

Females have stronger neurogenic response than males after non-specific nasal challenge in patients with seasonal allergic rhinitis

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ABSTRACT

Epidemiological studies show female predominance in the prevalence of non-allergic rhinitis (NAR) and local allergic rhinitis (LAR). Experimental studies show female patients with allergic rhinitis (AR) demonstrate higher levels of sensitivity to irritants and airway hyperresponsiveness than males. Bronchial asthma shows female predominance in post-puberty patients, and gender interaction with severe asthma endotypes. Fibromyalgia, chronic fatigue syndrome, migraine and chronic cough, syndromes, which are commonly related to neurokinin substance P (SP) in the literature, also show strong female predominance. Studies have demonstrated that sex hormones, primarily oestrogens, affect mast cell activation. Mast cell proteases can amplify neurogenic inflammatory responses including the release of SP.

Based on human epidemiological data and animal experimental data we hypothesized that female patients have different interaction between mast cell activation and neurogenic inflammation, i.e. substance P release, resulting in a different nasal symptom profile.

To test the hypothesis we performed allergen and non-specific nasal challenges in patients with seasonal allergic rhinitis (SAR) out of season and looked for gender differences in subjective and objective responses. The interaction between subjective and objective reactivity was evaluated through the comparison of subjective symptom scores, concentrations of neurokinin substance P (SP) and cellular markers in nasal lavages after low doses of nasal allergen challenges.

Female allergic subjects tended to have higher substance P (SP) concentrations both before and after non-specific challenges. The difference between post-allergen and post-hypertonic saline (HTS) challenge was highly significant in female patients ($p = 0.001$), while insignificant in male subjects ($p = 0.14$). Female patients had significantly stronger burning sensation after HTS challenge than male. These data indicate difference in the interaction between inflammatory cells and the neurogenic response, which is gender-related, and which may affect symptom profiles after challenges.

Different regulation of neurogenic inflammation in females may have impact on symptoms and endotyping in respiratory disorders, not only in allergic rhinitis, but also asthma, chronic rhinosinusitis and irritant-induced cough.

Introduction

Nasal hyperreactivity is an increased sensitivity of the nasal mucosa characterized by symptomatic response to otherwise harmless physical and chemical stimuli: changes in temperature, humidity and exposure to strong odors or irritants. It is a common feature of allergic (AR) and non-allergic rhinitis (NAR) [1]. Patients with allergic rhinitis who have a significant burden of reactivity to environmental triggers are usually

diagnosed as mixed rhinitis [2]. Patients with perennial nasal symptoms or symptoms triggered by irritants who have no evidence of sensitization to common aeroallergens, are usually diagnosed as non-allergic rhinitis (NAR), although some of them may suffer from local allergic rhinitis (LAR) [3].

The non-specific hyperreactive response in AR is often considered to be related to the severity of IgE mediated inflammatory response after allergen exposure. However, it may be present also in the absence of

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Table 1
Demographics of the patients in the study.

	Male	Female	All
No SAR patients	12	14	26
Age	38.41 ± 12.4	35.64 ± 9.49	36.92 ± 10.8
Ragweed allergic	5	11	16
Grass allergic	7	3	10
Mean ± S.D. ragweed specific IgE (I.U.)	22 ± 30.2	7.92 ± 9.54	12.57 ± 18.69
Mean ± S.D. grass specific IgE (I.U.)	4.5 ± 8.6	2.47 ± 1.96	3.93 ± 7.16

Demographics of the patients in the study. Distribution of the data related to sensitization and specific IgE with the gender distribution is presented in the table. S.D. – standard deviation.

allergen, as in patients with seasonal allergic rhinitis (SAR) out of season, where it is possibly a result of minimal persistent inflammation [4]. A marked late phase response and nasal eosinophilia were often blamed in the literature for enhancing nasal hyperreactivity [5]. However, in the early phase reaction, minutes after contact with allergen, and following degranulation of the mast cells, stimulation of sensory nerves occurs, resulting in the release of neuropeptides and neurokinins. They initiate neural responses characterized by cholinergic reflexes (lacrimation, sneezing, cough) and transitional receptor potential (TRP) channel activation, followed by substance P (SP) release (itch, burning sensation). It was demonstrated that patients with non-allergic rhinitis have transitional receptor potential vanilloid 1 (TRPV1) /SP pathway up-regulation, and have a good response to topical capsaicin treatment [6]. Such a phenomenon was not demonstrated in patients with mixed rhinitis, and no benefits of topical capsaicin were demonstrated in a randomized placebo controlled trial [7], suggesting that the mechanism is different.

