Eurasian J Pulmonol 2017; 19: 84-90

Effects of Omalizumab Treatment on Some Biomarkers in Severe Allergic Asthma Patients

Seda Tural Önür[1], Arzu Didem Yalçın[2], Saadet Gümüşlü[3], Gizem Esra Genç[3], Kemal Kiraz[4], Sedat Altın[1]

[1]Clinic of Chest Diseases, University of Health Sciences Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, İstanbul, Turkey
[2]Clinic of Allergy and Clinical Immunology, University of Health Sciences Antalya Training and Research Hospital, Antalya, Turkey
[3]Department of Medical Biochemistry, Akdeniz University School of Medicine, Antalya, Turkey
[4]Clinic of Chest Diseases, University of Health Sciences Antalya Training and Research Hospital, Antalya, Turkey

Cite this article as: Tural Önür S, Yalçın AD, Gümüşlü S, Genç GE, Kiraz K, Altın S. Effects of Omalizumab Treatment on Some Biomarkers in Severe Allergic Asthma Patients. Eurasian J Pulmonol 2017; 19: 84-90.

Abstract

Objective: The mechanism of biological treatment molecule called omalizumab used in asthma treatment is thought to be versatile; however, the mechanism still remains unknown. This study was undertaken in severe asthma patients underwent omalizumab treatment, in order to investigate the relationship between biomarker expression and disease characteristics related to the immune system.

Methods: Consecutive patients with severe asthma disease (n=15; Group IA, pretreatment and Group IB, post-treatment) underwent omalizumab treatment. Control group was age- and sex-matched including 25 healthy in Group II. Blood samples from both the groups were taken during their first visit (Group IA and II) and then after 12 months of treatment in asthmatic patients (Group IB). Serum levels of homocysteine (Hcy), eosinophil cationic peptide (ECP), 25-hydroxyvitamin D (25(OH)D), interleukin-1β (IL-1β), soluble OX_2 (sCD200) and clinical follow-up tests including fractional exhaled nitric oxide (FeNO), asthma control test (ACT), and pulmonary function tests were evaluated.

Results: After the treatment, 25(OH)D levels and pulmonary function tests, including forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) levels, were significantly increased. Furthermore, total immunoglobulin E (IgE), Hcy, ECP, FeNO, and sCD200 levels were dramatically diminished. Regression analysis revealed positive correlations between ACT-FEV_1 and ACT- FVC and between FeNO-age and FeNO-ECP for Group IA patients. Negative correlations were detected between ACT-IgE, age-FEV_1, FeNO-FEV_1, and FeNO-FVC for Group IA patients.

Conclusion: Our results suggest that the potential use of serum biomolecules in concordance to the clinical status of the asthmatic patients might be a follow-up tool for the omalizumab therapy.

Keywords: Asthma, biomarkers, omalizumab

Received Date: 05.09.2016
Accepted Date: 27.01.2017

DOI: 10.5152/ejp.2017.64935

Corresponding Author
Seda Tural Önür
E-mail: sedatural@yahoo.com

• Available online at www.eurasianjpulmonol.com

INTRODUCTION

Allergic asthma is a disease which mostly exists at the Mediterranean coast (1). Furthermore, previous studies engaged in the relationship between climate records and the disease stated that the number of patients visiting the clinic with increased health problems has increased with the increasing amount of pollen in the air (2). During the recent years, anti-immunoglobulin E (IgE) therapy (omalizumab) has been developed as a primary representative of new therapeutic class for allergic asthma and has become a crucial example for the biological treatments (3). Important guidelines for asthma, such as the Global Initiative for Asthma (GINA) and National Asthma Education and Prevention Expert Panel Report 3 (EPR-3), stated omalizumab can be used for severe asthma patients (4, 5).

The mechanism of action of anti-IgE has a decreasing effect on the free IgE and afterwards decreases the regulatory effect on the IgE receptors of basophils and mast cells. Hence, inhaled allergen reac-

tions are diminished (6). Throughout the regression, mast cell concerned effects are decreased, and mediators linking with the symptoms of patients with allergies and asthma are released. Besides, the reduction of allergic inflammation is also associated to the rush of eosinophilic granulocytes (7).

