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Q1 Aspirin desensitization for patients with 2 aspirin-exacerbated respiratory disease: 3 A randomized double-blind 4 placebo-controlled trial ☆☆☆

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KEYWORDS

Aspirin-intolerant asthma;
Desensitization;
Quality of life;
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Abstract The effect of aspirin desensitization (AD) on immunologic profile of patients with AERD has been poorly understood. This study is aimed at investigating the effect of AD on clinical and immunological markers of patients with AERD. This randomized double-blind placebo-controlled trial comprised 34 adult patients (67.6% female) with chronic rhinosinusitis, nasal polyps, and aspirin-intolerant asthma. The active group underwent AD over a 2-day period with increasing doses of aspirin (60, 125, 325, and 625 mg), followed by receiving aspirin 625 mg twice daily for 6 months. Symptom scores and medication needs of patients with AERD who have undergone AD were significantly lower compared to the placebo group after 6 months (7.5 ± 3.5 vs. 10.6 ± 3.8 and 9.3 ± 2.0 vs. 11.0 ± 3.1 , respectively, all $p < 0.05$). However, no significant difference was observed in serum concentration of IL-10, IFN- γ , and TGF- β between two groups neither at baseline nor at the end of study.

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Abbreviations: SNOT-22, Sino-Nasal Outcome Test-22; FEV1, Forced expiratory volume in 1 second; AERD, Aspirin-exacerbated respiratory diseases; CRSwNP, Chronic rhinosinusitis with nasal polyposis; IL-10, Interleukin-10; TGF- β , Transforming growth factor beta; IFN- γ , Interferon gamma; AD, Aspirin desensitization.

☆ Clinical trial registration: This study was registered in clinical trials. gov with identifier of "NCT01867281".

☆☆ Author contribution: HE helped randomize the patients into active and placebo groups, supervised the trial, and reviewed the final draft critically. MN was the chair study, had the concept, and reviewed the manuscript. ZA had the concept, analyzed the data, and wrote the primary draft of manuscript. SA, MF, and MHB collected the raw data, interviewed the patients (these investigators were blinded to each study group assignment), and reviewed the final draft critically. NM had the concept and helped the team prepare active and placebo capsules and sprays. Normal saline used as placebo spray and glucose was used as placebo capsules. The containers were exactly similar in size, appearance, and weight. Finally, all of the authors approved the final draft and its submission to the journal of Annals of Allergy Asthma and Immunology.

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1. Introduction

Aspirin-exacerbated respiratory disease (AERD) is characterized by chronic rhinosinusitis with nasal polyposis (CRSwNP), asthma, and aspirin intolerance [1]. Aspirin-tolerant asthma usually have an allergic basis and present in children [2], while aspirin-intolerant asthma as a distinctive entity usually presents in adults who are non-allergic to the aero-allergens and have genetic predisposition [3,4]. Aspirin desensitization is indicated in patients with AERD who have sub-optimally controlled asthma or sinusitis, those required multiple revision polypectomies, and patients who require cyclooxygenase-1 (COX-1) inhibitors for anti-platelet therapy [5]. Aspirin desensitization may improve sinusitis, asthma control, and quality of life the patients with AERD [5]. However, most reports in this regard were observational, non-randomized, or non-controlled studies [6,7]. In addition, the exact mechanism of aspirin desensitization is still unknown [3].

Inhibition of COX-1 pathway and abnormal shift toward lipoxygenase pathway as well as eosinophil and mast cell activation either by aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) have been supposed as the main pathologic features of AERD [8]. Up-regulation of cysteinyl-leukotriene (cys-LT) receptors has been reported in patients with AERD [9]. In addition, the distinct immunophenotype of patients with AERD may regulate expression of cys-LT receptors. Expression of interleukin (IL)-4, IL-13, IL-5, and interferon gamma (IFN- γ) is significantly higher in nasal tissue samples of patients with AERD compared to healthy subjects and those with chronic sinusitis [10]. IL-4 induces up-regulation of cys-LT receptors and IFN- γ enhances the production and release of cys-LTs [10]. IFN- γ also induces eosinophil maturation [10] and eosinophil cationic release following aspirin exposure [8]. IL-10 and transforming growth factor-beta (TGF- β) exert anti-inflammatory roles and might be ameliorative of the effects of IFN- γ and IL-4 [11]. Increased levels of IL-10 and TGF- β have been reported in patients with AERD [12,13]. It might be a compensatory increase or it itself might be a risk factor for promoting inflammation in sinuses and airway of susceptible patients as supposed by Kim and his colleagues [14]. Chronic aspirin exposure may ameliorate IL-4 effects and reduce IL-4 levels and thereby down-regulate cys-LT receptors [15,16]. However, the exact mechanism of tolerance induction by aspirin desensitization is not yet well understood. Here, we aimed at investigating the clinical efficacy and safety of aspirin desensitization in patients with AERD. This study is also aimed at investigating the effect of aspirin desensitization on serum IL-10, TGF- β , and IFN- γ concentrations in patients with AERD to shed light on mechanism of aspirin desensitization.

