ORIGINAL RESEARCH

Biomarkers for Overweight in Adult-Onset Asthma

Hiroki Tashiro[1]
Koichiro Takahashi [iD][1]
Hironori Sadamatsu[1]
Yuki Kurihara[1]
Tetsuro Haraguchi[1]
Ryo Tajiri[2]
Ayako Takamori[2]
Shinya Kimura[1]
Naoko Sueoka-Aragane[1]

[1]Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan; [2]Clinical Research Center, Saga University Hospital, Saga, Japan

Purpose: Overweight and obesity are associated with one of the severe phenotypes of asthma, with an increased rate of exacerbations, low level of lung function, and reduced response to corticosteroid therapy. The present study focused on identifying useful biomarkers of severity in overweight patients with adult-onset asthma using real-world data.

Patients and Methods: A total of 56 patients with adult-onset asthma who visited Saga University Hospital between 2018 and 2019 were retrospectively reviewed. Overweight was defined as a body mass index (BMI) greater than 25 kg/m^2. Blood eosinophils, cytokines, and chemokines were compared between non-overweight asthma and overweight asthma patients.

Results: Overweight asthma patients had a higher annual exacerbation rate, lower pulmonary function even when treated frequently with high-dose inhaled corticosteroids, and a significantly lower percentage of eosinophils and lower eosinophil count compared to non-overweight asthma patients (p<0.01, p=0.03). Moreover, the percentage of eosinophils was significantly negatively correlated with BMI (ρ=−0.38, p<0.01) (Figure 1). On serum cytokine and chemokine analyses, the overweight asthma group included significantly more patients with a lower level of tissue growth factor α (TGF-α) (1.1 pg/mL) and higher levels of hsIL-6 (2.5 pg/mL), RANTES/CCL5 (298.5 pg/mL), and vascular endothelial growth factor A (VEGF-A) (63.7 pg/mL), than the non-overweight asthma group (p=0.02, p<0.01, p=0.02, p=0.01, respectively).

Conclusion: The present study showed that overweight patients with adult-onset asthma were characterized by a higher rate of annual exacerbations and worse lung function despite treatment with high-dose inhaled corticosteroids and lower blood eosinophil counts than non-overweight patients with asthma. On blood cytokine and chemokine analyses, a low level of TGF-α and high levels of hsIL-6, RANTES/CCL5, and VEGF-A might be biomarkers reflecting the pathophysiology in overweight patients with asthma.

Keywords: asthma, overweight, biomarker

Introduction

Overweight and obesity are associated with one of the severe phenotypes of asthma, with an increased rate of exacerbations, low level of lung function, and reduced responses to corticosteroid therapy.[1–3] Because effective treatment for asthma with obesity is limited to reducing weight,[4] useful biomarkers associated with severity should be identified to help find new therapeutic targets. Recently, cluster analysis showed that the clinical features of asthma associated with obesity have at least two different phenotypes: the first phenotype includes patients with early-onset asthma usually triggered by allergens with eosinophilia and high serum immunoglobulin E (IgE) titers, which are exacerbated by obesity, and a second phenotype that includes patients with adult-onset asthma, who are predominantly female without

Correspondence: Koichiro Takahashi
Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga, Saga Prefecture 849-8501, Japan
Tel +81-952-34-2369
Fax +81-952-34-2017
Email takahak@cc.saga-u.ac.jp

type 2 markers such as eosinophils and IgE.[1,5,6] Considering the previous study by To et al, which reported significantly higher rates of annual exacerbations and frequent exacerbators in overweight asthma patients with adult-onset compared to patients with carryover or outgrowth from childhood asthma,[7] the present study focused on identifying useful biomarkers of severity in overweight patients with adult-onset asthma using real-world data.