Among the later described molecules in serum which have a regulatory effect on inflammation and immune reactivity, homocysteine (Hcy), 25-hydroxyvitamin D (25(OH)D), eosinophil cationic peptide (ECP), interleukin-1 β (IL-1β), and OX2 (sCD200) are more focused on. Already existing studies suggest that OX2 and its receptor OX2R interactions regulate the allergic inflammatory disease that IL-1β has important effects on the inflammation of the respiratory tract, and that increased ECP levels are correlated with asthma exacerbation (8,9). Moreover, 25(OH)D activates an Hcy-metabolizing enzyme. A significant study suggested a correlation between vitamin D status and Hcy levels (10). Some studies revealed that Hcy has an effect on inflammatory cytokine/chemokine production and it might be through monocytes and macrophages in serum (11, 12). Therefore, Hcy appears to have immunomodulating and pro-inflammatory activities.

As little is known about the potential relationship between all these inflammatory markers, the objective of our study is to consider the potential impact of immunomodulation related with biomolecules of Hcy, ECP, 25(OH)D, IL-1β, and OX2 concentrations together with clinical markers of fractional exhaled nitric oxide (FeNO), asthma control test (ACT), and pulmonary function tests in asthmatic patients who is undergoing the biological treatment of omalizumab.

METHODS

Subjects

Consecutive patients with severe asthma disease (n=15, Group I) without any other autoimmune and/or chronic disorders, older than 18 years, managed by the Clinical Immunology and Allergy Unit, were recruited for the study. Age- and sex-matched control group (n=25, Group II) was diagnosed to have any atopy, autoimmune disorder, or allergic disease and major systemic disorders attending the outpatient of the same clinic. None of the participants smoked cigarette. They were informed about the study and were asked for consent before starting the study. The study had local Ethical Committee approval.

Laboratory

In Group I, 7 males and 8 females were diagnosed with severe allergic asthma and allergic rhinitis. They all underwent omalizumab treatment with 300–375 IU/mL subcutaneous injection within every 2 weeks for 12 months. Serum specimens were collected in May, which is the highest air pollination period in our region when they were first diagnosed and were named as Group IA (pre-omalizumab), and after 12 months of treatment during the disease remission as Group IB (post-omalizumab) (1, 2). All the samples for serum soluble markers' evaluation were collected at approximately 8–10 AM. As a result of the effects on inflammatory markers, participants abstained from caffeinated drinks and food for 12 hours before testing. Pulmonary function tests and evaluation of FeNO were assessed on the same day of clinical visit. Clinical assessment and adverse effects, serum IgE and ECP levels with pulmonary function tests, ACT (Quality Metric Incorp.) and FeNO concentrations were assessed during each outpatient visits. The latest version of severity of asthma score (SOA) was used to diagnose asthma severity (13). FeNO was evaluated under the ATS/ERS guidelines (NObreath; Bedfont Scientific Ltd., Rochester, Kent, UK). Subjects first inhaled ambient air to near total lung capacity and then exhaled for 16 s at a constant flow rate through a mouthpiece into the device. Patients' daily medications, asthma severity, and control test scores were recorded (4, 14).

Serum samples were centrifuged at 3000 rpm for 10 minutes to separate them from cells. OX2 levels (Sino Biological Inc., Catalog Number: SEK10886) and IL-1β (Diaclone, Catalog No: 851.610.005) were counted in serum samples via enzyme-linked immunosorbent assay according to the manufacturer's guidance. Serum Hcy concentrations were measured using an Hcy assay kit (Roche). Serum IgE concentrations were quantified via fluoroenzyme immunoassay (FEIA) which uses an ImmunoCAP (Pharmacia, Uppsala, Sweden). All of the patients have high IgE levels. Cutoff value was 100 kU/L for total IgE concentration and 0.35 kU/L for specific IgE concentration for selected patients. Serum 25(OH)D concentrations were counted via a radioimmunoassay (Cobra Quantum, Packard, MN), and we categorized its concentration as sufficient (≥30 ng/mL), deficient (<20 ng/mL), or insufficient (20-30 ng/mL), based on previous suggestions (15). The doubled measurements were recorded from devices.

All patients underwent skin prick tests (SPT) since they are first diagnosed because of our regular clinical algorithm which uses standardized latex extract and a complete allergens set which includes 35 common (such as pollen and mite) and food allergens (Allergopharma, Reinbek, Germany) (Table 1). Total IgE levels were also administered within the correlation of SPTs.

