



Naso-ocular neuropeptide interactions in allergic rhinoconjunctivitis, rhinitis, and conjunctivitis

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ABSTRACT

Background: Ocular as well as nasal symptoms contribute to allergic response but remain poorly characterized. The aim of this study was to analyze the levels of substance P (SP), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) in tears and nasal secretions of patients with allergic rhinoconjunctivitis (ARC), allergic rhinitis, and allergic conjunctivitis, while exploring possible mechanisms of naso-ocular interactions.

Methods: A total of 21 patients with ARC, 17 with allergic rhinitis, 13 with allergic conjunctivitis, and 15 healthy controls were enrolled in the study. Nasal secretions and tears were collected. Patient demographics and clinical characteristics were recorded and levels of substance P, VIP, and CGRP were measured.

Results: SP levels in nasal secretions and tears were significantly higher in the ARC, AR, and AC groups. Similar results were obtained for VIP levels. CGRP levels in tears were also significantly higher in the 3 patient groups. The level of SP was significantly higher in the nasal secretions than in the tears of the ARC, AR, and AC patient groups. The level of VIP was significantly higher in the nasal secretions than in the tears in the ARC and AR groups. The level of CGRP was significantly higher in the nasal secretions than in the tears in the ARC and AC groups. Finally, both of the nasal and tear levels of SP and VIP but not CGRP were positively correlated with the visual analog scale (VAS) score in the patients with ARC.

Conclusion: The results of this study suggested that SP, VIP, and CGRP play important roles in the mechanism of ARC and that nasal neurotransmitters and neuropeptides might have more important roles than those of ocular origin.

Keywords: Allergic conjunctivitis, Calcitonin gene-related peptide, Substance P, Vasoactive intestinal peptide

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INTRODUCTION

Allergic rhinoconjunctivitis (ARC) is an allergic disease of the nose and eyes that affects approximately one-fifth of the total world population,¹ resulting in substantial socioeconomic burden and negatively impacting the work and daily life of affected individuals.² The main manifestations of ARC include nasal congestion, rhinorrhea, nasal itching, and sneezing accompanied by ocular allergic reactions such as lacrimation, conjunctival congestion, and eye itching.

The pathogenesis of allergic diseases involves a variety of factors, such as environmental, cellular, and molecular mechanisms, along with tissue remodeling.³ Notably, some recent studies have demonstrated that immune cells do not act alone in the pathogenesis of allergic diseases; rather, the cross talk and reciprocal regulation between neural and immune systems and neuroimmune interactions and neuropeptides also play important roles.⁴⁻⁸ Neural regulation in the upper airways is maintained by the sympathetic and the parasympathetic nervous systems, which innervate and interact in the nasal mucosa to regulate epithelial, vascular, and glandular processes in particular.⁵ Once stimulated, sensory and autonomic neurons release neurotransmitters and neuropeptides such as substance P (SP), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP).⁴ SP constitutes a short amphipathic peptide that is stored in dense-core vesicles and released upon calcium influx into peripheral nerve terminals. It induces mast cells to degranulate and release inflammatory mediators such as prostaglandin D₂, histamine, and leukotrienes.⁹ In comparison, VIP secreted by nerve endings has been shown to induce vasodilatation and mucus secretion, increase vascular permeability, and stimulate leukocyte extravasation.⁸ Its target receptors include VPAC-1, VPAC-2, CRTH2, and PAC1, which are expressed on several kinds of immune cells such as eosinophils, mast cells, neutrophils, and lymphocytes.¹⁰ CGRP is a kind of co-transmitter with SP. Even though CGRP has not been recognized as glandular secretion, it is probably involved in vasomotor responses.⁸ To date, both VIP and CGRP can also induce vasodilatation and increase vascular

permeability.¹¹ Moreover, several studies of allergic conjunctivitis (AC) and allergic rhinitis (AR) demonstrated that the expression of SP, VIP, and CGRP increased significantly in the tears of patients with AC and the nasal secretions of patients with AR caused by allergen stimulation.^{8,12,13}

Owing to the proximity of the anatomical locations of the nose and eye, a close connection exists between their vascular- and neuro-anatomy. However, previous surveys of allergies usually focused on nasal symptoms although ocular symptoms are also relevant. In addition, to our knowledge, no studies have explored the characteristics of neurotransmitter levels in the tears and nasal secretions of patients with ARC, AR, or AC. The aim of this study was, therefore, to analyze the levels of SP, VIP, and CGRP in the tears and nasal secretions of patients with these disorders, using a cross-sectional design, in order to shed light on possible mechanisms of naso-ocular interactions in allergic disease.

