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# The Effects of Combined Therapeutic Protocol on Allergic Rhinitis Symptoms and Molecular Determinants

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## ABSTRACT

Current medications to treat allergic rhinitis (AR) include antihistamines, corticosteroids, and anti-leukotrienes. In the present study, we investigated the effects of combination therapy; using these drugs, and evaluates the AR-related markers and parameters in an animal model.

After inducing BALB/c mice AR models, the animals were treated with either pranlukast, loratadine, fluticasone, loratadine + fluticasone, loratadine + pranlukast, fluticasone + pranlukast, or loratadine + fluticasone + pranlukast. Clinical symptoms, Immunoglobulin (Ig)G1, ovalbumin (OVA)-specific and total IgE, leukotriene (LT)B4, LTC4, histamine, thymic stromal lymphopoietin (TSLP) serum levels, and interleukin 4 levels in the nasal lavage fluid were determined. The expressions of *HRH1*, *CysLT1R*, *NLR3*, *Caspase-1*, and *MUC5a* were studied.

Allergic symptoms (nasal rubbing and sneezing), serum Igs (IgG1, total and OVA-specific IgE), eicosanoids (LTB4 and LTC4), histamine, TSLP, and IL-4 as well as gene expressions of *MUC5a*, *Caspase-1*, *NLR3*, *HRH1*, and *CysLT1R* were reduced in the animals receiving each of the therapeutic regimens; however, more pronounced effects were seen in the group treated with the triple combined protocol (loratadine + fluticasone + pranlukast).

The combination of the loratadine, fluticasone, and pranlukast can effectively control the symptoms of AR probably via modulating several related mechanisms at the early and late phases of allergic responses.

**Keywords:** Allergy and immunology; Histamine; Inflammation; Leukotrienes; Pathology; Prostaglandins; Steroids

## INTRODUCTION

Allergic diseases are dependent on Th2-mediated immunological responses. Allergic rhinitis is a common disease of the upper respiratory system with clinical symptoms such as rhinorrhea, nasal obstruction, sneezing, coughing, loss of smell, postnasal drainage, lacrimation, and nasal irritation.[1] The Allergic Rhinitis and its Impact on Asthma(ARIA) classification of AR have been shown in Figure 1.[1,2]

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There are available treatments for AR such as intranasal steroids, antihistamines, leukotriene (LTR) antagonists, and immunotherapy.[1,3] Allergy is still the main trigger of AR pathophysiology and this phenomenon is largely mediated by Th2 cytokines and immunoglobulin E (IgE) production. In allergic diseases, the sensitization phase is the primary step in which antigen-presenting cells (APCs); especially dendritic cells (DCs), take arrived allergens up and transport them to lymph nodes. The allergen is then presented to lymphocytes that are transformed into allergen-specific T and B cells and releaseTh2 cytokines, which finally stimulate B cells to switch Ig class and produce IgE. Afterward, IgE binds to FcεRs on mast cells and basophils and initiates the sensitization phase. When the allergen re-enters the body, it directly binds to IgE-bound FcεRs on mast cells and induces degranulation of these cells; resulting in the production of mediators such as histamine, serotonin, cytokines, chemokines, eicosanoids, etc. that orchestrate allergic responses.[1,4,5] Activated mast cells produce vasoactive and inflammatory molecules such as interleukin (IL)-1, IL-6, IL-8, TSLP, and tumor necrosis factor-alpha (TNF-α) via mitogen-activated protein kinases (MAPKs)/caspase-1/NF-κB signaling cascades. The activation of FcεRI on the surface of mast cells induces cellular degranulation, cytokine production, and secretion of prostaglandin D2 (PGD2) and LT mediators. Protein kinase C activator and calcium ionophore (PMACI) stimulate mast cells to produce mediators and cytokines via caspase-1/NF-κB/MAPKs signaling pathways which play a key role in AR development by inducing Th2 lymphocytes, vasculature beds, mucosal glands, smooth muscle cells, and inflammatory cells. Moreover, activation of the caspase-1 up-regulates inflammatory factors, and suppression of caspase-1 reduces allergic inflammatory reactions (Figure 1).[6]

