

Effect of aspirin desensitization on T-cell cytokines and plasma lipoxins in aspirin-exacerbated respiratory disease

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ABSTRACT

The pathogenesis of aspirin-exacerbated respiratory disease (AERD) is thought to be based on, mainly, overproduction of eicosanoid lipid mediators and on defective anti-inflammatory regulators. Aspirin desensitization treatment, the mainstay of controlling asthma and rhinitis in AERD patients, however, is the least understood aspect of the disease. The study was designed to determine the effect of aspirin desensitization on T-lymphocyte cytokine expression and on plasma lipoxin levels in AERD. Spirometry, skin-prick test and asthma control test were documented and intracellular cytokine expression in T lymphocytes and plasma lipoxin levels were measured in 23 AERD patients, 17 aspirin-tolerant asthmatic (ATA) patients, and 16 healthy controls. In the AERD group nasal symptom and smell scores were assessed. Of the 23 AERD patients 15 accepted to undergo aspirin desensitization protocol and 14 of them were desensitized successfully. In the desensitized AERD group, cytokine and lipoxin measurements were repeated after 1-month aspirin treatment. CD4⁺ IL-10 levels were higher in AERD patients than in healthy controls and CD4⁺ interferon (IFN) gamma levels were higher in AERD and ATA patients than in controls. Plasma lipoxin-A4 and 15-epi-lipoxin-A4 levels were similar among the three study groups. In the AERD group, subjects underwent aspirin desensitization followed by a 1-month aspirin treatment. Clinical parameters improved and CD4⁺ IFN-gamma levels decreased significantly. No significant change in lipoxin levels was recorded. CD4⁺ IFN-gamma and CD4⁺ IL-10 levels in AERD patients after 1-month aspirin desensitization treatment were similar to the healthy controls. The study confirms aspirin desensitization is effective clinically in AERD patients and suggests that IFN gamma and IL-10 expression in CD4⁺ T lymphocytes may be related to the mechanism of action.

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Aspirin-exacerbated respiratory disease (AERD) is a clinical entity that refers to inflammatory disease of the airways characterized by severe asthma and rhinitis attacks after ingestion of aspirin and most non-steroidal anti-inflammatory drugs (NSAIDs).¹ The patients present with nasal polyps, chronic rhinosinusitis, asthma, and sensitivity to cyclooxygenase 1-inhibiting medications.² To understand the pathogenesis of the disease, several studies have been performed and the results consolidate mainly on overproduction of eicosanoid lipid mediators^{3–5} and on defective anti-inflammatory regulators.^{6,7}

Treatment of AERD was planned according to the components of the disease present in the patients. Asthma management was planned consistent with the Global Initiative for Asthma (GINA).⁸ Chronic rhinosinusitis should also be treated according to guidelines

whereas nasal polyposis usually requires multiple polypectomies.^{9,10} Finally, for aspirin sensitivity either complete avoidance of aspirin and other NSAIDs or aspirin desensitization followed by aspirin treatment is required.¹⁰ Aspirin desensitization and daily aspirin treatment is effective in controlling upper and lower respiratory symptoms and it decreases postpolypectomy relapse rate. Aspirin desensitization is not only well documented to provide clinical benefit to patients with AERD, but has also been shown to be cost-effective.¹¹ Aspirin desensitization treatment, which is the mainstay of providing control of asthma and rhinitis in AERD patients, however, is the least understood aspect of the disease.¹² However, a wide variety of different possible mechanisms are still being investigated in AERD and altered interferon (IFN) γ expression in T lymphocytes is reported in relation to aspirin desensitization treatment.¹³

This study aimed to evaluate cytokine expression in CD4⁺ and CD8⁺ T lymphocytes and plasma lipoxin levels in AERD as well as evaluate the effect of aspirin desensitization treatment on these cytokine and lipoxin levels.