MATERIALS AND METHODS

Subject enrollment

A total of 21 patients with ARC, 17 with AR, 13 with AC, and 15 healthy controls (HCs) were enrolled from March to May 2019. Subjects suspected to have AR based on the presence of common symptoms of nasal obstruction, rhinorrhea, sneezing, and itching were recruited consecutively from the allergy-rhinology outpatient clinic of our Hospital. Each subject completed a questionnaire at recruitment to record their demographic data and nasal symptom severity, and was then assessed for sensitization to relevant aeroallergens by measurement of specific IgE (sIgE) in serum. Diagnosis of AR was based on criteria of the Allergic Rhinitis and its Impact on Asthma (ARIA) consensus statement.¹⁴ All patients with AR exhibited no ocular allergy symptoms and no history of ocular allergy.

The diagnostic criteria of patients in the AC group included ocular itching, burning sensation, tingling, lacrimation, and photophobia, along with physical examination showing bulbar conjunctiva hyperemia and edema.¹⁵ Patients classified in the AC group exhibited no nasal allergy symptoms

and no history of nasal allergy, but all patients demonstrated positive serum allergen-specific tests. The diagnostic criteria of ARC met both criteria of AR and AC.

Healthy subjects without any nasal and ocular allergic disease and patients with nasal septum deviation, cerebrospinal fluid leak, or pituitary tumor with normal nasal mucosa in the ethmoid sinus, but without chronic rhinitis, were recruited as HCs. Patients with any chronic or acute nasal and ocular disease, who had used topical or systemic anti-allergic agents or corticosteroids within 1 month, who wore contact lenses, or exhibited other allergic diseases especially asthma and other allergic diseases of the upper respiratory tract, were excluded.

Serum IgE

Serum-specific IgE testing was performed in all patients; patients with at least one positive allergen were considered to meet the ARC, AR, or AC group inclusion criteria. Serum sIgE levels toward common aeroallergens were determined using the fluoroenzyme immunosorbent assay (UniCAP, Uppsala, Sweden), with a value for serum sIgE ≥ 0.35 kU/L regarded as positive. The sIgE examination was performed using a panel of allergens including *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Candida albicans*, mugwort, *Penicillium notatum*, *Cladosporium*, *Alternaria*, *Aspergillus*, ragweed, and trees mixed. These allergens were selected on the basis that mite and mugwort are the 2 most prevalent aeroallergens in China.¹⁶ Furthermore, based on evidence from daily clinical practice during the period when this study was conducted, we noted that the fungal allergy rate was also high and thus the panel of fungal allergens was also investigated. Total IgE was also recorded.

Visual analogue scale (VAS)

The severity of nasal and ocular symptoms including nasal obstruction, running nose, sneezing, and nasal/eye itching lacrimation and redness, was recorded using a visual analogue scale (VAS) of 10 cm. Each symptom was categorized as "mild" (VAS: 0-3 cm), "moderate" (VAS: >3-7 cm), or "severe" (VAS >7 cm).

Nasal secretion and tear sample collection and analysis

Nasal secretions were collected using sinus packs and processed as described previously.¹⁷ All samples were collected from patients when they presented to our allergy outpatient clinic with symptoms, during or out of the specific allergen season. We obtained all samples during the symptomatic period. All sponges were stored at 4 °C for at least 2 h and then transferred to a 5 mL syringe. The bulk of the nasal secretion was forced out of the sponges using the piston of the syringe and centrifuged at 1500 g for 15 min at 4 °C. The supernatants were separated and stored in aliquots at -20 °C until analysis for the presence of sIgE and other inflammatory mediators. At the time of the assay, an aliquot was also assessed to ensure that there was no contamination.

