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Adipokine relations with Th17 and Th9 in asthma

Relationship of circulating chemerin and omentin levels with Th17 and Th9 cell immune responses in patients with asthma

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Abstract

Objective: Adipokines are correlated with immune response in asthma, but data on the roles of chemerin and omentin in asthma are limited. This study explored the relationship of chemerin and omentin levels with Th17 and Th9 cells in asthma. **Methods:** Seventy-six asthmatics were divided into the intermittent to mild persistent ($n = 28$), moderate persistent ($n = 26$) and severe persistent ($n = 22$) and enrolled in the study. Additionally, 20 healthy subjects were enrolled as controls. Clinical characteristics of the subjects, the Asthma Control Test (ACT), lung function, fractional exhaled nitric oxide (FeNO) score, plasma chemerin and omentin levels were evaluated, and the percentage of Th17 and Th9 cells were determined by flow cytometry.

Results: The percentage of Th17 and Th9 cells were higher in the moderate to severe persistent asthmatics than in the intermittent to mild persistent asthmatics or healthy controls ($p < 0.05$).

The severe persistent asthma group had higher chemerin level but lower omentin levels than

control group ($p < 0.05$). Chemerin levels were positively correlated with Th17 and Th9 cell percentages, while omentin levels were negatively correlated with Th17 and Th9 cell percentages ($p < 0.01$).

Conclusions: The regulatory functions of adipokines on immune responses may be associated with pathogenesis and processes of asthma.

Keywords

asthma; chemerin; omentin; Th17; Th9

Introduction

Growing evidence suggests that obesity is an independent risk factor for asthma. Adipokines provide a mechanistic link between obesity and asthma [1], which has augmented asthma incidence and severity [2] and influenced the efficacy of standard asthma treatments [3].

Adipokines are produced by adipose tissue and help regulate energy metabolism, but also influence chronic inflammatory conditions [4] and immune responses [5].

Adipokines (adiponectin and leptin) are known to influence asthma prevalence and severity [6], but there is limited information on the role of certain novel adipokines like chemerin and omentin in asthma and immune responses. Chemerin is a 18 kDa protein secreted by adipose tissue that modulates immune system function through binding to the chemerin receptor (Chemerin R, chemokine-like receptor 1 (CMKLR1), and G protein-coupled receptor) [7]. Omentin (also known as intelectin), a 34 kDa adipocytokine, is produced in visceral adipose tissue, and may direct the therapeutic development in inflammation related conditions [8]. Recent studies in murine models implicate chemerin [9] and omentin [10] in allergic asthma.

CD4⁺ T helper (Th) cells produce cytokines in response to antigen activity, and play key roles in the inflammatory process of asthma. For many years, Th2 cells and an imbalance in Th1/Th2 have been implicated in the pathogenesis of asthma [11], and more recent evidence suggests that Th17 cells [12] and Th9 cells [13] also are involved in these processes. Recently, leptin has been shown significant positive correlation with Th1-cytokine (interferon- γ) but not the Th2-cytokine (IL-4) in obese children with asthma [14]. On the other hand, in obese females with asthma, serum adiponectin positively correlated with markers of Th2 but negatively with

markers of Th1 in peripheral blood mononuclear cells (PBMCs) [15]. Interestingly, distinct interactions between adipokines and Th17 cytokines are involved in the pathogenesis of psoriasis [16].

Although classical adipokines have been implicated in asthma, whether novel adipokines are related to the Th17 and Th9 responses has not yet been established. Therefore, the aim of this study was to elucidate the relationship of circulating chemerin and omentin to Th17 and Th9 cells in asthma, to better understand the role of adipokines in the immune responses occurring in inflammatory diseases.

Methods

Subjects

Seventy-six asthmatic subjects (36 male and 40 female, age range: 23--68 years) were enrolled in this study at the Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China. Asthma was diagnosed by the physicians according to the criteria of the American Thoracic Society (ATS). These criteria included clinical symptoms of asthma and a 20% decrease in forced expiratory volume in 1 second (FEV1) after a methacholine challenge and/or bronchodilator reversibility in FEV1 of > 20% and >200mL from baseline [17]. Asthma severity was evaluated using the Global Initiative for Asthma (GINA)[18] Management Strategy. Intermittent to mild persistent asthmatics (n = 28) had episodic symptoms or day symptoms more than once a week but less than once a day, with as-needed inhaled short-acting beta₂-agonist alone or with low dose inhaled corticosteroids (ICS). Moderate persistent asthmatics (n = 26) had daily symptoms and nocturnal symptoms at

least once a week, and used low/medium dose ICS/long-acting beta₂-agonists. Severe persistent asthmatics (n = 22) had intense or frequent nocturnal symptoms and limited activity as a result of their asthma, and required combination therapy with high-dose ICS/long-acting beta₂-agonists with or without anti-leukotriene and/or theophylline treatment. Patients were considered non-eligible if they had other co-existing pulmonary diseases, inflammatory diseases, autoimmune disorders, pregnancy, or any language or cognitive deficits. Twenty healthy non-obese individuals with normal lung function (8 male and 12 female; age range: 23--65 years), who had no atopic disease history or immune disorders, and were negative for bronchial provocation tests were recruited and enrolled in the study. No participants had received systemic glucocorticoid or immune inhibitors medications within 4 weeks, or had an acute infection within 2 weeks. All subjects had their age, gender, height, weight and body mass index (BMI) recorded, and performed lung function tests. In addition, asthma patients described family history of asthma, smoking history, prevalence of allergic rhinitis, level of fractional exhaled nitric oxide (FeNO) and completed Asthma Control Test (ACT) scores.

