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# Glucocorticoids Decreased GATA-3 Expression but Increased FOXP3 Expression in Allergic Rhinitis Patients

## Glukokortikoidler, Alerjik Rinitli Hastalarda GATA-3 Ekspresyon Seviyesini Düşürdü, ancak FOXP3 Ekspresyon Seviyesini Artırdı

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### Abstract

**Objective:** Allergic rhinitis (AR) is one of the most common allergic diseases worldwide. T lymphocytes play an important role in the development and control of this disease. This study aimed to measure the levels of Th2, Th17, and regulatory T cell-specific transcription factors (GATA-3, RORγt, and FOXP3, respectively) in the blood of AR patients treated with glucocorticoids (GCs) compared to those in healthy controls.

**Materials and Methods:** Blood samples were collected from 32 patients with AR treated with GCs and 20 healthy individuals. RNA was extracted from the blood cells, and cDNA was synthesized. The expression levels of GATA-3, RORγt, and FOXP3 were measured by real-time polymerase chain reaction. Serum IgE levels in the patients and controls were measured using ELISA.

**Results:** Our results showed that AR patients expressed significantly higher levels of FOXP3 and lower levels of GATA-3 than healthy controls. However, the level of RORγt was not significantly different between the patients and healthy controls. In addition, serum IgE levels were higher in patients with AR than in the controls.

**Conclusion:** These results showed that treatment of patients with GCs increased FOXP3 expression and decreased GATA-3 expression but had no significant effect on RORγt expression.

**Keywords:** Allergic rhinitis, GATA-3, RORγt, FOXP3, IgE

### Öz

**Amaç:** Alerjik rinit (AR), alerjik hastalığın en yaygın şeklidir. T-hücreleri bu hastalığın gelişmesinde ve kontrolünde önemli bir rol oynamaktadır. Bu çalışmanın amacı glukokortikoidler (GC) ile tedavi edilen AR'li hastaların periferik kan hücrelerinde Th2, Th17 ve T düzenleyici hücrelere özgü transkripsiyon faktörlerinin (sırasıyla, GATA-3, RORγt ve FOXP3) ekspresyon düzeyini araştırmak ve sağlıklı kontrollerle karşılaştırılmasıdır.

**Gereç ve Yöntem:** Glukortikoid tedavisi alan 32 AR'li hastadan ve 20 sağlıklı kontrolden kan alındı. RNA, periferik kan hücrelerinden ekstrakte edildi ve daha sonra cDNA'ya dönüştürüldü. cDNA, gerçek zamanlı polimeraz zincir reaksiyon ve spesifik primerler kullanılarak GATA-3, RORγt ve FOXP3'ün ekspresyon seviyelerini ölçmek için kullanıldı. AR'li hastalarda ve sağlıklı kontrollerde serum IgE seviyeleri ELISA kullanılarak ölçüldü.

**Bulgular:** Sonuçlarımıza göre GC tedavisi alan AR'li hastalarda sağlıklı kontrollere göre FOXP3 ekspresyon seviyeleri anlamlı derecede yüksekken GATA-3 ekspresyon seviyeleri anlamlı derecede düşük bulunmuştur. Bununla birlikte, AR'li hastalarda ve sağlıklı kontrollerde RORγt ekspresyon seviyesi açısından fark saptanmamasına karşılık, AR'li hastalarda serum IgE düzeyleri sağlıklı kontrollere göre daha yüksek saptanmıştır.

**Sonuç:** Bu sonuçlar, hem hasta hem de kontrol gruplarında RORγt ekspresyon seviyesinin benzer olduğunu, GC'lerin GATA-3 ekspresyon seviyesini azalttığını ancak FOXP3 ekspresyon seviyesini artırdığını göstermiştir.