2. Methods

2.1. Study design

This study is a parallel double-blind randomized clinical trial (RDBCT) conducted on 34 subjects with CRSwNP, asthma, and aspirin hypersensitivity. A total of 80 patients with CRSwNP and asthma were screened for aspirin hypersensitivity. CRSwNP [17] and asthma [18] were diagnosed and

categorized in accordance with published guidelines. Aspirin challenge test was performed to establish the diagnosis of aspirin hypersensitivity [19]. A total of 39 patients with CRSwNP, asthma, and aspirin hypersensitivity were identified in screening. These 39 patients were monitored for 12 weeks prior to randomization to investigate asthma control and compliance of the patients to the monthly visits. Of them, 34 agreed to participate in the trial and they were randomized to active and placebo groups (Fig. 1). All the participants were visited at baseline, 1 month, and after 6 months (at the end of study). Forced expiratory volume in 1 second (FEV1), Sino-Nasal Outcome Test-22 (SNOT-22), symptom and medication scores were recorded at each visit. Computed tomographic (CT) scan and blood sampling for cytokine measurement were done at baseline and at the end of the study. All the patients were informed regarding the study protocols and possible adverse events and signed the informed consents. This study was done in accordance with the principles of the Helsinki Declaration. Appropriate institutional review board and local ethics committee approved the study protocol. This study was registered in clinical trials.gov with identifier of "NCT01867281."

2.2. Eligibility criteria

The inclusion criteria were (1) CRSwNP in accordance with endoscopic and CT findings, (2) stable asthma with no increase in baseline glucocorticoids for at least 3 months or no asthma attack in at least last 6 months, (3) aspirin hypersensitivity confirmed by a positive Intranasal Ketorolac and modified oral aspirin challenge test, and (4) age of greater than 18 years. Both sexes were eligible to participate. Exclusion criteria were (1) smoking, (2) pregnancy or current breastfeeding, (3) history of bleeding diathesis and gastrointestinal bleeding, (4) history of ischemic heart disease, stroke, and diabetes, (5) history of abnormal liver function, and (6) uncontrolled hypertension.

2.3. Intranasal Ketorolac and modified oral aspirin challenge test

Intranasal Ketorolac and modified oral aspirin challenge test was performed to identify and recruit the patients with CRSwNP and asthma who were aspirin intolerant [5]. Antihistamines, beta agonists, and corticosteroids were discontinued for at least 48 hours prior to aspirin challenge test. The challenge was performed in 2 consecutive days. On the first day, 4 increasing doses of Ketorolac were administered at 30-minute intervals. Subsequently, 2 doses of 60 mg aspirin were used and spirometry was done before each dose of aspirin. Patients with FEV1 < 70% of the best prior value at the end of first day were excluded [20]. On the second day, two increasing doses of aspirin from 150 to 325 mg were administered with a 180-minute interval and spirometry was repeated before each dose as needed to measure FEV1. Aspirin hypersensitivity was diagnosed if FEV1 decreased more than 20% of the baseline or 15–19% accompanied by naso-ocular reactions, urticaria, or angioedema. In each day of challenge test, patients were discharged from the clinic 3 hours after the last dose of aspirin.

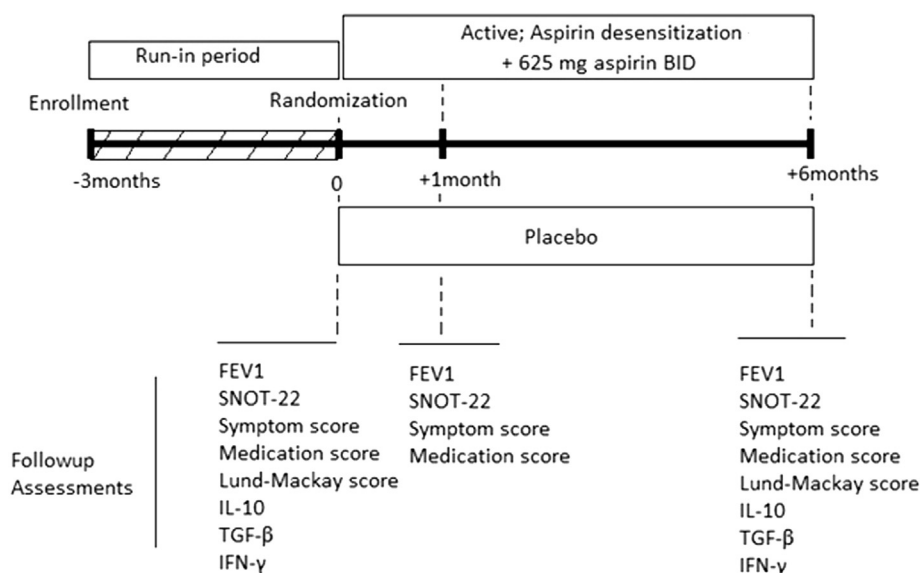


Figure 1 Study diagram.