A capillary method was used to collect tears from all participants by a skilled ophthalmologist.¹⁸ In brief, patient tears were collected using a sterile capillary tube at the outer canthus, taking care to touch the ocular surface as little as possible, and patients was asked to turn their eyes to the opposite side to avoid contact of the capillary with the cornea. The patient could blink normally during collection. A total of 40 μ L per participant was obtained, with up to 20 μ L of tear fluid being collected each time in each eye. To avoid the influence of diurnal variations in tear composition on experimental results, all samples were collected between 2-4 p.m. The collected tear samples were immediately stored in a freezer at -80 °C.

Measurement of inflammatory mediators

The concentration of total protein in each sample was measured using commercial enzyme-linked immunosorbent assay (ELISA) kits from Beyotime (Beijing, China). SP, VIP, and CGRP were evaluated using commercial ELISA kits from R&D Systems (Minneapolis, MN, USA). The concentration of each inflammatory mediator was expressed as ng/g protein. When the level of a mediator was below the detection level in the initial measurement, the results of that particular sample were excluded.

Ethical approval

This study was approved by the Ethics Committees of our Hospital and conducted in accordance with the ethical standards of the Committee on Human Experimentation of the institution or in accordance with the Helsinki Declaration of 1975 as revised in 1983. All patients were provided the relevant study information and instructions necessary for inclusion in the study, and all patients provided written informed consent prior to entering into the study.

Statistical analysis

All statistics are presented as the means \pm standard deviation. Variables were compared between 2 groups using one-way analysis of variance and the Tukey test using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was set at a p value < 0.05 .

RESULTS

The general data of subjects are shown in Table 1. The mean age of the ARC, AR, AC, and HC groups did not differ significantly. The sex ratio was generally balanced. The runny nose score was

significantly higher in the ARC and AR groups whereas the sneezing score was significantly higher in only the AR group. The lacrimation and redness scores were significantly higher in ARC and AC groups. There was no significant difference between ARC, AR, and AC groups with respect to the ocular itching score and total IgE level. Among the patients in the ARC group, 15 first-visits were to Otolaryngology and 5 were to Ophthalmology Departments.

The distribution of subject allergen types was determined according to the results of serum-specific IgE. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were the 2 most common allergen types (Fig. 1).

SP levels in nasal secretions and tears were significantly higher in the ARC, AR, and AC groups than those of the HC group but did not significantly differ between ARC, AR, and AC groups. Similar results were obtained for VIP levels. CGRP levels in tears were also significantly higher in the 3 patient groups than those in HCs. However, the nasal secretion CGRP levels were significantly higher in the ARC and AC groups than that in the AR group, and the tear CGRP levels did not differ between the ARC and AC groups (Fig. 2).

	ARC (n = 21)	AR (n = 17)	AC (n = 13)	Control (n = 15)	p value
Age, mean \pm SD (years)	34.4 \pm 3.1	40.3 \pm 2.7	35.7 \pm 5.1	32.9 \pm 4.8	ns
Sex (M/F)	8/13	10/7	8/5	8/7	ns
Nasal obstruction, mean \pm SD	5.9 \pm 1.2	6.2 \pm 1.1	2.4 \pm 0.9	-	ns
Runny nose, mean \pm SD	4.3 \pm 1.3*	5.3 \pm 2.2*	1.5 \pm 0.5	-	0.013
Nasal itching, mean \pm SD	4.1 \pm 1.5	4.5 \pm 2.1	2.7 \pm 1.2	-	ns
Sneezing, mean \pm SD	4.7 \pm 2.5*	4.9 \pm 2.4*	2.3 \pm 0.6	-	0.050
Lacrimation, mean \pm SD	7.3 \pm 2.5*	2.4 \pm 0.7	7.2 \pm 1.7*	-	0.044
Redness, mean \pm SD	5.3 \pm 1.4*	1.9 \pm 0.2	4.1 \pm 1.8*	-	0.031
Ocular itching, mean \pm SD	5.0 \pm 1.6	3.2 \pm 1.7	5.3 \pm 2.4	-	ns
Total IgE (kU/L) (range)	42.8 (0.35-67.6)	68.2 (0.35-112.1)	52.8 (0.35-89.5)	-	ns

Table 1. Demographic and clinical characteristics of the patients A t-test was used to determine the equality of means (two-tailed); ARC, allergic rhinoconjunctivitis; AR, allergic rhinitis; AC, allergic conjunctivitis; SD, standard deviation; M, male; F, female; *, p < 0.05 ; ns, not significant