The study protocol was approved by the Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University and informed consent was obtained in writing from all participants at entry into the study.

Lung function testing

Lung function parameters including FEV₁, forced vital capacity (FVC), FEV₁/FVC ratio, and peak expiratory flow (PEF) were measured in all participants by using a Master Screen spirometer (Jaeger, Hoechberg, Germany) according to ATS criteria [17]. These spirometric

parameters were expressed as a percentage of the predicted healthy individual with the same age, sex, and height [19].

Measurement of FeNO

Levels of FeNO were evaluated in asthmatic patients with a chemiluminescence analyzer (NIOX MINO, Aerocrine AB, Solna, Sweden) in accordance with the ATS and European Respiratory Society standards [20]. FeNO values were expressed as parts per billion (ppb).

Asthma control evaluation

ACT has the advantages of its validity and convenience, and was used to evaluate asthma control. ACT includes five questions about the impact of asthma on daily activities, sleeping, frequency of dyspnea, and use of rescue medications, as well as a self-assessment of asthma control during the past four weeks [21]. Each question has five options for the answer, and total scores can range from 5 to 25 with higher scores meaning better asthma control.

Blood sample preparation

A 4-mL sample of venous blood was drawn from an antecubital vein of each subject, and collected in a sodium heparin tube. Plasma was separated by centrifuging at 4°C for 10 min at $3000 \times g$ and stored at -80°C for detection of adipokines. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Hypaque density gradient centrifugation at 2000 r/min for 25 min at room temperature (Tianjin Hao Yang Biological Technology Co., Ltd, Tianjin, China). PBMCs were cultured at a density of 2×10^6 cells/mL in Roswell Park Memorial Institute

medium 1640 supplemented with 100U/mL penicillin, 100µl/mL streptomycin, 2mM glutamine, and 10% heat inactivated fetal bovine serum (FBS, Cat. No: p30-3302, PAN-Biotech, Germany).

Flow cytometric (FCM) analysis of Th17 and Th9 cells

In vitro activation of T lymphocytes is required to elicit cytokine production and secretion, because levels of intracellular cytokines from unstimulated cells are so low that they cannot be detected [22, 23]. Following established protocols [24, 25], Th17 and Th9 cells were stimulated with 4µl/mL phorbol 12-myristate 13-acetate (12.5µg/mL)/ionomycin (0.25 mg/mL) mixture for 6h. This activates T cells nonspecifically [26]. Then brefeldin A (0.75 mg/mL)/monensin (0.35mg/mL) mixture (MultiScience Biotech Co., Ltd, Hangzhou, China) was added into the 24 well plates at 37°C in a 5% CO₂ environment for the final 2h. After harvesting into tubes, the cells were washed twice with phosphate buffered saline. The cells were incubated with the surface markers phycoerythrin-cy5 (PE-cy5)-conjugated anti-human CD3 (Clone HIT3a, Biolegend, San Diego, CA, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-human CD8a (Clone HIT8a, Biolegend, San Diego, CA, USA) at room temperature in the dark for 30 min. Then stained cells were fixed and permeabilized using FIX & PERM Kit (MultiScience Biotech Co., Ltd, Hangzhou, China) according to the manufacturer's instructions. Intracellular staining was conducted for interleukin (IL)-17A (eBioscience, San Diego, CA, USA) and IL-9 (eBioscience, San Diego, CA, USA) at room temperature for 30 min in dark. Isotype controls were purchased from eBioscience to avoid nonspecific binding. Cell samples were analyzed by BD FACSVantage cytometer with CellQuest software (BD Biosciences, USA). Because the stimulation of PMA/Ionomycin could down-regulate the level of CD4 expression, we selected

CD3+CD8- as a marker for CD4+ T cell according to previous studies [27-29]. Consequently, in our study, CD3+CD8-IL-17+ T cells were classified as Th17 cells, while CD3+CD8-IL-9+ T cells were reclassified as Th9 cells.

Enzyme-linked immunosorbent assays (Elisa) analyses

Plasma was thawed at room temperature for 2h before detection. Adipokines were measured by double-antibody sandwich Elisa kits following the manufacturer's protocols. The minimum detectable dose of chemerin (E-EL-H0698c, Elabscience, Wuhan, China) is 0.1ng/mL and omentin (E-EL-H2028c, Elabscience, Wuhan, China) is 0.38 ng/mL, for both the coefficient of variation was <10%. All the samples were tested in duplicate.