Finally, aspirin-intolerant patients were followed for 12 weeks as the washing out period prior to randomization.

2.4. Randomization and masking

Eligible patients were randomized with equal proportion into active or placebo arms. Block randomization was performed using a computer-generated list of random numbers. The block size was 4. Neither of participants nor investigators was aware of the arm assignments. The randomization list was implemented by the unblinded study director (HE).

2.5. Interventions

With using 80 and 325 mg tablets of aspirin and a pill cutter, capsules with different doses of aspirins were provided. Similar capsules containing glucose were used as placebo for use in placebo group. Besides study intervention, participants were allowed to take standard medications to control their respiratory and naso-ocular symptoms.

Following randomization, active group underwent aspirin desensitization as described previously [5]. The desensitization was initiated by administering the intranasal ketorolac spray and aspirin capsules in 2 consecutive days. On the first day, 4 increasing doses of Ketorolac were administered at 30-minute intervals. The placebo arm used placebo sprays containing normal saline. Subsequently, 2 doses of 60 mg aspirin at 90 minute intervals were used and spirometry was done before each dose of aspirin. For the placebo arm, similar placebo capsules containing sugar was used. On the second day, two increasing doses of aspirin from 150 to 325 mg were administered with a 180-minute interval, and spirometry was repeated before each dose as needed to measure FEV1. Following initial 2 days of desensitization, the active arm received 650 mg aspirin twice daily for 1 month and 325 mg twice daily for 6 months. The placebo arm underwent similar procedure except that they received capsules containing glucose instead of aspirin.

2.6. Safety monitoring

Patients were hospitalized for 2 days to monitor the unpredictable reactions during aspirin challenge and desensitization. Complete blood counts, alanine aminotransferase (ALT), prothrombin time (PT), and partial thromboplastin time (PTT) were assessed once prior to aspirin challenge test and once during run-in period. They were repeated after randomization only if a patient had symptoms relevant to a bleeding disorder or liver dysfunction. In addition, patients were asked for possible side effects in each visits and weekly phone contacts. Asthma exacerbations leading to emergency department visits during the study were recorded as asthma attacks.

2.7. Clinical evaluations

For sino-nasal-related quality of life, the patients were assessed by SNOT-22 [21] at each visit (Supplementary file 1). Lower scores of SNOT-22 indicate higher quality of life of the patients with naso-ocular symptoms. In addition, an expert physician filled two tables scoring the symptoms and medication needs of the patients, separately (supplementary file 2). FEV1 was measured using standard spirometry (Sensor Medics, CA). Lower symptom and medication scores denote to lower symptoms and medication needs.

CT scan was obtained at baseline and at the end of study to calculate the Lund-Mackay score [22]. Briefly, opacification of each sinus and the ostiomeatal complex were scored from zero to two by an expert radiologist.

2.8. Immunological evaluations

Blood samples were taken at baseline and 6 months later at the end of study. The sera of all the participants were kept at -20°C until the running of the assays. IL-10, IFN- γ and TGF- β were assessed using enzyme-linked immunosorbent assay (ELISA kit, Biosciences, United States) in

accordance to the manufacturer's instructions. Results of cytokine assay were expressed as international unit per milliliter (IU/ml). In addition, white blood cells, platelets, and eosinophil counts were investigated.

2.9. Outcome measures

Primary outcome measures of this study include (1) effect of aspirin desensitization on quality of life of the patients with AERD, (2) effect of aspirin desensitization on anti-inflammatory cytokines of IL-10, and TGF- β , and IFN- γ .

Secondary outcome measures of this study include effect of aspirin desensitization on symptoms, Lund-Mackay score, and lung function and medication needs of the patients with AERD.

2.10. Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences, Version 16 (SPSS Inc, Chicago, Illinois, USA). We set the type 1 error α as 0.05, type 2 error β as 0.2, and thus the power of 80%. With 16 participants in each arm, we could demonstrate 0.56 differences in mean of outcome measures between two arms. We also considered 5% dropout rate and started the study with 34 participants. Categorical variables are expressed as frequency and compared between the active and placebo arms using Fisher exact test. Quantitative variables are expressed as mean $\pm$ standard error and compared between the two arms using Mann-Whitney U test. Baseline and final values of cytokines were compared using Wilcoxon matched-pair signed-rank test. Error bars were employed to demonstrate change in variables through the study. All of the tests were two tailed, and the probability of less than 0.05 was considered statistically significant.

3. Results

3.1. Demographics

Of 34 participants at baseline, 23 women and 9 men completed the 6-month trial. Fig. 2 shows the flowchart of the study and the reasons of lost to follow-up. Baseline characteristics of the participants have been demonstrated in Table 1. Participants in the active and placebo arms were similar with respect to the age and gender distribution. However, participants assigned to the placebo arm had significantly better SNOT-22 and Lund-Mackay scores compared with those assigned to the active arm which was unintended.