Treatment protocol

Patients were questioned so as to decide their treatment protocol for maintaining asthma diaries, and the use of total oral corticosteroid dose per month was recorded. Patients were also questioned to emergency unit visits for asthma exacerbation. They were also told, preferably, to go to our outpatient allergy clinic during working hours instead of going to emergency room outside the working hours, in order to keep the treatment under control. Best standard care was conducted following medications with inhaled salmeterol 50 mg bid, inhaled fluticasone 500 mg bid, and oral methyl prednisolone according to the suggestions from GINA. Omalizumab treatment was started patients at least 12 months according to GINA. Oral steroid doses were decreased if the patient clinical signs were stable. Oral steroid was reduced to 2 mg/week in the following weeks. Four of our patients used oral steroid, and we could stop them from using oral steroid by gradually reducing their medication.

Statistical Analysis

The data were presented as means ± SD. Data were analyzed by descriptive and inferential statistics using the SPSS 13.0 version (Statistical Package for the Social Sciences Inc.; Chicago, IL, USA). In comparison of groups, the Student t test was used according to the normality of distribution of the variables in dependent groups. Pearson correlation analysis was used for correlations between variables. p-value meant statistically significant when it was less than 0.05.

RESULTS

The general features and laboratory test results of the patients and control group are summarized in Table 2. ACT score is shown in Fig-

Table 1. Skin prick test results and omalizumab dose schemes in asthmatic patients

Patient no.	Number of injections (total times/ dose, mg/2 weeks)	SPT	Daily oral steroid doses (mg), Group IA	Daily oral steroid doses (mg), Group IB	Daily inhalant steroid doses (µg), Group IA	Daily inhalant steroid doses (µg), Group IB
1	22 / 375	Wheat, tree, mold, cockroach	4	0	1000	500
2	23 / 375	Cockroach, mold, wheat	0	0	1000	400
3	20 / 225	Mite, grass, olive, mold	4	0	2500	1000
4	24 / 300	Grass, tree, mite	6	0	600	600
5	19 / 225	Olive, mite, cockroach, dog	0	0	2000	500
6	22 / 375	Cockroach, olive, latex mite, mold, wheat	8	0	1500	1000
7	21 / 150	Mold, grass, tree, latex, cat	0	0	2000	500
8	18 / 150	Mite, olive, horse, sesame, kiwi	0	0	1000	400
9	21 / 150	Grass, wheat, olive, tree, mite	4	0	2000	500
10	24 / 300	Grass, wheat, tree, mold, mite, cockroach, honeybee, dog, cat	12	4	1200	1000
11	23 / 300	Mite, tree, cat, olive, honeybee	4	0	2000	500
12	21 / 300	Grass, tree, mite, dog	8	0	800	400
13	20 / 300	Mite, cockroach, mold	0	0	1000	400
14	25 / 150	Wheat, mold, olive, mite	0	0	1500	500
15	18 / 225	Mite, tree, mold	4	0	1000	400

SPT: Skin prick tests

Table 2. Clinical and laboratory characteristics of the patients (group IA and IB) and controls (group II)

	Group IA (n=15)	Group IB	Group II (n=25)	p
Age (y)	44.47±8.20		41.88±9.20	p>0.05
Gender (female/male)	8 / 7		15 / 10	p>0.05
BMI (kg/m^2)	30.59±7.3	30.2±6.9	29.28±6.60	p>0.05
Smoker/non-smoker	2 / 13		3 / 22	p>0.05
ACT	14.31±3.1	22.47±1.45		p<0.0001
FEV_1 (%)	59.53±6.45	89.82±5.27		p<0.0001
FVC (%)	64.5±12.56	93.42±5.33		p<0.0001
25(OH) D (ng/mL)	13.33±2.85	18.04±2.02	16.98±2.15	p<0.05
Hcy (mmol/mL)	28.98±3.46	23.22±1.87	19.17±3.24	p<0.05
Total IgE (IU/mL)	555.90±100.75	348.30±96.00		p<0.05
ECP (ng/mL)	42.56±8.24	29.45±13.00		p<0.05
FeNO (ppb)	38.55±5.10	26.70±3.05		p<0.0001
SOA	21.34±2.97	7.53±2.2		p<0.0001
sCD200 (pg/mL)	61.80±3.15	33.25±6.30	21.57±5.55	p<0.0001
IL-1β (pg/mL)	45.27±0.6864	42.83±0.8175	44.32±0.842	p<0.05

The p value of 25 (OH) D is a comparison between Group 1A and Group 1B. The p value of Hcy is a comparison between Group 1A and Group 1B. The p value of sCD200 is a comparison between Group 1A and Group 1B.

