFBP-4	Corrected model	—	<0.001 ^b	0.294	0.993
	Intercept	0.79 (−2.23, 3.82)	0.602	0.005	0.081
	Age	0.12 (0.04, 0.20)	0.005 ^b	0.129	0.814
	Group	2.07 (0.46, 3.69)	0.013 ^a	0.104	0.714

^a*p* < 0.05. ^b*p* < 0.01.

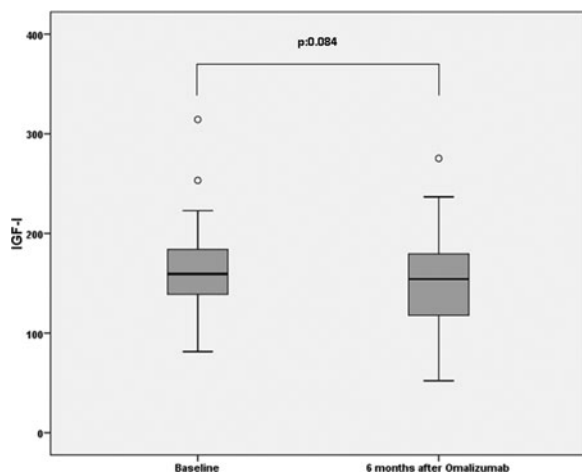


Figure 2. IGF-I levels of asthmatic patients before and after omalizumab.

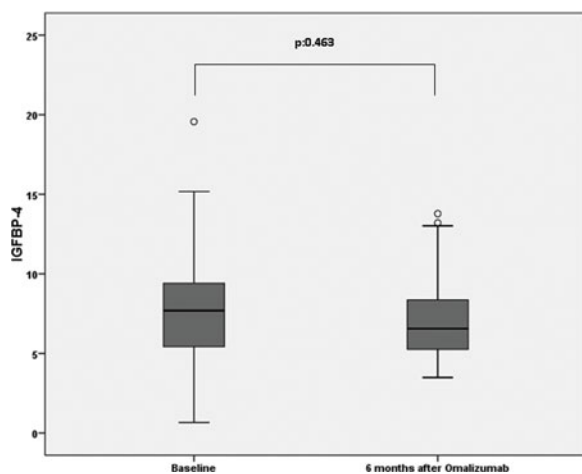


Figure 3. IGFBP-4 levels of asthmatic patients before and after omalizumab.

omalizumab treatment (Table 5, $p > 0.05$). In addition, there was no statistically significant correlation between the PFT, ACT, number of asthma attacks, exacerbations, and the baseline serum PAPP-A, IGF-I, and IGFBP-4 values in patients with allergic asthma ($p > 0.05$), except for FEV1/FVC and baseline PAPP-A there was a significant correlation ($r = -0.422$, $p = 0.010$) (Table 6).

Table 3. PFT changes of participants before and after treatment in patients with severe asthma.

	Before treatment ($n = 36$)	After treatment ($n = 36$)	p	Effect size
FEV1 (%)	54.44 $\pm$ 22.39	76.56 $\pm$ 19.96	<0.001** ^c	1.376
FEV1 (mL)	1722.78 $\pm$ 709.91	2323.89 $\pm$ 796.74	<0.001** ^c	1.239
FVC (%)	67.06 $\pm$ 21.19	89.97 $\pm$ 18.66	<0.001** ^c	1.392
FVC (mL)	2573.89 $\pm$ 1082.22	3363.61 $\pm$ 1123.08	<0.001** ^c	1.312
FEV1/FVC	67.64 $\pm$ 12.14	75.08 $\pm$ 12.56	0.003** ^c	0.540
FEF 25–75 (%)	33.25 $\pm$ 21.73	45.61 $\pm$ 22.09	<0.001** ^c	0.835
FEF 25–75 (mL)	1278.06 $\pm$ 809.80	1769.72 $\pm$ 878.08	<0.001** ^c	2.924

^cPaired t test.

** $p < 0.01$.

Table 4. Clinical changes of participants before and after treatment in patients with asthma.

	Before treatment	After treatment	p	Effect size
Attack frequency in a month	3.5 (1, 6.5)	0 (0, 0)	<0.001** ^d	0.953
Attack frequency in a year	13.5 (5, 40)	0 (0, 1.5)	<0.001** ^d	0.938
Hospitalization in a year	0 (0, 2)	0 (0, 0)	0.002** ^d	0.445
ACT score	7.47 $\pm$ 3.10	20.44 $\pm$ 4.11	<0.001** ^c	2.924

^cPaired t test.

^dWilcoxon signed-ranks test.

** $p < 0.01$.

Discussion

In the present study, PAPP-A and IGFBP-4 levels were higher in patients with asthma than in controls. After treatment with omalizumab for 6 months, significant improvements were detected in the PAPP-A, IGF-1, and IGFBP-4 levels. In addition, FEV1, FVC, the FEV1/FVC ratio, forced expiratory flow 25–75% (FEF 25–75), and ACT scores were significantly higher after treatment. A significant decrease in the frequency of acute exacerbations and hospitalization was revealed after treatment at the 1-year follow up.