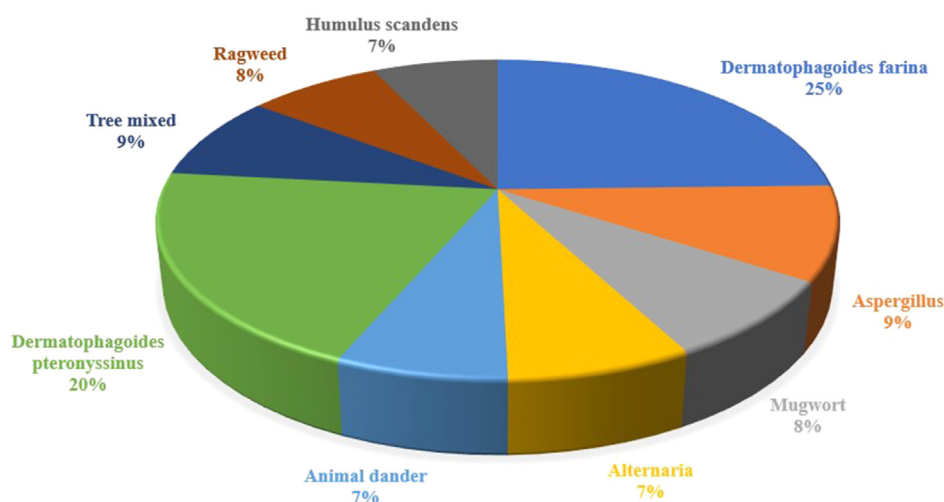


Fig. 1 Distribution of allergen types of the patients

Comparison of the levels of the separate neurotransmitters in the nasal and ocular samples revealed that the level of SP was significantly higher in the nasal secretions than in the tears of the ARC, AR, and AC patient groups. The level of VIP was significantly higher in the nasal secretions than in the tears in the ARC and AR groups. The level of CGRP was significantly higher in the nasal secretions than in the tears in the ARC and AC groups (Fig. 3).

In this study, the neuromediators we focused on were all highly associated with nasal-ocular stimulating symptoms. Therefore, we calculated the average of the total scores of itching and ocular itching (VAS). Finally, both of the nasal and tear levels of SP and VIP but not CGRP were positively correlated with the VAS score in the patients with ARC (Fig. 4).

DISCUSSION

ARC is a common medical condition but its underlying mechanism has not been fully unraveled. From an anatomical perspective, the nasal cavity and ocular conjunctival sac communicate through the nasolacrimal duct and constitute continuous mucosal epithelial tissues. The sensation in both the eye and nose is innervated by the trigeminal nerve and the parasympathetic nerves of both the eye and nose arise from the facial nerve and pass through the pterygomandibular ganglion to reach the corresponding site. Therefore, the proximity of the nose and eye anatomically and the close relationship between their

nervous systems provide a theoretical basis and research strategy for the mechanistic study of naso-ocular comorbid diseases.

Another mechanism that has drawn considerable attention is naso-ocular reflex, the presence of which has been confirmed the improvement of ocular symptoms by intranasal medication corroborates to the presence of nasal-ocular reflex.¹⁹ Moreover, several earlier studies have found that histamine released by mast cells in the nasal mucosa can cause symptoms of AR and AC.²⁰⁻²² However, controversies also exist regarding the theory of naso-ocular reflexes. For example, it has been postulated that the phrase "nasal-ocular reflex" could be replaced by terms such as "nasal-ocular interaction" as being more appropriate, because nasal irritation does not always cause ocular symptoms. Therefore, we consider that the link between nasal and ocular allergy and its mechanism warrants further investigation.