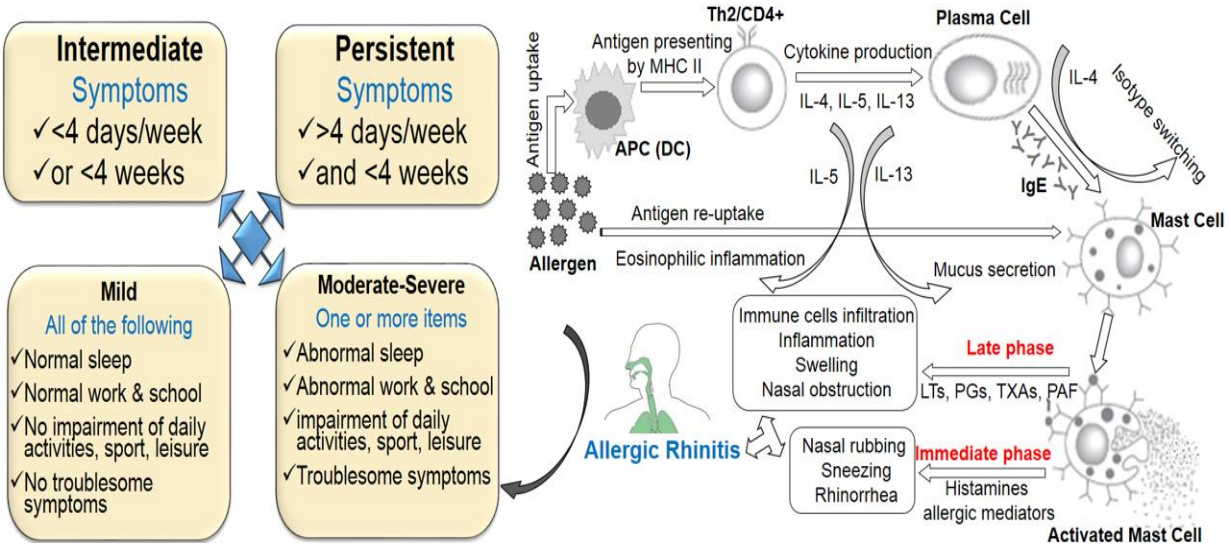


**Figure 1. Allergic rhinitis (AR) pathophysiology and Allergic Rhinitis and its Impact on Asthma (ARIA classification): In susceptible patients, AR is mediated by type 2 immune responses. Several genes have important roles in the initiation and severity of AR. HRH1, CysLT1R, NLR3, Caspase-1, and MUC5a were analyzed in this study. When the allergen enters the airways, it is taken up by antigen-presenting cells (APCs); especially dendritic cells (DCs), then is transported to lymph nodes and presented to Th2 cells by major histocompatibility complex (MHC) II molecules and then specific B cells. Th2 cells produce and release type 2 cytokines (IL-4, 5, and 13). IL-4 forces B cells to produce IgE (via Ig isotype switching). Produced IgE binds to its specific receptors on mast cells and basophils (sensitization). When the allergen re-enters the body, it binds to receptor-bound IgE on mast cells, triggering their degranulation and releasing allergic and inflammatory mediators (such as histamine), which orchestrate allergic responses (immediate phase). Activated immune cells produce inflammatory molecules such as cytokines, thymic stromal lymphopoietin (TSLP), platelet-activating factor (PAF), and leukotriene (LT)and lead to local infiltration by inflammatory cells (i.e., the late phase), culminating in allergic inflammatory symptoms. Regarding AR symptoms, the Allergic Rhinitis and its Impact on Asthma (ARIA) consider four categories as indicated.**


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AR pathogenesis follows a complex phenomenon and is strongly related to the production of various cytokines and the activation of immune cells. IL-4 is related to Th2 differentiation, IgE synthesis, IgE receptors' up-regulation, and mucus hyper-secretion.[7] The late phase reactions caused by the accumulation of inflammatory cells, cytokines, chemokines, and other related mediators usually occur within 4 to 6 hours. AR leads to mucus secretion in the upper airways and is characterized by wheezing, itchiness, sneezing, congested or runny nose, watery eyes, and swelling around eyes.[1,4,5]

Current treatment options for AR include anti-histamines, vasoconstrictors, mast cell stabilizers, and steroidal anti-inflammatory drugs.[8] Clinical remedies used for controlling AR are classified into three types: second-generation antihistamines, intranasal corticosteroids, and anti-leukotrienes.[3,9] The main treatment of AR includes nasal corticosteroids that deliver maximum efficacy usually within a week. Anti-histamines, as standard therapeutics for AR, have lower impacts on nasal inflammation and congestion. The role of leukotriene receptor (LTR) antagonists in treating AR is related to their impacts on the LT pathway of allergic inflammatory cascades.[10]

Montelukast, zafirlukast, and pranlukast are LTR antagonists; reducing inflammatory mediators' production and inhibiting allergic immune reactions.[11,12] Loratadine belongs to the second-generation antihistamine family that is used for managing AR symptoms. The drug also attenuates the itching associated with Kimura's disease. Loratadine acts as a selective inverse agonist of histamine $H_1$ receptors.[13] Fluticasone, an anti-inflammatory corticosteroid, is also inhaled via the intranasal route to control AR.[14,15]

Studying the effects of combinational therapies on the bio-factors released during hypersensitivity reactions and determining optimal therapeutic regimens in AR are necessary to effectively manage the disease. In the present study, we aimed to use combinations of anti-histamine, anti-leukotriene, corticosteroid, anti-prostaglandin, and anti-inflammatory drugs to investigate their effects on AR symptoms in mice models. Considering growing observational and experimental evidence for the effectiveness of combinational therapeutic protocols in AR, we here evaluated if combinational protocol could affect AR-related allergic and immunologic bio-factors and symptoms.