Statistical Analysis

Data were analyzed using Prism 5.0 (GraphPad Software, La Jolla, California) and SPSS 20.0 Software (IBM, Armonk, NY, USA). Normal distribution was verified with the Shapiro-Wilk test. Continuous variables were expressed as mean $\pm$ standard deviation (SD). Differences were compared with the one-way analysis of variance (ANOVA) followed by Newman-Keuls test for parametric values and the Kruskal-Wallis test for non-parametric data. Categorical variables were assessed with the Chi-square test. Correlations were analyzed by Pearson's test. A value of $p < 0.05$ was defined as statistically significant.

Results

Characteristics of subjects

According to the GINA guidelines, a total of 76 patients with allergic asthma were divided into three subgroups: 28 with intermittent to mild persistent asthma, 26 with moderate persistent asthma, and 22 with severe persistent asthma. No significant differences in age, gender or BMI occurred between the asthmatic subjects and the healthy controls, and no differences in smoking history, allergic rhinitis or family history of asthma were found between the three subgroups of asthmatic patients. As expected, the asthmatics had decreased FEV1 (% predicted), FVC (% predicted), FEV1/FVC (%) and PEF (% predicted) compared to the healthy controls. No significant differences occurred in FEV1 (% predicted), FVC (% predicted), FEV1/FVC (%) between the subjects in the 3 subgroups of asthmatic patients. However, PEF (% predicted) was significantly lower in patients with moderate to severe persistent asthma compared to those with intermittent to mild persistent asthma. Moreover, there was no significant difference in PEF (% predicted) between the moderate persistent and severe persistent asthma groups. The FeNO levels were significantly higher in the groups with moderate and severe persistent asthma than with intermittent to mild persistent asthma, whereas ACT scores were lower. The characteristics of the study participants are presented in Table 1.

Th17 and Th9 cells are associated with FeNO levels in asthmatic subjects

A representation of the intracellular cytokine staining of the Th17 and Th9 cells percentage from each group is shown in Figure 1. We gated on CD3⁺CD8⁻ in PBMCs and then the intracellular expression of IL-17 and IL-9 was measured by gating CD3⁺CD8⁻ T cells. As shown in Figure 2, in PBMCs, both moderate ($1.24 \pm 0.53\%$) and severe persistent asthmatics ($1.69 \pm 0.52\%$) had higher percentages of Th17 cells than subjects with intermittent to mild persistent asthma

($0.72 \pm 0.31\%$) and the healthy controls ($0.53 \pm 0.17\%$, $p < 0.05$). There was no significant difference between the results for the moderate ($1.24 \pm 0.53\%$) and severe persistent asthmatics ($1.69 \pm 0.52\%$, $p > 0.05$). The percentages of Th9 cells were progressively higher in subjects with intermittent to mild ($0.74 \pm 0.40\%$), moderate ($1.04 \pm 0.33\%$) and severe persistent asthma ($2.11 \pm 0.44\%$) than in the healthy subjects ($0.51 \pm 0.36\%$, $p < 0.05$). In addition, the percentage of Th17 cells in asthmatics was positively correlated with their FeNO levels ($r = 0.43$; $p < 0.01$) (Figure.3 (a)). The percentage of Th9 cells was similarly positively correlated with FeNO values ($r = 0.53$; $p < 0.01$) (Figure.3 (b)).

Plasma chemerin and omentin levels in asthmatic patients

The levels of chemerin were significantly higher in asthmatic patients (0.33 ± 0.26 ng/mL) than in control subjects (0.18 ± 0.06 ng/mL, $p = 0.028$). As shown in Figure. 4 (a), severe persistent asthmatics had significantly increased chemerin concentrations (0.45 ± 0.36 ng/mL) compared to the control subjects (0.18 ± 0.06 ng/mL, $p < 0.05$). However, there were no chemerin differences between the intermittent to mild (0.24 ± 0.15 ng/mL) and moderate persistent asthmatics (0.30 ± 0.19 ng/mL) and the control subjects (0.18 ± 0.06 ng/mL, $p > 0.05$). In contrast, omentin levels were significantly lower in asthmatic subjects (4.82 ± 3.50 ng/mL) than in controls (9.74 ± 4.87 ng/mL, $p < 0.05$). Plasma omentin concentrations were lower in the moderate persistent asthma (4.07 ± 2.91 ng/mL) and severe persistent asthma (3.72 ± 2.81 ng/mL) groups than in the control group (9.74 ± 4.87 ng/mL, $p < 0.01$), whereas no significant differences occurred between intermittent to mild persistent asthma patients (6.62 ± 4.04 ng/mL) and controls

(9.74 ± 4.87 ng/mL, $p > 0.05$) (Figure 4 (b)). Similarly, we found no significant differences in chemerin or omentin levels in the subgroup analysis.

Association of chemerin and omentin with Th17 and Th9 cells in patients with asthma

We assessed whether the levels of plasma chemerin and omentin were associated with the Th17 and Th9 percentages of the asthmatic subjects. As shown in Figure 5, we found a significant positive correlation between plasma levels of chemerin and Th17 ($r = 0.62$, $p < 0.01$) (a) and Th9 ($r = 0.71$, $p < 0.01$) (b). Conversely, a negative correlation was found between omentin levels and Th17 ($r = -0.50$, $p < 0.01$) (Figure 5 (c)). A similar correlation was seen between the omentin levels and Th9 ($r = -0.66$, $p < 0.01$) (Figure 5 (d)).