MATERIALS AND METHODS

Study Population

Twenty-three patients with AERD, 17 patients with aspirin-tolerant asthma (ATA), and 16 aspirin-tolerant

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healthy subjects were enrolled in the study between 2009 and 2012. All patients included in the study were followed in our immunology and allergy clinic. AERD patients who received an asthma diagnosis according to GINA criteria were included if they had a history of asthma attacks related to acetylsalicylic acid (ASA) or other NSAIDs and they had nasal polyposis or had been operated on for it or who experienced chronic persistent nasal congestion. ATA patients who received an asthma diagnosis according to GINA criteria were included if they had a history of taking ASA or other NSAID medication with different indications without any nasal, naso-ocular, or respiratory reactions.⁸ Asthmatic patients with (a) chronic persistent nasal congestion and rhinorrhea, (b) nasal polyposis or history of nasal polypectomy, (c) pansinusitis, and/or (d) history of severe asthma attack requiring treatment in the intensive care unit without any relevant reason were not included in the ATA group because of the possibility of underlying AERD. Other exclusion criteria for AERD and ATA patients were (a) pregnancy, (b) having an attack in the last 4 weeks, (c) psychiatric disease, (d) unwillingness of patient, (e) use of β -blocker therapy, (f) severe systemic or gastrointestinal diseases, and/or (g) blood dyscrasias. Healthy controls included in the study had no known diseases including the respiratory system and were all nonatopic and were aspirin tolerant.

The study protocol was approved by the Ethics Committee of Eskişehir Osmangazi University Medical Faculty (21.05.2010-41/PR-10-03-19-41) and both written and verbal informed consent were obtained from each participant before study entry.

Clinical Assessment

Clinical assessments included a complete medical history including drug reactions, detailed physical examination, and pulmonary function tests. Skin-prick tests with common aeroallergens (ALK-Abello, Madrid, Spain) were performed on all subjects. Additionally, asthma control test, nasal symptom scores, and smell scores were assessed in AERD patients. Venous blood samples for intracellular cytokine and plasma lipoxin measurements were drawn from all subjects at the same time of the day (9:00 A.M.) in fasting state. Of the 23 AERD patients, 15 consented to undergo aspirin desensitization. Fourteen of these patients were successfully desensitized, with one desensitization failure due to severe anaphylaxis during the desensitization procedure. The AERD patients desensitized successfully were assessed after 1 month of aspirin treatment at 2×650 mg daily with reevaluation of pulmonary function tests, nasal symptom scores, asthma control scores, and smell scores and blood was redrawn to repeat the intracellular cytokine and plasma lipoxin measurements.

Skin-Prick Tests

Skin-prick tests were performed by using a common panel including *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; grass, tree, and weed pollens; animal dander (cat and dog); animal feathers; and molds (including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*) allergen extracts (ALK). Positive and negative controls were histamine and phenolated glycerol saline, respectively. A mean wheal diameter of 3 mm or greater than that obtained with the control solution was considered as positive.

Smell Scores

Subjective symptom scores for sense of smell were scored as follows: 0, no sense of smell; 1, intermittent partial sense of smell; 2, intermittent complete sense of smell; 3, partial sense of smell the majority of the time; 4, complete sense of smell the majority of the time; and 5, perfect and continuous sense of smell.¹⁴ We scored the sense of smell before desensitization and 1 month after aspirin treatment.

Nasal Symptom Score

Nasal symptoms of each participant including obstruction, rhinorrhea, itching, and sneezing were questioned. Each symptom was scored using the following scale: 0, no symptoms; 1, mild (symptom was present but was of short duration and not annoying or troublesome); 2, moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep); or 3, severe (symptom was troublesome and interfered with normal daily activity or sleep). The total symptom score was the sum of the scores for the individual symptoms. Total symptom score values (0–12) were categorized as mild (0–4), moderate (5–8), or severe (9–12).¹⁵ Nasal symptom scores were assessed before desensitization and 1 month after aspirin treatment.