Patients and Methods

A total of 56 patients with asthma who visited Saga University Hospital between 2018 and 2019 were retrospectively reviewed. All patients were diagnosed with asthma by two expert pulmonary physicians referring to the Global Initiative for Asthma (GINA) guidelines. This study was approved by the ethics committee of Saga University Hospital (approval number: 2019-08-02, approval date: December 27, 2019) and was performed in accordance with the 1964 Declaration of Helsinki. To focus on adult-onset asthma, patients who developed asthma when they were older than 20 years were enrolled; patients with a history of childhood asthma were excluded from the present study. Overweight was defined as a body mass index (BMI) greater than 25 kg/m^2 referring to a previous study.[7] The asthma control test (ACT) score, the details of therapy for asthma, pulmonary function test results, fractional exhaled nitric oxide (FeNO), and blood examinations including cytokines and chemokines were evaluated in the stable phase, defined as no additional use of oral corticosteroids or antibiotics, no unscheduled doctor's visits, or no hospitalizations due to exacerbations of asthma in the past 4 weeks. Treatments for asthma were selected at the physicians' discretion. Doses of inhaled corticosteroid (ICS) were divided into 3 groups, low, moderate, and high, referring to the GINA guidelines. FeNO was measured using the NIOX VERO (CHEST Inc., Tokyo, Japan). Forty-two serum cytokines and chemokines were measured by multiplex assay (Eve Technologies, Calgary, Canada) and ELISA (for high-sensitivity interleukin-6 (hsIL-6)) (R&D Systems, Minneapolis, MN); however, results for 13 were excluded because they were below the detection limit, and 29 cytokines and chemokines were examined. To accurately evaluate the interactions between asthma pathophysiology and blood biomarkers, patients treated with systemic corticosteroids and/or molecular targeting drugs were excluded from the examinations of serum cytokines and chemokines. Therefore, 20 non-overweight patients with asthma and

11 overweight patients with asthma were evaluated after obtaining their informed consent. As comorbidities, gastroesophageal reflux disease (GERD), diabetes mellitus, hyperlipidemia, hypertension, and sleep apnea syndrome were diagnosed by physicians. Eosinophilic chronic rhinosinusitis (ECRS) was diagnosed using the JESREC score. Briefly, the JESREC score includes 4 factors, including bilateral lesion sites (3 points), nasal polyps (2 points), ethmoid-dominant lesion on computed tomography (CT) (2 points), and the percentage of blood eosinophils (4 points for >2% to ≤5%, 8 points for >5% to ≤10%, and 10 points for >10%). ECRS was diagnosed when the total JESREC score was ≥11.[8] The clinical data were analyzed by the Mann–Whitney U-test for continuous variables or the chi-squared test for categorical variables. For correlation analysis, Spearman's rank correlation coefficient between the percentage of blood eosinophils and BMI was calculated to determine whether it was zero or not. For comparison analyses of serum cytokines and chemokines, the chi-squared test was performed referring to the previous report[9] because of the small sample size, which might lead to low power for continuous comparisons. Cut-off values of 29 cytokines and chemokines were set the value where Youden's index is the maximum by drawing the receiver operating characteristic (ROC) curve. In addition, Benjamini and Hochberg false discovery rate adjustments were performed for multiple comparisons on biomarker analysis. Multivariate analysis was not performed because of the small sample size. Statistical analysis was performed with JMP Pro version 14.2.0 software (SAS Institute Inc., Cary, NC).

Results

The data of 56 adult-onset asthma patients were analyzed. To evaluate the clinical impact of overweight in adult-onset asthma, the 56 patients were divided into 39 non-overweight patients with asthma (BMI≤25 kg/m^2) and 17 overweight patients with asthma (BMI>25 kg/m^2). There were more female patients in the overweight group than in the non-overweight group, but the difference was not significant. Smoking history and ACT scores were not different between the two groups. The annual exacerbation rate was significantly higher, at 1.5 times per year, in overweight patients than in non-overweight patients (0.4 times per year; p=0.04). Use of long-acting β_2 adrenergic agonists, long-acting muscarinic antagonists, leukotriene receptor antagonists, oral corticosteroids, and molecular targeted drugs was not different between the two groups.