3.2. Clinical outcomes

No significant change in clinical outcomes was observed after 1 month of follow-up. After 6 months, participants in the active arm had significantly higher FEV1 compared to the baseline (87.1 ± 1.7 vs. 79.1 ± 1.9 , $p = 0.001$) and participants in the placebo arm (87.1 ± 1.7 vs. 80.1 ± 1.8 , $p = 0.015$). Participants in the active arm had better quality of life (25.8 ± 2.9 vs. 35.9 ± 2.7 , $p = 0.015$) and symptoms score compared to the placebo arm (7.5 ± 0.8 vs. 10.6 ± 0.9 , $p = 0.014$). There was a trend toward better medication scores in patients

assigned to active arm compared to placebo arm (9.3 ± 0.5 vs. 11.0 ± 0.7 , $p = 0.138$). No significant change was observed in quality of life, symptoms, and medication needs of the participants in the placebo arm after 6 months. By contrast, quality of life, symptoms, and medication scores of participants in active arm were significantly improved compared to their baseline values (Fig. 3). Following the aspirin desensitization and aspirin consumption for 6 months, Lund-Mackay score reduced significantly in participants of active arm (11.1 ± 0.5 vs. 15.1 ± 0.7 , $p < 0.001$). Fig. 4 shows detailed data on CT changes and Lund-Mackay scores through the study.

3.3. Immunological outcomes

Serum concentration of IL-10 decreased following aspirin desensitization (8.3 ± 0.7 vs. 7.7 ± 0.4 , $p = 0.270$). Same results were observed regarding IFN- γ (13.7 ± 1.5 vs. 13.4 ± 1.1 , $p = 0.338$) and TGF- β (13.7 ± 1.5 vs. 13.4 ± 1.1 , $p = 0.474$). There was no significant difference between two arms of the study with respect to the serum concentration of IL-10, IFN- γ , and TGF- β (Fig. 5). At baseline, eosinophilia (counts $> 4.5 \times 10^8/L$) were found in 3 persons in the active versus 2 in the placebo arm ($p = 0.500$). Following 6 months, eosinophilia was present only in 2 participants in the active arm and 2 in the placebo arm ($p = 0.700$).

3.4. Asthma attacks and adverse events

Eight patients with AERD experienced asthma exacerbations in the placebo arm, while only 4 patients in the placebo arm experienced asthma exacerbations through the study (relative risk (RR), 2.0; 95%CI, 0.79–5.06, $p = 0.137$). On the other hand, chronic aspirin consumption led to severe gastrointestinal bleeding in one participant who discontinued the study (Fig. 2).

4. Discussion

In this RDBCT, we confirmed that aspirin desensitization leads to significant improvement of symptoms, quality of life, and lung function of patients with AERD independent of serum IL-10, TGF- β , and IFN- γ concentration. To date, several observational, non-randomized, or non-controlled studies investigated the effect of aspirin desensitization on patients with aspirin desensitization [6,12,15,23,24]. However, the number of RDBCTs is limited. To the best of our knowledge, only 3 RDBCT investigated the clinical efficacy of aspirin desensitization on patients with AERD thus far [25–27]. Stevenson et al. were the first to conduct a randomized double-blind cross-over trial on 25 patients with AERD and reported improvement in nasal symptoms following aspirin desensitization and maintenance treatment with 1300 mg aspirin per day [26]. However, they found no significant improvement in lower respiratory tract symptoms, lung function, and anti-asthma medications [26]. Fruth et al. designed an RDBCT and investigated the effect of aspirin desensitization with maintenance dose of 100 mg daily on 70 patients with AERD. Of these 70 patients, only 31 completed the study with 55.7% dropout rate. They found that those assigned to aspirin group had better quality of life, general health condition, and nasal symptoms compared with placebo group

