The association between allergic rhinitis and airway dysfunction and nasal endothelial damage and oxidative stress*

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Abstract

Background: Although lower airway hyperresponsiveness is present in approximately one in three patients with allergic rhinitis (AR), the underlying mechanism remains unclear. To evaluate nasal patency and pulmonary functions in AR independently of the presence of asthma and to investigate the relationships between these and nasal oxidative stress parameters and endothelial damage.

Methodology: Seventy adolescents with AR (AR group - 27 with asthma and 43 without asthma) and 30 healthy controls (HC group) were included in this prospective, cross-sectional study. Endocan and oxidative biomarkers [total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI)] in nasal lavage fluid specimens; peak nasal inspiratory flow (PNIF); fractional exhaled nitric oxide (FeNO), and impulse oscillometry (zR5, zR20, and R5-20 for resistance and zX5 and zX20 for reactance) were investigated.

Results: Nasal endocan, TOS, and OSI values were higher in the AR group and TAS in the HC group. There was no difference between AR groups with and without asthma in terms of nasal endocan and oxidative biomarkers. FeNO levels and airway resistance (zR5, zR20, and R5-20) were higher in the AR group than in the HC group. However, there was no difference between the groups in PNIF. X5 was higher among the AR without asthma than in the other groups. Correlation between OSI and R5-20 was observed in the AR group. In the linear regression model, (logged) OSI was significantly predicted (logged) R5-20.

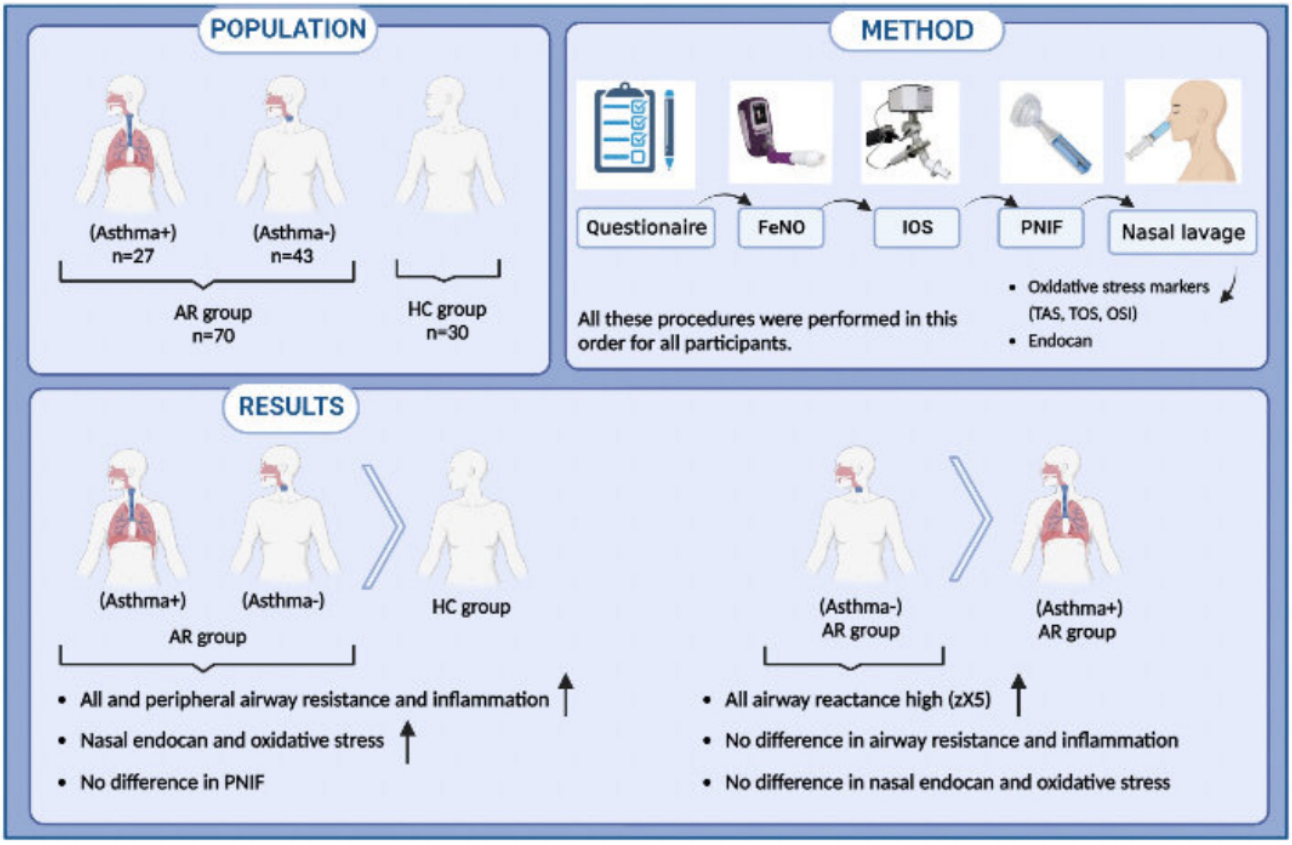
Conclusions: The airways of adolescents with AR without asthma were as much affected as those of the AR with asthma, and this effect was associated with nasal endothelial damage and an increase in oxidative stress.

Key words: allergic rhinitis, endocan, impulse oscillometry, nasal peak inspiratory flow, oxidative stress

Introduction

Allergic rhinitis (AR) is a chronic inflammatory disease characterized by IgE-mediated nasal mucosal inflammation and symptoms following exposure to allergens[(1)]. According to the results of cohort studies, asthma was reported in 15% to 38% of patients with AR, and accompanying rhinitis in 6% to 85% of asthmatic patients[(2)]. AR is an important risk factor for the development of asthma and affects its control and severity[(3–5)]. According to the 'one airway' concept, efforts have been made to explain the relationship between AR and asthma in terms of nasal dysfunction, nasobronchial reflex, bronchonasal reflex, secretion drainage, and the role of chemokines caused by Th2 inflammation[(6–9)].