**Anahtar Kelimeler:** Alerjik rinit, GATA-3, RORγt, FOXP3, IgE

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## Introduction

Allergic rhinitis (AR) is an IgE-mediated hypersensitivity reaction of the upper respiratory tract to innocent environmental substances (plant pollen, mites, molds, and animal dander which are called allergen) (1). AR symptoms result from the synthesis and release of inflammatory mediators from mast cells, eosinophils, and T-cells in response to allergens (2,3). $CD4^+$ T-cells play an important role in promoting or controlling allergic diseases (4,5). T-helper type 2 (Th2) cells induce IgE against allergens by B-cells through the production of interleukin (IL)-4 and IL-13, and recruit and activate eosinophils by producing IL-5. The hallmark of Th2 cells is GATA-3 as a main transcription factor (6,7). In contrast, numerous studies have reported that Th17 cells, which express RORγt as a major transcription factor, are also involved in allergic diseases (8-10). In contrast, $FOXP3^+$ regulatory T-cells (Tregs), which make up approximately 5-10% of human $CD4^+$ T-cells, are crucial for maintaining the immune balance and suppressing allergic diseases (11-13). These cells prevent the differentiation and activity of mast cells, basophils, eosinophils, and other cell types involved in allergic reactions (14). Tregs also inhibit the production of IgE and induce IgG4 production and can suppress the immune response to allergens (15). This study evaluated the mRNA levels of Th2-, Th17-, and Treg-related transcription factors in patients with AR treated with glucocorticoid (GCs).

## Materials and Methods

### Study Subjects

This study included 32 AR patients treated with GCs (16 males and 16 females, age 34.08±1.8 years) and 20 healthy controls (12 males and 8 females, age 33.3±2.6). AR was diagnosed on the basis of the history of patients' symptoms (clear rhinorrhea, sneezing, nasal congestion, nasal itching, and/or postnasal drainage) and positive skin prick test (SPT). The SPT was performed for 60 different allergens, including house dust, weed, grass, tree, mold, food, cat and dog dander. Patients with rhinitis symptoms were positive. SPT reactions to at least one allergen were considered for the AR group. Patients treated with oral or intranasal GCs such as dexamethasone, prednisone, and prednisolone were selected. The controls were healthy volunteers without a history of respiratory disease or allergies.

Peripheral blood (6 mL) was collected from each AR patient and healthy control. Four milliliters of blood were transferred to an EDTA-containing tube to measure the expression of GATA-3, RORγt, and FOXP3 mRNA, and the remainder was transferred to a hemolysis tube to measure the level of serum IgE. The demographic data of the patients and controls were also recorded.

### Measuring Serum IgE Levels

The serum IgE, in AR patients and controls, was measured by ELISA, using an ELISA kit (Abnova, Taiwan), and expressed in IU/mL. All measurements were performed in duplicate.

### RNA Extraction and Complementary DNA (cDNA) Synthesis

Total RNA was extracted from the blood samples of patients and controls using an RNA purification kit (TAKARA Biotechnology, China). The extracted RNA was used to synthesize the first cDNA strand using a cDNA Synthesis Kit (TAKARA Biotechnology, China).

### Relative Real-time PCR

The cDNA was subjected to real-time polymerase chain reaction (PCR) to evaluate the expression of GATA-3, RORγt, and FOXP3 mRNA using SYBR green master mix (TAKARA Biotechnology, Dalian, China) and the following primers: (fwd: 5'-AGAAGAGAGAGAGACGGAGGG-3', reverse: 5'-CCTCGGGTCACCTGGGT-3') for GATA-3, (fwd: 5'CCTGCTGAGAAGGACAG-3, reverse: 5'-GATCCCAGACGACTTGTC-3') for RORγt, and (fwd: 5'-CAGCACATTCCCAGAGTTC-3', reverse: 5'-CGTGGCGTAGGTGAAAG-3) for FOXP3.

The conditions for real-time PCR were as follows: stage 1, 30 s at 95°C; stage 2, 5 s at 95°C followed by 20 s at 60°C (40 repeats); stage 3, 1 s at 95°C, followed by 15 s at 65°C and 1 s at 95°C.

The relative expression of each gene was calculated using the comparative threshold cycle method (Pfaffl formula: fold change = $(E\ target)^{\Delta Ct\ target(control\ sample)}$ / $(E\ ref)^{\Delta Ct\ ref(control\ sample)}$ (16). The housekeeping gene used for normalization was glyceraldehyde-3-phosphate dehydrogenase.

### Statistical Analysis

Unpaired t-tests were performed for the statistical analysis of the data using SPSS and GraphPad Prism version 7.01. The chi-square test was performed for quality analysis of data. The statistical relationship between the variables was measured using Pearson's tests. Statistical significance was set at p-value $\leq 0.05$.