Discussion

It is now accepted that adipose tissue is not only involved in energy storage, but is also a key endocrine organ that releases multiple bioactive substances, known as adipokines. Dysregulation and dysfunction of these adipokines can alter immune responses and contribute to disease processes [4]. To understand these factors, hundreds of adipokines were divided into sub-groups that have pro- or anti-inflammatory activities [5].

Both pro- and anti-inflammatory roles of chemerin and CMKLR1 in immune processes were documented in studies on animal- and cell-based models [7]. A growing body of literature has associated high chemerin levels with inflammation in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis [30], as well as in serum of patients with chronic pancreatitis [31], chronic obstructive pulmonary disease [32], psoriasis [33] and inflammatory

bowel disease (ulcerative colitis and Crohn's disease) [34]. In our study, plasma chemerin levels were elevated in asthmatic patients, but only the severe persistent asthmatics had a statistically significant increase compared to the controls. Thus, chemerin appears to have a role in the systemic inflammatory response. However, Doyle et al. [35] reported that activation of chemerin receptors decreased allergic airway inflammation in a mouse model of asthma, which was apparent both histologically and by reduced eosinophil counts in bronchoalveolar lavage fluid. The conflicting results on the effects of chemerin may be attributed to varying roles played by chemerin in different phases of inflammation [7].

Omentin is a pleiotropic adipokine associated with inflammation. Lower levels of omentin in plasma have been reported in individuals with several chronic inflammatory diseases, such as inflammatory bowel disease [36], coronary artery disease [37] and obstructive sleep apnea [38]. But little is known of the circulating omentin level in asthma. In the present study, patients with asthma had significantly lower plasma omentin levels than healthy controls. However, a previous study demonstrated that omentin expression in airway epithelium was significantly increased in 30 subjects with asthma [39]. It has been reported recently that omentin is a prominent protein in pathologic mucus of fatal asthma as well as in acute severe asthma [40]. That study did not evaluate circulating omentin levels. Nevertheless, these findings suggest that omentin may contribute to the pathogenesis of asthma. The local and systematic actions of omentin may be different, and it may participate in different pathways of inflammation.

Th17 and Th9 cells play a role in the inflammatory processes of allergic diseases, and Th17 cells tend to increase with the severity of the disease in allergic asthmatics [41]. Hoppenot et al.

[42] suggested that the percentage of peripheral blood Th9 cells was increased in allergic asthmatics. Our results support these findings, because in this study Th17, Th9 cells and FeNO levels in the subjects with severe persistent asthma were all significantly higher than in those with those from intermittent to mild persistent asthma or the healthy controls, suggesting that systemic and local airway inflammation occur simultaneously in patients with persistent asthma. Both Th17 and Th9 cells are positively correlated with FeNO levels. This finding supports the hypothesis that higher Th17/Th9 cell levels are associated with T cell-mediated systemic and airway inflammation.

The data suggest that adipokines and Th17-related cytokines (IL-6, IL-22, and tumor necrosis factor- α) are involved in the pathogenesis of psoriasis [16]. Chemerin may be involved in the initiation and resolution of inflammation, indicating that altered levels may contribute to a disturbed immune response [34]. Furthermore, omentin up-regulation is a feature of “Th2-high” asthma with eosinophilic airway inflammation [40]. Our results suggest that both chemerin and omentin contribute to immune responses and inflammation in asthma, beyond the model based on Th1 and Th2 imbalances. However, the roles of chemerin and omentin in allergic asthma are controversial. In murine models of allergic asthma, chemerin plays a protective role by suppressing recruitment of inflammatory dendritic cells through the inhibition of chemokine ligand 2 secretions [9]. Nevertheless, our data are consistent with a positive association of chemerin with the Th17 and Th9 cell percentages in asthmatics, although the precise molecular mechanism of chemerin in the pathogenesis of asthma needs to be further explored. The pro-inflammatory effects of chemerin were documented by Demoor et al. [43] in a murine model of chronic obstructive pulmonary disease. Specifically, subacute cigarette smoke exposure caused

inflammatory responses that were severely attenuated in chemerin-receptor 23 knockout mice [43]. Interestingly, data from humans describing the properties of chemerin indicate that it may be shift from a pro-inflammatory to an anti-inflammatory adipokine in pathological conditions [16]. Chemerin is activated when a 6-amino acid peptide at the carboxyl-terminal of pro-chemerin is cleaved of by various proteases, and its biological activity depends on the protease. The resulting chemerin shows pro-inflammatory activity when processed by serine and inhibitory effects when processed by cysteine [44]. On the other hand, other sources [8] indicate that omentin exerts anti-inflammatory effects in lung injury, and potentially has a therapeutic role in inflammatory conditions, although it has possible pro-inflammatory effects as well. Pemberton et al. [45] concluded that there is a possible association between a single-nucleotide polymorphism in omentin and asthma susceptibility. In addition, omentin participates in allergen-induced airway inflammation in a mouse model of asthma [10]. Due to sample size and specimen selection differences, our data demonstrated that omentin levels declined and a negative correlation occurs with Th17 and Th9 cells in asthmatics. The dominant activity of omentin during the lung injury is yet to be revealed and its elucidation will await the results of future research.