Even though the neurotransmitters play an important role in the pathogenesis of patients with nasal allergy and indicated the presence of a correlation between the increase of neurotransmitter levels and the occurrence of symptoms, we were unable to confirm whether the ocular allergic symptoms observed in the patients with ARC included in this analysis were "secondary to AR" or constituted "primary AC". To further determine the possible mutual influence between factors contributing to nasal and ocular allergies, we therefore performed the experiment, in which ARC

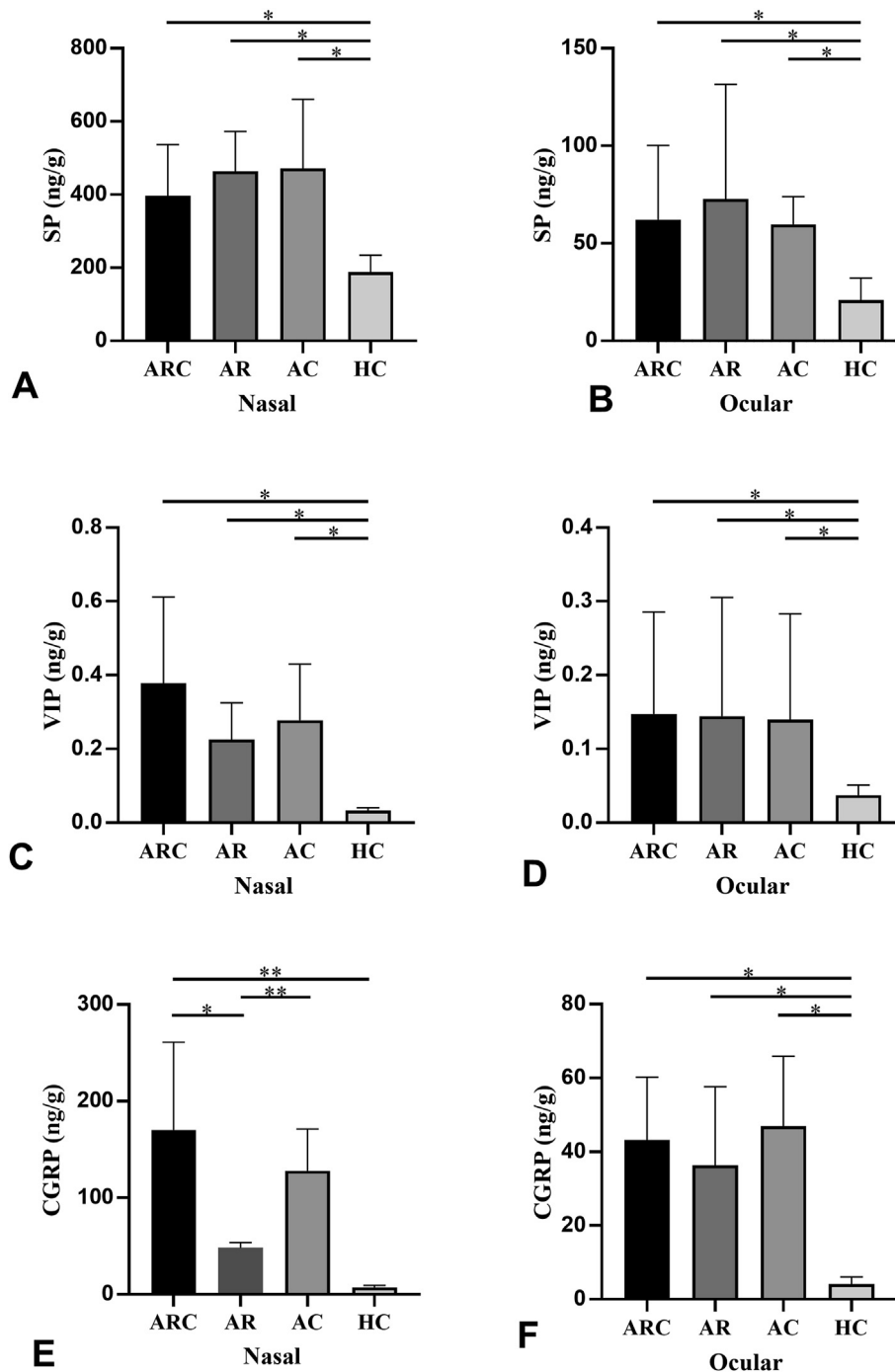


Fig. 2 Levels of SP (A, B), VIP (C, D), and CGRP (E, F) in nasal secretions and tears of patients with ARC, AR, AC and healthy controls. SP: substance P, VIP: vasoactive intestinal peptide, CGRP: calcitonin gene-related peptide, ARC: allergic rhinoconjunctivitis, AR: allergic rhinitis, AC: allergic conjunctivitis

results were compared with those from patients with AC or AR alone.

