

Serum eosinophil cationic protein level in Chinese subjects with nonallergic and local allergic rhinitis and its relation to the severity of disease

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ABSTRACT

Background: Nonallergic rhinitis (NAR) is a common disease, and local allergic rhinitis (LAR) is a newly described entity that is called "entopy" in recent years. However, the etiology and incidence of NAR and LAR are uncertain. This study evaluates the incidence of LAR in Chinese subjects and serum eosinophil cationic protein (ECP) levels in patients with NAR and LAR and its relationship with symptom scores.

Methods: Positive skin-prick tests (SPT), nasal allergen provocation testing (NAPT) to *Dermatophagoides farinae* (DF), serum Phadiatop tests, and symptom score evaluations were performed in 147 patients with nonatopic persistent rhinitis. LAR was diagnosed by positive NAPT to DF results. The serum ECP level was measured using the UniCAP100 automated system by enzyme-linked immunosorbent assay (Pharmacia Diagnostics, Uppsala, Sweden).

Results: Twelve patients were diagnosed with LAR, and 135 patients were diagnosed with NAR. There was no statistical difference of the visual analog scale scores between the two groups. Serum ECP was significantly increased in the patients with NAR. Serum ECP in the LAR group showed no difference compared with the NAR group or the controls. In the NAR group, nasal obstruction, rhinorrhea, and total scores had close and statistically significant relationships with serum ECP level.

Conclusion: The present study provides the first evidence that LAR exists in Chinese subjects. This study also suggests that eosinophils and ECP take part in the pathogenesis of NAR. A local examination for ECP and specific IgE in nasal lavage fluid or secretions in patients with LAR should be performed in a future study.

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Persistent rhinitis (PR) is a common disease, involving up to 20% of people and increasing gradually each year.¹ It is classically divided into two types: allergic rhinitis (AR) and nonallergic rhinitis (NAR).² Patients with AR manifest nasal obstruction, rhinorrhea, itching, or sneezing; have increased serum-specific IgE (sIgE); and have positive skin-prick tests (SPTs) to relevant allergens.³ Subjects with NAR have the same clinical symptoms as those with AR in the absence of systemic sIgE and are negative for SPT. NAR currently affects ~200 million individuals globally,⁴ and its diagnosis depends on exclusion of AR. NAR can be classified into many subtypes: drug-induced rhinitis, hormone-induced rhinitis, NAR with eosinophilia syndrome, occupational rhinitis, and vasomotor rhinitis.⁵ However, the etiology and pathogenesis in most patients with NAR is unclear.⁶

In recent years, some investigators have detected local allergen sIgE in nasal mucosa and positivity to nasal allergen provocation testing (NAPT) in a number of patients previously diagnosed with NAR and is characterized by a nasal Th2 inflammatory response with local sIgE production and a positive response to NAPT without evidence of atopy.^{7–10} Similarly, the etiology and incidence of local allergic rhinitis (LAR) are uncertain.

Eosinophil cationic protein (ECP), also known as ribonuclease 3, is a single cationic polypeptide chain comprising 133 amino acids.¹¹ It is mainly present in the granules of eosinophils, and small amounts can be found in neutrophils and monocytes. ECP can be detected in serum, plasma, tear fluid, nasal lavage fluid, nasal secretions, saliva, and sputum.¹¹ It exhibits various biological activities and is associated with several pathological conditions, including microbial infections,

allergic inflammation, and tumors. Many clinical studies have been performed to investigate the relationship between ECP and atopic disease. Serum ECP has been taken to be a potential marker of asthma and is associated with disease activity and severity, especially with alterations caused by inhaled corticosteroids.¹² Serum ECP has been less investigated in upper airway disease, including PR. Some authors suggest that serum ECP be used to monitor the efficacy of immune therapy in patients with AR.¹³ In our previous study, serum ECP was elevated in subjects with inhaled AR and had positive correlations with symptom scores.¹⁴ However, it remains controversial. Some studies showed no elevation in serum ECP levels in NAR and LAR, but increased levels of ECP in nasal lavage fluid.⁷ However, few studies have reported serum ECP levels in nonatopic rhinitis.

The purpose of this study was to evaluate the incidence of LAR in Chinese subjects and serum ECP levels in patients with NAR and LAR. To our knowledge, this is the first study on LAR in Chinese patients. The relationship between serum ECP and symptom scores was also investigated.

METHODS

Subjects

This study included a total of 147 patients with nonatopic PR in our hospital from September to November 2011 (91 male and 56 female subjects; aged 9–78 years; mean age, 36.6 years). SPT, NAPT to *Dermatophagoides farinae* (DF), serum Phadiatop tests, and symptom score evaluations were performed in all patients. The diagnosis of NAR was based on clinical symptoms (continuing for >3 months) and negative SPT to prevalent allergens, NAPT to DF, and serum Phadiatop test results. LAR was diagnosed by clinical symptoms, negative SPT and serum Phadiatop test results, and positive NAPT to DF results.

Patients with asthma, acute upper respiratory infections, anatomic nasal disorders, or nasal polyps and those using nasal or oral corticosteroids or decongestants and antihistamines within the previous 4 weeks were excluded.

A control group of 20 healthy nonatopic subjects with negative SPT, serum Phadiatop, and NAPT to DF was included.