Impulse oscillometry (IOS) is a non-invasive standardized respiratory function test that yields information about resistance and reactance in the peripheral and other airways in the early period of life[(10)]. Few studies have used IOS to evaluate potential airway damage in the early period of AR in children. Those studies showed that the presence of reversible airway obstruction and



Graphical abstract.

- An elevation was observed in nasal endothelial damage (endocan levels) and oxidative stress (TOS and OSI), independently of asthma status, in the cases with AR.
- The airways of adolescents with AR without asthma were as much affected as those of the AR with asthma.
- The airway dysfunction was associated with nasal endothelial damage and an increase in oxidative stress.

Abbreviations: AR, allergic rhinitis; FeNO, fractional exhaled nitric oxide; HC, healthy control; IOS, impulse oscillometry; n, number; OSI, oxidative stress index; PNIF, peak nasal inspiratory flow; TAS, total antioxidant status; TOS, total oxidant status; X, reactance, z, z-score.

an inverse association between nasal patency and airway resistance in children with AR[11,12].

Oxidative stress has been investigated in the context of the complex pathophysiology of AR[13–15]. Reactive oxygen species (ROS), endogenous nitric oxide (NO), and NO-derived ROS are known to mediate airway inflammation[13,16,17]. Fractional exhaled NO (FeNO) is a non-invasive method that shows type 2 inflammation in the airway epithelium[18]. In the context of comorbid airway diseases, FeNO has also been reported to increase in patients with AR without asthma[19–22].

Endocan, formerly known as endothelial cell-specific molecule 1, is a dermatan sulfate proteoglycan specific to the human endothelial cell[23]. Endocan plays an important role in the upregulation of adhesion molecules, the rearrangement of the endothelial cell skeleton, and the emergence of proinflammatory responses such as leukocyte adhesion and migration through interaction with NF-κB[24]. Endocan has been associated with several allergic diseases such as asthma, atopic dermatitis, and hereditary angioedema and also to be associated with disease activity in asthma[25–28].

The purpose of the present study was to evaluate nasal patency and pulmonary functions in AR in adolescents independently of the presence of asthma and investigate the relationships between these and nasal oxidative stress parameters and endothelial damage and compare the data with the values of healthy adolescents.

Materials and methods

Patient selection and study design

This prospective cross-sectional study was performed between 1 Dec 2021 and 1 Dec 2022 in the pediatric allergy and immunology clinic of a tertiary hospital. Seventy adolescents with AR aged 12-17 (AR group, 43 with asthma, 27 without) and 30 healthy

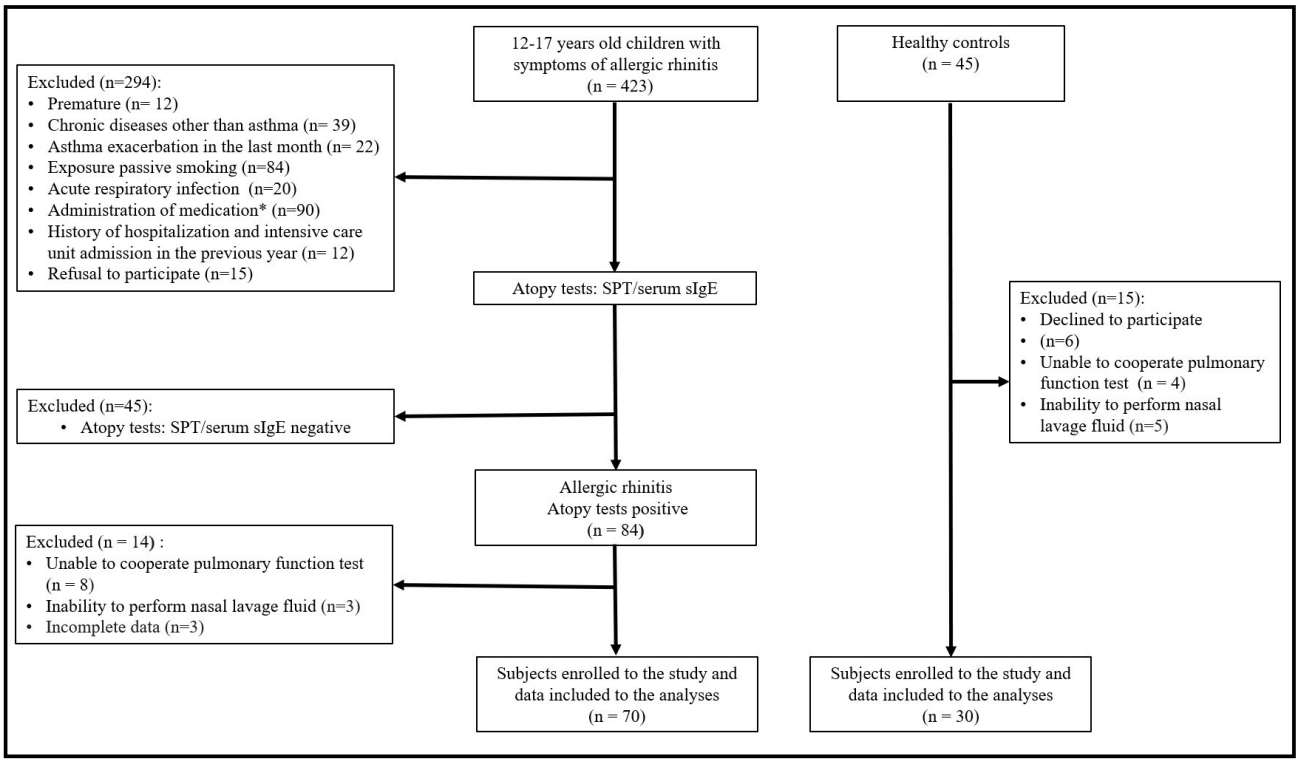


Figure 1. Study flowchart. * Participants with drug use (nasal, systemic, or high-dose inhaled corticosteroids, short- or long-acting beta-agonists, leukotriene receptor antagonists, and antihistamines) were excluded from the study, except for the use of step II inhaled corticosteroids for asthma.

controls (HC group) were included. Individuals with treatment-naive, moderate-severe persistent AR were enrolled in the study. Diagnosis of AR was based on clinical symptoms compatible with ARIA guidelines (nasal discharge, sneezing, nasal itching, and obstruction)[4].