## MATERIALS AND METHODS

### Ethics Board Approval

Animal study methods have been approved by the ethical committee of the animal house of ix.med.vet.dep, 2021 (No.IX.MED.VET.DEP.REC.2021.300010.0)

### Allergic Rhinitis Model and Treatment Schedule

Producing AR mice models is relatively straightforward and fast, and also inducing allergic reactions in this model is easy and generally takes a short time.

Female 8-week-old BALB/c mice were kept under the standard condition (12 h light/dark cycle, 23±2ºC, 55±5% humidity, free access to the water and food) in an animal house for one week. All protocols were done according to the Laboratory Animal Care ethical guidelines. Forty-five mice were divided into 9 groups (n=5) including negative control that received phosphate-buffered saline (PBS) alone, AR-induced mice, AR-induced mice receiving pranlukast (0.1 mg/kg, intraperitoneally), AR-induced mice receiving loratadine (10 mg/kg, oral administration), AR-induced mice receiving fluticasone (100 µg/mL, 20-min inhalation by an ultrasonic nebulizer), AR-induced mice receiving loratadine and fluticasone, AR-induced mice receiving loratadine + pranlukast, AR-induced mice receiving fluticasone + pranlukast, and finally AR-induced mice receiving loratadine + fluticasone + pranlukast.

To produce AR models, mice were sensitized and challenged by OVA (Figure 2) as previously described.[16] Briefly, 0.3mg OVA and 30mg alum adjuvant were daily injected intraperitoneal (IP) for 7 days. Then, 2 mg OVA in 20 µL PBS was continuously instilled via daily intratracheal (IT) injection to maintain the phenotype (Figure 2). The negative control group was sensitized and challenged with PBS. The treatment groups received drugs on days 8 to 30, and at the end of this period on day 31$^{st}$, blood and nasal lavage fluid (NLf) samples were obtained after euthanizing the mice. Finally, the AR-related factors (markers and genes) related to survival[1,3,4,5,14,16] and nasal rubbing (nasal itching), and sneezing, as the main AR signs, were screened according to previous studies.

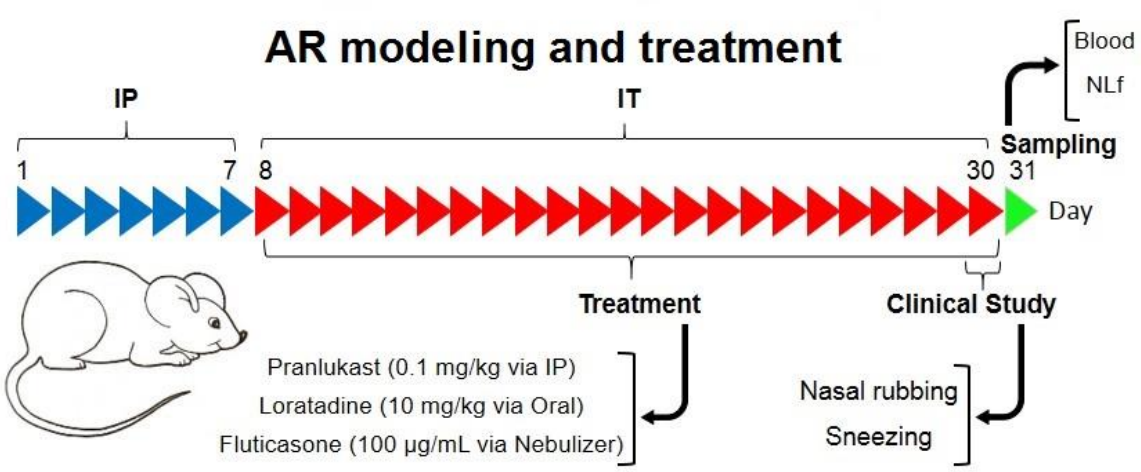


**Figure 2. Animal models of Allergic rhinitis (AR). Mice were sensitized by injected intraperitoneal (IP) injection of ovalbumin (OVA)+ alum. Then, the sensitized mice were challenged by the intratracheal (IT) inhalation of OVA solution that was aerosolized by a nebulizer. Clinical symptoms were investigated on day 30, and sampling was done on day 31.**

**Clinical Investigation**

On day 30, nasal rubbing and sneezing were investigated in all mice by counting the episodes the mice perpetrated such behaviors 15 min after the last intranasal challenge of OVA.