Epidemiological studies have indicated a female predominance in the prevalence of both NAR and LAR [8,3]. Experimental studies show female AR subjects demonstrate higher level of airway hyperresponsiveness [9] and sensitivity to irritants than males [10,11]. High nasal responsiveness to a broad range of environmental triggers and irritants may interact with multiple chemical sensitivity (MCS). MCS includes non-immune mediated response to a contact with low concentrations of variable chemical compounds, which may induce a broad range of vague and non-specific symptoms, including those related to the airways. This disorder is more common in females, but also in allergic and asthmatic individuals.

Bronchial asthma shows female predominance in post-puberty patients, and gender interaction with severe asthma endotypes. Fibromyalgia, chronic fatigue syndrome, migraine, depression and chronic cough, syndromes, which are commonly related to stress exposure and neurokinin substance P (SP) in the literature, also show strong female predominance [12]. Studies have demonstrated that sex hormones, primarily oestrogens, affect mast cell activation [13]. Mast cell proteases can amplify neurogenic inflammatory responses, including the release of SP, probably by the activation of protease activated receptors 2 (PAR-2). Trypsin from the activated mast cells interacts with neural PAR-2 in skin, gut and airways which leads to the release of SP and calcitonin gene related peptide (CGRP), promoting neurogenic inflammation. However, trypsin and chymase from the mast cells may also prevent or decrease neurogenic inflammation by degrading excessive neuropeptides, acting as a bio-feedback mechanism [14].

It is not clear whether gender differences in the prevalence and severity of non-specific airway hyperreactivity reflect the difference in neuro-allergic response in challenge tests.

Hypothesis. Based on human epidemiological data and animal experimental data we hypothesized that female patients have different interaction between mast cell activation and neurogenic inflammation, i.e. substance P release, resulting in a different nasal symptom profile. We have attempted to explore these areas in a

preliminary study which includes gender comparison of the severity of neurogenic inflammation and inflammatory cell activation after allergen and non-specific challenges in patients with isolated SAR out of season, to avoid priming and perennial allergen exposure.

Subjects and methods

After signing informed consent forms, patients who were referred to the Rhinology diagnostic laboratory in the University Hospital Centre “Sestre milosrdnice”, Zagreb School of Medicine, for the evaluation of their nasal hyperreactivity, were recruited for the study. The study was approved by the Ethics Committee of the University Hospital Centre “Sestre milosrdnice”, Zagreb School of Medicine.

The subjects consisted of 26 grass and/or ragweed pollen allergic volunteers. Demographics of the patients is shown in Table 1. Inclusion criteria were: the history of AR symptoms in at least two past pollen seasons, skin prick test positivity to either grass or ragweed pollen and/or serum specific IgE to the respective allergen. Patients with sensitization to perennial allergens, acute rhinitis, chronic rhinosinusitis, nasal polyps, bronchial asthma, significant septal deformity, and those who were taking topical or systemic medication in the past 4 weeks which may interfere with the response to provocation were excluded. Patients who suffered from non-specific nasal hyperreactivity to environmental stimuli during and out of season, or throughout the year, were not excluded.

Recruited patients filled in a questionnaire on their history during past pollen seasons and visual analog scale (VAS) for nasal and ocular symptoms. They also filled in the irritant index scale questionnaire.