The adolescents with AR and asthma were selected from patients receiving only inhaled corticosteroids as step II therapy for at least three months and whose asthma symptoms were under control[29]. The diagnosis of asthma and asthma control were based on Global Initiative for Asthma guidelines[29].

Exclusion criteria

Patients with histories of low birth weight/premature birth, neonatal mechanical ventilation, chronic respiratory disease other than asthma, chronic heart and neuromuscular disease, malignancy, immune deficiencies, obstructive sleep apnea, or acute airway disease were excluded from the study. Patients with asthma exacerbation in the previous four weeks, using medications including nasal, systemic, or high-dose inhaled corticosteroids, short- or long-acting beta-agonists, leukotriene receptor antagonists, and antihistaminics, with histories of admission to hospital and intensive care in the previous one year, patients exposed to active or passive smoking, and patients experiencing airway infection in the preceding two weeks were also excluded.

The control group was selected from healthy children presenting to our hospital for routine controls, with no chronic lung disease (such as asthma, bronchopulmonary dysplasia, and bronchiectasis), systemic disease (congenital heart disease, musculoskeletal system diseases, neurological disease, immune deficiency, etc.), allergic diseases (eczema, AR or food allergy), or growth retardation, with no history of prematurity, not hospitalized due to any respiratory disease, not exposed to active or passive smoking, and with no airway infection in the preceding two weeks.

The case report form and data collection

All participants underwent basic physical examinations, anthropometry, nasal lavage sample collection, and respiratory function tests (FeNO, IOS, and peak nasal inspiratory flow (PNIF)]. Patients' demographic characteristics (age, sex, and date of birth), symptoms, parental history of atopy, and domestic factors (such as humidity, heating stoves, and the presence of pets) were recorded.

Atopy evaluation

Serum allergen-specific immunoglobulin E (IgE) testing was evaluated using ImmunoCAP (Phadia ImmunoCAP; Pharmacia Diagnostics, Uppsala, Sweden), and a value >0.35 kU/L was regarded as positive. The skin prick test (SPT) was applied to the

Table 1. Comparison of demographic characteristics and domestic factors of adolescents with allergic rhinitis and healthy control group.

	AR group (n=70)	HC group (n=30)	p value
Gender, n (%) Male	39 (55.7)	14 (46.7)	0.406*
Age, year; median (IQR)	14.47 (12.95-15.94)	15.13 (13.38-16.72)	0.136**
BMI (kg/m^2) (z scores) (median, IQR)	21.55 (18.50-24.24)	21.81 (19.14-25.86)	0.525**
Parenteral atopy history (%)	38 (54.3)	7 (23.3)	**0.004***
Domestic Factors Exposure to humidity at home, n (%) Household pets, n (%) Stove heating at home (%), n (%)	 8 (11.4) 28 (40) 12 (17.1)	 3 (10) 12 (40) 10 (33.3)	 0.834* N/A 0.073*

AR, allergic rhinitis; BMI, body mass index; HC, healthy control; IQR, interquartile range; N/A, not avaliable; n, number; %, percentage. *Comparison of categorical variables was performed using the χ^2 test, **Comparison of non-normally distributed continuous variables was performed using the Mann-Whitney U test, p<0.05 value is significant.

volar surface of the forearm, in line with international guidelines. Commercially available standard solutions (Alk-Abello®, Hørsholm, Denmark) were used for SPTs. Positivity was defined as swelling at least 3 mm greater than that of the negative control[30].

Respiratory function tests

All lung function tests were performed on all patients in the pediatric allergy and immunology respiratory laboratory, in the form of FeNO, IOS, and finally PNIF during the same screening visit. The study protocol and procedures were explained to the patients in detail. They were advised to avoid excessive exercise and not to consume products containing theophylline and caffeine for 2 hours before FeNO and the subsequent measurements.

Fractionated exhaled nitric oxide (FeNO)

FeNO was measured based on American Thoracic Society/European Respiratory Society (ATS/ERS) criteria using a commercial, portable electrochemical sensor NObreath® device (Bedfont Scientific Ltd., Maidstone, UK) at a steady flow rate of 50 ml/s, and the results were expressed as parts per billion (ppb). At measurements performed in line with the ATS/ERS guidelines, FeNO values < 5 ppb were regarded as low, 5-20 ppb as normal, 20-35 ppb as moderate, and 35 < ppb as high[31].

Impulse oscillometry

The Jäeger MasterScreen IOS system (Jäeger, Wurzburg, Germany) was employed to measure respiratory system impedance. Respiratory oscillometry was performed in line with ERS technical standards[32]. The patient was prevented from breathing through the nose through manual occlusion with nasal clips, a mouthpiece was installed, and the child's chin and hands were supported by the hands to reduce the upper airway shunt. At least three valid measurements were taken for each child. Data collection lasted 60 s, with 30 s intervals between measurements. The mean value of three acceptable curves was selected for analysis from least three or more repeated measurements (at least three artifact-free measurements) where two technically acceptable measurements resulted in the same resistance and reactance values[32].