Comparison between ARC, AR, and AC groups revealed that SP levels in nasal secretions and tears were significantly higher in the 3 patient groups than those of the HC group, whereas levels did not

significantly differ between patient groups for either sample type. Meanwhile, the nasal and tear levels of SP were positively correlated with the VAS score in the ARC patients. Our results were in accordance with some former studies. For example, a previous study indicated that the SP level of patients with AR was higher than that of

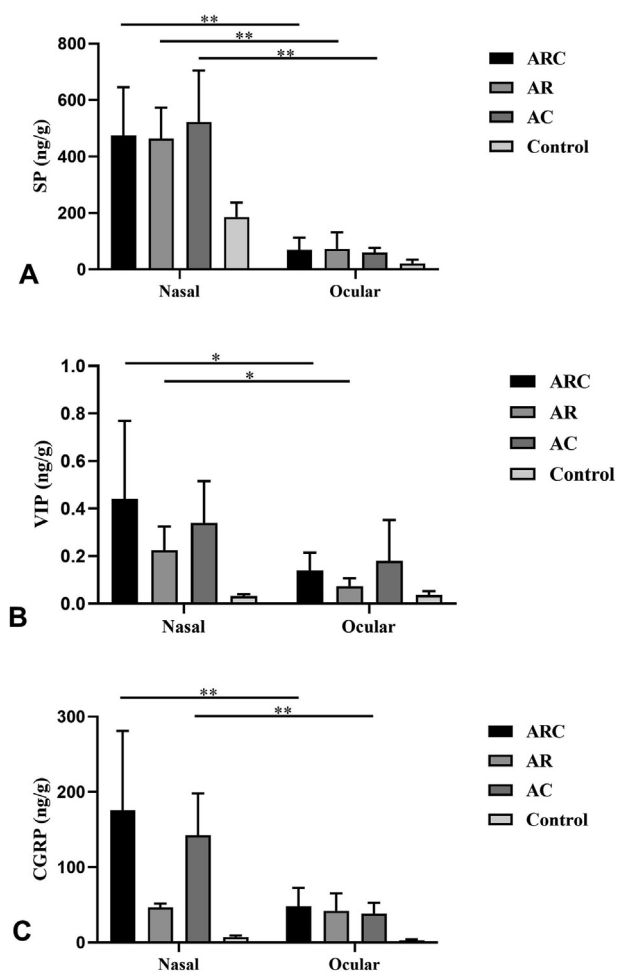


Fig. 3 Comparison between nasal secretions and tears with respect to the level of SP (A), VIP (B), and CGRP (C) in patients with ARC, AR, AC, and healthy control. SP: substance P, VIP: vasoactive intestinal peptide, CGRP: calcitonin gene-related peptide, ARC: allergic rhinoconjunctivitis, AR: allergic rhinitis, AC: allergic conjunctivitis

HCs.¹³ In addition, SP was found in nasal secretions following allergen challenge, which suggested that SP is released in humans during allergic reactions.¹² In turn, the level of SP in tears is increased together with the induction of a conjunctival allergic reaction following conjunctival (ocular) allergen provocation; moreover, increased tear and plasma SP levels are observed in patients with vernal keratoconjunctivitis and increased tear SP levels are evident in patients with seasonal AC.⁸ Similarly, SP was found to influence tear secretion and goblet cell function in rats by inducing mucus secretion and increasing vascular permeability,²³ which suggested that SP might contribute to the pathogenesis and severity of AC. Animal experiments also showed that SP

released by sensory nerves can selectively activate the secretory function of goblet cells,²⁴ and can induce mast cells to release histamine, promote eosinophil migration, and stimulate T cell proliferation.^{25,26}

VIP is secreted by parasympathetic nerves and previous studies have shown that the mRNA levels of VIP receptors (*VPAC1R* and *VPAC2R*) were upregulated in the nasal mucosa of patients with AR compared with those having normal nasal mucosa.²⁵ In the present study, VIP levels in nasal secretions and tears were significantly higher in the ARC, AR, and AC groups than that of the HC group and the VIP level in nasal secretion and tear was positively correlated with the VAS score in the ARC patients. These results are consistent with previous studies, which indicated that increased expression of VIP receptors may underlie nasal hyper-responsiveness in patients and that locally-released VIP might participate in modulating the allergic response of the ocular surface.^{8,27} In addition, a previous study indicated that increased VIP receptor expression on lacrimal gland cells results in the secretion of water through increased cAMP intracellular levels,²³ which is in line with our observed correlation between VAS and SP level. Alternatively, it was previously shown that the levels of VIP in tears did not differ significantly between patients with dry eye and HCs.²⁸ Therefore, the role of VIP in allergic diseases requires further investigation.