### Ethics Statement

Our study was approved by the Ethics Committee of the First Affiliated Kermanshah University of Medical Sciences (KUMS) (approval number: IR.KUMS.REC.1394.305). Informed consent was obtained from all participants. This information included the aims and process of the study, method of sampling, diagnostic method of SPT, and prescribed drugs.

## Results

### Clinical Finding of Participants

The demographic and clinical data of the AR and control groups are presented in Table 1. As the table shows, all patients had a long disease duration (mean: 7 years and 9 months) which demonstrates the chronic state of disease in the patient group. Additionally, clinical data showed that the common clinical symptoms of patients with AR were sneezing, rhinorrhea, itchy nose, itchy and watery eyes, dyspnea, nasopharyngeal drip, and urticaria. The prevalence rate of each symptom was 70.3%, 83.8%, 16.2%, 86.5%, 24.3%, 48.7%, and 27.0%, respectively.

### Serum IgE Levels

The mean level of total serum IgE in the AR and control was 156.29 ± 22.59 and 69.81 ± 15.49 IU/mL, respectively. As depicted in Figure 1, the total serum IgE level in AR patients was significantly higher than that of controls ($p<0.03$). No correlation was found between the total serum IgE levels, demographic data, and clinical symptoms.

### Expression of T-cell Specific Transcription Factors

As shown in Figure 2, AR patients expressed significantly lower levels of GATA-3 mRNA than healthy controls (p=0.008). However, AR patients expressed significantly higher levels of FOXP3 mRNA than the control group (p=0.0001) (Figure 3). However, the relative expression of RORγt mRNA in AR patients and healthy individuals was not statistically different (p=0.17) (Figure 4).

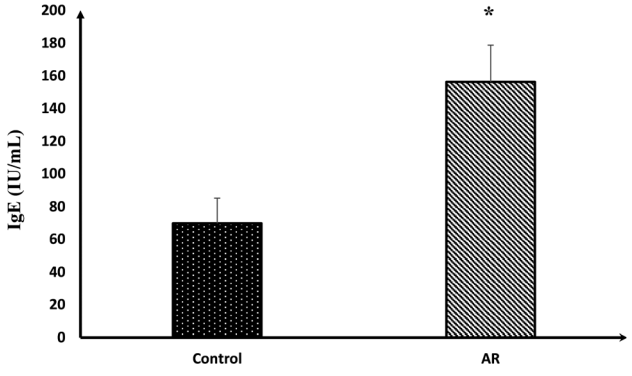


**Figure 1.** Total serum IgE level in allergic rhinitis (AR) patients with treatment by GCs compared to healthy controls. The level of total serum IgE in AR patients with treatment by GCs and healthy controls was measured by use of ELISA kit. Results showed that the level of total serum IgE in AR patients was significantly higher than that of control group ($p<0.03$). Data show mean ± SEM. *: P-value <0.05.

## Discussion

This study explored the expression of T-cell specific transcription factors in the blood cells of AR patients treated with GCs.

Our results revealed that AR patients treated with GCs express higher levels of FOXP3 mRNA than healthy controls. Regulatory T-cell specific transcription factor (FOXP3) modulates inflammation by suppressing Th2 and Th17 cells (17,18). It has been shown that FOXP3 expression decreased in allergic diseases (19). For modulating chronic inflammation, induced $FOXP3^+$ Tregs suppress Th2 and Th17 cells (17,18). Therefore, in this study, we found that the FOXP3 mRNA level was higher in AR patients treated with GCs than in normal individuals. It has been reported that treatment with inhaled or systemic GCs or both can upregulate FOXP3 mRNA and increase Treg cells activity (20,21). It has also been reported that after administration of GCs in allergic diseases, the number of Tregs and the expression of FOXP3 and IL-10 increased (22,23). GCs can also help express FOXP3 ectopically in non-regulatory

