A New Experimental Allergic Rhinitis Model in Mice

Farelerde Oluşturulan Yeni Bir Alerjik Rinit Modeli

Şükran Köse ⓘ
Tuba Tatlı Kış ⓘ
Gulden Diniz ⓘ
İlkay Akbulut ⓘ
Başak Göl Serin ⓘ
Canberk Yılmaz ⓘ
Mine Özyazıcı ⓘ
Mesut Arıcı ⓘ
Aysu Yurdasiper ⓘ
Osman Yılmaz ⓘ

Tuba Tatlı Kış
Silopi Devlet Hastanesi,
Enfeksiyon Hastalıkları ve
Klinik Mikrobiyoloji Kliniği,
Şırnak, Türkiye
✉ tubatatlii@hotmail.com
ORCID: 0000-0001-6952-3748

Şükran Köse 0000-0002-4228-1213
Sağlık Bilimleri Üniversitesi, İzmir Tepecik Eğitim ve Araştıma Hastanesi, Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği, İzmir, Türkiye

Gülden Diniz 0000-0003-1512-7584
İzmir Demokrasi Üniversitesi, Patoloji Anabilim Dalı, İzmir, Türkiye

İlkay Akbulut 0000-0002-4840-6865
Gümüşhane Kelkit Devlet Hastanesi, Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği, Gümüşhane, Türkiye

Başak Göl Serin 0000-0003-4810-3239
Balıkesir Şehir Hastanesi, Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği, Balıkesir, Türkiye

Canberk Yılmaz 0000-0002-0049-7614
Foça Deniz Üs Komutanlığı 1. Basamak sağlık Hizmetleri, İzmir, Türkiye

Mine Özyazıcı 0000-0002-3288-0685
Mesut Arıcı 0000-0002-4054-4868
Aysu Yurdasiper 0000-0001-6551-4334
Ege Üniversitesi Eczacılık Fakültesi, Farmasötik Teknoloji Anabilim Dalı, İzmir, Türkiye

Osman Yılmaz 0000-0001-7817-7576
Dokuz Eylül Üniversitesi Tıp Fakültesi, Laboratuvar Hayvanları Bilimi Anabilim Dalı, İzmir, Türkiye

ABSTRACT

Objective: Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa mediated by IgE after exposure to an allergen. The most well known related comorbidity of AR is asthma. This study was planned due to the need for an animal model for studies on AR-asthma coexistence. In this study, the frequency of AR accompanying in the asthma model created in mice,and the usability of the related model in AR studies will be investigated.

Methods: In our study, 6-8 week-old, 18-20 g BALB/c mice were used. Chicken egg ovalbumin (OVA Grade V, Sigma) was administered through intraperitoneal (IP) route at doses of 10 µg on days 0 and 14. Mice were exposed to aerosolized 2.5% ovalbumin solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). After exposure to OVA, mice were observed for typical signs of AR including sneezing, runny nose, and nasal itching. The final diagnosis of AR was made by histopathological examination of the rhinotracheal tissues of mice.

Results: In our study, all mice exposed to ovalbumin received histopathologic diagnosis of AR. Increased number of capillaries lymphocytes, polymorphonuclear leukocytes and eosinophilsper square millimetre of rhinotracheal tissues were calculated in the murine model of AR compared to the the control group.

Conclusion: This study introduced a new AR model, not cited in the literature, and induced with the longest-term ovalbumin exposure in the literature. It was concluded that this model, known as the asthma model, can also be used to induce an AR model and can be used in studies investigating coexistence of allergic rhinitis and asthma.

Keywords: Allergic rhinitis, asthma, mouse

ÖZ

Amaç: Alerjik rinit (AR), allerjenle karşılaştıktan sonra IgE aracılığı ile oluşan burun mukozasının inflamatuvar hastalığıdır. AR' in en iyi bilinen ve üzerinde en fazla durulan ilişkili komorbiditesi astım'dır. AR-astım birlikteliği üzerine yapılacak çalışmalar için hayvan modeli ihtiyacından dolayı bu çalışma planlanmıştır. Bu çalışmada farelerde oluşturulan astım modelinde AR'in ne sıklıkta eşlik ettiği araştırılacak olup, ilgili modelin aynı zamanda AR çalışmalarında da kullanılabilirliği araştırılacaktır.