Low-dose ICS tended to be used more in the non-overweight than in the overweight patients with asthma, and high-dose ICS was used significantly more in the overweight than in the non-overweight patients with asthma (p=0.05 and p<0.01, respectively) (Table 1). The duration of asthma and the rate of aspirin sensitivity were not different between the two groups. In terms of commodities, including those related to allergic diseases, there were more patients with sleep apnea syndrome in the overweight than in the non-overweight patients with asthma (p<0.01), but the rates of GERD, diabetes mellitus, hyperlipidemia, hypertension, allergic rhinitis, atopic dermatitis, food allergy, drug allergy, and ECRS were no difference between the two groups (Table E1). On pulmonary function testing, forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) tended to be lower in overweight than in non-overweight patients with asthma (p=0.05 and p=0.06, respectively) (Table E1). On hematology, the percentage of eosinophils and the eosinophil count were significantly lower in overweight patients, at 3.8% and 247.0/mL, respectively, than in non-overweight patients with asthma, at 7.6% and 512.3/mL, respectively (p<0.01, p=0.03) (Table 1). Moreover, the percentage of eosinophils was significantly negatively correlated with BMI (ρ=−0.38, p<0.01) (Figure 1). Immunoglobulin E (IgE) was lower in overweight than in non-overweight patients with asthma, but there was no significant difference (Table 1). On serum cytokine and chemokine analyses, the overweight group included significantly more patients with a lower level of tissue growth factor α (TGF-α) (1.1 pg/mL) and higher levels of hsIL-6 (2.5 pg/mL) RANTES/CCL5 (298.5 pg/mL), and vascular endothelial growth factor A (VEGF-A) (63.7 pg/mL), than the non-overweight group (p=0.02, p<0.01, p=0.02, and p=0.01, respectively) even after adjustment by the Benjamini and Hochberg method (Table 2).

Discussion

The clinical characteristics of overweight patients with asthma in the present study provide evidence that overweight is associated with a severe phenotype of asthma, especially in adult-onset cases.[1,7] Overweight patients with asthma showed a higher rate of annual exacerbations and worse lung function (Table 1, Table E1), which are clinical factors indicating severe asthma, compared to non-overweight patients with asthma.[1,2] Importantly, high-dose ICS was more frequent in the overweight group than in the non-overweight group (Table 1), which suggested a reduced response to corticosteroids, one of the features associated with severity in overweight and obese patients with asthma.[10] Exploring biomarkers including blood eosinophils in overweight and obese patients with asthma is challenging because of the clinical heterogeneities reported by the cluster analysis.[6] In fact, a previous report of overweight asthma patients in Japan showed no difference in blood eosinophils compared to non-overweight patients with asthma, even though the exacerbation rate was significantly higher in the overweight population with asthma when targeting adult-onset asthma.[7] Thus, the present study focused only on adult-onset asthma and showed that blood eosinophil counts were significantly lower in overweight patients with asthma than in non-overweight patients with asthma (Table 1), and they were negatively
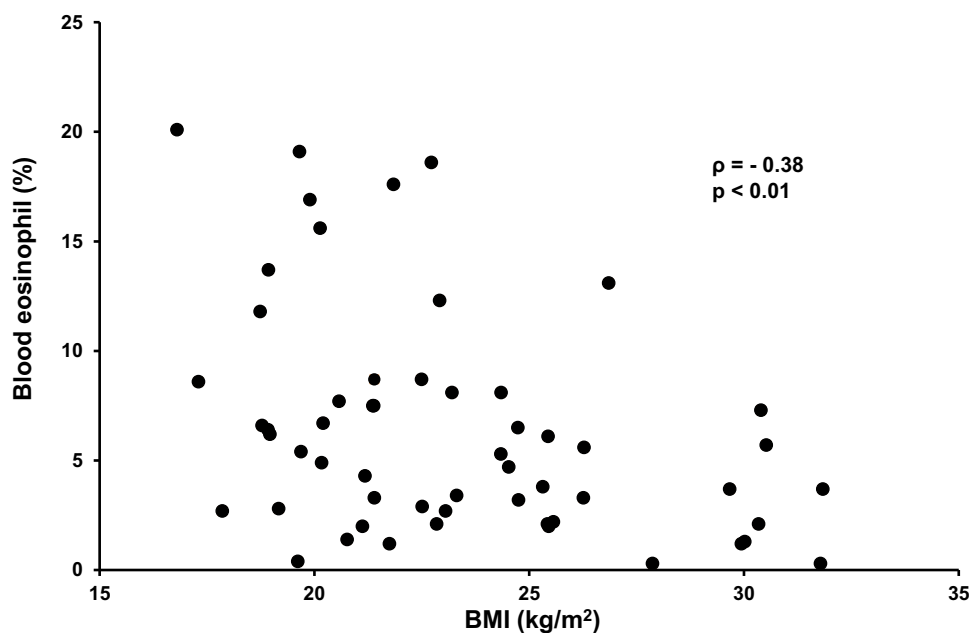
Table 1 Comparison of Clinical Characteristics and Laboratory Data Between Non-Overweight and Overweight Asthma Patients