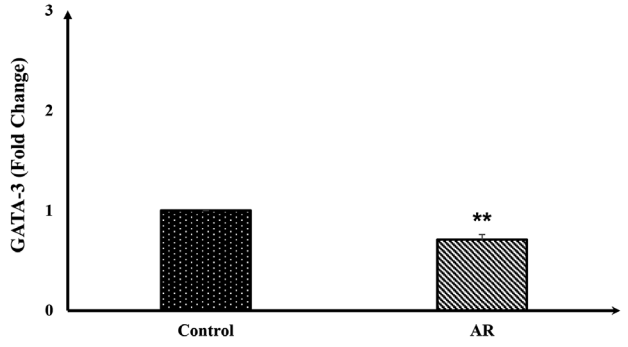


**Figure 2.** The relative expression of GATA-3 mRNA in peripheral blood cells of allergic rhinitis (AR) patients with treatment by GCs and healthy controls. RNA was extracted from peripheral blood cells of AR patients with treatment by GCs and healthy controls and transcribed to cDNA. The cDNAs were subjected to qPCR to measure the levels of GATA-3 mRNA expression. Data analysis of qPCR results showed that the level of GATA-3 mRNA expression in AR patients was significantly lower than that of control group ($p<0.008$). Data represent mean values ± SEM. **: P-value <0.01.

**Table 1.** Demographic and clinical data of AR patients and healthy controls.

| Group | Sex (F/M) | Age (year) | Disease duration (mean) | Seasonal/ perennial % | Asthma % | Smoking % | Polyp% |
|---|---|---|---|---|---|---|---|
| **AR (n=32)** | 16/16 | 34.08 ± 1.8 | 7 years & 9 months | 56.8/43.2 | 13.51 | 13.51 | 27.02 |
| **Cont. (n=20)** | 12/8 | 33.3 ± 2.6 | - | - | - | - | - |

AR: Allergic rhinitis, Cont.: Control, F: Female, M: Male, GCs: Glucocorticoids

T-cells (20,22,24). Most AR patients who participated in our study had consumed GCs for a long time. Therefore, the enhancement of FOXP3 mRNA levels in AR patients may be, in part, due to the usage of GC drugs by these patients. It has also been reported that in the chronic state of upper airway inflammation such as AR, the level of TGF-β produced by eosinophils increases. This increase in TGF-β is not affected by GC treatment (25-27). Therefore, the enhancement of TGF-β in tissue microenvironments may also affect the augmentation of FOXP3 levels in AR patients.

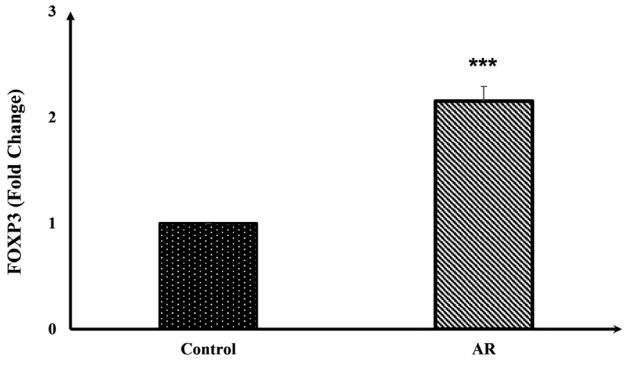


**Figure 3.** The relative mRNA expression of FOXP3 in peripheral blood cells of allergic rhinitis (AR) patients with treatment by GCs and healthy controls. RNA was extracted from peripheral blood cells of AR patients with treatment by GCs and healthy controls and transcribed to cDNA. The cDNA was subjected to qPCR to measure the levels of FOXP3 mRNA expression. Data analysis of qPCR results showed that the level of FOXP3 mRNA expression in AR patients was significantly higher than that of control group (p-value=0.00012). Data show mean ± SEM. ***: P-value <0.001.