Yöntem: Çalışmamızda 6-8 haftalık 18-20 gr ağılığında, BALB/c fareler kullanılmıştır. Farelere 0. ve 14. günlerde 10 µg intraperitoneal tavuk yumurtası ovalbumini (OVA Grade V, Sigma) uygulanmıştır. Son uygulamadan 7 gün sonra (21. gün) başlamak üzere, günde 30 dakika süre ile haftanın 3 günü, 8 hafta boyunca steril serum fizyolojik içindeki %2,5'lik ovalbumin solüsyonundan oluşan aerosol inhale ettirilmiştir. İnhalasyon uygulamaları tüm vücut inhalasyon sistemi ile yapılmıştır. OVA'ya maruz kaldıktan sonra fareler hapşırma, burun akıntısı, burun kaşınması dahil AR'nin tipik belirtileri açısından gözlenmiştir. AR kesin tanısı farelerin rinotrakeal materyallerinin histopatolojik incelenmesi ile konulmuştur.

Bulgular: Çalışmamızda ovalbumine maruz bırakılan tüm fareler histopatolojik olarak AR tanısı almıştır. Bu farelerin rinotrakeal dokusunda kontrol grubundaki farelerin rinotrakeal dokusuna kıyasla AR lehine milimetrekarede artmış kapiller sayısı, lenfosit, polimorfonükleer lökosit ve eozinofil sayısı saptanmıştır.

Sonuç: Bu çalışmanın literatürde en uzun süreli ovalbümin maruziyeti ile oluşturulan yeni bir AR modeli olduğu saptanmıştır. Astım modeli olarak bilinen bu modelin AR oluşturulmak için de kullanılabileceği ve astım-AR birlikteliği üzerine yapılacak çalışmalarda bu modelin kullanılabileceği sonucuna ulaşılmıştır.

Anahtar kelimeler: Alerjik rinit, astım, fare

INTRODUCTION

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa occurring after exposure to the allergen and mediated by immunoglobulin E (IgE) [1]. The prevalence of AR in the world is 10-25% in adults and up to 40% in children, and millions of people have allergic rhinitis [2]. The most well known and emphasized related comorbidity of AR is asthma, and up to 85% of asthmatic patients have AR [1]. AR and asthma are comorbidities that occur as a result of inflammation of the respiratory tract mucosa associated with similar cells and mediators, but the mechanisms by which AR and asthma occur in the same patient are still unclear.

As far as is known, in AR, the antigen binds to the IgE receptors and causes the release of histamine, prostaglandin, leukotriene and protease from the mast cells, revealing inflammation that will cause runny nose, sneezing, nasal itching, and congestion [3]. This binding to IgE receptors in asthmatic patients creates acute bronchoconstriction in smooth muscles of the lower respiratory tract.

Animal models of AR are extremely important for research studies on AR. To date, guinea pigs, mice, rats and rabbits have been used in animal models of AR [4-8]. Firstly, guinea pigs were used, and BALB/c type mice have been used mostly in recent years [4]. The reasons why mice are used in AR models are that they produce human-like IgE and are relatively cheap [9]. In various studies ovalbumin, pollen, dust mites, fungi, and schistosoma antigens have been used as allergens [4,6]. These agents were also administered as nasal drops, inhalation or multipoint subcutaneous injections to increase sensitivity [4].

In our study, the animal model, which was created by Temelkovski J. et al. [10] in BALB/c type mice and defined as an asthma model, was used. Our aim was to investigate whether AR also coexisted in these asthma-induced mice and whether the asthma model induced can also be used as an AR model in future studies. At the end of this study, the frequency of coexistence of AR in the murine model of asthma model, and the usability of the relevant model in AR studies and/or studies on asthma-AR coexistence will be investigated.

The aim of this study is to contribute to the literature by using a new murine model of AR. If at the end of the study, AR is induced in mice, then this animal model of AR will be defined as an AR model with the longest-term ovalbumin exposure in the literature.

MATERIAL and METHOD

A total of 22 BALB/c female 6-8- week- old mice weighing 18-20 g were used in the study. Before starting the study, ethics committee approval (decision no: 40/2018 date: February 24, 2021) was obtained from Dokuz Eylül University Experimental Animals Local Ethics Committee. Mice were kept individually in plastic cages on a 12 hour light/12 hour dark cycle, at 22±2°C room temperature and 40-50% relative humidity.