On Day-1, 500B.U. per nostril in 0.2 ccm of aqueous solution with lyophilized allergen (Croatian Immunological Institute, Zagreb, Croatia), either the specific grass, according to SPT (Phleum pratense, Dactylis glomerata) or ragweed pollen (Ambrosia elatior), respectively, were applied to both inferior turbinates with micropipette. On the next day 80 mcg of histamine was applied per nostril in the same fashion. More than 48 h after histamine challenge, nasal provocation was done with 2 ml of 2% hypertonic saline (HTS). Before allergen, and HTS provocation, visual analog scale (VAS) subjective scores of nasal and ocular symptoms and baseline nasal lavage were done. VAS scores, and nasal lavage were done 15 min after each challenge.

Evaluation of nasal and ocular symptoms was done using a VAS questionnaire. Symptoms evaluated were: nasal obstruction, hypersecretion, nasal itch, burning sensation in the nose, ocular itch and lacrimation.

Nasal lavage and mediator analysis

Nasal lavage was performed 15 min after each challenge with 3 ccs of warmed saline per nostril. The subjects were seated with the head extended 30 degrees from horizontal, abstaining from breathing and swallowing. After 20 s the patients expelled the lavage into the container which was kept on ice until completion of the experiment. The recovery of fluid was 50–80%. The lavages were centrifuged at 1600 × g

for 10 min (Heraeus Sepatech, Verifuge 3.OR). The supernatant was kept at -80°C until analysis.

Concentrations of tryptase and eosinophil cationic protein (ECP) were analysed with fluoro- enzyme immunoassay using ImmunoCAP technology (Phadia AB, Uppsala, Sweden on UniCAP 100). The sensitivity of the tryptase assay was $< 1\text{ mcg/mL}$ and ECP $2\text{--}200\text{ mcg/mL}$.

Substance P levels were measured in nasal lavage using a competitive enzyme-linked immunoabsorbance assay with sensitivity to 3.9 pg/mL (Cayman, MI, USA).

Standard curves were done for each assay. Values were corrected for the fraction recovered.

Statistical analysis

The distribution of the data was calculated using the Kolmogorov-Smirnov test. Statistical analysis was performed depending on the normality of the distribution by either the paired *t*-test or the Wilcoxon signed rank test to compare pre- and post provocation total VAS nasal and ocular symptoms scores, concentrations of substance P, tryptase and ECP.

The Mann-Whitney *U*-test was then used for between gender comparison and correlations were tested using the Spearman rank correlation test. The Friedman test for related samples was used to compare the responses to different provocations in the same individuals, and post hoc comparison was done with Wilcoxon Sign rank test. All conclusions were based on a significance level of $p < 0.05$.

Results

Symptom scores

Patients had a significant increase in all individual nasal and ocular symptoms in VAS after allergen and histamine challenge, but not after HTS. The change in VAS scores after challenges for almost all symptoms tended to be higher in female patients, however, the difference was significant only for burning sensation in the nose after HTS challenge (Table 2).

Substance P levels

Fig. 1 shows gender differences in SP concentrations in SAR subjects' nasal lavages at baseline, 15 min after allergen and 15 min after 2% HTS: female patients tended to have higher SP concentrations both before and after challenges. This was significant post-HTS, $p = 0.03$.

Table 2

Changes in VAS scores after different challenges.

		VAS 0–10		ALLERGEN		HISTAMINE		HTS	
				Mean	S.D.	Mean	S.D.	Mean	S.D.
Δ VAS obstruction	SARM			1.71	1.50	1.43	0.98	1.14	1.46
	SARF			3.27	4.69	2.09	1.14	0.91	1.81
Δ VAS secretion	SARM			1.71	1.11	1.57	1.27	1.57	1.72
	SARF			4.36	4.01	2.91	1.64	2.64	2.77
Δ VAS nose itch	SARM			0.71	1.38	0.71	0.76	0.71	0.95
	SARF			3.18	3.09	1.91	2.81	1.73	2.20
Δ VAS nose burning	SARM			1.43	1.40	1.71	1.50	0.5714	0.79
	SARF			2.73	3.47	1.45	1.63	3.55*	2.42
Δ VAS eye itch	SARM			1.57	1.81	2.14	1.95	1.43	1.62
	SARF			1.73	1.95	1.18	2.48	0.45	1.37
Δ VAS lacrimation	SARM			1.86	1.86	1.57	2.07	1.00	1.00
	SARF			3.64	3.20	4.00	3.32	3.64	3.56

Changes in VAS scores after different challenges presented as mean and standard deviation, separately showing SAR male (SARM) and SAR female (SARF) score differences. Although the mean responses to allergen were consistently higher in females the only statistically significant gender-related difference was the change in nose burning sensation after HTS challenge (*: $p = 0.04$).