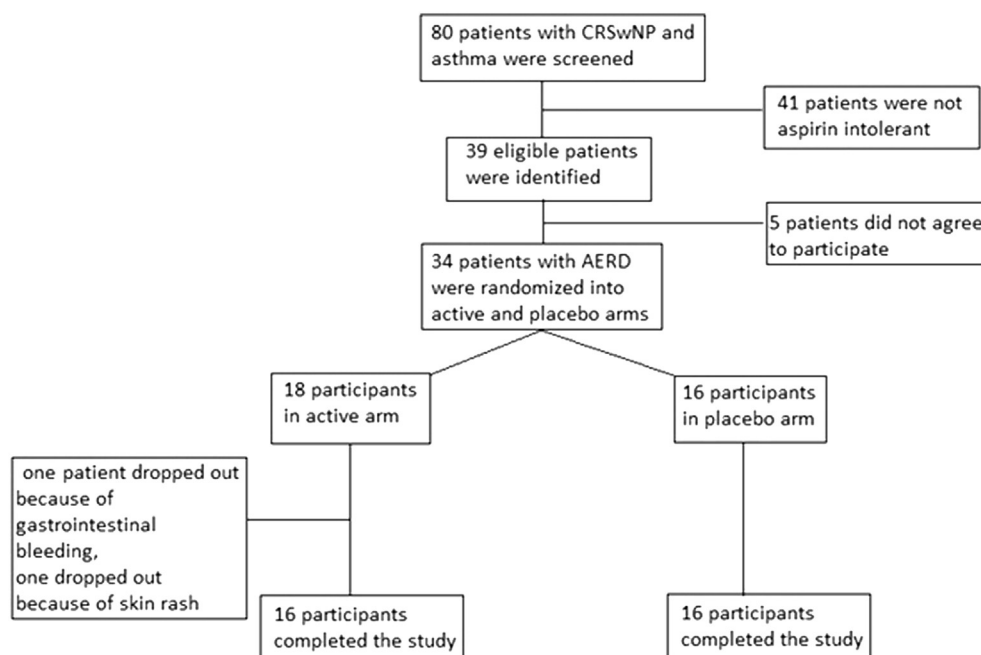


Figure 2 Flowchart, A total of 80 patients with CRSwNP and asthma were screened for aspirin hypersensitivity and 39 were identified. Of them, 34 agreed to participate in this double-blind randomized clinical trial. Eighteen patients with AERD were assigned to the active arm and sixteen patients were assigned to the placebo arm. Two participants in the active discontinued the study because of aspirin adverse events. At the end, 32 participants completed the study.

[25]. Recently, another RDBCT has been published in which 20 patients with AERD were assigned to aspirin desensitization or placebo groups. At the end of the study 8 and 7, patients in active and placebo groups completed the study, respectively. Aspirin desensitization led to significant improvement in quality of life, asthma control, inhaled corticosteroid use, and peak nasal inspiratory flow of active arm compared to placebo group [27]. Reduction in symptoms of the patients concurrent with medication needs results in greater quality of life expressed by the patients [25,27]. In line with the literature, we found that those assigned to aspirin group had significantly improved quality of life, reduced symptoms and higher FEV1 compared to placebo group.

In addition to measurement of FEV1 as an objective evaluation of lung function, we performed CT scans at baseline and at the end of the study to provide an objective measurement of nasal inflammation after aspirin

desensitization. Interestingly, Lund–Mackay scores of patients with AERD in the active group decreased significantly through the study (15.1 ± 0.7 vs. 11.1 ± 0.5 , $p < 0.001$). However, Lund–Mackay scores of the active and the placebo groups did not differ significantly at the end of study. One reason might be the higher scores of participants in the active group at baseline that was unintended (15.1 ± 0.7 vs. 12.4 ± 0.3 , $p = 0.015$). There exists only one study that investigated sinus CT scan changes of patients with AERD who underwent aspirin desensitization. In contrast to our results, they found no improvement in Lund–Mackay scores [27]. As period of follow-up was the same, non-significant results of that study might be attributable to the low number of the participants. They analyzed data of only 8 patients with AERD who underwent aspirin desensitization [27].

One of the main pathologic features of AERD is the overexpression of leukotriene C4 synthase in biopsies taken from nasal polyp and lung tissues [28]. It leads to surge in release of cys-LTs following exposure to COX-1 inhibitors particularly from eosinophils and mast cells [10,28]. Influx of calcium facilitated by dihydropyridine receptors are implicated in leukotriene release [29–31]. Inflammatory and broncho-constrictor prostanoids of prostaglandin (PG)D2 and PGF-2 α are overexpressed in patients with AERD, while anti-inflammatory prostanoids such as PGE2 are under-expressed [28]. Patients with AERD suffer from chronic eosinophilic inflammation of upper and lower respiratory tract as well [32]. Interestingly, aspirin desensitization leads to reduction in cys-LT receptors on inflammatory cells of nasal mucosa as well as marked reduction in urinary excretion of cys-LTs [33]. Distinct immunophenotype of patients with AERD might underlie overexpression of cys-LTs [10]. Patients with AERD [10] have components of increased

Table 1 Characteristics of participants at baseline.

Variable	Active (N = 18)	Placebo (N = 16)	p-value
Age (years)	31 $\pm$ 4.3	27 $\pm$ 5.5	0.571
Female (%)	13 (72%)	10 (62%)	0.433
Lund–Mackay score	15.1 $\pm$ 0.7	12.4 $\pm$ 0.3	0.015
Medication score	13.1 $\pm$ 0.3	11.5 $\pm$ 0.6	0.056
Symptom score	14.5 $\pm$ 1.1	11.3 $\pm$ 0.9	0.056
SNOT-22 score	52.8 $\pm$ 4.1	37.6 $\pm$ 2.7	0.014
FEV1 (% of predicted)	79.1 $\pm$ 1.9	83.8 $\pm$ 2.0	0.138
Sino-Nasal Outcome Test-22 (SNOT-22), Forced Expiratory volume in 1 second (FEV1),			