The IOS system creates a small pressure swing to determine lung impedance (Z). The components of Z are pulmonary reactance (X) and resistance (R). The principal IOS parameters include resistances (R5, R10, R15, and R20), reactance (X5, X10, X15, and X20), R5-R20 (resistance at 5 Hz minus resistance at 20 Hz), resonance frequency (Fres), and reactance area (AX). Resonance frequency (Fres) is the point at which reactance is zero and is measured as Hertz (1/s). AX is calculated by integrating the reactance curve from 5 Hz to Fres and represents low-frequency respiratory reactance. Higher R frequencies (~20 Hz) represent larger airways and represent the resistance in the main airways. Lower R frequencies (~5Hz) provide information about all the airways, large and small. Peripheral (small) airway resistance is defined with R5-20[32,33]. Lower X frequencies (~ 5 Hz) provide information about total airway reactance. AX, "the Goldman Triangle," represents the size of the integrated low-frequency respiratory reactance between 5 Hz and Fres. The dead space was < 70 mL, including the bacterial filter. Acceptable intra-session coefficient of variation (CoV) was ≤ 15%[32]. Consistency thresholds were set to ≥ 0.6 at 5 Hz and ≥ 0.8 at 20 Hz[34]. R5, R20, X5, and X20 were converted to z-scores from reference values to adjust for age and gender[35]. R5-20, AX, and Fres were used as raw values due to the absence of reference values.

Nasal resistance/peak nasal inspiratory flow (PNIF)

PNIF is an easily applied, non-invasive method widely employed to evaluate nasal patency. A portable peak flow meter equipped

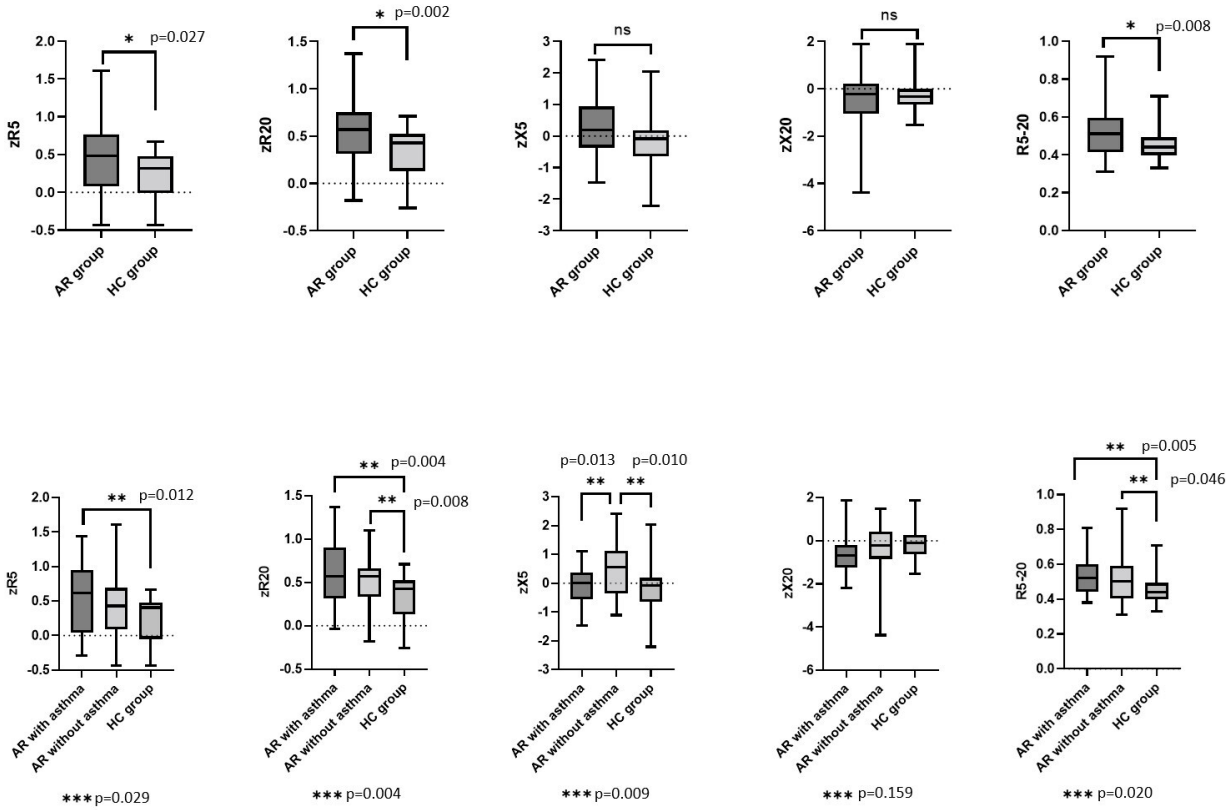


Figure 2. Comparison of impulse oscillometry parameters between AR and HC groups. Box and whisker plots were generated in GraphPad Prism 9. AR, allergic rhinitis; HC, healthy control; ns, not significant; R, resistance; X, reactance; z, z-score; * The Mann-Whitney U test; ** The Bonferroni-corrected Mann-Whitney U test; *** The Kruskal-Wallis test.

with face masks (In-check DIAL; Clement Clarke International, Essex, UK) was used. Participants were given careful instruction in a standard technique. The mask was installed following deep expiration, with participants in an upright position, and they were then asked to perform a deep and rapid inspiration. This activity was performed in triplicate, and the highest of the three values was recorded. The results were expressed as liters/minute (L/min)[36,37].