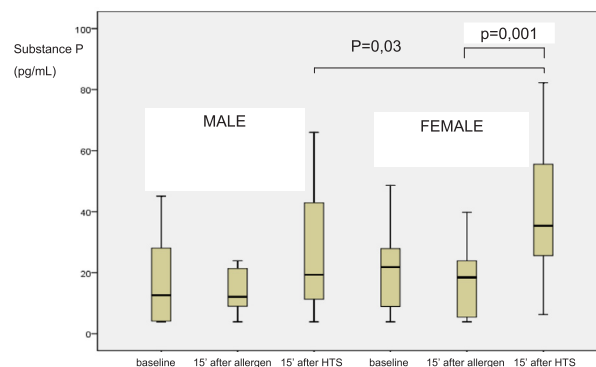


Fig. 1. Concentrations of substance P at baseline, 15 min after allergen and after hypertonic saline challenges, presented as box plots (median and 75% confidence intervals), separately for male and female, respectively. The difference in concentrations of SP after allergen and hypertonic saline (HTS) is significantly higher in female, but not in male. The concentration of SP after HTS challenge is significantly higher in females.

As shown in the figure, there is a highly significant difference in females when SP concentrations are compared after allergen and HTS, not shown in male subjects.

In the SAR group SP 15 min after allergen correlated significantly with that 24 h later ($\rho = 0.62$, $p = 0.004$), and with SP concentrations after histamine ($\rho = 0.75$, $p = 0.0001$) and HTS ($\rho = 0.47$; $p = 0.04$).

Allergic mediators

Tryptase was significantly higher in post-provocation samples after allergen compared to baseline tryptase concentrations (which were 0 mcg/mL). Gender differences in tryptase concentrations are not significant (Table 3).

ECP concentration 15 min after allergen was significantly higher than in baseline lavage. There was no statistically significant gender-related difference in mediator release (Table 3).

Discussion

There is epidemiological, clinical and biological evidence supporting gender differences in prevalence, clinical severity and inflammatory cell activation in allergic diseases. There is a clear female predominance in the prevalence of allergic respiratory diseases after puberty, opposite to its prevalence before puberty. Gender differences in the pattern and severity of allergic symptoms, (including respiratory, food allergy and anaphylaxis), were demonstrated clinically and in animal experiments. Most of the research results suggest a role for estrogen and its receptors on inflammatory cells involved in the allergic reaction [13]. There is evidence in cultivated mouse bone marrow mast cells that estradiol, through estrogen receptor alpha, induced mast cell degranulation (*per se*) and also enhanced IgE mediated mast cell degranulation, resulting in a shift in allergen dose response [15].

Our results, albeit in a small sample did not demonstrate any significant difference between the sexes in VAS scores, nor in the release of tryptase (a mast cell mediator) or ECP (eosinophil-related) post extra-seasonal allergen challenge.

On the other hand, similar gender differences have been found in the prevalence of non-allergic rhinitis and in sensitivity to airway irritants and strong odours [8]. Females are also overrepresented in pain conditions involving neurogenic inflammation. Oestradiol mediates different substance P release after subcutaneous formalin injection in male and female rats [16]. Gender difference was found in the level of inflammatory response, measured by plasma extravasation, after

Table 3
Cellular markers at baseline and after challenges.