ACT: Asthma control test; BMI: body mass index; ECP: eosinophil cationic peptid; FeNO: exhaled nitric oxide; FEV_1: forced expiratory volume 1; FVC: forced vital capacity; Hcy:homocysteine; IL-1β: interleukin (IL)-1β; sCD200: soluble CD200; SOA: severity of asthma score; 25(OH)D: 25-hydroxyvitamin D

ure 1a with p<0.001. Forced expiratory volume 1 (FEV_1) is shown in Figure 1b with p<0.001 and forced vital capacity (FVC) in Figure 1c with p<0.001. Serum 25(OH)D (ng/mL) levels are represented in Figure 2 with p<0.05. ACT scores, FEV_1, FVC, and serum 25(OH)D levels were increased statistically significantly after omalizumab treatment; however, serum Hcy (mmol/mL) concentrations were statistically sig-

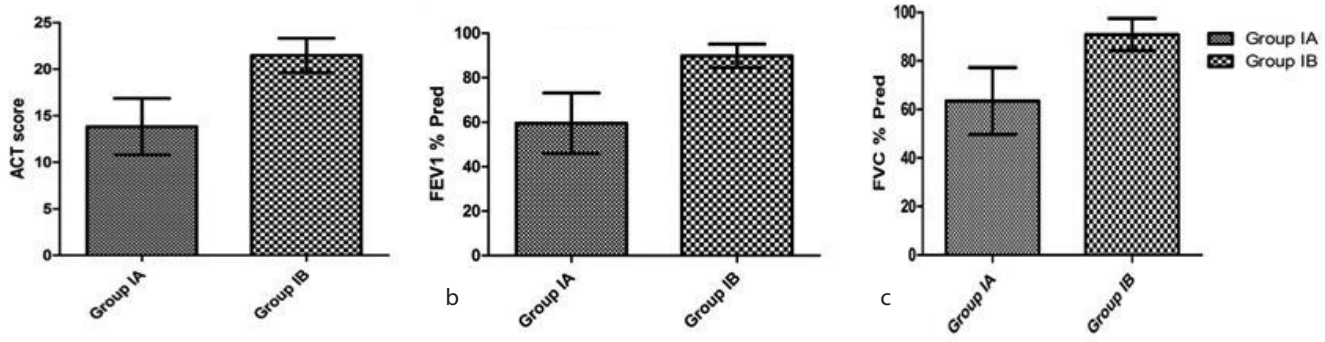

Figure 1.a-c. Comparison of pulmonary function test results and ACT scores between Groups IA and IB. (a) ACT scores. (b) FEV_1% rates. (c) FVC% of patients
ACT: Asthma control test; FEV_1: forced expiratory volume 1; FVC: forced vital capacity

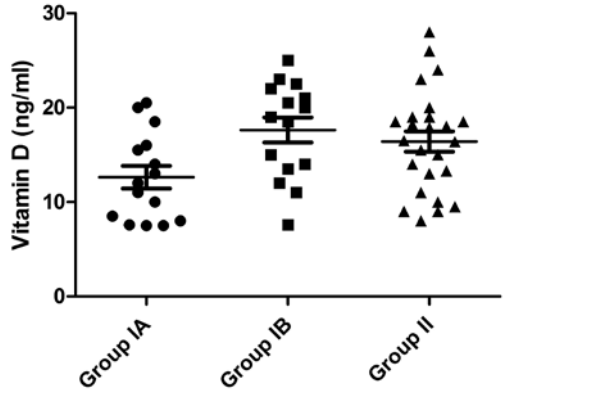

Figure 2. Comparison of vitamin D (25(OH)D) levels between groups IA and IB
25(OH)D: 25-hydroxyvitamin D

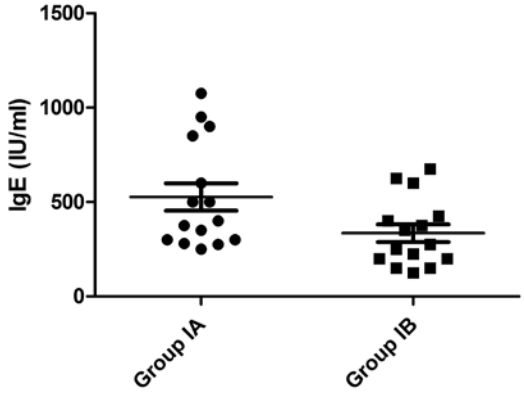

Figure 3. Comparison of total IgE levels between groups IA and IB

nificantly decreased after omalizumab treatment ($p<0.05$). Total IgE is shown in Figure 3 with $p<0.05$. ECP is demonstrated in Figure 4 with $p<0.05$ and FeNO in Figure 5 with $p<0.001$. SOA is demonstrated in Figure 6 with $p<0.001$ and sCD200 in Figure 7 with $p<0.0001$. On the contrary, total IgE, ECP, FeNO, SOA, and sCD200 were all significantly decreased in the post-omalizumab group. However, IL-1β concentrations in the patients and control groups have no statistically significant variation which is demonstrated in Figure 8.