**NLf Collection**

NLf samples were collected 24 h after the last challenge. After anesthesia, the trachea was ligated at the upper tract, and a catheter was guided into the nasopharynx. Nasal passages were gently perfused with 1 mL PBS and collected into a tube. Then NLf was centrifuged (700×g for 10 min at 4°C) to separate cells from thesupernatant.[17,18]

**Serum Immunoglobulin Levels**

After euthanizing the mice by CO2, blood samples were collected from the heart and centrifuged (700×g for 10 min at 4°C) to separate serum IgG1 and OVA-specific, and total IgE levels were measured by the enzyme-linked immunosorbent assay (ELISA) method (Dainippon Sumitomo Pharma, Osaka, Japan).

**Serum Leukotrienes (LTs) Levels**

Serum was assayed in duplicate for LTB4 and LTC4; using specific ELISA kits (Cayman Chemical, Ann Arbor, USA) according to the manufacturer's instructions.

**Serum Histamine and TSLP Levels**

The serum levels of histamine and TSLP were determined by relevant ELISA Kits (Biocompare, USA).

**IL-4 Measurement**

The level of IL-4 in NLf was determined; using a specific ELISA kit according to the manufacturer's instructions.

**Real-time Polymerase Chain Reaction (Real-time PCR)**

As a cost-effective and rapid method, real time-PCR was used to determine the expressions of the genes encoding HRH1, CysLT1R, NLR3, Caspase-1, and MUC5a. NLf was centrifuged, and the resulting cell suspension was used for RNA extraction by the TRI reagent. Then, cDNA was synthesized and the target genes expressions were studied; using SYBR Green Master Mix (Bio-Rad) and specific primers.

**Statistical Analysis**

All statistical analyses were performed in SPSS Version 20.0. The effects of treatments were analyzed by one-way ANOVA (with Tukey's post hoc test). Correlations between serum bio-factors (histamine, LTs, etc.) were assessed; using the Pearson's correlation test. All data were presented as mean±SD

from at least three independent experiments, and differences were considered to be statistically significant when p values were less than 0.05. Graphs were drawn in GraphPad prism (Version 6).

## RESULTS

### Clinical Symptoms

Main allergic symptoms including nasal rubbing and sneezing were surveyed in all experimental groups (Figure 3). Both sneezing and nasal rubbing significantly increased in the AR group compared with the control group; while these symptoms remarkably decreased in all the AR mice treated with the therapeutic regimens ($p$<0.05) except for those receiving pranlukast alone ($p$>0.05).

### Serum Immunoglobulins Levels

In the AR group, significant elevations were seen in the serum levels of IgG1 as well as total and OVA-specific IgE (68±5, 2590±270, and 225±30 ng/mL, respectively) compared to the control group (9±1, 124±15, and 0±0 ng/mL, respectively) ($p$<0.05). IgG1as well as total and OVA-specific IgE levels significantly decreased in the AR groups treated with fluticasone (59±3, 1420±190, and 156±21 ng/mL, respectively), loratadine+ fluticasone (51±3, 1459±183, and 138±19 ng/mL, respectively), fluticasone+ pranlukast (50±1,1510±210, and 140±25 ng/mL, respectively), and loratadine+ fluticasone+ pranlukast (47±2, 1402±221, and 133±22 ng/mL, respectively) ($p$<0.05) compared to the non-treated AR group (Table 1).

### Serum Eicosanoid

The serum levels of LTB4 and LTC4 significantly increased in the AR group (101±11 and 233±41 pg/mL, respectively) compared with the control group (64±7 and 72±28 pg/mL, respectively) ($p$<0.05). Treatment with pranlukast (75±9 and 81±26 pg/mL, respectively), loratadine + pranlukast (76±8 and 80±23 pg/mL, respectively), fluticasone + pranlukast (70±7 and 77±25 pg/mL, respectively), and loratadine + fluticasone + pranlukast (68±12 and 75±29 pg/mL, respectively) significantly decreased LTB4 and LTC4 serum levels compared to the non-treated AR group ($p$<0.05) (Table 1).