Serum PAPP-A and IGFBP-4 levels were higher among patients with asthma than in controls; omalizumab significantly reduced these levels, as well as those of IGF-1. Although PAPP-A levels are elevated in patients with acute coronary syndrome (23), the clinical significance of PAPP-A is not limited to cardiac events (7,25). We previously found that PAPP-A levels were higher in patients with asthma than in controls (7). Similarly, Talay et al. reported that PAPP-A levels were higher in patients with chronic obstructive pulmonary disease than in controls (25).

Airway inflammation in patients with asthma causes tissue injury and subsequent structural changes, which is referred to as remodeling. Contributory factors include growth factors, such as IGF-I. PAPP-A regulates IGF-1 availability by cleaving IGFBP-4 at a particular site,

Table 5. The correlation between the delta changes (between baseline and 6 months) of spirometry values and the delta changes of serum PAPP-A in patients with allergic asthma.

Variables	r^a	p
Delta change of FEV1 (mL)	−0.096	0.595
Delta change of FEV1 predicted (%)	−0.130	0.472
Delta change of FVC (mL)	−0.077	0.669
Delta change of FVC predicted (%)	−0.152	0.399
Delta change of FEF 25–75 (mL)	−0.031	0.864
Delta change of FEF 25–75 predicted (%)	−0.138	0.444

^aPearson correlation coefficient. FEV1: forced expiratory volume in 1 second; FVC: forced volume capacity; FEF 25–75%: forced expiratory flow in 25–75% of 1 second.

Table 6. The correlation between the PFT, ACT, number of asthma attacks, exacerbations, and the baseline serum PAPP-A, IGF-I, and IGFBP-4 values in patients with allergic asthma.

	PAPP-A		IGF-I		IGFBP-4	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
FEV1 (%)	−0.258	0.129	−0.139	0.419	−0.053	0.760
FEV1 (mL)	−0.086	0.616	−0.061	0.723	−0.114	0.507
FVC (%)	−0.103	0.551	−0.081	0.638	−0.164	0.338
FVC (mL)	0.094	0.585	−0.020	0.906	−0.150	0.383
FEV1/FVC	−0.422	0.010 ^b	−0.119	0.488	0.121	0.481
FEF 25–75 (%)	−0.249	0.142	−0.119	0.489	−0.099	0.567
FEF 25–75 (mL)	−0.218	0.201	−0.075	0.664	−0.131	0.447
Attack frequency in a month	−0.046	0.792	−0.055	0.752	−0.002	0.992
Attack frequency in a year	0.136	0.430	0.057	0.741	−0.079	0.646
Hospitalization in a year	0.056	0.744	0.157	0.362	−0.190	0.266
ACT score	−0.063	0.714	0.196	0.253	−0.293	0.082
IgE	−0.115	0.504	0.144	0.401	0.160	0.351

^a*r*: Pearson correlation coefficient.^b*p* < 0.05.

thereby lowering the affinity of the protein for IGF (26,27).

Reversing airway remodeling is critical to prevent disease progression. We found significant improvements in PFT parameters, ACT scores, and asthma attacks, and hospitalization frequency rates in the year following 6 months of omalizumab treatment. The fall in PAPP-A level evident after treatment suggests that the protein may play a role in airway remodeling.

IgE plays a central role in the pathophysiology of asthma and allergic inflammation. Roth et al. reported an association between IgE levels and airway remodeling (28). Kang et al. showed that omalizumab reduces the levels of remodeling markers (peribronchial collagen III/IV and smooth muscle actin- α) (29). Hoshino et al. (30) reported that 16 weeks of omalizumab treatment significantly reduced sputum eosinophilia and significantly increased FEV1. Tajiri et al. (31) also reported significant reductions in induced sputum eosinophilia and the fraction of exhaled nitric oxide at the 50 mL/s level. Similarly, we noted significant improvements in PFT values, including FEV1, FVC, the FEV1/FVC ratio, and FEF 25–75 after 6 months of omalizumab treatment. This drug very effectively improved the clinical parameters of patients with severe allergic asthma. We also found significant decreases in monthly and annual asthmatic attack frequency rates, and in the annual hospitalization rate of such patients. The ACT score also rose significantly after treatment.

Careful patient selection is essential for omalizumab treatment because the drug is expensive and patients must be monitored during treatment. As our understanding of asthma pathophysiology and airway remodeling improves, targeted therapies will become more cost-effective (32). The level of the extracellular matrix protein galectin-3 is correlated with the modulation of airway remodeling by omalizumab. We previously

reported that the PAPP-A level predicts the response to treatment and may also serve as an index of treatment efficacy.

Limitations of the study

Our study had certain limitations. Firstly, our sample size was small. Secondly, we did not histologically assess airway remodeling (or the lack thereof). However, our results may stimulate further work in the field.

Conclusion

PAPP-A levels were higher in patients with asthma than in controls, and fell after 6 months of omalizumab treatment; this suggests that the PAPP-A level can be used to predict modulation of airway remodeling, and could also serve as an index of the treatment response. Further studies are required to elucidate the exact role played by PAPP-A in patients with severe asthma and to determine the significance of the PAPP-A level during management of such patients.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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