Notably, a recent study reported that immunocytes interfere with the function of adipokines [5]. In this study, we found that chemerin levels were positively associated with Th17 and Th9 cells, while omentin levels were negatively associated with Th17 and Th9 cells. However, these results revealed a relationship in asthmatics. The adipokines may block or exacerbate inflammation and the immune response, adding complexity to our understanding of the pathogenesis of asthma.

There are limitations to our study. It had a cross-sectional in design, and thus can establish correlations but not evidence for cause and effect relations. Patient selection bias cannot be ruled out. There are several possible explanations for the clinical characteristics of the asthmatic subjects. There was no statistically significant difference in BMI in subgroups of asthmatics. The majority of the study population was not obese, and the levels of adipokines would be different in obese individuals. Severe asthma patients usually have more symptoms, and this may be caused by poor compliance with the treatment regimen, inadequate inhaled corticosteroid dosage, or overwhelming inflammatory activity. Asthma symptoms are positively and independently associated with FeNO [46]. Moreover, the presence of allergic rhinitis lead to increased FeNO [47], and may explain to the presence of high FeNO levels in severe persistent asthma patients. There is a high prevalence of allergic rhinitis in patients with intermittent to mild persistent asthma in China [48]. This study was based on outpatient research, and may not be fully representative of the general asthma population. Nonetheless, the results of this study support a relationship of the adipokines chemerin and omentin with the immune response involved in the pathogenesis of asthma. However, given the relatively small sample size, these results are preliminary and need to be confirmed in a larger population. Finally, the mechanism by which adipokines interact with the immune response needs to be explored in the future.

Conclusions: In summary, our study suggests that chemerin levels were positively correlated with the percentages of Th17 and Th9 cells, while omentin levels were negatively correlated with those cell percentages in asthma. The findings are consistent with a potential regulatory function of adipokines to the immune responses in the pathogenesis of asthma. Elucidating the

mechanisms by which adipokines link to asthma may ultimately lead to new diagnostic or therapeutic target

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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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Table 1. Clinical characteristics of patients

Characteristics	Intermittent to mild persistent	Moderate persistent	Severe persistent	Healthy Controls
Number	28	26	22	20
Age, years	49.29±14.24	50.92±8.90	43.41±13.89	43.00±12.27
Gender,				
Male/Female	13/15	12/14	11/11	8/12
BMI, Kg/m ²	23.81±3.57	23.59±3.08	23.88±3.39	22.28±1.88
Smoking history				
Never, n(%)	20 (71.43)	19(73.07)	12(54.55)	
Former, n(%)	4(14.29)	3(11.54)	4(18.18)	
Current, n(%)	4(14.29)	4(15.38)	6(27.27)	
Allergic rhinitis, n(%)	13 (46.43)	13 (50)	15 (68.18)	
Family history of asthma, n(%)	9 (32.14)	8 (30.77)	6 (27.27)	
FEV1, % predicted	79.61±12.17 [†]	78.65±16.17 [†]	69.56±22.21 [†]	103.50±11.64
FVC, % predicted	97.60±4.82	102.78±4.45	94.73±3.80	106.84±3.82
FEV1/FVC,%	72.45±14.48 [†]	67.09±10.61 [†]	64.90±19.21 [†]	93.01±8.37
PEF, % predicted	87.66±26.41 [†]	81.55±18.81 ^{†‡}	75.53±26.09 ^{†‡}	104.79±17.02
FeNO, ppb	26.54±8.42	54.47±18.10 [‡]	77.50±37.33 [‡]	

ACT	20.43±2.27	15.92±2.65 [†]	14.09±3.21 ^{†*}	
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[†] $p < 0.05$ vs healthy controls;

[‡] $p < 0.05$ vs intermittent to mild persistent;

^{*} $p < 0.05$ vs moderate persistent.