Mice acclimated to the environment for one week were fed ad libitum with standard pellets and tap water under optimum conditions throughout the study. The asthma model developed by Temelkovski J. et al. [10] in BALB / c mice was used.

To induce mice model of asthma, chicken egg ovalbumin (OVA Grade V, Sigma) was administered through intraperitoneal (IP) route at doses of 10 µg on days 0 and 14. Female BALB/c mice inhaled an aerosol solution consisting of 2.5% OVA in sterile saline for 8 weeks, 3 days a week for 30 minutes a day, starting 7 days after the last IP injection of OVA (day 21). Inhalation applications were performed with the whole- body exposure inhalation system.

OVA was given in a closed glass chamber through a jet nebulizer using whole-body exposure inhalation system and the particle size was below 4 µm with a concentration of 10-20 mg/m^3. After the mice models were created, the mice were sacrificed and the rhinotracheal tissue samples were evaluated histopathologically.

Randomly selected mice were divided into 3 groups.

Group 1: Sham (control) group (n=6).

Group 2: Mice model of asthma was induced, and the group was sacrificed on the 74th day (n=7).

Group 3: The asthma model was formed and the group was sacrificed on the 81st day (n=9).

Evaluation of AR Symptoms

After sensitization following exposure to OVA, mice were observed for typical signs of AR including sneezing, runny, and itching nose. Nasal itch was defined as perinasal rubbing with one or both forelimbs.

Histopathological evaluation

Rhinotracheal tissue samples were evaluated histopathologically after the mice were sacrificed. All tissue samples were first fixed in a 10% formaldehyde solution for 24 hours. Following this process, the tissue samples were treated with conventional grades of alcohol (70%, 80%, 90%, and 100%) to remove the water within the tissues. The tissue samples were then passed through xylol and embedded in paraffin. Four-to-five micron sections were cut from the paraffin blocks and stained with hematoxylin-eosin. An experienced pathologist examined the histological preparations under a light microscope (Olympus BX51), and photographs were taken with an Olympus DP72 camera. Histopathological evaluation was performed by this pathologist blinded to the allocation of study groups.

Number of capillaries, lymphocytes, polymorphonuclear leukocytes (PMNLs), eosinophils per mm² were calculated in histopathological examination.

RESULTS

Clinical evaluation

In all mice exposed to OVA, increased sneezing, rubbing perinasal area with one or both forelegs, significant increase in nasal discharge were observed compared to the control group, all of which suggested clinical symptoms of AR.

Histopathological findings

In our study, diagnosis of AR was made histopathologically in all mice exposed to OVA in Groups 2 and 3. In Groups 2 and 3, increased number of capillaries, lymphocytes, PNLs and eosinophils were found per mm² of the rhinotracheal tissue of mice in favor of AR compared to the control group (Table 1). The increase in lymphocyte, PNL and eosinophil counts in Group 3 compared to Group 2 could be attributed to the longer exposure to ovalbumin. Although the number of capillaries in Group 3 decreased compared to Group 2, there was no statistically significant difference between the two groups. All mice that were sacrificed on the 74th and 81st days after exposure to OVA received the histopathologic diagnosis of AR. Considering the cost of the experiment, we thought that it will be

Figure 1. Cross-sectional area of nasal cavity (HEx40).

Table 1. Number of capillaries, lymphocytes, PNLs and eosinophils calculated per mm² of rhinotracheal tissue samples of mice in Groups 1, 2 and 3.

	Number of capillaries/HPF mean (min-max)	Lymphocytes/mm² mean (min-max)	PNLs/mm² mean (min-max)	Eosinophils/mm² mean (min-max)
Group 1	5.3 (4-7)	18.3 (10-30)	0.5 (0-2)	0.16 (0-1)
Group 2	9.8 (7-13)	57.2 (35-70)	6.2 (4-10)	3.7 (2-5)
Group 3	8 (6-10)	64.4 (40-90)	8.2 (4-15)	4 (3-5)

HPF: high - power field; PNL: polymorphonuclear leukocyte.

appropriate for the AR model to terminate the experiment on the 74th day.