	Tryptase mean (mcg/L)	S.D.	Range		ECP Mean (mcg/L)	SD	Range
SAR baseline	0	0	0	SAR baseline	4.69	3.61	2–13
SAR allergen all	5.06*	10.66	0–45.3	SAR allergen all 15 m	16.479*	23.15	2–75
SAR allergen male	4.70*	7.57	0–9.4	SAR allergen male 15 m	15.82	20.26	2–54
SAR allergen female	8.14*	14.85	0–45.3	SAR allergen female 15 m	16.47	23.15	2–75
SAR histamine all	0.21	0.50	0–1.6	SAR 24 h all	12.80	22.47	2–96.4
SAR histamine male	1.58	2.86	0–1.6	SAR 24 h male	20.74	32.01	2–96.4
SAR histamine female	0.00	0.00	0	SAR 24 h female	6.84	9.11	2–35.2
SAR HTS all	0.44	1.04	0–3.7	SAR HTS all	26.97	63.61	2–34.6
SAR HTS male	0.41	1.23	0–3.7	SAR HTS male	14.44	17.44	2–56.7
SAR HTS female	0.50	0.96	0–2.92	SAR HTS female	15.21	16.97	2–52.7

Data for tryptase at baseline and after challenges, and for ECP at baseline, 15 min and 24 h after allergen, and 15 min after histamine and HTS in SAR. There was no significant difference in tryptase nor in ECP release between males and females. Abbreviations: ECP = eosinophil cationic protein; SD = standard deviation. SAR = seasonal allergic rhinitis.

* $p < 0.05$. The differences in tryptase and ECP concentrations are significant only compared to baseline concentrations, but not between male and female samples.

capsaicin and heat, but not direct substance P application. These stimuli evoked stronger inflammatory responses in female rats, dependent on the sex cycle [17]. It was suggested that estrogens may up-regulate TRPV-1 expression in rats [18]. Estrogen receptors are also present on trigeminal ganglia. Estradiol stimulates prolactin which increases phosphorylation of the nociceptor transducer (TRPV1) [19,20]. It reduces not only TRPV1, but also TRPA1 and TRPM8 activation thresholds [21]. Progesterone also increased the receptor for neural growth factor (NGF) in an in vitro animal experiment [22]. Activation of NGF through this receptor increased membrane expression of TRPV1 [23]. To summarize, all female hormones (estrogen, prolactin, progesterone) through their receptors interact with the activation of TRP sensory proteins. Most of these animal experiments indicate the interaction of neurogenic inflammation and female steroid hormones and help to explain upregulated pain perception in females.

Our results do indicate a tendency for stronger SP release in female SAR patients after saline lavage (i.e. at baseline) and after histamine and HTS challenge, however, only hypertonic saline challenge resulted in a statistically significant difference. The only challenge after which no gender difference in SP release was noted was the allergen challenge.

The main striking gender difference is in SP concentrations after allergen and HTS challenges, which shows a highly significant increase after HTS challenge, compared to the concentration after allergen, in females ($p = 0.001$), but not in males ($p = 0.14$). The one symptom possibly related to the increased SP concentrations in nasal lavage after HTS challenge in females compared to males, was the increase in the burning sensation. Neurokinins like SP give burning or pain in the nose. However, there was no correlation between subjective scores for burning sensation and SP concentrations in nasal secretions.

The SP release after histamine and HTS challenge is the immediate neural reflex response, after histamine in the primed patients, and after HTS due to C-fibre stimulation with hyperosmolar challenge. It was previously reported that histamine challenge does not lead to SP release, whilst allergen does, however, in that experiment histamine challenge was not done in allergen-primed patients [24]. In general, interaction between neurogenic inflammation and mast cell degranulation based on different stimuli may be different in different target organs, like skin, gut, nose or bronchi. On the other hand, it may be also difference in sampling methods and timing of collecting samples. SP may induce histamine release from mast cells in the skin; it was not demonstrated in the nose after in vivo SP challenge [25], but it was demonstrated in vitro from the nasal mucosal samples of AR and NAR patients [26]. Antihistamine leads to reduced SP release after allergen challenge in PAR patients [27]. Nasal histamine challenge in SAR patients does not lead to increase in SP concentrations in nasal lavage, even if samples were preserved with protease inhibitors, but after allergen challenge SP concentrations increase significantly [24].