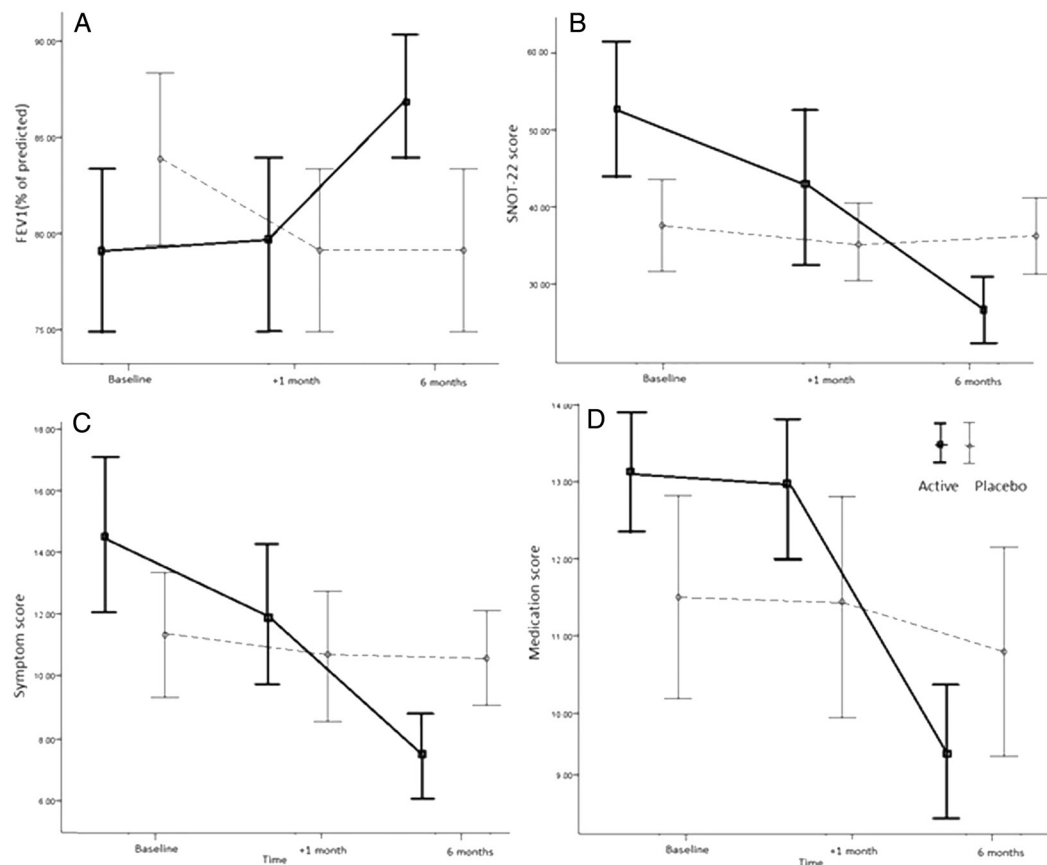


Figure 3 Clinical outcomes, A) FEV1 improved following aspirin desensitization and aspirin consumption for 6 months in active arm (87.1 ± 1.7 vs. 79.1 ± 1.9 , $p = 0.001$). In addition, participants in the active arm had significantly higher FEV1 compared with participants in placebo arm at 6 months follow up (87.1 ± 1.7 vs. 80.1 ± 1.8 , $p = 0.015$). B) SNOT-22 score improved following aspirin desensitization and aspirin consumption for 6 months in active arm (35.9 ± 2.7 vs. 52.8 ± 4.1 , $p < 0.001$). In addition, participants in active arm had significantly lower SNOT-22 scores compared to participants in placebo arm at 6 months follow up (25.8 ± 2.9 vs. 35.9 ± 2.7 , $p = 0.015$). C) Symptom score of participants in active arm reduced significantly following 6 months (7.5 ± 0.8 vs. 14.5 ± 1.1 , $p = 0.001$). Active arm had significantly lower symptom score compared to placebo arm after 6 months (7.5 ± 0.8 vs. 10.6 ± 0.9 , $p = 0.014$). D) Medication score of participants in the active arm reduced significantly following 6 months (9.3 ± 0.5 vs. 13.1 ± 0.3 , $p = 0.001$). There was a trend toward lower medication score in active arm compared to placebo arm after 6 months (9.3 ± 0.5 vs. 11.5 ± 0.6 , $p = 0.138$). Error bars show the mean value of variables with 95% confidence interval.