Full-thickness sections were prepared from nasal specimens obtained from mice (Figure 1). In the control, and AR groups, capillary vessels in the nasal tissue samples were counted per high-power field
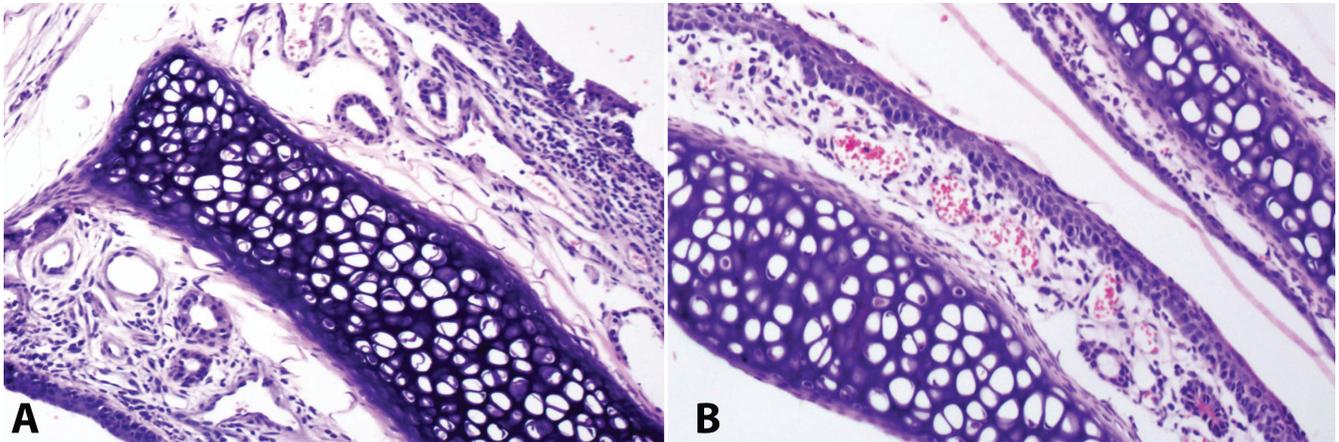
Figure 2. Capillary vessels per high power field (HPF) were counted. A) A rat from the control group with 3-4 capillary vessels per HPF and B) A rat from the allergic rhinitis group with 13 capillary vessels per HPF (HEX200).
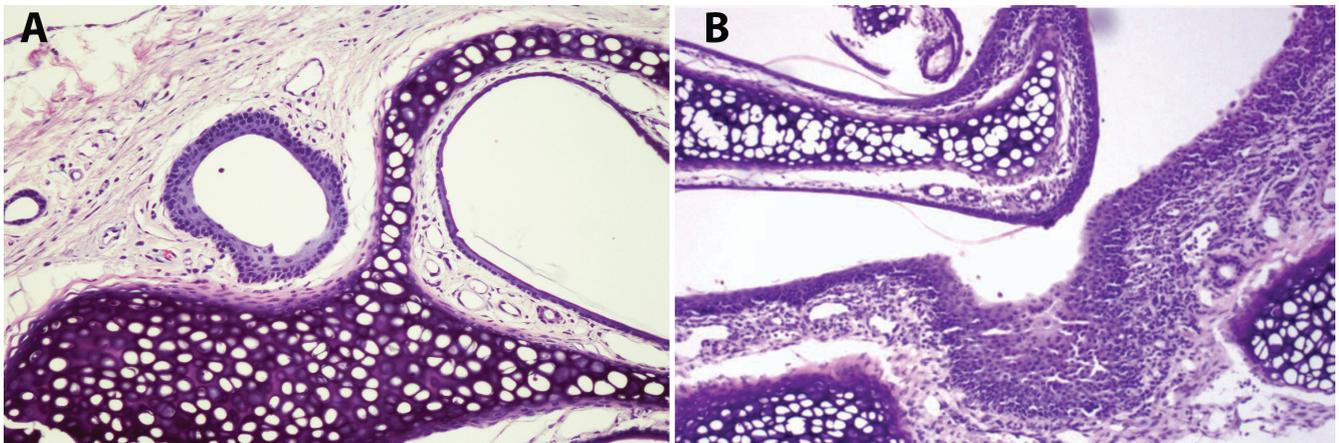