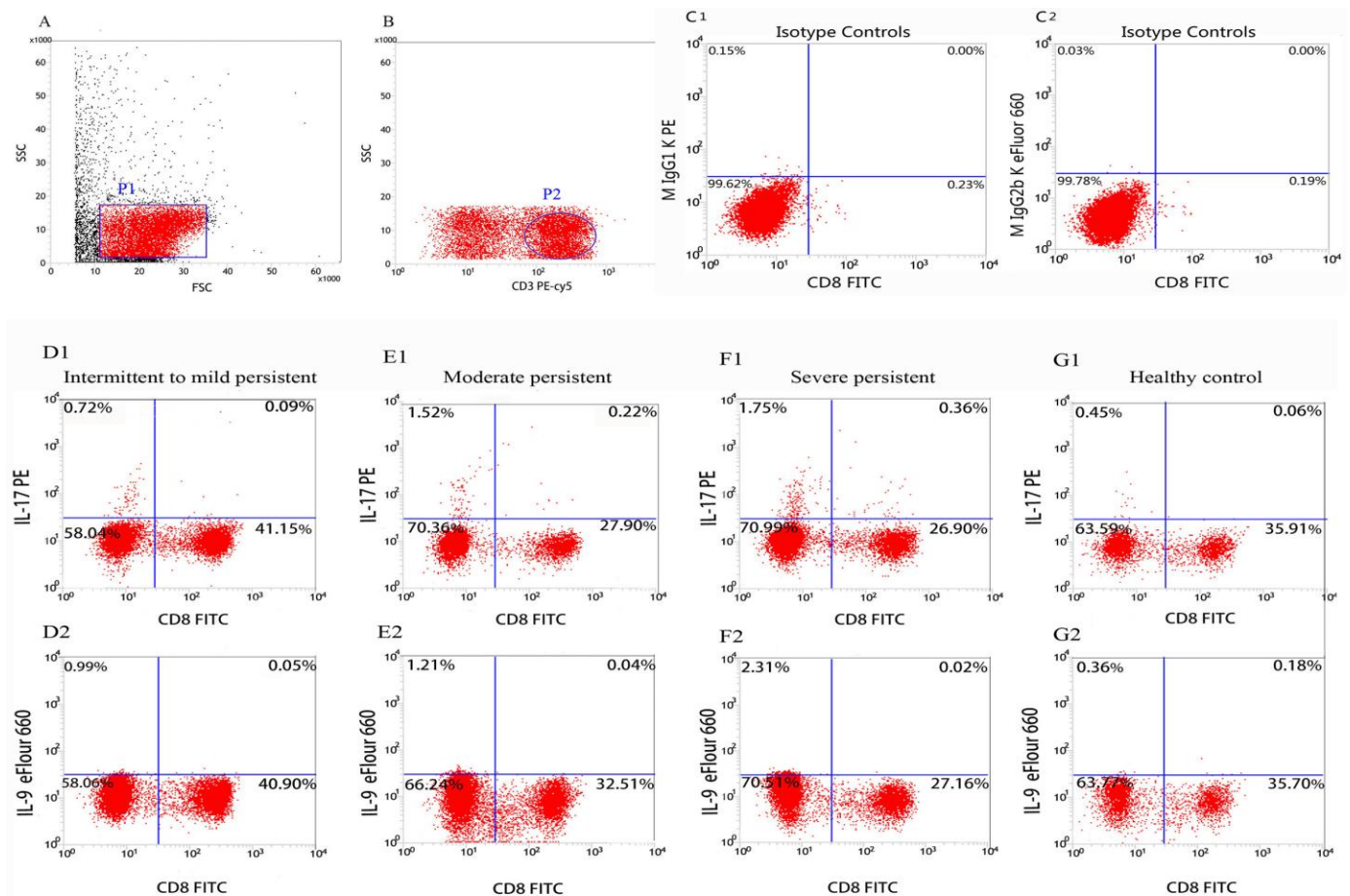


Figure 1 A: Lymphocytes were restricted via forward scatter (FSC) and sideward scatter (SSC) in PBMCs. P1 represents lymphocytes. B: dot plots representing anti-CD3 were carried out to establish CD3+ and CD3- lymphocyte gate. P2 represents CD3+ lymphocytes. C: We used anti-CD8 to sort CD8+ and CD8- cells gated on the CD3+ cells. IL-17 (C1) and IL-9 (C2) isotype controls were used to determine the positive cells and all the values were gated on the CD3+CD8- cells. Representative plots are shown. D1: CD3+CD8-IL-17+ T cells were presented in

intermittent to mild persistent asthma; D2: CD3+CD8-IL-9+ T cells were presented in intermittent to mild persistent asthma; E1: CD3+CD8-IL-17+ T cells were presented in moderate persistent asthma; E2: CD3+CD8-IL-9+ T cells were presented in moderate persistent asthma; F1: CD3+CD8-IL-17+ T cells were presented in severe persistent asthma; F2: CD3+CD8-IL-9+ T cells were presented in severe persistent asthma; G1: CD3+CD8-IL-17+ T cells were presented in healthy controls; G2: CD3+CD8-IL-9+ T cells were presented in healthy controls.