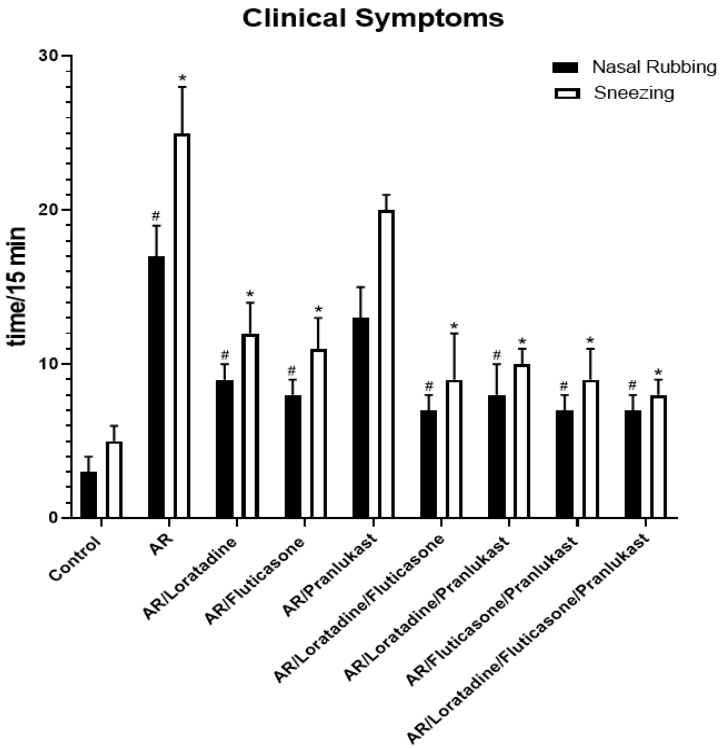


**Figure 3. Clinical symptoms. Main clinical symptoms in allergic rhinitis (AR) mice included nasal rubbing and sneezing which were observed on day 30 in all the studied groups (* and # =$p$<0.05 between treated and non-treated AR groups).**

**Table 1. Results of the immunoglobulins (Igs), Leukotrienes (LTs), histamine, thymic stromal lymphopoietin (TSLP), and interleukin (IL)-4 in studied groups**

| Groups | total IgE (ng/mL) | specific IgE(ng/mL) | IgG1 (ng/mL) | LTB4 (pg/mL) | LTC4 (pg/mL) | Histamine (ng/mL) | TSLP (ng/mL) | IL-4 (pg/mL) |
|---|---|---|---|---|---|---|---|---|
| **Control** | 124±15 | 0±0 | 9±1 | 64±7 | 72±28 | 88±7 | 0.4±0.1 | 39±8 |
| **AR** | 2590±270 | 225±30 | 68±5 | 101±11 | 233±41 | 589±21 | 2.7±0.2 | 87±18 |
| **AR/Loratadine** | 1994±216 | 203±23 | 67±2 | 98±5 | 230±33 | 189±32 | 2.6±0.1 | 80±13 |
| **AR/Fluticasone** | 1420±190 | 156±21 | 59±3 | 90±8 | 202±37 | 439±29 | 1.3±0.2 | 57±14 |
| **AR/Pranlukast** | 1822±206 | 192±25 | 63±2 | 75±9 | 81±26 | 566±26 | 1.7±0.2 | 65±11 |
| **AR/Loratadine/Fluticasone** | 1459±183 | 138±19 | 51±3 | 89±10 | 198±35 | 178±34 | 1.2±0.1 | 59±10 |
| **AR/Loratadine/Pranlukast** | 1735±173 | 182±30 | 61±1 | 76±8 | 80±23 | 170±30 | 1.6±0.2 | 64±16 |
| **AR/Fluticasone/Pranlukast** | 1510±210 | 140±25 | 50±1 | 70±7 | 77±25 | 342±24 | 1±0.1 | 55±7 |
| **AR/Loratadine/Fluticasone/Pranlukast** | 1402±221 | 133±22 | 47±2 | 68±12 | 75±29 | 166±19 | 0.9±0.1 | 49±8 |

### Serum Histamine

Serum histamine levels significantly increased in the AR group (589±21 ng/mL) compared to the control group (88±7 ng/mL) ($p<0.05$). Compared to untreated AR mice, all treated AR groups showed significant reductions in serum histamine levels ($p<0.05$) except for the group treated with pranlukast (566±26 ng/mL) (Table 1).

### Serum TSLP

Serum TSLP level significantly increased in the AR group (2.7±0.2 ng/mL) compared to the control group (0.4±0.1 ng/mL) ($p<0.05$). Compared to control untreated AR mice, TSLP level also significantly ($p<0.05$) increased in the AR mice treated with fluticasone (1.3±0.2 ng/mL), pranlukast (1.7±0.2 ng/mL), loratadine + fluticasone (1.2±0.1 ng/mL), loratadine + pranlukast (1.6±0.2 ng/mL), fluticasone + pranlukast (1±0.1 ng/mL), and loratadine + fluticasone + pranlukast (0.9±0.1 ng/mL) but not in the animals that received loratadine alone (2.6±0.1 ng/mL) (Table 1).