Oral Aspirin Provocation and Desensitization

Oral aspirin provocation and desensitization protocol started with placebo three times a day on the 1st day. Minimum forced expiratory volume in 1 second (FEV₁) was 1.5 L and was >60% of predicted to start the protocol. On the 2nd day protocol continued with 20 mg of aspirin and advanced every 3 hours. We measured FEV₁ every hour and waited 3 hours between doses. If at any time a reaction occurred in the larynx or the bronchi, reactions were treated before continuation and the provocation dose was then repeated. If the reaction was limited to the nasal membranes, oxymetazoline nasal spray was used and the challenge was then continued with the next dose of aspirin. After a reaction had been treated and was resolved we repeated the ASA provoking dose. Aspirin

Table 1 Demographic data of the study groups

	AERD Patients (n = 23)	ATA patients (n = 17)	Healthy controls (n = 16)	p Value
Age (yr)	50.6 ± 10.5	44.9 ± 10.7	40.2 ± 12.0	0.015*
Gender (M/F; n)	5/18	4/13	10/6	0.017**

Data are given as mean ± SD unless stated otherwise.

*Mean age of AERD patients was significantly higher than that of healthy controls.

** Male/female ratio was significantly higher in healthy controls compared to AERD and ATA groups.

AERD = aspirin-exacerbated respiratory disease; ATA = aspirin tolerant asthma.

desensitization was completed at 325 mg of ASA and we gave 650 mg as a first dose and then treated with 650 mg twice daily.²

Measurement of Intracellular Cytokine Levels

Heparinized whole blood was diluted with RPMI (Sigma-Aldrich Corp., St. Louis, MO) and incubated for 4 hours at 37°C and 5% CO₂. Stimulated whole blood was distributed in 100-μL aliquots into tubes and lysis of red cells was performed by adding 2 mL of Pharm Lyse (BD, Franklin Lakes, NJ) followed by vortexing and incubation for 10 minutes at room temperature in the dark and centrifuged at 500 × g for 5 minutes. Samples were washed with 2 mL of stain buffer at 500 × g for 5 minutes. Antihuman CD4-FITC (eBioscience, San Diego, CA) and CD3-PerCp (BioLegend, San Diego, CA) were added to four of the eight aliquots, and CD8-FITC (BioLegend) and CD3-PerCp were added into other four aliquots, and then incubated for 15 minutes at room temperature in the dark and washed with stain buffer. To fix and permeabilize cells, aliquots were treated with 500 μL of Cytofix/Cytoperm (BD), followed by vortexing and incubation for 20 minutes at room temperature in the dark and centrifuged at 500 × g for 5 minutes and then 2 mL of Perm/Wash buffer (BD) followed by vortexing and incubation for 10 minutes at room temperature in the dark and centrifuged at 500 × g for 5 minutes. The intracellular staining procedure included addition of anti-IL-4, IL-5, IL-10, and IFN-γ monoclonal antibodies (BD). The flow cytometric assessment with Becton-Dickinson FACSCalibur flow cytometry (BD) was performed on the same day. Acquisition was gated on CD3⁺CD4⁺ and CD3⁺CD8⁺ cells and then the percentage of CD3⁺CD4⁺ and CD3⁺CD8⁺ cells producing IL-4, IL-5, IL-10, and IFN-γ was determined on dot plots. Acquisition and analysis were performed with the Cell Quest software package (BD).

Measurement of Plasma Lipoxins

Venous blood samples for plasma lipoxin level measurements were drawn from all subjects into tubes containing EDTA, after which samples were immedi-

ately centrifuged at 1000 × g, 2–8°C for 15 minutes and obtained plasma samples were immediately frozen at –80°C until the time of analysis. Plasma concentrations of lipoxin-A4 and 15-epi-lipoxin-A4 were measured using Human Lipoxin A4 and Human 15-epi-Lipoxin-A4 ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, China), respectively.