In a study of respiratory allergic diseases, it was found that CGRP can act as a chemokine and recruit CD4+T cells, CD8+T cells, eosinophils, and dendritic cells, causing proliferation of the respiratory epithelium.²⁹ In the present study, we found that CGRP levels in tears were significantly higher in the ARC, AC, and AR groups than in the HC group, and those in nasal secretions were significantly higher in the ARC and AC groups than in the AR group. In comparison, a previous study found that allergic airway inflammation significantly increased the numbers of CGRP-expressing neurons in the jugular-nodose ganglion complex in comparison to those of controls.⁸ Similarly, another study suggested that the CGRP level of patients with hay fever was significantly higher than that in HCs,³⁰ which is in accordance with the results of the present study. Moreover, levels of CGRP in tears are decreased in patients

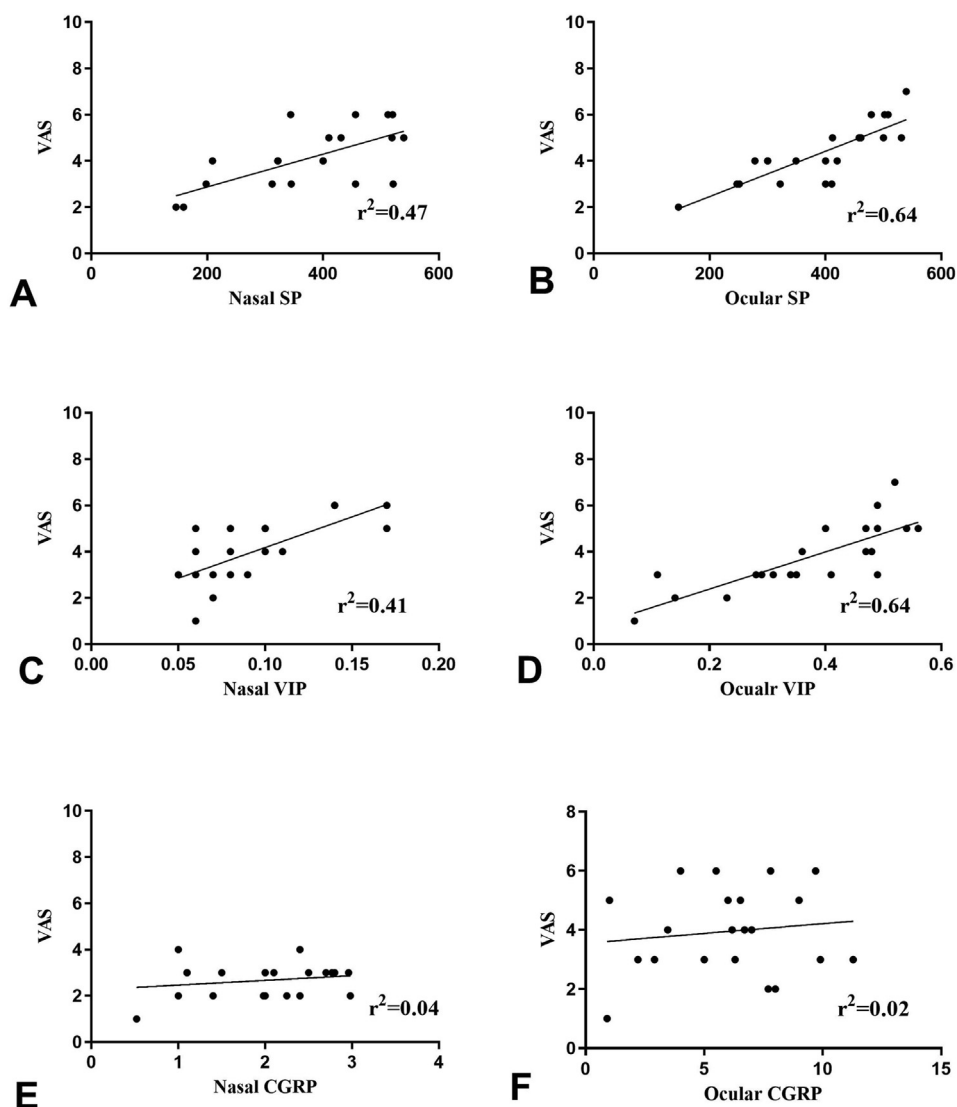


Fig. 4 Correlation between VAS (the average of the total scores of itching and ocular itching) and level of SP (A, B), VIP (C, D), and CGRP (E, F) in of nasal secretions and tears of patients with ARC. VAS: visual analogue scale, SP: substance P, VIP: vasoactive intestinal peptide, CGRP: calcitonin gene-related peptide, ARC: allergic rhinoconjunctivitis

with dry eye, whereas they increased following topical instillation of cyclosporine,²⁸ which indirectly confirms that CGRP plays an important role in the regulation of ocular disease. Contrasted to SP and VIP, the nasal and tear levels of CGRP was not correlated with the VAS score in the ARC patients. This was interesting because CGRP was a kind of co-transmitter with SP.⁸ This result might be due to 2 following reasons: firstly, the sample size in the present study was small ($n = 21$); secondly, the concentration of the allergen caused the patients allergy was not high enough. Based on an earlier study in AR patients, the CGRP level did not increase significantly after histamine challenge

with concentration of 1000 AU/ml for 3 min. However, when the histamine concentration was higher than 10,000 AU/ml, the CGRP level significantly increased.¹²

Together, these findings suggest that a two-way interaction exists between nasal and ocular allergies; ie, nasal allergies alone can simultaneously lead to increased ocular neurotransmitter levels, and ocular allergies alone can also lead to increased nasal neurotransmitter levels. This differs from the theory of naso-ocular interaction, which states that AR can have an effect on the eye with a unidirectional mechanism of action.³¹ However, for patients with single-site allergy, the significant