There is also a difference between gender representation in phenotypes of chronic rhinosinusitis. Females are dominant in the phenotype without polyps (CRSsNP), while males are predominant in phenotype with polyps (CRSwNP). In CRSsNP pain syndromes (facial pain, headache) and chronic fatigue are dominant symptoms, and are more highly expressed in female patients. CRSwNP phenotype has more asthmatic comorbidity and higher inflammation. Dominant symptoms are nasal obstruction and smell disorders, while pain is significantly lower than in CRSsNP phenotype, despite strong inflammation in nasal polyps [28]. It has been shown that CRSwNP patients release fewer neurokinins than controls and patients with allergic rhinitis [29]. The authors hypothesized that decreased release of neuropeptides may be involved in the pathogenesis of nasal polyps. Gender differences were established in the endotypes of asthma, where a specific cluster was formed by obese females, with high symptoms and low eosinophilic inflammation [30]. Substance P has also been linked to obesity. There is an experimental evidence of a beneficial effect of SP antagonists in obesity and asthma [31].

Although epidemiological data are sometimes controversial regarding gender prevalence, fibromyalgia, chronic fatigue syndrome, migraine and chronic irritant cough, also show female predominance. These disorders are commonly related to neurokinin substance P (SP) in the literature, although its role is not clearly proven [32–34]. There may be also a central role in gender bias related to persisting pain. The possible pathway may also be primed spinal glia and subsequent neurogenic inflammation, which may act as a contributing factor leading to peripheral inflammation. It may be a possible predisposing factor for females in developing persistent pain conditions [35].

Although the data are controversial, it seems that there is also a female predominance in MCS. This condition is often related to voltage gated ligand channels (like TRPV1 and TRPA1), although there is no current evidence for this interaction. TRPV1 is a ligand-gated cation channel which responds to heat, protons, and irritants. It is expressed on sensory neurons and plays a major role in pain associated with tissue damage, inflammation, and nerve lesions, as mentioned previously [36]. Transient receptor potential ankyrin 1 (TRPA1) is coexpressed with TRPV1. TRPV1 overexpression is found in patients with non-allergic rhinitis, but not in patients with allergic rhinitis and controls [6]. Although patients with allergic rhinitis also experience non-specific hyperreactivity to irritants and chemicals, which are subjectively evaluated in the Cincinnati irritant index scale [37], patients with NAR have higher scores in irritant index scale than patients with SAR out of season, but lower than patients with severe PAR (our own data unpublished). It is not clear whether patients with MCS have overexpression of TRP sensory proteins, like patients with NAR. However, higher prevalence of MCS was found in patients with allergic disease, however, only in the younger age group (30–40 years) [38]. On the other hand, MCS patients show inflammatory profile closer to Th1 than

Th2 cytokines (IL-2 and IL-4 upregulated, IL-13 downregulated) [39].

The major weakness of our research is that we analysed several subjective and objective parameters in a relatively small sample in a highly selected group of patients. However, we regard this as a pilot experiment showing a probable gender difference related to neurogenic inflammation, involving SP release significantly more expressed in females with rhinitis, which is worth further exploration.

The biggest obstacle in the research on substance P release in the clinical setting is its instability, sensitivity to degradation by proteases released by inflammatory cells in allergy and infection and easy contamination of the samples, as SP is ubiquitous. Our pilot experiment indicated that sex hormones should be taken into account (estrogen levels, time in the cycle, age), protease inactivation with inhibitors should be controlled to measure the actual SP concentration after its release, but inflammatory cells protease concentration may be altered if using protease inhibitors. Despite our small sample, and not taking into account hormone levels, we have managed to show that females release more substance P after hypertonic saline. Our data need confirmation in an improved experimental model, on a larger sample, and if proven, may be used to explain gender differences in the symptom profile of allergic (and maybe non-allergic) upper respiratory disorders.

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Conflict of interest statement

All of the authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mehy.2018.04.021>.