T-helper 2 responses as well as increased IFN- γ expression [10], while it was not observed in aspirin-tolerant patients with CRSwNP [34]. IFN- γ may promote inflammatory responses in AERD as it induces eosinophil maturation and degranulation and enhances expression of genes involved in leukotriene synthesis [8,10]. Aktas et al. assessed CD4+ T cell release of IL-2, IL-4, and IFN- γ prior to and 1 month after aspirin desensitization. They found no significant change in CD4+ T cell release of IL-2, IL-4, and IFN- γ 1 month after desensitization compared to baseline values [13]. Shome et al. studied one patient with AERD and found that aspirin desensitization led to an increase in IFN- γ expression by CD4+ lymphocytes and a decrease in IFN- γ expression by CD8+ lymphocyte probably due to inhibition of PGE2 [35]. We found non-significant reduction in serum IFN- γ following aspirin desensitization. It is possible the receptors of IFN- γ rather than IFN- γ are overexpressed in patients with AERD and aspirin desensitization may regulate the expression of IFN- γ receptors. On the other hand, tolerance induction by chronic aspirin digestion may be a consequence of modulation

of expression of regulatory cytokines of like IL-10 and TGF- β . We also found non-significant reduction in serum IL-10 and TGF- β following desensitization. IL-10 and TGF- β inhibit most functions of T cells, and antigen presenting cells and mast cells, while promoting B cell responses [36]. However, at present, the role of regulatory cytokines in pathogenesis of AERD seems to be few. Taken together, there should be further investigations on the role of the regulatory cytokines in pathogenesis and treatment of AERD.

During aspirin challenge, the effects of aspirin on airway exhaled nitric oxide, eosinophilia, and subsequent asthma exacerbations are dose dependent [15]. Similarly, control of the sinusitis and asthma-related symptoms of patients with AERD requires intake of at least 100 mg aspirin per day [25]. There exists controversy on the efficacious and safe aspirin maintenance dose [6,23,25]. Some suggested daily dosage of 100 mg [25], while the others recommended 300 mg [7,23], 625 mg [27], and 1300 mg [15,26] aspirin as maintenance dose. The higher the dosage of aspirin the more side effects

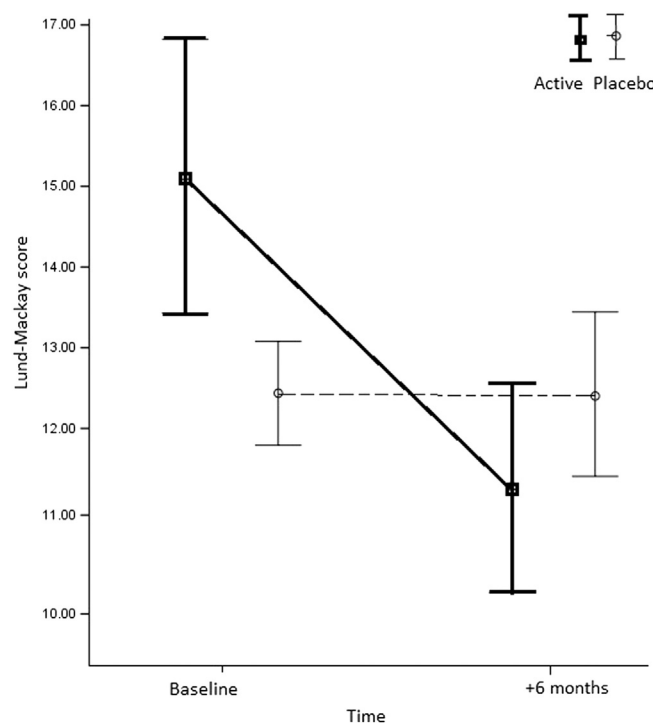


Figure 4 Lund-Mackay score; at baseline, participants in the active arm had significantly higher Lund-Mackay scores compared to the placebo arm (15.1 ± 0.7 vs. 12.4 ± 0.3 , $p = 0.015$). Following the aspirin desensitization and aspirin consumption for 6 months Lund-Mackay score reduced significantly in participants of active arm (11.1 ± 0.5 vs. 15.1 ± 0.7 , $p < 0.001$). At the end, active arm had lower Lund-Mackay score compared to the placebo arm that did not attain significance (11.1 ± 0.5 vs. 12.1 ± 0.4 , $p = 0.102$). Error bars show the mean value of variables with 95% confidence interval.

may happen. On the other hand, low dose of 100 mg could not reduce nasal polyp extend and need for revision surgeries [7]. Lee et al. suggested initial dose of 625 mg aspirin with subsequent tapering to the lowest effective dosage of 325 mg per day [6]. Despite reports on significant in sinusitis and asthma symptoms after 1 month of follow-up, we did not find any significant change after 1 month [19]. Long-term treatment with aspirin may be necessary to observe the effects of aspirin desensitization and maintenance treatment.