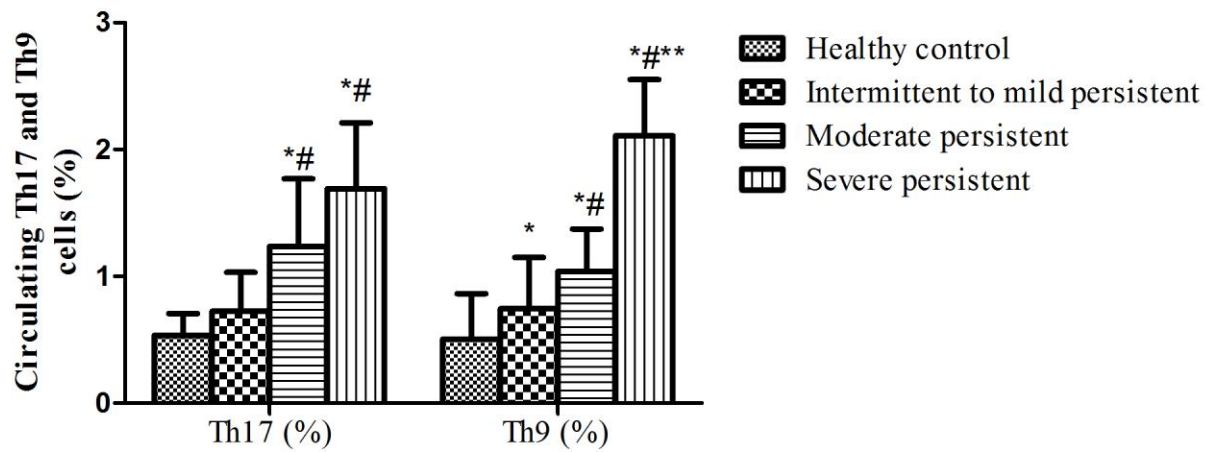


Figure 2 Percentages of Th17 and Th9 cells in subgroups of asthmatic patients (n = 16/11 per group for Th17/Th9, respectively) and healthy subjects (n = 20 for Th17/Th9). Data are presented as mean $\pm$ SD. * p < 0.05 versus healthy control, # p < 0.05 versus intermittent to mild persistent asthma, ** p < 0.05 versus moderate persistent asthma.

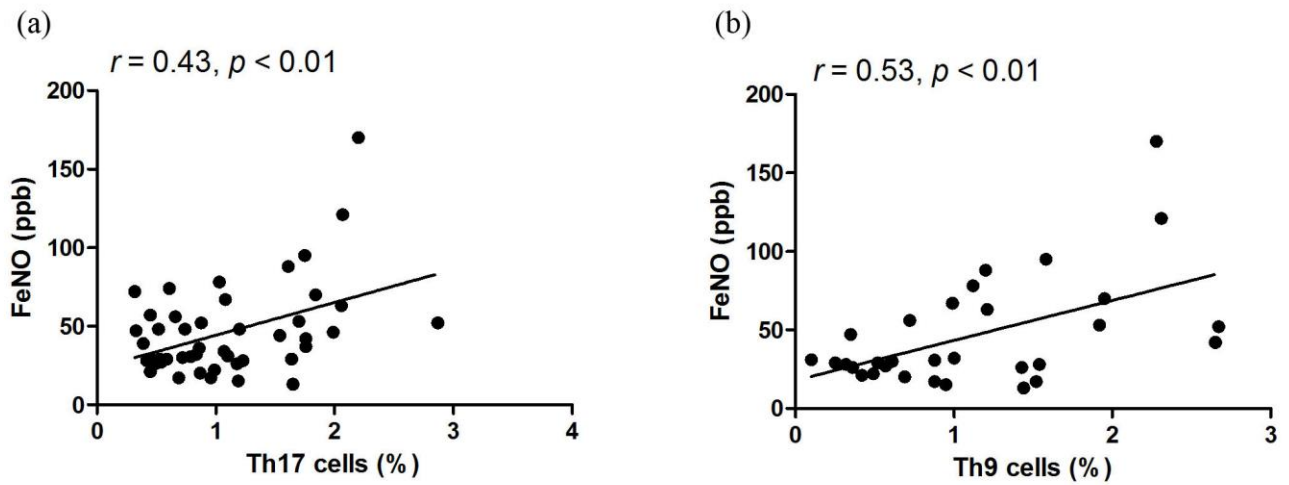


Figure 3 (a) The relationship between FeNO and Th17 cells in asthmatic subjects ($n = 48$); (b) The relationship between FeNO and Th9 cells in asthmatic subjects ($n = 33$). The Spearman's test was used to calculate the strength of correlation. r , Spearman's rho correlation coefficient.

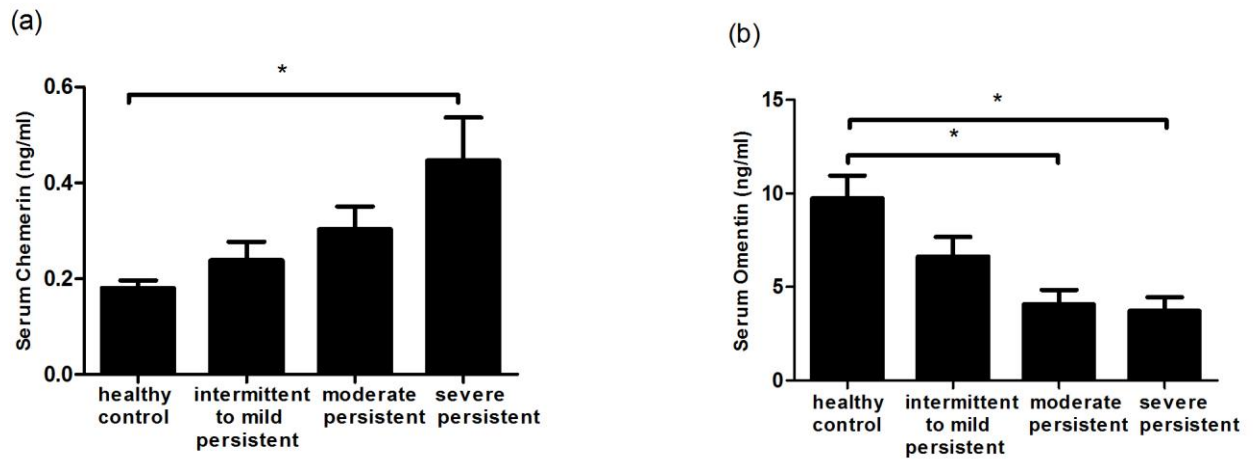


Figure 4 Plasma levels of chemerin (a) and omentin (b) in asthma patients with intermittent to mild persistent (n = 28), moderate persistent (n = 26), severe persistent (n = 22) and healthy subjects (n = 20). * $p < 0.05$ versus healthy controls.