Statistical Analysis

Continuous variables were expressed as means ± SD and categorical variables were expressed as numbers and percentages. For comparison of the groups, an independent Student's *t*-test or one-way ANOVA with *post hoc* least significant difference test was applied for continuous variables if normally distributed, Mann Whitney *U* test or Kruskal Wallis test for data with abnormal distribution. The χ^2 -test was used for categorical variables. To compare data in AERD patients before and after aspirin desensitization, paired samples *t*-test was applied for continuous variables if normally distributed, Wilcoxon signed-rank test for data with nonnormal distribution. All statistical tests were two-sided and a value of *p* < 0.05 was considered statistically significant in all analyses. The analyses were assessed with the Statistical Package for Social Sciences (SPSS) Version 15 (SPSS, Inc., Chicago, IL) for Windows system.

RESULTS

A total of 23 AERD patients, 17 ASA patients, and 16 aspirin-tolerant healthy subjects were recruited in the study. Mean age was significantly higher in patients with AERD compared with healthy controls. Significant male predominance was found in healthy controls compared with AERD and ATA groups. Demographic data of the study groups is shown in Table 1.

The clinical characteristics of patients with AERD and ATA including the duration, severity, and control level of the disease, as well as the pulmonary functions and atopy status, were similar. Asthma severity was evaluated and asthma patients enrolled in the study were grouped as severe and mild/moderate according

Table 2 Clinical characteristics of patients with AERD and ATA

	AERD Patients (n = 23)	ATA Patients (n = 17)	p Value
Duration of disease (yr)	13.4 ± 8.8	14.8 ± 9.0	>0.05
Asthma Control Test score	16.4 ± 5.8	16.1 ± 5.3	>0.05
Nasal symptom score	6.9 ± 2.6	0 ± 0	<0.001*
Smell score	0.9 ± 1.5	5.0 ± 0	<0.001*
FEV ₁ (mL)	2201.3 ± 1031.8	2247.7 ± 843.9	>0.05
FEV ₁ (predicted, %)	81.5 ± 23.8	80.5 ± 22.3	>0.05
Atopic patients (n, %)	8 (35)	8 (47)	>0.05
Severe asthmatic patients (n, %)	14 (61)	11 (65)	>0.05

Values are expressed as mean ± SD, except otherwise stated.

*Statistical significance at $p < 0.001$ level.

AERD = aspirin-exacerbated respiratory disease; ATA = aspirin tolerant asthma; FEV₁ = forced expiratory volume in 1 s.

Table 3 Intracellular cytokine levels expressed in CD4⁺ and CD8⁺ T lymphocytes and plasma lipoxin levels in the study groups

	AERD Patients (n = 23)	ATA Patients (n = 17)	Healthy Controls (n = 16)	p Value
CD4 ⁺ IL-4	3.6 ± 2.1	2.8 ± 1.1	2.8 ± 0.9	>0.05
CD4 ⁺ IL-5	1.8 ± 1.2	1.5 ± 0.6	1.2 ± 0.5	>0.05
CD4 ⁺ IL-10	2.1 ± 1.2	1.6 ± 0.8	1.3 ± 0.5	0.015*
CD4 ⁺ IFN-γ	20.3 ± 6.7	19.9 ± 3.1	16.1 ± 5.2	0.046*
CD8 ⁺ IL-4	1.8 ± 1.3	1.4 ± 0.7	1.3 ± 0.6	>0.05
CD8 ⁺ IL-5	1.2 ± 0.7	0.9 ± 0.5	0.8 ± 0.4	>0.05
CD8 ⁺ IL-10	0.9 ± 0.5	0.7 ± 0.6	0.7 ± 0.5	>0.05
CD8 ⁺ IFN-γ	21.2 ± 6.8	21.6 ± 10.0	18.3 ± 7.0	>0.05
Lipoxin-A4	1360.5 ± 2290.5	1742.3 ± 2979.5	1529.5 ± 2765.0	>0.05
15-epi-lipoxin-A4	579.0 ± 760.5	654.6 ± 639.7	641.3 ± 822.4	>0.05

Values are expressed as mean ± SD.