Reduction of polyp revision surgeries is one of the main benefits of aspirin desensitization in patients with AERD. Rozsasi et al. reported no need to revision surgeries in those who continued daily aspirin intake for about 2 years [7]. Increased risk of gastrointestinal bleedings is the main obstacle of long-term high-dose intake of aspirin [27]. In the present study, 2 patients in the active group (2/18, 11.1%) experienced aspirin adverse effects and discontinued the study in which one was due to gastrointestinal bleeding and another was due to

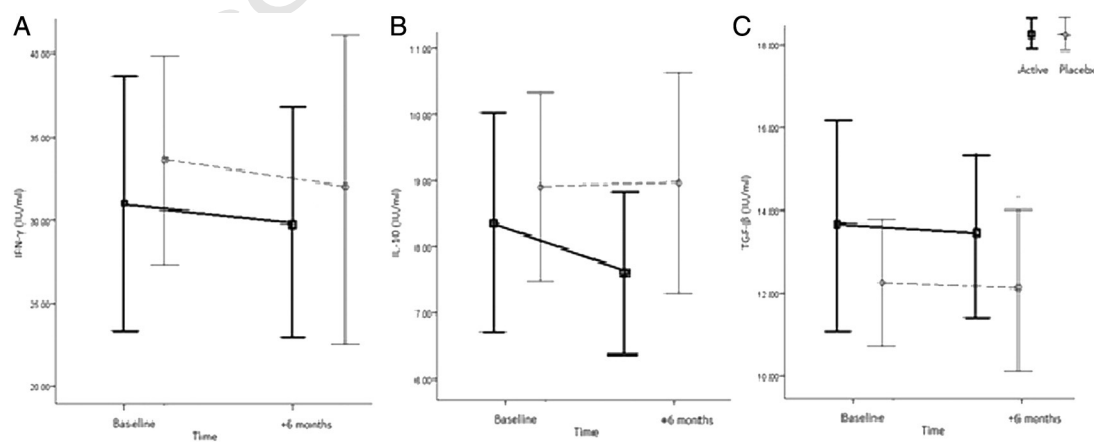


Figure 5 Aspirin desensitization did not affect significantly on serum concentration of IFN- γ , IL-10, and TGF- β . However, there was a trend toward reduction of serum concentration of IFN- γ , IL-10, and TGF- β . Error bars show the mean value of variables with 95% confidence interval.

skin rashes. Overall, most agree that at least 325 mg aspirin per day is needed to reduce the symptoms of patients with AERD, significantly [6]. Such a high dose of aspirin may cause adverse events when consumed for a long period of time.

One of the limitations of present study is the low number of participants with 80% power to detect 0.56 differences in tested variables. It was hard to find patients willing to undergo aspirin challenge and about half of those underwent aspirin challenge were eligible to participate. In addition, high-dose aspirin intake and possible adverse events made patients reluctant to participate. Another weakness of this study was our limitation to obtain sputum samples and absence of exhaled nitric oxide measurement. However, this study, benefited from a randomized double-blind design, enabled us to reduce the bias. We had acceptable follow-up and investigated both subjective and objective variables to assess the clinical efficacy of aspirin desensitization. Finally, we tried to investigate the mechanism of aspirin desensitization through a well-designed prospective trial by measuring anti-inflammatory cytokines and IFN- γ for the first time. IFN- γ deemed to play a crucial role in AERD [10].

5. Conclusions

In conclusion, we confirmed clinical efficacy of aspirin desensitization in improving symptoms, medication needs, quality of life, opacification of sinuses, and lung function of patients with AERD. We also showed that serum level of anti-inflammatory cytokines do not change significantly following aspirin desensitization. Absence of assessment of the cytokine expression in sinus and airway tissue of patients with AERD justifies further studies to draw certain conclusions.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clim.2015.05.012>.

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Supl 1.

Patients were asked to score the severity of following 22 items from 0 (No problem) to 5 (severe problem).

- 1- need to blow nose,
- 2- sneezing,
- 3- dripping nose,
- 4- cough,
- 5- postnasal drip,
- 6- dense nasal drip,
- 7- ear fullness,
- 8- dizziness,
- 9- ear pain,
- 10- facial pain/pressure,
- 11- difficulty falling asleep,
- 12- wake up at night,
- 13- lack of a good night's sleep,
- 14- wake up tired,
- 15- fatigue,
- 16- reduced productivity,
- 17- reduced concentration,
- 18- feeling frustrated or restless or irritable,
- 19- sadness,
- 20- embarrassment,
- 21- decrease in smell and taste,
- 22- nasal obstruction

Supplementary 2.1

symptoms		1	2	3
Nasal symptoms	Itching			
	Sneezing			
	Nasal discharge			
	Nasal obstruction			
Eye symptoms	Itching			
	Redness			
Bronchial symptoms	cough			
	Difficulty in breath			
	Wheezing			
Total point				

Scoring: 0: no symptom; 1: mild; 2: moderate; 3: severe symptoms

Supplementary 2.2

Drugs	Score
local	
Systemic	
Total point	

local (both nasal sprays and eye-drops) 1 point for use of each one.

systemic (systemic antihistamines, inhaled b₂-agonists, inhaled steroids, and theophylline); 2 points for use of each one.

Score for the use of each drug will be multiplied by two in case of use of maximum dose.