Data are presented as mean $\pm$ SD.

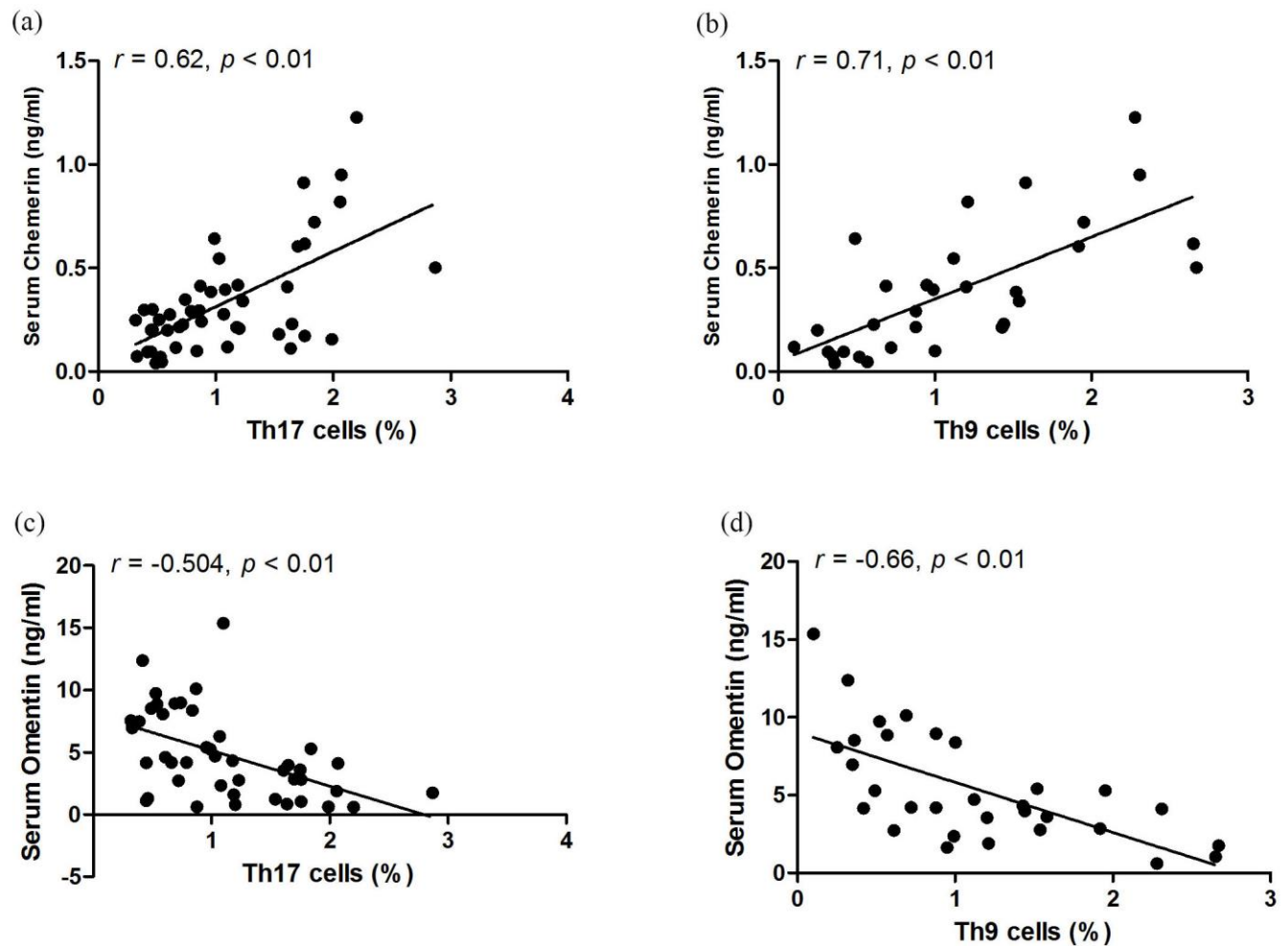


Figure 5 (a) Correlations analysis between plasma chemerin levels and Th17 percentages in patients with asthma ($n = 48$); (b) Correlations analysis between plasma chemerin levels and Th9 percentages in patients with asthma ($n = 33$); (c) Correlations analysis between plasma omentin levels and Th17 percentages in patients with asthma ($n = 48$); (d) Correlations analysis between plasma omentin levels and Th9 percentages in patients with asthma ($n = 33$). The Spearman's test

was used to calculate the strength of correlation. r , Spearman's rho correlation coefficient.