Figure 3. A) A case from the control group with mild inflammation B) A case from allergic rhinitis group with diffuse inflammation (Hex 200).
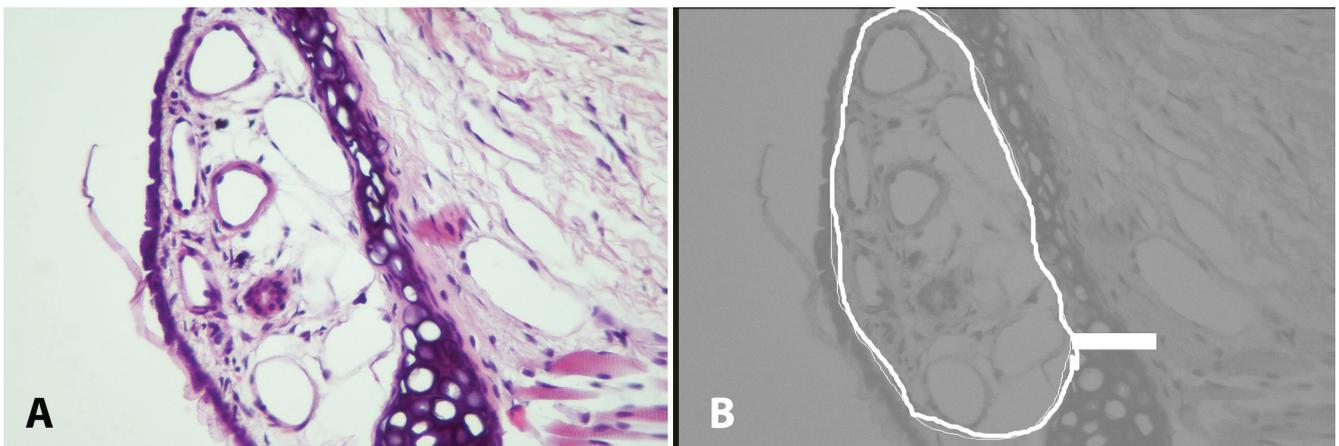
Figure 4. In all samples, a demonstrative area was selected, marked, and the inflammatory cells per 0.1 mm² were calculated. A) A microscopic area with minimal inflammation B) Demonstration of the same area in dark-field microscopy (HEX400).
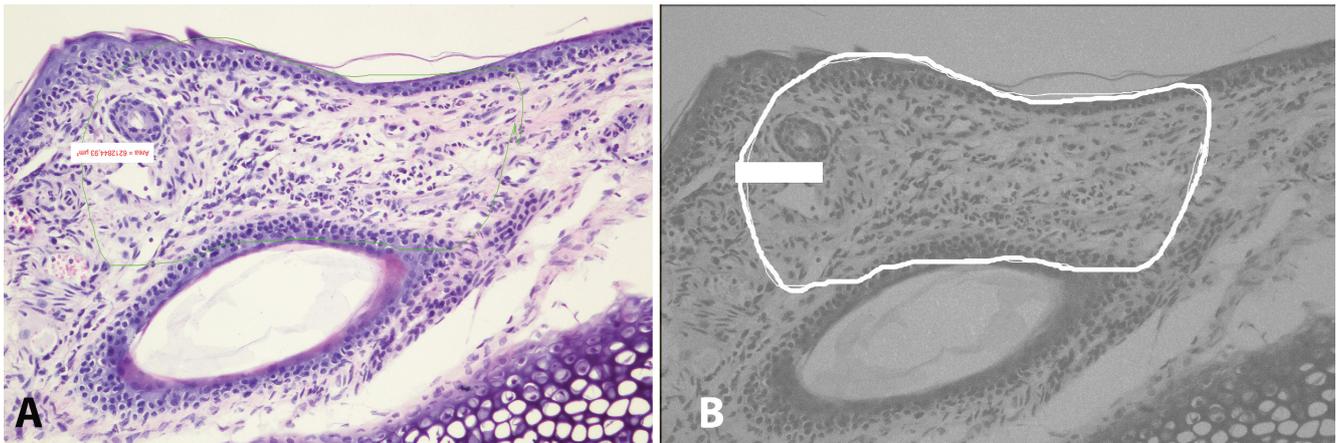
Figure 5. A) A microscopic area with severe inflammation B) Demonstration of the same area in dark-field microscopy (HEX200).