Moreover, we evaluated whether there is a correlation in clinical markers. In group IA, there were positive correlations between ACT and FEV_1 ($r=0.534$, $p<0.05$), ACT and FVC ($r=0.616$, $p<0.05$), FeNO and age ($r=0.562$, $p<0.05$), FeNO and ECP ($r=0.480$, $p<0.05$), FeNO and SOA ($r=0.601$, $p<0.05$), and IL-1β and SOA ($r=0.507$, $p<0.05$). Also, negative correlations in group IA were detected between ACT and IgE ($r=-0.533$, $p<0.05$), age and FEV_1 ($r=-0.491$, $p<0.05$), FeNO and FEV_1 ($r=-0.542$, $p<0.05$), and FeNO and FVC ($r=-0.489$, $p<0.05$).
In group IB, age and FeNO have a positive correlation ($r=0.562$, $p<0.05$) and body mass index (BMI) and IgE have a negative correlation ($r=-0.499$, $p<0.05$). Also, negative correlations in group IB were detected between IL-1β and FEV_1 ($r=-0.567$, $p<0.05$) and ACT and IgE ($r=-0.620$, $p<0.05$).

DISCUSSION

The major pathophysiological mechanisms of omalizumab treatment in patients with asthma are still being investigated by many groups, while some systematic reviews show that omalizumab has efficiency as an additional treatment option in allergic patients with asthma (16). There is no knowledge about serum soluble biomarkers related to inflammation including Hcy, OX2, and 25(OH)D concentrations in asthmatics and their potency to guide a therapeutic approach by omalizumab. Our study showed that these are able to guide a therapeutic approach by omalizumab.

Cell membrane--connected OX2 and serum-soluble form of OX2 are novel immune-efficient molecules which have dual effects as a pro-inflammatory through its receptor and an immunomodulatory drug (8). In a previous study, OX2 levels were altering owing to the clinical condition of patients that might be on account of playing the role of pathogenesis of autoimmune/inflammatory diseases (17, 18). Our observations on the patients seem to be supporting the role of OX2 as a pro-inflammatory molecule such as IL-1β (8, 19, 20). Thus, the increased levels of OX2 seen in our study with active disease (Group 1A) presumably represent a manifestation of a homeostatic response directed to the control of inflammation. The diminished levels of OX2, seen in the post-omalizumab group, reflect the successful suppression of the inflammatory response.

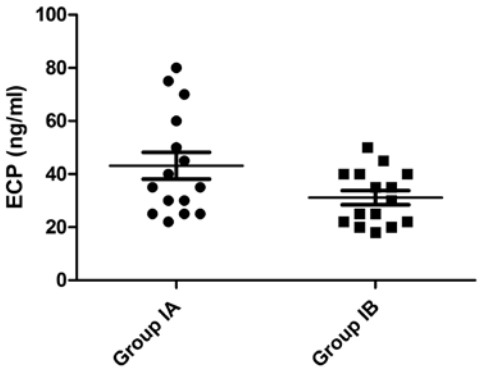

Figure 4. Comparison of ECP levels between groups IA and IB
ECP: Eosinophil cationic peptid

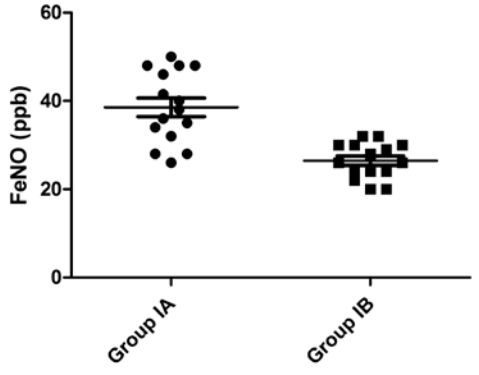

Figure 5. Comparison of FeNO concentrations between groups IA and IB
FeNO: Exhaled nitric oxide