### IL-4 Level

IL-4 significantly increased in the AR group (87±18 pg/mL) ($p<0.05$) as compared to the healthy control group (39±8 pg/mL). Although treatment with loratadine (80±13 pg/mL), fluticasone (57±14 pg/mL), pranlukast (65±11 pg/mL), loratadine +fluticasone (59±10 pg/mL), loratadine + pranlukast (64±16 pg/mL) decreased IL-4 level, none of these were statistically significant ($p>0.05$). In the AR mice models treated with fluticasone + pranlukast (55±7 pg/mL) and loratadine + fluticasone + pranlukast (49±8 pg/mL), the IL-4 level was decreased. However, these reductions were significant ($p<0.05$) compared with the non-treated AR group (Table 1).

### Real-time PCR

The mRNA expression of *MUC5a* was significantly decreased in all treated AR groups compared with the untreated AR group ($p<0.05$). The mRNA expression of *Caspase-1* was also significantly decreased in all treated AR groups except for the loratadine-treated group. *NLR3* gene expression showed significant reductions in the AR mice treated with loratadine +fluticasone (4.6±0.4), fluticasone + pranlukast (4.5±0.5), and loratadine + fluticasone + pranlukast (3.9±0.6) compared with control untreated AR mice (6.8±1.5). In addition, *HRH1* gene expression was significantly decreased in the animals receiving loratadine + fluticasone + pranlukast (1.3±0.2) compared with the untreated AR group (2.2±0.5). Finally, the mRNA expression of *CysLT1R* was significantly decreased in the loratadine + fluticasone + pranlukast (1.4±0.1) compared to the untreated AR (2.7±0.6) group ($p<0.05$, Figure 4).


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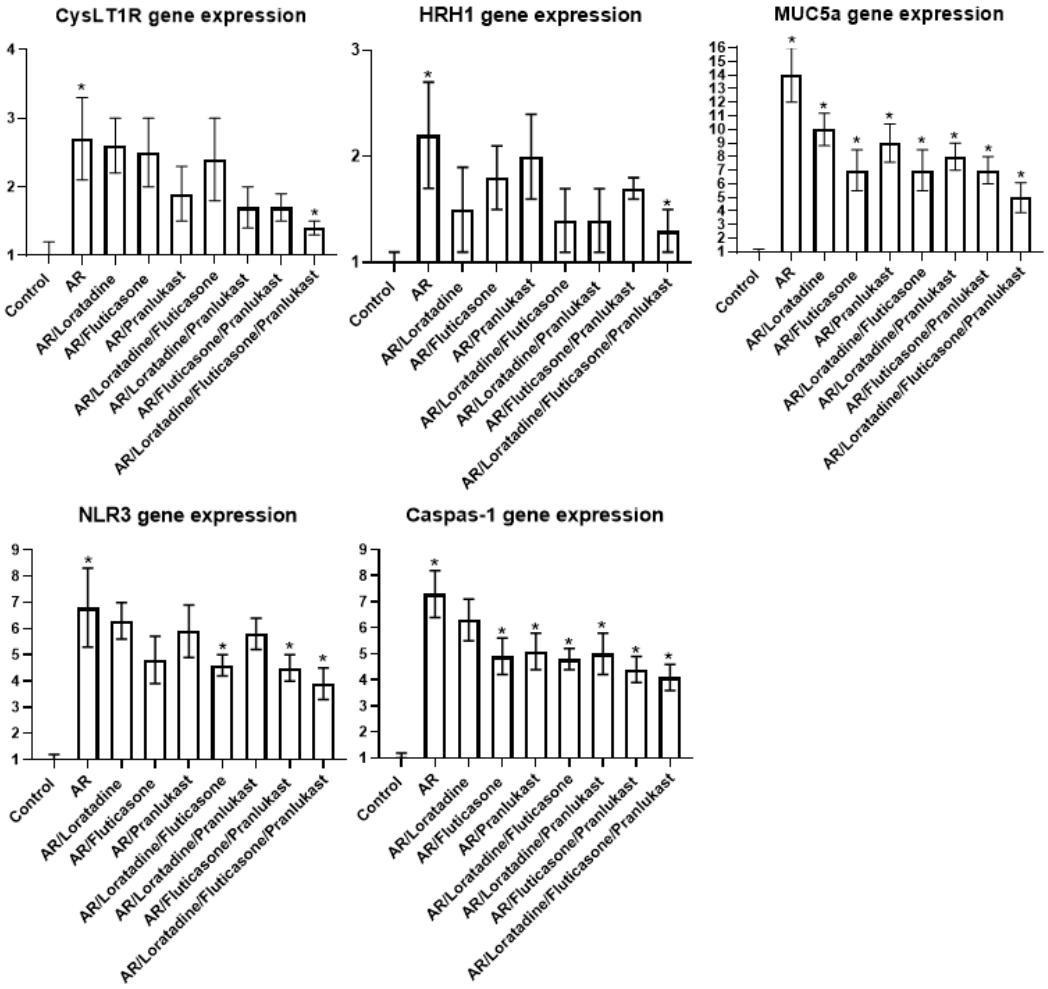