increase of neurotransmitters at another site may not cause symptoms. For example, a study by McCary et al.³² found that SP could down-regulate the expression of high-affinity IgE receptor (FcεRI) mRNA and protein on the surface of human mast cells and lead to decreased mast cell activation. However, in mast cells that are activated by IgE-mediated mechanism to the action of SP, the expression of FcεRI does not decrease in response to SP. This indicates that in the case of non-allergy, SP can inhibit the action of mast cells. Nevertheless, further studies are needed to explore the underlying causes of this phenomenon.

Notably, the levels of SP, VIP, and CGRP were generally significantly higher in the nasal secretions when compared between nasal and ocular samples. These results indicated that nasal rather than ocular neurotransmitters and neuropeptides might have a more important role in the mechanisms underlying ARC. Consistent with this, the majority of patients were first evaluated in the Otolaryngology Department. It might also be speculated that the nasal symptoms and associated adverse effects on work and daily life in patients with ARC are more severe than those from ocular symptoms.

CONCLUSIONS

ARC is an allergic disease of the nose and eyes that affects a large population worldwide. The cross talk and reciprocal regulation between neuroimmune interactions and neuropeptides play important roles in the pathogenesis of allergic diseases. Owing to the proximity of the anatomical location of the nose and eye, a close connection exists between its vascular- and neuro-anatomy. Our findings suggested that SP, VIP, and CGRP, in particular, play important roles in the mechanism of ARC and that nasal neurotransmitters and neuropeptides might have more important roles in allergic diseases than those of ocular origin. The results might provide a new target for the treatment of nasal-ocular allergic diseases in the future.

Abbreviations

SP, substance P; VIP, vasoactive intestinal peptide; CGRP, calcitonin gene-related peptide; ARC, allergic rhinoconjunctivitis; AC, allergic conjunctivitis; AR, allergic rhinitis; HC, healthy control; SIgE, specific immunoglobulin E; ARIA, Allergic Rhinitis and its Impact on Asthma; VAS, visual analogue scale.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Consent for publication

All authors agreed to publication of the work.

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Ethics approval

The study was approved by the Medical Ethics Committee of Beijing TongRen Hospital (version 1.0). All patients provided written informed consent before enrollment and data collection.

Declaration of competing interest

The authors report no competing interests.

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