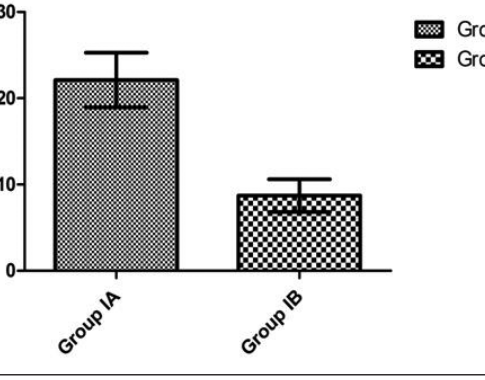

Figure 6. Comparison of SOA between groups IA and IB
SOA: Severity of asthma score

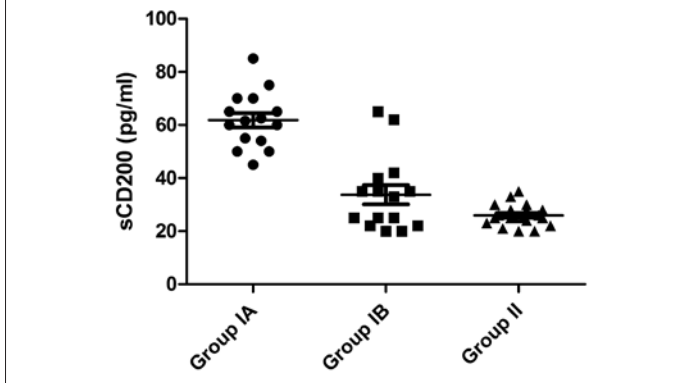

Figure 7. Comparison of sCD200 between groups IA and IB
sCD200: Soluble CD200; IL-1β: interleukin (IL)-1β

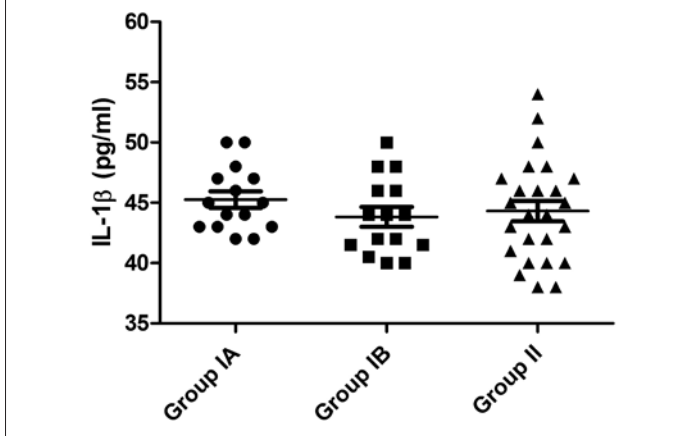

Figure 8. Comparison of IL-1β levels between groups IA and IB

25(OH)D which is a steroid hormone is approved to have immunomodulatory effects both in vitro and in vivo. Data from previous researches suggest that 25(OH)D has important effects on the inflammation of the respiratory tract and is a predictive marker for pulmonary function (9). Nonetheless, it is scarcely unknown that whether this effect is on natural or adaptive immune system or is connected to cytokines. Its association with asthma severity in relation to different treatment modalities is also unknown (15, 19-21). Hence by our study, we investigated the serum 25(OH)D concentrations in patients with asthma with other inflammatory molecules in relation to omalizumab treatment. 25(OH)D studies were increased in the last decade, and many studies reported that its deficit is contrarily associated with BMI. We concluded that the increase in 25(OH)D might be due to a decreased use of systemic steroids with accomplished biological treatment modality (20). The other probable explanation is, as reported earlier, the seasonal factors relating the vitamin D levels. It decreases from summer to winter (22). In this study, we also had the samples from the patients during the highest pollination time in our region during May within the average temperature of 25°C with high humidity percentage as in mid-summer season in many other countries. This data however propose a new target to study, air pollination and seasonal effects.

Asthma is linked to eosinophils which are found in increased levels in our body. Elevated levels of ECP have been observed in asthmatics. Additionally, there is an association between increased ECP concentrations and asthma aggravation as a biomarker (23, 24). We observed a correlation between ECP and FeNO and FeNO and age in the pretreatment group (Group IA) in our study.

The effect of IL-1β on asthma pathology was earlier defined and linked with inflammation (25). Fascinatingly, Group IA has a positive correlation between IL-1β levels and SOA, while in Group IB, there is a

negative correlation between IL-1β levels and FEV_1. Conversely, there was no change in IL-1β levels between the groups and omalizumab had no effect on IL-1β levels. Controversial to prior studies showing a decrease on IL-1β levels in severe asthmatics, no change in the concentration might be caused in patients having asthma and shorter treatment periods (19, 20, 26).