*Statistical significance at $p < 0.05$ level.

AERD = aspirin-exacerbated respiratory disease; ATA = aspirin tolerant asthma; IFN = interferon.

to American Thoracic Society criteria.¹⁶ As expected, nasal symptom scores were significantly higher and smell scores were significantly lower in patients with AERD because asthmatic patients who had nasal symptoms were not included in ATA group. Clinical characteristics of patients with AERD and ATA are summarized in Table 2.

Measurements of intracellular cytokine levels expressed in CD4⁺ and CD8⁺ T lymphocytes revealed higher CD4⁺ IL-10 levels in patients with AERD compared with healthy controls and higher CD4⁺ IFN-γ levels in patients with AERD and ATA compared with healthy controls. IL-4 and IL-5 levels expressed in CD4⁺ T lymphocytes as well as IL-4, IL-5, IL-10, and IFN-γ levels expressed in CD8⁺ T lymphocytes and plasma lipoxin-A4 and 15-epi-lipoxin-A4 levels were similar among the three study groups (Table 3).

ASA desensitization was performed in 15 AERD patients but was discontinued on one of them because of severe anaphylactic reaction. ASA desensitization was

completed successfully in the rest of the patients. Among these 14 AERD patients, during the desensitization procedure, classic reaction (naso-ocular reaction and at least 15% decline in FEV₁) was observed in one-half of them and naso-ocular reaction in the other half.

In AERD patients who underwent aspirin desensitization followed by a 1-month daily aspirin treatment the clinical parameters including asthma control test, nasal symptom scores, and smell scores improved significantly, although increase in FEV₁ measurements were not statistically significant (Table 4).

CD4⁺ IFN-γ levels decreased significantly in AERD patients after aspirin desensitization treatment. No significant change was observed in IL-4, IL-5, and IL-10 levels expressed in CD4⁺ T lymphocytes or in IL-4, IL-5, IL-10, and IFN-γ levels expressed in CD8⁺ T lymphocytes after aspirin desensitization treatment (Table 5). No significant change was observed in

Table 4 Clinical characteristics of AERD patients before and after aspirin desensitization

	AERD Patients before Aspirin Desensitization (n = 14)	AERD Patients after Aspirin Desensitization (n = 14)	p Value
Asthma Control Test score	16.4 ± 4.7	23.6 ± 1.7	<0.001*
Nasal symptom score	7.6 ± 2.5	1.6 ± 1.2	<0.001*
Smell score	0.6 ± 1.2	3.7 ± 1.8	<0.001*
FEV ₁ (mL)	2235.7 ± 744.7	2361.4 ± 528.6	>0.05
FEV ₁ (predicted, %)	84.2 ± 16.8	93.6 ± 12.2	>0.05

Values are expressed as mean ± SD.

*Statistical significance at $p < 0.001$ level.

AERD = aspirin-exacerbated respiratory disease; ATA = aspirin tolerant asthma; FEV₁ = forced expiratory volume in 1 s.

Table 5 Intracellular cytokine levels expressed in CD4⁺ and CD8⁺ T-lymphocytes and plasma lipoxin levels in AERD patients prior to and after aspirin desensitization