The nasal fluid sampling method and the enzyme-linked immunosorbent test

Nasal lavage fluid was collected from all the cases taking part in the study. This was done by dripping 5 mL of sterile, room temperature, 0.9% normal saline solution into each nostril using a tube-connected syringe. The participants remained in this position for a few seconds without swallowing while the nasopharynx was deliberately closed by the soft palate. The nasal lavage fluids were then placed into clean, plastic collection jars. They were then transferred to tubes and centrifuged at 3000xg for 10 min for supernatant separation. The resulting supernatants were then stored at -80°C. Total antioxidant status (TAS) and total oxidant status (TOS) levels were measured using a commercial kit (Rel Assay Diagnostics, Mega Tip, Gaziantep, Türkiye) in line with the manufacturer's instructions. The oxidative stress index (OSI) was defined as the ratio of TOS to TAS and was calculated as follows: OSI = (TOS, μmol H_2O_2 Eq/mg protein)/(TAS, mmol Trolox Eq/mg protein). This is often used to quantify the net oxidative stress in the body. Endocan levels were measured using a commercial ELISA kit (Fine Biotech Co. Ltd., Wuhan, PR China) following the manufacturer's instructions. Protein levels were measured using Bradford reagent, and the results were calculated by dividing by mg protein.

Ethics

This study was approved by our institutional Ethics Committee (decision no. 07, protocol no. 2021/199). The research was conducted in conformity with the principles of the Declaration of Helsinki. Informed consent was obtained from the children and their parents or legal guardians.

Statistical analysis

Statistical analyses were performed on SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed using the Kolmogorov-Smirnov test and descriptive

Table 2. Comparison of IOS, FeNO, PNIF and nasal biomarker measurements in adolescents with allergic rhinitis and healthy control group.

Parameters	AR group (n= 70)	HC group (n=30)	p value
Lung Function Tests			
zR5 (median, IQR)	0.47 (0.04-0.74)	0.31 (-0.01-0.47)	**0.027**
zR20 (median, IQR)	0.56 (0.30-0.75)	0.42 (0.13-0.52)	**0.002**
zX5 (median, IQR)	0.18 (-0.37-0.92)	-0.09 (-0.64-0.18)	**0.052**
zX20 (median, IQR)	-0.21 (-1.05-0.21)	-0.31 (-0.65-0.00)	0.749
R5-20 (median, IQR)	0.51 (0.41-0.59)	0.44 (0.39-0.49)	**0.008**
Fres (median, IQR)	14.35 (11.51-18.91)	13.79 (11.65-16.41)	0.679
AX (median, IQR)	0.45 (0.27-0.69)	0.48 (0.31-0.70)	0.527
FeNO, ppb (median, IQR)	6 (2-16.25)	4 (1-6.5)	**0.038**
PNIF, L/min (median, IQR)	87.5 (78.75-110)	100 (80-110)	0.157
Nasal biomarkers			
Endocan, pg/mg protein (median, IQR)	268.86 (225.56-329.76)	186.51 (157.92-211.20)	**<0.001**
TAS, mmol trolox Eq/mg protein (median, IQR)	0.33 (0.17—0.39)	0.43 (0.23-0.51)	**0.001**
TOS, µmol H2O2 Eq/mg protein (median, IQR)	20.33 (18.29-22.50)	18.20 (16.16-21.15)	**0.007**
OSI (median, IQR)	61.29 (48.71-115.53)	42.20 (32.58-58.45)	**<0.001**

AR, allergic rhinitis; AX, reactance area; FeNO, fractional exhaled nitric oxide; Fres, resonance frequency; IQR, interquartile range; HC, healthy control; n, number; IOS, impulse oscillometry; OSI, oxidative stress index; PNIF, peak nasal inspiratory flow; R, resistance; TAS, total antioxidant status; TOS, total oxidant status; X, reactance; z, z-score; %, percentage. Group comparisons were held by Mann Whitney U test and the significant (bold) value was accepted as $p < 0.05$.

statistics. Categorical variables were expressed as case number and percentage values. Depending on normality of distribution, continuous variables were expressed as median and interquartile range (IQR – 25th and 75th percentile). Qualitative data were compared between the groups using the chi-square test, and quantitative data using the Mann-Whitney U or Kruskal-Wallis tests. In cases where the Kruskal-Wallis test yielded a statistical significance, post hoc analysis was performed to identify the groups, which showed differences, by Bonferroni-corrected Mann-Whitney U test; the cut-off level of α error was reduced to 0.05/(number of tests; Bonferroni correction). Spearman's correlation analysis was applied for continuous variables. After variables had been identified at univariate analysis, they were subjected to multivariate linear regression analysis in order to determine potential risk factors for IOS resistance and reactance in the study participants. Non-normally distributed variables were converted logarithmically. p values <0.05 were regarded as statistically significant for group comparisons.

Results

Patient characteristics

The median age of the AR group was 14.47 (12.95-15.94) years, and 38 (55.7%) were boys. There was no age or gender difference between the AR and HC groups (p>0.05). A history of parenteral atopy was present in 54% of the AR group, higher than in the HC group (p=0.004). A comparison of the demographic characteristics between the AR and the HC groups is shown in Table 1. The flowchart of the study is shown in Figure 1.

Among AR group, 32.6% (16/49) and 76.3% (33/49) were monosensitized and polysensitized, respectively. The monosensitized patients had sensitivity for 62.5% pollen, 25% house dust mites, and 12.5% mold. Polysensitized patients had 36% pollen, 20.2% mold, 19% cat, 17% house dust mite, and 5.9% dog sensitivity. Perrenial symptoms were observed in 8 (50%) and 16 (48.4%) patients, respectively, in monosensitized and polysensitized patients.

Respiratory Function Analyses

- ***FeNO***

FeNO levels were higher in the AR group than in the HC group (p=0.038) (Table 2). No difference in FeNO levels was observed when the AR groups with and without asthma were compared with the HC group (p=0.106) (Table 3).

- ***IOS***

The IOS parameters zR5, zR20, and R5-20 were higher in the AR group than in the HC group (p<0.05 for all) (Table 2).