References

- [1] Segboer CL, Holland CT, Reinartz SM, et al. Nasal hyper-reactivity is a common feature in both allergic and nonallergic rhinitis. *Allergy* 2013;68(11):1427–34.
- [2] Bernstein JA, Levin LS, Al-Shuik E, Martin VT. Clinical characteristics of chronic rhinitis patients with high vs low irritant trigger burdens. *Ann. Allergy Asthma Immunol.* 2012;109(3):173–8.
- [3] Rondón C, Campo P, Galindo L, et al. Prevalence and clinical relevance of local allergic rhinitis. *Allergy* 2012;67(10):1282–1288.
- [4] Canonica GW, Compalati E. Minimal persistent inflammation in allergic rhinitis: implications for current treatment strategies. *Clin. Exp. Immunol.* 2009;158(3):260–71.
- [5] de Graaf-in't Veld C, Garrelds IM, van Toorenbergen AW, Gerth van Wijk R. Nasal responsiveness to allergen and histamine in patients with perennial rhinitis with and without a late phase response. *Thorax* 1997;52(2):143–8.
- [6] Van Gerven L, Alpizar YA, Wouters MM, et al. Capsaicin treatment reduces nasal hyperreactivity and transient receptor potential cation channel subfamily V, receptor 1 (TRPV1) overexpression in patients with idiopathic rhinitis. *J. Allergy Clin. Immunol.* 2014;133(5):1332–9.
- [7] Gerth Van Wijk R, Terreehorst IT, Mulder PG, Garrelds IM, Blom HM, Popering S. Intranasal capsaicin is lacking therapeutic effect in perennial allergic rhinitis: a house dust mite. A placebo-controlled study. *Clin. Exp. Allergy* 2000;30(12):1792–8.
- [8] Settiani RA. Epidemiology of vasomotor rhinitis. *World Allergy Organ. J.* 2009;2(6):115–8.
- [9] Leynaert B, Bousquet J, Henry C, Liard R, Neukirch F. Is bronchial hyperresponsiveness more frequent in women than in men? A population-based study. *Am. J. Respir. Crit. Care Med.* 1997;156(5):1413–20.
- [10] Shusterman D, Murphy MA, Balmes J. Differences in nasal irritant sensitivity by age, gender, and allergic rhinitis status. *Int. Arch. Environ. Health* 2003;76(8):577–83.
- [11] Andersson L, Lundberg C, Astrom J, Nordin S. Chemosensory attention, habituation and detection in women and men. *Int. J. Psychophysiol.* 2011;79:316–22.
- [12] Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. *Front. Neuroendocrinol.* 2014;35(3):303–19.
- [13] Chen W, Mempel M, Schober W, Behrendt H, Ring J. Gender difference, sex hormones, and immediate type hypersensitivity reactions. *Allergy* 2008;63(11):1418–27.
- [14] Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol. Rev.* 2007;217:141–54.
- [15] Zaitis M, Narita S, Lambert KC, et al. Estradiol activates mast cells via a non-genomic estrogen receptor- α and calcium influx. *Mol. Immunol.* 2007;44(8):1977–85.
- [16] Nazarian A, Tenayuca JM, Almasarweh F, Armendariz A, Are D. Sex differences in formalin-evoked primary afferent release of substance P. *Eur. J. Pain.* 2014;18(1):39–46.
- [17] Carmichael NM, Charlton MP, Dostrovsky JO. Sex differences in inflammation evoked by noxious chemical, heat and electrical stimulation. *Brain Res.* 2009;18:103–11.
- [18] Yamagata K, Sugimura M, Yoshida M, et al. Estrogens exacerbate nociceptive pain via up-regulation of TRPV1 and ANO1 in trigeminal primary neurons of female rats. *Endocrinology* 2016;157(11):4309–17.
- [19] Xu S, Cheng Y, Keast JR, Osborne PB. 17 β -estradiol activates estrogen receptor beta-signalling and inhibits transient receptor potential vanilloid receptor 1 activation by capsaicin in adult rat nociceptor neurons. *Endocrinology* 2008;149(11):5540–8.
- [20] Diogenes A, Patwardhan AM, Jeske NA, et al. Prolactin modulates TRPV1 in female rat trigeminal sensory neurons. *J. Neurosci.* 2006;26(31):8126–36.