**Figure 4. Real-time gene expression analysis. The gene expressions of *MUC5a, Caspase-1, NLR3, HRH1,* and *CysLT1R* were studied in all groups by real time-PCR [*$p$<0.05 between treated and non-treated allergic rhinitis (AR) groups].**

## DISCUSSION

Allergic diseases are caused by Th2-mediated immunological responses. AR is a common allergic disease of the upper respiratory system. The clinical symptoms of AR include rhinorrhea, nasal congestion, sneezing, coughing, loss of smell, postnasal drainage, lacrimation, and nasal irritation.[1,3] Signaling through FcεRI on the surface of mast cells induces cellular degranulation, cytokine production, the release of PGD2, and LT mediators' secretion which all play key roles in AR development. Moreover, infiltration by inflammatory cells and release of leukotrienes, histamine, pro-inflammatory cytokines, intercellular adhesion molecule-1 (ICAM-1), *Cyclooxygenase-2* (COX-2), and macrophage inflammatory protein-2 (MIP-2) exaggerate further allergic reactions.[6] Currently, the main AR treatment includes using nasal corticosteroids, offering maximum efficacy within a week. Anti-histamines, as standard AR therapeutics, have lower impacts on nasal inflammation and congestion. LTR antagonists; on the other hand, modulate LT-mediated allergic inflammatory pathways.[10] In this study, the drugs belonging to three different families (antihistamine, LTR antagonist, and corticosteroids) were used to control the clinical symptoms of AR.

Our results showed that sneezing and nasal rubbing were significantly decreased in the AR mice that were treated with these drugs. Nevertheless, pranlukast, as an LTR antagonist, could not control AR symptoms as effectively as other treatments. Among all treatment

groups, pranlukast (when administered alone) showed the lowest effects on the controlling of sneezing and nasal rubbing. The combination of loratadine, fluticasone, and pranlukast offered the best results in terms of reducing sneezing and nasal rubbing compared to other therapeutic regimens. These beneficial effects can be explained by the role of these drugs (loratadine and fluticasone) in suppressing allergic mediators and inflammatory markers. Similar to our findings, Shen et al reported that loratadine, montelukast, and nasal steroids combinational therapy improved sneezing and reduced serum histamine and LTD4 levels, as well as nasal congestion.[3]

CysLTs are derived from arachidonic acid via the enzymatic action of 5-lipoxygenase and synthesized by basophils and mast cells during the early phase of allergy and by eosinophils and macrophages during the late phase. They can stimulate mucous glands and increase microvascular permeability; inducing rhinorrhea and tissue edema. Nasal edema is an important contributor to nasal congestion, and in AR, LTs are more important than histamine in inducing nasal congestion.[11] In the present study, serum levels of LTB4 and LTC4 were significantly decreased in the AR mice that were treated with pranlukast + loratadine + pranlukast, fluticasone + pranlukast, and loratadine + fluticasone + pranlukast. This indicates that the late phase of allergic responses can be controlled; using combinational therapeutic protocols. All the pranlukast, loratadine + pranlukast, fluticasone + pranlukast, and loratadine + fluticasone + pranlukast therapeutic combinations decreased the levels of LTC4 and LTB4 in comparable thresholds. Also, loratadine, loratadine + fluticasone, loratadine + pranlukast, and loratadine + fluticasone + pranlukast treatment protocols notably decreased serum histamine levels. In the AR untreated group, serum IgG1 and total and OVA-specific IgE were significantly enhanced; leading to sensitization and the release of inflammatory mediators. On the other hand, the levels of these Igs were significantly decreased after treatment with fluticasone, loratadine + fluticasone, fluticasone + pranlukast, and loratadine + fluticasone + pranlukast. Therefore, combinational therapy can control the clinical symptoms of AR via modulating the sensitization phase and relevant molecular pathways. Nevertheless, loratadine, fluticasone, and the combination of loratadine +fluticasone + pranlukast had lower effects on the levels of allergic Igs (IgE and IgG1). So, these may have lower impacts on allergic mechanisms. Overall, these protocols seem to better control the early phase of allergic responses.

Intranasal corticosteroids and antihistamines are standard therapies for AR.[19] We also observed that treatment with loratadine and fluticasone, either alone or combined with other drugs could reduce serum histamine levels. On the other side, pranlukast had no significant effect on serum histamine levels. Overall, antihistamines and corticosteroids can control the immediate allergic phase of AR; while corticosteroids and LTR antagonists are more effective in managing the late phase of AR.