	AERD Patients before Aspirin Desensitization (n = 14)	AERD Patients after Aspirin Desensitization (n = 14)	p Value
CD4 ⁺ IL-4	2.8 ± 1.2	2.8 ± 1.6	>0.05
CD4 ⁺ IL-5	1.3 ± 0.7	1.4 ± 0.7	>0.05
CD4 ⁺ IL-10	1.8 ± 1.1	1.7 ± 1.2	>0.05
CD4 ⁺ IFN- γ	19.6 ± 7.4	17.5 ± 7.7	0.040*
CD8 ⁺ IL-4	1.4 ± 1.0	1.2 ± 0.6	>0.05
CD8 ⁺ IL-5	1.0 ± 0.7	0.8 ± 0.2	>0.05
CD8 ⁺ IL-10	0.7 ± 0.3	0.8 ± 0.4	>0.05
CD8 ⁺ IFN- γ	18.5 ± 6.5	16.1 ± 8.1	>0.05
Lipoxin-A4	1361.6 ± 1708.9	2093.7 ± 1918.3	>0.05
15-epi-lipoxin-A4	698.7 ± 957.6	650.4 ± 1018.4	>0.05

Values are expressed as mean ± SD.

*Statistical significance at $p < 0.05$ level.

AERD = aspirin-exacerbated respiratory disease; ATA = aspirin tolerant asthma; IFN = interferon.

plasma lipoxin levels after aspirin desensitization treatment (Table 5).

CD4⁺ IFN- γ and CD4⁺ IL-10, which are the intracellular cytokines that were significantly different between AERD patients and healthy controls, were similar in AERD patients after 1-month daily aspirin treatment with the levels in healthy controls (Figs. 1 and 2).

DISCUSSION

The present study indicated that aspirin desensitization treatment is clinically effective in AERD patients, improving nasal symptom scores, smell scores, and asthma control test scores, in accordance with some previous studies. Additionally, the present study showed that CD4⁺ IL-10 and CD4⁺ IFN- γ levels are higher in patients with AERD compared with healthy controls and they were similar in AERD patients after 1-month daily aspirin treatment with the levels in healthy controls.

Recently, determining the pathogenesis of AERD and mechanism of action of aspirin desensitization

treatment has been the focus of the studies. Increased thromboxane, prostaglandin D₂ (PGD₂), and cysteinyl leukotriene synthesis,^{3,4,5,17} as well as decreased lipoxin and PGE₂ levels,^{6,7,18,19} are the most encountered findings of several reports. However, these findings have not been enough to describe completely the mechanism of the disease and the effective therapeutic options performed readily in allergy clinics. Intracellular cytokine expression in T lymphocytes might also be potentially involved in the pathogenesis of AERD based on a recent report by Shome *et al.* They have shown low CD4⁺ IFN- γ levels in an AERD patient that rose to levels of healthy subjects after aspirin desensitization treatment.¹³ This study involved a larger number of AERD patients and has shown higher CD4⁺ IFN- γ levels that decrease significantly after aspirin desensitization treatment in these patients. IFN- γ is a critical cytokine in inhibiting allergic immunity because of its capacity to inhibit the isotype switch to IgE and IL-4-mediated expression of low-affinity IgE receptors.²⁰ Accordingly, it was suggested by the authors