A comparison between the cases with and without asthma in the AR group and the HC group revealed differences in terms of zR5, zR20, zX5, and R5-20 (p=0.029, p=0.004, p=0.009, and

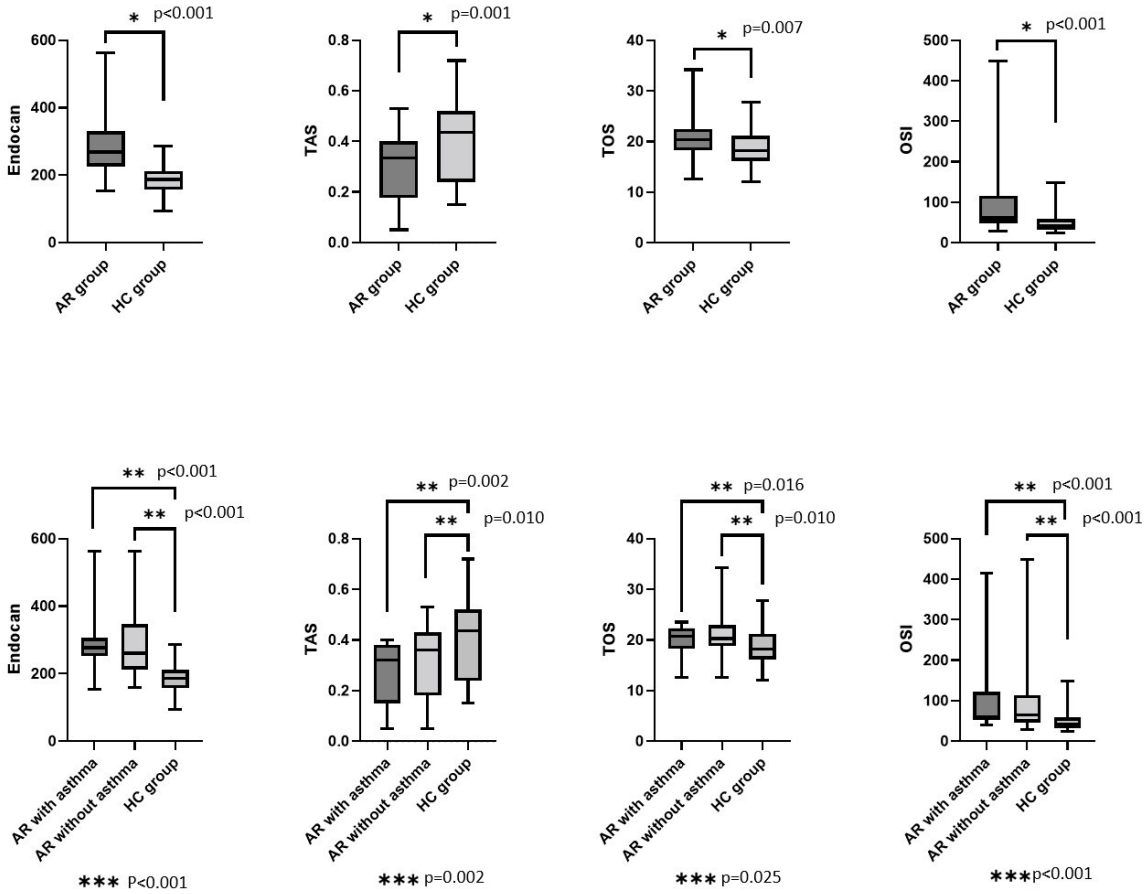


Figure 3. Comparison of nasal biomarkers between AR and HC groups. Box and whisker plots were generated in GraphPad Prism 9. AR, allergic rhinitis; HC, healthy control; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status; * The Mann-Whitney U test; ** The Bonferroni-corrected Mann-Whitney U test; *** The Kruskal-Wallis test.

p=0.020, respectively). Subgroup analyses revealed higher zR5, zR20, and R5-20 in the AR group with asthma compared to the HC group (p=0.012, p=0.004, and p=0.005, respectively), while zR20, zX5, and R5-20 were higher in the AR group without asthma compared to the HC group (p=0.008, p=0.010, and p=0.016, respectively). zX5 values were higher in the AR group without asthma than in the AR group with asthma and the HC group (p=0.013 and p=0.010, respectively), while no difference was observed in the other IOS parameters (p>0.05) (Table 3) (Figure 2).

- ***PNIF***

No difference in PNIF was found between the AR and HC groups (p=0.157) (Table 2), nor between the patients with and without asthma in the AR group and the HC group (p=0.083) (Table 3).

Nasal oxidative biomarkers

The nasal oxidative biomarkers TOS and OSI were higher in the AR group, while TAS was higher in the HC group (p<0.05 for all) (Table 2). No difference in terms of nasal oxidative biomarkers was observed between individuals with and without asthma in the AR group (p>0.05) (Table 3; Figure 3).

Nasal endocan levels

Nasal endocan was higher in the AR group than in the HC group (p<0.001) (Table 2). No difference in terms of nasal endocan was observed between individuals with and without asthma in the AR group (p>0.05) (Table 3; Figure 3).

Significant correlation was determined between OSI and R5-20 in the patients with AR (r=0.341, p=0.04). When (logged) OSI was entered as a continuous variable in linear regression models, it was found to significantly predict (logged) R5-20 (β = 0.128, p = 0.006).