- [21] Patil MJ, Ruparel SB, Henry MA, Akopian AN. Prolactin regulates TRPV1, TRPA1, and TRPM8 in sensory neurons in a sex-dependent manner: contribution of prolactin receptor to inflammatory pain. *Am. J. Physiol. Endocrinol. Metab.* 2013;305(9):E1154–64.
- [22] Lanlua P, Decorti F, Gangula PR, Chung K, Tagliatalata G, Yallampalli C. Female steroid hormones modulate receptors for nerve growth factor in rat dorsal root ganglia. *Biol. Reprod.* 2001;64(1):331–8.
- [23] Zhang X, Huang J, McNaughton PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *EMBO J.* 2005;24(24):4211–23.
- [24] Mosimann BL, White MV, Hohmann RJ, Goldrich MS, Kaulbach HC, Kaliner MA. Substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide increase in nasal secretions after allergen challenge in atopic patients. *J. Allergy Clin. Immunol.* 1993;92:95–104.
- [25] Braunstein G, Buvry A, Lacroix J, Desjardins N, Frossard N. Do nasal mast cells release histamine on stimulation with substance P in allergic rhinitis? *Clin. Exp. Allergy* 1994;24(10):922–9.
- [26] Hanf G, Schierhorn K, Brunnée T, Noga O, Verges D, Kunkel G. Substance P induced histamine release from nasal mucosa of subjects with and without allergic rhinitis. *Inflamm. Res.* 2000;49(10):520–3.
- [27] Shirasaki H, Watanabe K, Kanaizumi E, et al. Effects of cetirizine on substance P release in patients with perennial allergic rhinitis. *Ann. Otol. Rhinol. Laryngol.* 2004;113(12):941–5.
- [28] Gregurić T, Trkulja V, Baudoin T, Grgić M, Šmigovec I, Kalogjera L. Differences in the sino-nasal outcome test 22 and visual analog scale symptom scores in chronic rhinosinusitis with and without nasal polyps. *Am. J. Rhinol. Allergy* 2016;30(2):107–12.
- [29] Gungor A, Baroody FM, Naclerio RM, White SR, Corey JP. Decreased neuropeptide release may play a role in the pathogenesis of nasal polyps. *Otolaryngol. Head Neck Surg.* 1999;121(5):585–90.
- [30] Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE. Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* 2008;178:218–24.
- [31] Ramalho R, Almeida J, Beltrão M, et al. Substance P antagonist improves both obesity and asthma in a mouse model. *Allergy* 2013;68(1):48–54.
- [32] Evengard B, Nilsson CG, Lindh G, et al. Chronic fatigue syndrome differs from fibromyalgia. No evidence for elevated substance P levels in cerebrospinal fluid of patients with chronic fatigue syndrome. *Pain* 1998;78(2):153–5.
- [33] Lim KG, Rank MA, Kita H, Patel A, Moore E. Neuropeptide levels in nasal secretions from patients with and without chronic cough. *Ann. Allergy Asthma Immunol.* 2011;107(4):360–3.
- [34] Silberstein SD. TRPV1, CGRP and SP in scalp arteries of patients suffering from chronic migraine. Some like it hot! Chronic migraine increases TRPV1 receptors in the scalp. *J. Neurol. Neurosurg Psychiatry* 2015 Apr;86(4):361.
- [35] Dodds KN, Beckett EA, Evans SF, Grace PM, Watkins LR, Hutchinson MR. Glial contributions to visceral pain: implications for disease etiology and the female predominance of persistent pain. *Transl Psychiatry.* 2016 13;6(9):e888.
- [36] Bessac BF, Jordt SE. Breathing TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda)* 2008 Dec;23:360–70.
- [37] Bernstein JA. Characteristics of Nonallergic Vasomotor Rhinitis WAO Journal 2009; 2:102–105.
- [38] Jeong I, Kim I, Park HJ, Roh J, Park JW, Lee JH. Allergic diseases and multiple chemical sensitivity in Korean adults. *Allergy Asthma Immunol. Res.* 2014;6(5):409–14.
- [39] Dantoft TM, Elberling J, Brix S, Szecsi PB, Vesterhauge S, Skovbjerg S. An elevated pro-inflammatory cytokine profile in multiple chemical sensitivity. *Psychoneuroendocrinology* 2014;40:140–50.