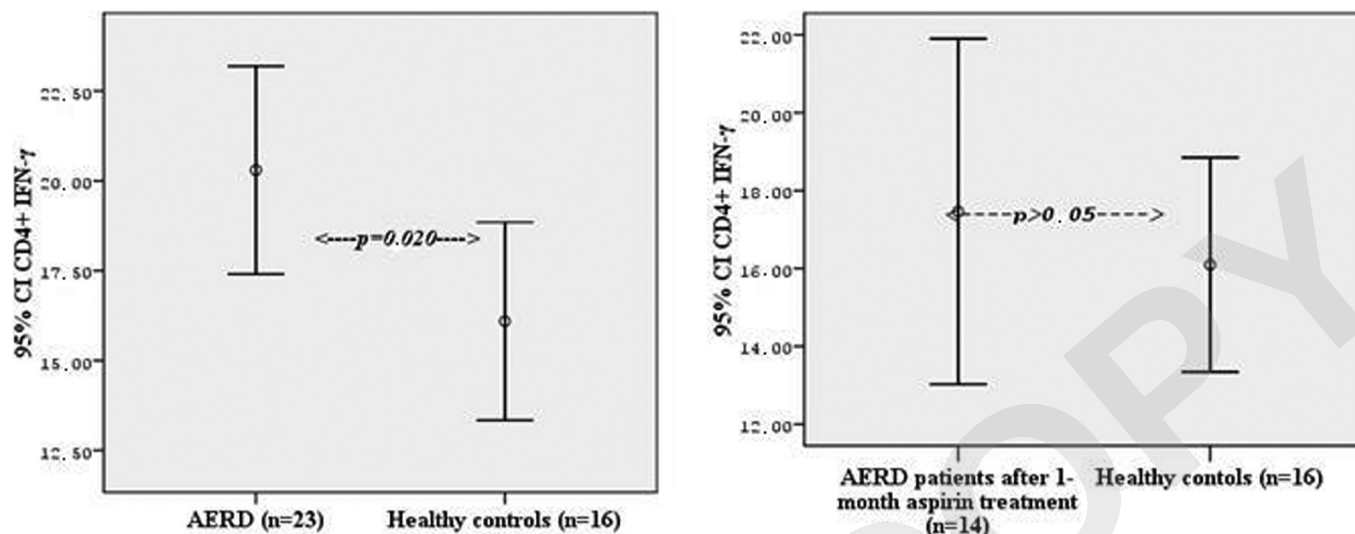


Figure 1. CD4⁺ interferon (IFN) γ levels were significantly different between aspirin-exacerbated respiratory disease (AERD) patients and healthy controls. After 1 month of aspirin treatment, CD4⁺ IFN- γ levels decreased and were similar to the healthy controls.

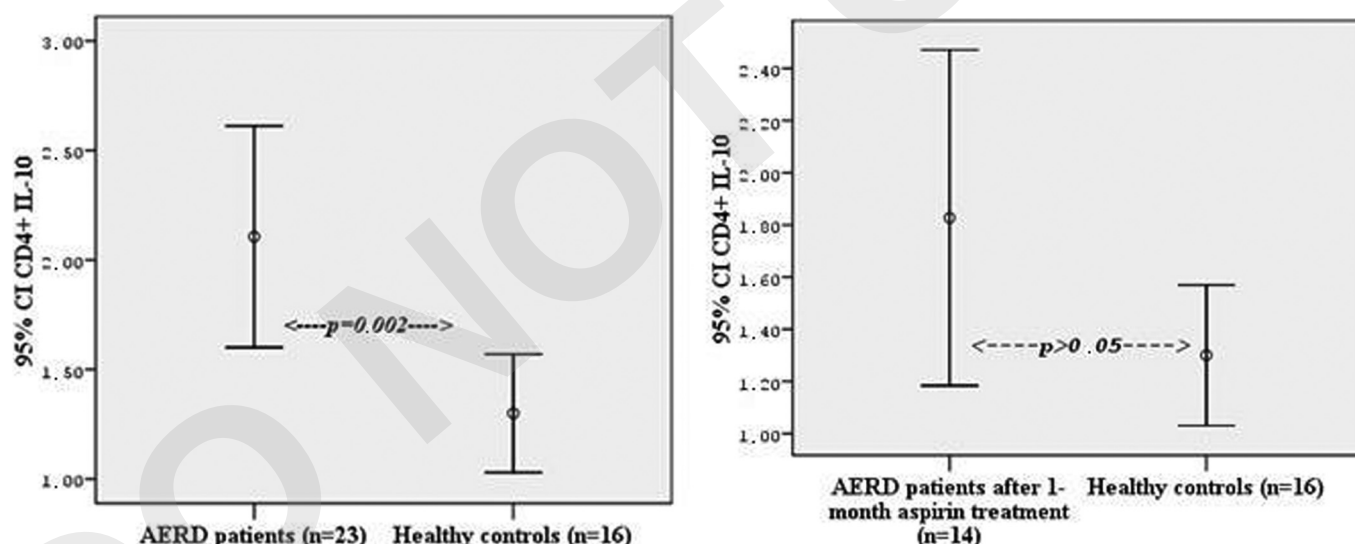


Figure 2. CD4⁺ IL-10 levels were significantly different between aspirin-exacerbated respiratory disease (AERD) patients and healthy controls. After 1 month of aspirin treatment, CD4⁺ IL-10 levels decreased and were similar to the healthy controls.