Discussion

Although the mechanisms underlying the pathology of AR and its relationship with the lower airways have previously been investigated, much about the disease is still uncertain, and

Table 3. Comparison of IOS, FeNO, PNIF and nasal marker measurements of AR group with and without asthma and healthy control group

Parameter	AR with asthma (n=27)	AR without asthma (n=43)	HC group (n=30)	p value	Subgroup analysis
Lung Function Tests					
zR5 (median, IQR)	0.62 (0.04-0.95)	0.43 (0.08-0.09)	0.31 (-0.01-0.47)	0.029	1-2=0.117 **1-3=0.012** 2-3=0.126
zR20 (median, IQR)	0.57 (0.30-0.89)	0.56 (0.33-0.66)	0.42 (0.13-0.52)	0.004	1-2=0.220 **1-3=0.004** **2-3=0.008**
zX5 (median, IQR)	0.00 (-0.55-0.37)	0.55 (-0.36-1.11)	-0.09 (-0.64-0.18)	0.009	**1-2=0.013** 1-3=0.665 **2-3=0.010**
zX20 (median, IQR)	-0.65 (-1.26- -0.21)	-0.21 (-0.84-0.42)	-0.31 (-0.65-0.00)	0.159	
R5-20 (median, IQR)	0.52 (0.44-0.60)	0.50 (0.40-0.59)	0.44 (0.39-0.49)	0.020	1-2=0.448 **1-3=0.005** **2-3=0.016**
Fres (median, IQR)	14.91 (11.64-19.09)	14.31 (11.33-18.85)	13.79 (11.65-16.41)	0.809	
AX (median, IQR)	0.46 (0.31-0.76)	0.41 (0.25-0.58)	0.48 (0.31-0.70)	0.433	
FeNO, ppb (median, IQR)	6 (2-19)	6 (2-16)	4 (1-6.5)	0.106	
PNIF, L/min (median, IQR)	90 (80-120)	80 (70-100)	100 (80-110)	0.083	
Nasal biomarkers					
Endocan, pg/mg protein (median, IQR)	276.97 (251.33-305.63)	260.60 (212.64-346.83)	186.51 (157.92-211.20)	<0.001	1-2=0.395 **1-3<0.001** **2-3<0.001**
TAS, mmol trolox Eq/mg protein (median, IQR)	0.31 (0.14-0.37)	0.36 (0.18-0.42)	0.43 (0.23-0.51)	0.002	1-2=0.086 **1-3=0.002** **2-3=0.010**
TOS, µmol H2O2 Eq/mg protein (median, IQR)	20.70 (18.27-22.36)	20.27 (18.83-22.92)	18.20 (16.16-21.15)	0.025	1-2=0.617 **1-3=0.016** **2-3=0.010**
OSİ (median, IQR)	60 (53.40-121.90)	64.43 (46.44-114.12)	42.20 (32.58-58.45)	<0.001	1-2=0.530 **1-3<0.001** **2-3<0.001**

AR, allergic rhinitis; AX, reactance area; FeNO, fractional exhaled nitric oxide; Fres, resonance frequency; IQR, interquartile range; n, number; IOS, impulse oscillometry; OSI, oxidative stress index; PNIF, peak nasal inspiratory flow; R, resistance; TAS, total antioxidant status; TOS, total oxidant status; X, reactance; z, z-score; %, percentage. Group comparisons were held by Kruskal-Wallis test and the significant (bold) value was accepted as $p < 0.05$. Subgroup analysis was held by Bonferroni-corrected Mann-Whitney U test and and the significant (bold) value was accepted as $p < 0.017$.

further research is now required[6–9]. The most striking findings of the present study is the elevation in nasal endothelial damage (endocan levels) and oxidative stress (TOS and OSI), independently of asthma status, in the cases with AR. All (zR5), main airway (zR20), peripheral airway resistances (R5-20) and lung inflammation (FeNO) were also high in these patients. Nasal airway reactance (zX5) was higher in the AR cases without asthma compared to those with asthma, while no difference was determined in terms of endothelial damage (endocan and oxidative stress parameters) and airway inflammation (FeNO), resistance, or reactance.

FeNO is a non-invasive method showing type 2 inflammation in the airway epithelium[18]. While some studies have reported higher FeNO levels in conditions when asthma accompanies AR, no difference has also been reported in terms of the presence or absence of rhinitis[20,38,39]. Several studies have also reported that FeNO increases in cases of AR without asthma[19–22]. Similarly in the present study, FeNO levels were higher in the AR group than in the HC group. This may have been due to AR exacerbating airway inflammation in the lung. Similar FeNO elevation was present in patients with AR only and those controlled asthma accompanying AR. This may be due effects of the asthma being under control and their regular use of steroid inhaler therapies for at least three months in our study population.

There have been few studies investigating the respiratory functions of pediatric patients with AR using the IOS method, and

their findings are inconsistent[11,12,40]. In 226 Korean children with rhinitis aged seven years, and observed negative correlation between airway resistance (R5) and nasal patency[12]. Another study reported high X5 and AX% change values in children with AR with bronchial hyperreactivation and showed reversible airway obstruction in these patients[11]. However, a cohort study investigating the effect of rhinitis on pulmonary functions in 16-year-old adolescents reported no difference in terms of spirometry or IOS parameters in AR groups with and without asthma[40]. The effect of AR on asthma in adults has been investigated using IOS, with higher resistance and reactance being observed in individuals with asthma only compared to cases of AR accompanying asthma[41]. Although the doses of inhaled corticosteroids and asthma control levels used by asthmatic patients in that study were similar, the older age of the adults with asthma only may have contributed to the worsening of lung function. In the present study, resistance in all, main, and peripheral airways (zR5, zR20, and R5-20 values) were higher in the AR group than in the HC group. The higher zX5 value in AR patients without asthma compared to those with asthma shows that AR affects airway reactance independently of asthma. A higher zR5 value was determined in the AR group with asthma than in the HC group, but no difference was observed between the two AR subgroups. A high zR5 value is an expected finding in asthmatic adolescents; interestingly, it was found to be similarly high in AR without asthma, although the difference was not statistically significant. We suggest that one of the potential reasons for this result is the use of ICS in asthmatics or increased airway resistance in AR without asthma. Our study demonstrated that despite significant differences in oxidative stress and endocan levels in nasal lavage measurements in AR group, the difference in IOS measurements was a relatively small effect size.