A few researches have pointed out that Hcy may lead to the advancement of atherosclerosis, which is an important risk factor of cardiovascular diseases. Nevertheless, mechanisms of endothelial dysfunction induced by Hcy are not yet exactly known. Also, Hcy has a pro-inflammatory property which was proven by studies. According to our results, we might speculate that it is an endothelial protection mechanism which might be together with an increase in IgE levels (27).

After all, our study is the first one to investigate the significance of distinct levels of serum sCD200, 25(OH)D, and Hcy in allergic asthma patients. The correlation of these levels with clinical features was performed after the omalizumab treatment. As shown in our data and previous studies, clinical follow-up markers are extremely associated with increased levels of immunoregulatory functioning 25(OH)D and decreased levels of Hcy, OX2, and ECP (20). Furthermore, the decrease in OX2 and Hcy serum levels and increase in 25(OH)D levels promote the probable mechanism of vascular endothelial protection. The increased 25(OH)D concentrations and decreased levels of ECP are reliable with the hypotheses that state these molecules play a significant part in reducing disease progression and pathology, respectively.

The number of research studies seems to be a small group. However, it is difficult to identify such large study groups when considering new biological treatments within the same clinical status. In addition, omalizumab treatment is not a cheap treatment option in our country due to the fact that a large number of patient series are needed.

In conclusion, these data recommend that the measurement of Hcy, 25(OH)D, and sCD200 might be a useful biomarker to get supplementary information about patients' inflammatory status to traditional clinical measures and this study might be done using more study groups because different groups might guide a way to an optimal therapeutic approach by omalizumab.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Akdeniz University, Turkey.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.T.Ö., A.D.Y., S.G., G.E.G., K.K., S.A.; Design - S.T.Ö., A.D.Y., S.G., G.E.G., K.K., S.A.; Supervision - S.T.Ö., A.D.Y., S.G., G.E.G., K.K., S.A.; Resources - A.D.Y., K.K.; Materials - A.D.Y., S.T.Ö.; Data Collection and/or Processing - S.G., G.E.G.; Analysis and/or Interpretation - S.A., S.T.Ö.; Literature Search - K.K., G.E.G.; Writing Manuscript - S.T.Ö., A.D.Y.; Critical Review - S.A., S.T.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Abadoğlu Ö, Aydın Ö, Başyiğit İ, Bavbek S, Bayındır Ü, Bayram H, et al. Turkish Thoracic Society Asthma Diagnose and Treatment Guideline, 2016.
2. Yalcin AD, Basaran S. The effects of climate and aero allergens changes in allergic rhinoconjunctivitis and allergic asthma patients in mediterranean region between 2011 and 2012. Med Sci Monit 2013; 19: 710-11. [CrossRef]
3. Chang TW, Shiung YY. Anti IgE as a mast cell stabilizing therapeutic agent. J. Allergy Clin. Immunol 2006; 117: 1203-12. [CrossRef]
4. Global Initiative for Asthma: GINA Report, Global Strategy for Asthma Management and Prevention - updated 2015. Available at: (www.ginasthma.org).
5. Expert Panel Report (EPR-3): guidelines for the Diagnosis and management of Asthma-summary Report 2007. J Allergy Clin Immunol 2008; 120 (5 Suppl): S94-138.
6. Noga O, Hanf G, Brachmann I, Klucken AC, Kleine-Tebbe J, Rosseau S, et al. Effect of omalizumab treatment on peripheral eosinophil and T-lymphocyte function in patients with allergic asthma. J Allergy Clin Immunol 2006; 117: 1493-9. [CrossRef]
7. Holgate S, Buhl R, Bousquet J, Smith N, Panahloo Z, Jeminez P. The use of omalizumab in treatment of severe allergic asthma: a clinical experience update. Respir Med 2009; 103: 1098-113. [CrossRef]
8. Gorczynski RM. CD200:CD200R-Mediated Regulation of Immunity. ISRN Immunology 2012, 682168: 1-18. [CrossRef]
9. Norton R, O'Connell MA. Vitamin D: potential in the prevention and treatment of lung cancer. Anticancer Res 2012; 32: 211-21.
10. Kriebitzsch C, Verlinden L, Eelen G, van Schoor NM, Swart K, Lips P, et al. 1,25-dihydroxyvitamin D3 influences cellular homocysteine levels in murine preosteoblastic MC3T3-E1 cells by direct regulation of cystathionine β-synthase. J Bone Miner Res 2011; 26: 2991-3000. [CrossRef]
11. Dalal S, Parkin SM, Homer-Vanniasinkam S, Nicolaou A. Effect of homocysteine on cytokine production by human endothelial cells and monocytes. Ann Clin Biochem 2003; 40: 534-41. [CrossRef]
12. Lazzerini PE, Selvi E, Lorenzini S, Capecchi PL, Ghittoni R, Bisogno S, et al. Homocysteine enhances cytokine production in cultured synoviocytes from rheumatoid arthritis patients. Clin Exp Rheumatol 2006; 24: 387-93.
13. Eisner MD, Yegin A, Trzaskoma B. Severity of Asthma Score Predicts Clinical Outcomes in Patients With Moderate to Severe Persistent Asthma Severity of Asthma Score Predicts Outcomes. Chest 2012; 141: 58-65. [CrossRef]
14. Anderson WJ, Short PM, Williamson PA, Lipworth BJ. Inhaled corticosteroid dose response using domiciliary exhaled nitric oxide in persistent asthma: the FENOtype trial. Chest 2012; 142: 1553-61. [CrossRef]
15. Korn S, Hübner M, Jung M, Blettner M, Buhl R. Severe and uncontrolled adult asthma is associated with vitamin D insufficiency and deficiency. Respir Res 2013; 14: 25. [CrossRef]
16. Bousquet J, Cabrera P, Berkman N, Buhl R, Holgate S, Wenzel S, et al. The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe persistent asthma. Allergy 2005; 60: 302-8. [CrossRef]
17. Yalcin AD, Genc GE, Celik B, Gumuslu S. Anti-IgE monoclonal antibody (omalizumab) is effective in treating Bullous Pemphigoid and effects on soluble CD200. Clin Lab 2014; 60: 523-4. [CrossRef]
18. Akman-Karakas A, Yalcin AD, Koc S, Gümüşlü S, Şenol YY, Özkesici B, et al. There might be a role for CD200 in the pathogenesis of autoimmune and inflammatory skin disorders. Med Sci Monit 2013; 19: 888-91. [CrossRef]
19. Yalcin AD, Ucar S, Gumuslu S, Strauss L. Effects of Omalizumab on Eosinophil Cationic Peptid, 25-Hydroxyvitamin-D, IL-1β, and sCD200 in a cases of Samter's syndrome: 36 Months follow-up. Immunopharmacol Immunotoxicol 2013; 35: 524-7. [CrossRef]
20. Yalcin AD, Cilli A, Bisgin A, Strauss L, Herth F. Omalizumab is effective in treating severe asthma in patients with severe cadiovascular complica-