Suppressors of cytokine signaling (SOCS) play the main role in the negative regulation of cytokine signaling. Clinically, elevated *SOCS1* expression is associated with AR pathogenesis via inhibiting IFN-γ mediated Th1 responses. Additionally, SOCS1 upregulation is associated with elevated GATA3 and IL-4 and reduced T-bet expressions.[20,21] The inhibition of *p-STAT6* down-regulates *SOCS1* expression and suppresses the GATA3/T-bet pathway; modulating Th1/Th2 cytokine balance and IgE induction. Th2 differentiation is induced by *GATA-3* and *STAT6;* while Th1 cells are induced by T-bet. Thus, alteration in *GATA-3* and *T-bet* expressions leads to an imbalance of Th1/Th2 contributing to AR. The modulation of *GATA-3* and *T-bet* expressions during T cell differentiation changes the ratio of IL-4/IFN-γ which is an important parameter in allergic diseases. Intranasal challenge with allergens in AR upregulates *GATA-3* and down-regulates *T-bet* and therefore increases the IL-4/IFN-γ ratio.[21] Therefore, harnessing *GATA-3* to suppress IL-4 is necessary to effectively treat AR and especially manage the immediate phase of the disease. IL-4 is the main allergic cytokine that is markedly increased in AR. All therapeutic protocols in this study decreased the IL-4 level. When the anti-AR drugs were used together as a combinational therapy, the inhibition of cytokine release was stronger and more effective; attenuating the clinical symptoms of AR. The combination of loratadine + fluticasone + pranlukast had the best effect in reducing IL-4 levels compared to other treatment groups.

NLRs are intracellular recognition receptors involved in immediate responses against pathogens or allergens during inflammation. *NLR3* (cryopyrin or NLR3 inflammasome) is an essential factor in allergic and inflammatory responses. *NLR3* and *Caspase-1* are

up-regulated in inflammation and allergy. *NLR3* inflammasome is composed of NLR3 protein, ASC, and procaspase-1. When NLR3 is activated, ASC forms a complex activating Caspase-1 and promotes IL-1β and IL-18; plays a substantial pro-inflammatory role.[22,23,24] *NLR3* inflammasome and *caspase-1* are important regulators of inflammation and the late phase of allergy. We observed that the mRNA expression of *Caspase-1*was decreased in all treated groups except for loratadine. On the other hand, the combination of loratadine +fluticasone + pranlukast caused the lowest expression of *Caspase-1*. The mRNA expression of *NLR3*was also significantly decreased in the groups treated with loratadine +fluticasone + pranlukast, fluticasone + pranlukast, and loratadine + fluticasone combinational protocols. Pranlukast had no significant effect on *NLR3* expression when applied alone. Furthermore, the gene expressions of *HRH1* and *CysLT1R* were significantly decreased only in the AR mice receiving the triple combinational therapy of loratadine + fluticasone + pranlukast compared to other treatments. Therefore, combination therapy seems to be a strong protocol to persistently control AR symptoms via modulating the late phase of allergic reactions. Finally, loratadine + fluticasone+ pranlukast, as well as fluticasone + pranlukast combinations reduced the expression of *MUC5a* which is the gene responsible for mucus production compared to other therapeutic protocols.

TSLP plays an important role in the pathogenesis of AR. TSLP is highly expressed on nasal epithelial cells in patients with AR. Also, it is produced in lymph nodes as an immune response center.[25,26,27,28,29] On the other hand, Caspase-1 plays an important role in NF-κB activation. Moreover, Caspase-1 -induced NF-κB activation; resulting in *TSLP* gene upregulation in airways. Furthermore, Caspase-1 and NF-κB inhibitors downregulate *TSLP* and *Caspase-1*via an upstream pathway to NF-κB.[25,28,29] In this study, TSLP level was decreased in all treated AR groups, except for loratadine; while combinational therapies were more effective than mono-drug regimens. TSLP reduction was particularly significant in fluticasone + pranlukast and loratadine + fluticasone + pranlukast treated groups. Fluticasone, either alone or in combination with other drugs, could decrease *Caspase-1* gene expression probably via modulating TSLP level or in this way, mitigates the pathological features of AR.

The goal of AR treatment is to improve AR symptoms and increase the patient's quality of life. Long-lasting AR can affect nasofacial anatomy in children; leading to anomalies of nasal bone and cartilage. The AR treatment protocols used in this study; especially the combination of loratadine, fluticasone, and pranlukast, could effectively regulate the early and late phases of allergic reactions. Although avoiding allergens is always the best way to manage AR; combinational pharmacological therapies are often necessary to prevent the progression of symptoms.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not Applicable.

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