that high CD4⁺ IFN- γ levels might inhibit the IL-4-related effects and the decrement in the levels after aspirin desensitization treatment might suggest that this treatment option may be effective *via* this mechanism. Moreover, because it is known that PGE₂ inhibits IFN- γ synthesis,²¹ the authors might also suggest that high IFN- γ levels in AERD are related to low PGE₂ levels characteristically seen in AERD. Increased levels of IFN- γ in AERD patients in the present study are in accordance with that of Steinke *et al.*²² The most interesting finding of the present study is the decrease of IFN- γ levels after aspirin desensitization treatment, which might be important for understanding the pathogenesis of this effective treatment.

The present study revealed significantly high levels of CD4⁺ IL-10 in AERD patients. Second, CD4⁺ IL-10 levels reevaluated after 1 month of daily aspirin treatment in the AERD group who underwent aspirin desensitization were found to be similar with healthy subjects. These findings support that IL-10 promoter polymorphisms contribute to the development of AERD suggested previously by Kim *et al.*²³ On the other hand, it is known that upper and lower airway inflammation is more prominent in aspirin-induced asthmatic patients compared with asthmatic patients and healthy subjects.²⁴ High CD4⁺ IL-10 levels in AERD patients in the present study might be an indirect result of more prominent inflammation in these

patients. Moreover, as CD4⁺ IL-10 levels in AERD patients are at similar levels to that of healthy controls after aspirin desensitization treatment may be indicative of diminished inflammation. Because IL-10 is an immunomodulator cytokine its level decreases with the control of inflammatory state. The authors think that this finding is important in explaining the mechanism of aspirin desensitization treatment.

Although it was previously reported that IL-4 production decreases with aspirin treatment in AERD patients,²⁵ in the present study we did not find any relationship between IL-4 levels and aspirin desensitization treatment. This finding is not surprising because in the present study it was seen that IFN- γ levels expressed in CD4⁺ T lymphocytes were increased. The high levels of IFN- γ in AERD patients may suppress IL-4 levels.

In the present study cytokine levels were measured in peripheral blood samples. However, local cytokine levels in respiratory mucosa were not studied because invasive procedures were required and were not planned in the study design because of ethical reasons.

In severe asthmatic patients lipoxin levels are lower than in mild asthmatic patients according to some recent reports.^{26,27} In animal asthma models lipoxins decrease allergic airway inflammation and inhibit inflammatory cell infiltration in allergic airway mucosa.²⁸ Defective lipoxin signaling is assumed to be responsible for increased airway response in severe asthmatic patients. The study of Planaguma *et al.* supports this hypothesis, showing that lipoxin-A4 levels are decreased in bronchoalveolar lavage in severe asthmatic patients.²⁹ However, Menzies *et al.*, in their studies that investigated the potential effect of aspirin treatment on 15-epi-lipoxin-A4 levels, failed to show any change in 15-epi-lipoxin-A4 levels in mild-to-moderate asthmatic patients.³⁰ In the present study, no potential effect of lipoxins in AERD or ATA patients or any difference in lipoxin levels in severe asthmatic patients is noted. Second, in AERD patients lipoxin levels did not change after aspirin desensitization treatment.

Finally, the present study confirms that aspirin desensitization treatment is an effective therapeutic option in patients with AERD and suggests that IFN- γ and IL-10 expression in CD4⁺ T lymphocytes might be related to pathogenesis of AERD and mechanism of action of aspirin desensitization treatment.

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