tions and its effects on sCD200, d- dimer , CXCL8 and IL-1β levels. Expert Opin Biol Ther 2013; 13: 1335-41. [CrossRef]
21. Chambers E, Nanzer A, Richards D. Serum 25-dihydroxyvitamin D levels correlate with CD4 (+) Foxp3 (+) T-cell numbers in moderate/severe asthma. J Allergy Clin Immunol 2012; 130: 542-4. [CrossRef]
22. Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C, Colucci G. Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study.Eur J Vasc Endovasc Surg 2012; 44: 307-12. [CrossRef]
23. Zietkowski Z, Skiepko R, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A. Airway inflammation and eotaxin in exhaled breath condensate of patients with severe persistent allergic asthma during omalizumab therapy. Adv Med Sci 2011; 56: 318-22. [CrossRef]
24. Kim CK. Eosinophil-derived neurotoxin: a novel biomarker for diagnosis and monitoring of asthma. Korean J Pediatr 2013; 56: 8-12. [CrossRef]
25. Krause K, Metz M, Makris M, Zuberbier T, Maurer M. The role of interleukin-1 in allergy-related disorders. Curr Opin Allergy Clin Immunol 2012; 12: 477-84. [CrossRef]
26. Yalcin AD, Bisgin A, Gorczynski RM. IL-8, IL10, TGF-β and GCSF levels were increased in severe persistent allergic asthma patients with the anti-IgE treatment. Mediators Inflamm 2012; 2012: 720976. [CrossRef]
27. Finkelstein JD, Martin JJ. Homocysteine. Int J Biochem Cell Biol 2000; 32: 385-9. [CrossRef]