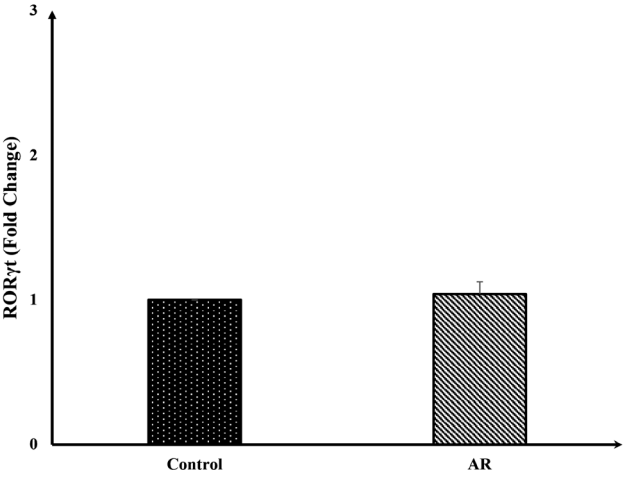


**Figure 4.** The relative mRNA expression of RORγt in peripheral blood cells of allergic rhinitis (AR) patients with treatment by GCs and healthy controls. RNA was extracted from peripheral blood cells of AR patients with treatment by GCs and healthy controls and transcribed to cDNA. The cDNA was subjected to qPCR to measure the levels of RORγt mRNA expression. Data analysis of qPCR results showed that the level of RORγt expression in AR patient and healthy controls wasn't significantly different (p-value=0.17). Data show Mean values ± SEM. Statistical significance was accepted at p-value <0.05.

Th2 cells play an important role in the development of allergic diseases. These cells produce signature cytokines such as IL-4, IL-5, IL-9, and IL-13, which are involved in the production of allergen-specific IgE, differentiation and activation of eosinophils, increased mucus secretion, and development of airway hyper-responsiveness (3,28,29). It has been shown that the level of GATA-3, a Th2-specific transcription factor, is enhanced in AR and that patients with severe AR express a higher level of GATA-3 than patients with mild AR (19). However, our findings revealed that GCs reduce GATA-3 mRNA levels in AR patients compared to controls. It has been reported that GCs reduce the production of Th2 cytokines and IgE secretion (7,30). The inhibitory effects of GCs involve inhibiting GATA-3 mRNA, GATA-3 protein production, and cAMP-induced GATA-3 phosphorylation (31). Since GCs could affect the expression of GATA-3 mRNA, the reduced GATA-3 mRNA expression in these patients, in part, could be due to patients' consumption of corticosteroids. However, the presence of higher levels of serum IgE in patients with AR could be due to the presence of long-lived plasma cells, in the bone marrow or inflamed tissue, which produces IgE for a long time (32).

Because the activation of $FOXP3^+$ Treg cells suppresses the induction of Th2 (33,34), it can be concluded that the reduction in GATA-3 expression in the AR group could be due to the enhancement of FOXP3 expression in this group. This notion was supported by the finding of Shi et al. (35) that the suppressive function of increased $CD4^+$ $CD25^+$ cells in PBMC of patients with exacerbated atopic asthma suppressed the production of Th2 cytokines (IL-4 and IL-13), but was not sufficient to suppress asthma development. It was also shown that TGF-β-producing Treg cells can inhibit GATA-3 expression in Th2 cells (36).

Our study showed that the levels of RORγt in the patient and healthy control groups were not significantly different. Consistent with this finding, Liu et al. (37) found that although nasal samples of persistent AR expressed higher levels of RORγt than the control group, the difference was not statistically significant. However, another study showed that RORc expression was significantly higher in the nasal mucosa from the AR group than in the control group (19); therefore, it may be the result of treatment with GCs in the patient group or an increase of FOXP3 expression level. However, since the source of samples in our study was different from that in other studies, it is acceptable that the results differ, and this controversy among different studies could be resolved by cohort studies with larger sample sizes.

## Conclusion

Taken together, our findings showed that treatment of patients with chronic AR with GCs increased FOXP3 expression and decreased GATA-3 expression in peripheral blood cells, but had no significant effect on RORγt expression. This may be mainly due to the consumption of GCs by AR patients and also to some extent due to the chronicity of the disease in these patients. In chronic AR, Treg cells are generated to control the immune response by suppressing the Th2 response. Therefore, it seems necessary to evaluate the effect of treatment with GCs on the expression of Th cell-specific transcription factors as well as their specific cytokines in patients with seasonal allergic rhinitis.

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### Ethics

**Ethics Committee Approval:** Our study was approved by the ethics committee of the first affiliated Kermanshah University of Medical Sciences (KUMS) (approval number: IR.KUMS.REC.1394.305).

**Informed Consent:** Written informed consent was obtained from our subjects.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Concept: S.B., S.H.M., F.S., A.G., Design: S.B., S.H.M., F.S., A.G., Data Collection or Processing: S.B., S.H.M., F.S., A.G., Analysis or Interpretation: S.B., F.S., A.G., Literature Search: S.B., A.G., Writing: S.B., S.H.M., F.S., A.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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