(Figure 2). A case from the control group with mild inflammation in the rhinotracheal tissue and the case from the allergic rhinitis group with intense inflammation are shown in Figure 3. In all samples, a demonstrative area was selected and marked, then inflammatory cells per mm² were counted. Cross-sectional areas with minimal (HEX400), and diffuse (HEX200) inflammation are shown in dark-field microscopy (Figures 4, and 5, respectively).

DISCUSSION

In our study, all mice exposed to OVA were clinically and histopathologically diagnosed with AR. AR models have been defined using different living species in the literature. AR has been induced by Nabe et al. [11] by making guinea pigs to inhale the pollen of Japan cedar flower [12]. In this study, AR had been induced by dropping pollen extracts into the nostrils of guinea pigs twice a day for a total of 7 days [11]. The researchers used lidocaine inhalation anesthesia to prevent the antigen from being removed by nasal ciliary activity [11]. Chen et al. [5] reported that AR was induced by IP injection of OVA on days 1, 3, 5, 7, 14 and 21, and then instillation of intranasal OVA for seven consecutive days.

In the rabbit model of AR defined by Güneş et al. [7] 30 mg of OVA in 100 mL of saline was administered intraperitoneally to rabbits every other day for 14 days. Then AR was induced in rabbits by intranasal application of OVA twice a day between the 14th and

18th days. In another rabbit model of AR, AR was induced by IP administration of OVA on days 0, 2, 4 and 6, followed by its intranasal application for seven days [13]. In the literature, AR models have been described in rats induced by a total of 7 IP injections of OVA once a day on days 1, 3, 5, 7, 9, 11 and 13, followed by its once daily intranasal applications for 14 days [6,14-16].

AR models are important for experimental studies performed on diagnosis and treatment of AR. Mice have been the most widely used animal species in AR models because they can be easily raised for the purpose, and often produce IgE allergens similar to humans at almost no expense [19]. AR has been induced in mice after IP injections of OVA on days 0, 7 and 14, followed by its intranasal applications twice a day for three weeks [8,17]. Bisphenol A (BPA) has been reported to exacerbate allergic inflammation in an ovalbumin-dependent AR mouse model [18]. In this study, BPA was given to mice after they received ovalbumin IP on days 0, 7 and 14. it was reported that allergic symptoms of AR were exacerbated and serum levels of OVA-specific IgE levels increased in mice [18]. In a study that investigating the effect of mesenchymal stem cells of human umbilical cord in AR treatment, IP ovalbumin was given to mice on the 0,7, and 14th days to induce AR [19]. In this study, AR was induced by instilling nasal ovalbumin in mice on days 15 and 21 [19]. In our study, 10 µg chicken egg ovalbumin (OVA Grade V, Sigma) was administered intraperitoneally to BALB/c mice on days 0 and 14.

Then the mice were exposed to inhaled aerosolized 2.5% OVA solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). As a result, AR was induced in all mice. It has been concluded that this murine model of AR can be used in experimental studies on asthma-AR coexistence.

We think that the model we defined can be used in experimental treatment studies on AR. Kan et al. [19] used the AR model that created in mice while investigating the effect of stem cells of the umbilical cord in the treatment of AR. In this model, 25 μg IP OVA was given to Balb/c strain mice on days 0, 7 and 14, followed by intranasal OVA was administered on days 15, and 21 [19]. Another study investigated the potential therapeutic effect of Metagonimus yokogawai extract on the OVA-induced allergic rhinitis model [20]. In this study, 25 μg OVA were given IP 4 times on days 0, 7 and 14 to Balb/c type mice [20]. Then, AR was induced by giving intranasal OVA every day from the 25th to the 29th day [20]. In another study investigating the role of topical red onion extract in AR treatment, the murine AR model was used [21]. In this model, Balb/c strain mice received 25 μg OVA IP on days 0,7 and 14 [21]. Intranasal 100 μg of OVA was administered five times a week between the days 21 and 41 [21]. In another study, an AR mouse model was used to investigate the therapeutic effect of Astragalus membranaceus plant on AR [22]. In this model, IP 75 μg OVA was given on days 0,7 and 14 [22]. AR model was created by intranasal administration of 200 μg IP OVA on days 21 and 42 [22].

In our study, 10 μg chicken egg ovalbumin (OVA Grade V, Sigma) was administered to BALB/c type mice via intraperitoneal route on days 0 and 14. Mice were made to inhale aerosolized 2.5% OVA solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). As a result of this application, which was used to create a murine model of asthma in the literature, AR diagnosis was made in all mice based on clinical and histopathological findings. It was concluded that this model can also be used to create an AR model whichcan be used in studies investigating asthma-AR coexistence.

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