	Non-Overweight Asthma	Overweight Asthma	p value
n	39	17	
Body mass index (kg/m^2)	21.1 ± 0.3	28.2 ± 0.6	
Age	63.9 ± 1.9	63.9 ± 3.0	0.93
Sex (M/F)	13/26	3/14	0.22
Smoking history (>10 pack-year)	10 (25.6%)	3 (17.6%)	0.51
Annual exacerbation rate	0.4 ± 0.1	1.5 ± 0.5	0.04
Score of asthma control test	22.6 ± 0.5	21.5 ± 0.9	0.25
Asthma therapy			
Low dose of ICS	5 (12.8%)	0 (0.0%)	0.05
Moderate dose of ICS	29 (74.4%)	9 (52.9%)	0.12
High dose of ICS	5 (12.8%)	8 (47.1%)	<0.01
LABA	30 (76.9%)	16 (94.1%)	0.09
LAMA	5 (12.8%)	5 (29.4%)	0.15
LTRA	17 (43.6%)	10 (58.8%)	0.29
Daily use of OCS	6 (15.4%)	2 (11.8%)	0.72
Molecular targeting drugs	3 (7.7%)	4 (23.5%)	0.11
Peripheral Blood			
White blood cell (/mL)	6235.9 ± 297.3	6470.6 ± 358.8	0.47
Eosinophil (%)	7.6 ± 0.9	3.8 ± 0.8	<0.01
Eosinophil count (/mL)	512.3 ± 78.4	247.0 ± 52.4	0.03
IgE (IU/mL)	1607.4 ± 699.3	314.9 ± 70.6	0.34
CRP (mg/dl)	0.2 ± 0.0	0.2 ± 0.0	0.46

Note: Data are presented as mean ± standard deviation.
Abbreviations: ICS, inhaled corticosteroid; LABA, long-acting β₂ adrenergic agonist; LAMA, long-acting muscarinic antagonist; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroid; IgE, immunoglobulin E; CRP, C reactive protein.

Figure 1 Correlation between percentage of eosinophils and body mass index. The percentage of eosinophils is significantly negatively correlated with body mass index (BMI) (ρ=−0.38, p<0.01).

correlated with BMI (Figure 1), consistent with low type 2 inflammation, as described in previous reports.[6] Although it was not significantly different, levels of serum IgE, which is one of the markers of atopic and type 2 inflammation status, were also lower in overweight patients with asthma than in non-overweight patients with asthma, which is also consistent with previous reports indicating the non-atopic features of asthma with obesity.[1] The phenotype of low type 2 inflammation in overweight patients with adult-onset asthma in the present study was also supported by the results of the blood cytokine and chemokine analyses (Table 2). For example, low levels of IL-9, which are considered type 2 cytokines,[11] tended to be more common in overweight patients than in non-overweight patients with asthma. A low level of TGF-α, which is expressed on human eosinophils,[12] was also significantly more common in overweight patients with asthma. In addition, a high level of hsIL-6, reflecting severity in obese patients with asthma through systemic inflammation,[13] was significantly more common in overweight patients with asthma. It was recently reported that plasma IL-6 is significantly higher in asthmatic patients than in control subjects, and BMI is significantly higher in the IL-6-high asthma group than in the IL-6-low asthma group.[14] Additionally, the IL-6 level was not correlated with the cellular characteristics of type 2 asthma, such as greater

blood eosinophils and total serum IgE,[15] which is also consistent with the present results. RANTES/CCL5, which is upregulated in adipose tissue of obese individuals[16] and associated with obesity-induced pathophysiology, such as atherosclerosis and sleep apnea syndrome,[17,18] was significantly higher in overweight asthma patients than in non-overweight asthma patients. RANTES/CCL5 is also associated with airway inflammation and airway hyperresponsiveness, as we and others previously reported,[19,20] which is also supported by the present results. Interestingly, a high level of VEGF-A, which is correlated with decreased pulmonary function,[21] was also significantly more common in overweight patients with asthma, supporting the lower FVC and FEV1, compared to non-overweight asthma patients.

There are two limitations in this study. First, the present study compared biomarkers between 2 groups, non-overweight asthma and overweight asthma groups, without comparison of non-overweight and overweight healthy controls, which might show that differences in biomarkers are related to overweight itself, but not asthma with overweight. Second, the present study involved a small number of patients at a single hospital with limited ethnic diversity. To confirm the validity of the present results, multicenter, prospective studies designed with appropriate controls and larger numbers of patients should be performed.