Endocan has been shown to elicit pro-inflammatory responses such as epithelial hyperpermeability, reorganization of the cytoskeleton, upregulation of cellular adhesion molecule expression, and leukocyte adhesion and migration[23]. Its role in pulmonary disorders with underlying endothelial dysfunction has therefore attracted particular attention[23]. In addition, endocan disrupts the NO production balance in endothelial cells, activates NFkB/iNOS signaling, and induces oxidative stress in the endothelium[42]. In the light of these findings, the present study therefore investigated endocan levels in nasal specimens in AR alone and in AR with asthma, and levels were higher in AR group than in the HC group. Additionally, no difference in endocan levels was observed in the AR groups with and without asthma. To date only one study has shown a relationship between increasing endocan levels in asthmatic children and severity of disease and impaired lung functions[25]. The present research is the first to show that nasal endocan levels rise in AR and that this increase is unaffected by the presence of asthma. Since endocan is known to play a role in the pathogenesis of disease, it may be concluded that it may reflect part of the pathophysiological processes described above in diseases such as asthma and AR and be capable of use as a useful marker in predicting endothelial damage in the nasal cavity and airways. However, the fact that the asthmatic patients' disease was under control in this study and their regular use of inhaled corticosteroid (ICS) may have prevented asthma affecting nasal endocan levels. The use of ICS may have hindered the prediction of endocan as a risk factor for lower airway resistance. Nonetheless, the study data suggest that nasal endocan levels can be used in the diagnosis of AR, and perhaps in the follow-up of treatment, and are capable of employment as a biomarker reflecting endothelial damage.

Although oxidative stress is known to play an important role in the pathogenesis of asthma, the numbers of studies of its effects on AR are limited[13,15,43,44]. Emin et al. detected a significant increase in TAS and TOS in children with AR[13]. Similarly, Sim et al. determined serum TAS, TOS, and OSI elevation in children with AR[14]. An association has been found between asthma and AR and increasing oxidative stress in the airways, although the co-presence of the two diseases has been found not to result in a greater increase in oxidative stress[15]. The present study shows the presence of increasing nasal oxidative stress, not only in adolescents with asthma accompanying AR, but also in those without asthma, compared to the HCs. No difference in nasal oxidative stress parameters was observed between the asthmatic and non-asthmatics with AR. Our results show that the presence of controlled asthma does not result in a further increase in nasal oxidative stress, may be due to regular ICS usage or the concept of 'one airway, one disease' in asthma and rhinitis.

Although PNIF has been reported to be successfully used in the response to treatment in AR in children and adolescents[45,46], there are also studies that have raised questions about the sensitivity of PNIF measurements and suggesting a limited role for this method in showing nasal patency[47]. Although measurements were taken by a single individual in this study, using the same instructions, and on participants from the same age group, no difference was detected in PNIF values, independently of the presence of asthma, between the cases with AR and the HCs, and no association was found with other biomarkers and respiratory function tests. We suggest that PNIF is not sufficiently sensitive to measure nasal patency in adolescents, or that there is no association between nasal patency and inflammation and respiratory functions.

The particular strengths of this study include its prospective nature, the fact that AR and asthma were diagnosed by pediatric allergy specialists, all lung function measurements were performed by the same physician and nurse in order to ensure

standardization. PNIF measurements and nasal lavage specimen collection were performed after lung function tests to avoid affecting the respiratory function test results. To avoid the risk of directly affecting asthma exacerbations, and particularly nasal inflammatory and oxidative markers and pulmonary function tests, care was taken to ensure that all patients' asthma was under control, that they had no active infection, and that they received step II ICS therapy. Additionally, the study was conducted during the same pollen season, before the treatment was started, when patients were symptomatic, as most patients had pollen allergies.

The principal limitation of this study is that the results cannot be generalized to the entire population due to the small sample size and the single-center and cross-sectional nature of the research. Another limitation in terms of inability to provide additional information about lung physiology is the absence of spirometry measurements, another respiratory function method. Endocan and oxidative stress may also be increased in bronchial tissue biopsy or bronchial fluid aspirate, but we could not examine this by bronchoscopy in children because it is impractical, costly, invasive, and requires infrastructure facilities. It is at this point unknown whether the nasal epithelium is also a source or modulator of the found endocan levels in the nasal lavage samples. Moreover, patients being evaluated with a single measurement limited our data concerning change over time.

Conclusion

This study shows, for the first time, that the airways of adolescents with AR without asthma were as much affected as those of the patients with asthma, and that airway dysfunction was associated with nasal endothelial damage and an increase in oxidative stress. At this time when the relationship between the two diseases is still not fully understood, it appears possible to conclude that the presence of AR alone raises nasal endocan levels and oxidative stress and exacerbates airway inflammation to the same extent as combined asthma and AR and affects resistance in the airways. Only airway reactance was higher in the patients with AR only compared to those with accompanying asthma. Nasal oxidative stress was found to be associated with peripheral airway resistance in patients with AR. Our findings support the concept of 'one airway, one disease' in asthma and rhinitis. In addition, the present study will contribute significantly to the literature as the first to show nasal endocan levels in cases of AR, and that endocan might be a part of the pathophysiology of AR by reflecting endothelial damage. Understanding the molecular pathways underlying oxidative stress and endothelial damage may be a useful strategy for reducing oxidative stress in asthma and AR through the inhibition of endocan-NO signaling and their potential association with airway resistance. We think that further, multi-center cohort studies with larger populations are now needed to strengthen these speculations.

Authorship contribution

ZGK carried out the data acquisition and analysis and drafted the manuscript with PU. OE and OC participated in the design and planning of the study, as well as in the interpretation of the results. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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