Table 2 Comparison of Blood Cytokines and Chemokines Between Non-Overweight and Overweight Asthma Patients

Pg/mL	Non-Overweight Asthma	Overweight Asthma	p value
EGF ≥162.3	7/20 (35%)	2/11 (18.2%)	0.31
FGF-2 ≥18.4	15/20 (75%)	6/11 (54.5%)	0.25
Eotaxin ≥78.6	3/20 (15%)	5/11 (45.5%)	0.07
TGF-α ≥1.1	14/20 (70%)	3/11 (27.3%)	0.02
GM-CSF ≥30.8	15/20 (75%)	8/11 (72.7%)	0.89
IFNα2 ≥17.5	16/20 (80%)	5/11 (45.5%)	0.05
IFNɣ ≥3.8	10/20 (50%)	4/11 (36.4%)	0.46
GRO α (CXCL1) ≥988.6	12/20 (60%)	4/11 (36.4%)	0.21
IL-10 ≥1.2	6/20 (30%)	2/11 (18.2%)	0.46
MDC (CCL22) ≥1217.9	6/20 (30%)	2/11 (18.2%)	0.46
PDGF-AA ≥848.0	13/20 (65%)	4/11 (36.4%)	0.12
PDGF-BB ≥1984.9	18/20 (90%)	8/11 (72.7%)	0.22
IL-15 ≥1.4	12/20 (60%)	4/11 (36.4%)	0.21
sCD40L ≥1356.4	12/20 (60%)	8/11 (72.7%)	0.47
IL-1Rα ≥14.5	14/20 (70%)	5/11 (45.5%)	0.18
IL-9 ≥0.4	12/20 (60%)	4/11 (36.4%)	0.21
IL-3 ≥0.4	12/20 (60%)	5/11 (45.5%)	0.33
IL-5 ≥2.5	1/20 (5%)	2/11 (18.2%)	0.25
hsIL-6 ≥2.5	2/20 (10%)	6/11 (54.5%)	<0.01
IL-7 ≥2.0	13/20 (65%)	4/11 (36.4%)	0.12
IL-8 ≥9.4	8/20 (40%)	3/11 (27.3%)	0.47
IP-10 (CXCL10) ≥204.7	1/20 (5%)	3/11 (27.3%)	0.08
MCP-1 (CCL2) ≥226.5	12/20 (60%)	10/11 (90.9%)	0.05
MIP-1α (CCL3) ≥7.9	7/20 (35%)	1/11 (9.1%)	0.09
MIP-1β (CCL4) ≥139.6	8/20 (40%)	1/11 (9.1%)	0.05
RANTES (CCL5) ≥298.5	4/20 (20%)	7/11 (63.6%)	0.02
TNFα ≥7.4	19/20 (95%)	9/11 (81.8%)	0.25
VEGF-A ≥63.7	7/20 (35%)	9/11 (81.8%)	0.01
IL-18 ≥208.7	2/20 (10%)	1/11 (9.1%)	0.93

Abbreviations: EGF, epithelial growth factor; FGF-2, fibroblast growth factor 2; TGF-α, tissue growth factor-α; GM-CSF, granulocyte macrophage colony-stimulating factor; IFNα2, interferon-α2; IFNɣ, interferon-ɣ; GROα, growth regulated oncogene-granulocyte macrophage colony-macrophage derived chemokine; PDGF, platelet-derived growth factor; sCD40L, soluble CD40 ligand; IL-1Rα, interleukin-1 receptor-α; hsIL-6, high-sensitivity interleukin-6; IP-10, interferon ɣ inducible protein 10; MCP-1, monocyte chemotactic protein-1; MIP, macrophage inflammatory protein; TNFα, tumor necrosis factor-α; VEGF-A, vascular endothelial growth factor-A.

Conclusion

The present study showed that overweight patients with adult-onset asthma were characterized by a higher rate of annual exacerbations and worse lung function despite treatment with high-dose ICS and lower blood eosinophil counts than non-overweight patients with asthma. On blood cytokine and chemokine analyses, a low level of TGF-α and high levels of hsIL-6, RANTES/CCL5, and VEGF-A might be biomarkers reflecting the pathophysiology in overweight patients with asthma.

Disclosure

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