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The study was approved by the local Ethics Committee of the First Affiliated Hospital of Zhejiang University. Each subject or parents of the children gave an informed consent.

Skin-Prick Test

The SPT was performed with a wide panel of the most prevalent allergens, including *Dermatophagoides pteronyssinus* (DP), DF, *Blomia tropicalis*, *Artemisia* spp., ragweed, pollens mix I, pollens mix IV, molds mix IV, *Blattella germanica*, the American cockroach, and animal epithelia (dog and cat; ALK-Abelló, Høsholm, Denmark).

Serum Phadiatop Test

The sIgE was measured by the *in vitro* Phadiatop test, performed according to the manufacturer’s instructions (Pharmacia Diagnostics, Uppsala, Sweden) using the UniCAP100 automated system, as described previously.¹⁵ This test includes >95% of aeroallergens. The test gives a qualitative result: either positive or negative according to the amount of fluorescence relative to a reference value. The cutoff value of the sIgE assay was 0.35 kU/L.

Nasal Allergen Provocation Testing

A saline challenge was first performed to exclude nasal hyperactivity. If negative, NAPT to DF was performed after 1 week using allergen solutions of DF with progressive concentrations of 10, 1000, and 100,000 U/mL SQ (ALK-Abelló). A filter paper soaked with the solution was applied to the left inferior turbinate for 10 minutes using a gun-shaped forceps. A positive result was considered to be an elevation of >30% in the total visual analog scale (VAS) and a decrease of 30% in volume at 2–6 cm as determined by acoustic rhinometry, as described previously.¹⁶ We had performed NAPT to DF in 27 patients with AR who were SPT⁺ or sIgE to DF by using this method. All showed positive results.

Symptom Scores

Symptoms of nasal obstruction, rhinorrhea, itching, and sneezing were recorded on a VAS of 100. The total VAS was the sum of the four VAS scores (range, 0–400). A severity graduation of individual symptoms was applied in “mild,” 0–30 mm; “moderate,” 31–70 mm; and “severe,” 71–100 mm.

Serum ECP

The serum ECP level was measured using the UniCAP100 automated system by enzyme-linked immunosorbent assay (Pharmacia Diagnostics). The normal serum ECP range was 4–20 µg/L.

Statistical Analysis

All statistical analyses were performed using the software SPSS for Windows, Version 18.0 (SPSS, Inc., Chicago, IL). Because of the non-normal distribution of parameters, we used *M* (25–75%) to represent the symptom scores and serum ECP levels. Differences in the symptom scores and serum ECP levels between the groups were compared by the rank-sum test, and correlations were evaluated by the Kendall test. A value of *p* < 0.05 was considered statistically significant.

RESULTS

Subjects and NAPT to DF

No patients or healthy individuals had a positive response to the saline challenge. NAPT to DF was negative in all healthy controls. Twelve patients with positive NAPT to DF results were diagnosed with LAR (8.2%; 10 male and two female subjects; mean age, 21.1 years), and 135 patients with negative results were diagnosed with NAR (91.8%; 81 male and 54 female subjects; mean age, 37.9 years).

Table 1 VAS of NAR and LAR patients

	NAR	LAR	Z Value	p Value
Nasal obstruction	50 (40–60)	55 (50–67.5)	–1.228	0.222
Rhinorrhea	50 (40–60)	60 (42.5–70)	–1.352	0.179
Itching	40 (20–60)	40 (20–60)	–0.363	0.724
Sneeze	40 (30–60)	50 (40–57.5)	–0.702	0.489
Total	180 (150–220)	195 (190–232.5)	–1.458	0.147

LAR = local allergic rhinitis; NAR = nonallergic rhinitis; VAS = visual analog scale.

Table 2 The severity graduation of individual symptoms of the NAR

	Mild	Moderate	Severe
Nasal obstruction	31	98	6
Rhinorrhea	22	102	11
Itching	38	92	5
Sneeze	46	82	7

NAR = nonallergic rhinitis.

Table 3 The severity graduation of individual symptoms of the LAR

	Mild	Moderate	Severe
Nasal obstruction	0	12	0
Rhinorrhea	1	9	2
Itching	5	6	1
Sneeze	2	10	0

LAR = local allergic rhinitis.

Table 4 Serum ECP level of the study subjects

Groups	NAR	LAR	Control
Serum ECP (µg/L)	16.3 (9.8–22.5)	11.6 (7.8–15.7)	8.8 (5.4–20.2)
Z value	–1.553*	–0.528#	–2.215§
p value	0.122*	0.617#	0.026§

*The difference between the NAR and control groups was statistically significant.

#The difference between the LAR and control groups was not statistically significant.

§The difference between the NAR and LAR groups was not statistically significant.

LAR = local allergic rhinitis; NAR = nonallergic rhinitis; ECP = eosinophil cationic protein.

Table 5 Relations between VAS and Serum ECP in NAR and LAR patients

VAS/Serum ECP	NAR		LAR	
	r Value	p Value	r Value	p Value
Nasal obstruction	0.575	<0.001	–0.481	0.051
Rhinorrhea	0.514	<0.001	0.016	0.944
Itching	–0.006	0.923	–0.350	0.125
Sneeze	–0.044	0.480	0.083	0.723
Total	0.341	<0.001	–0.362	0.119

LAR = local allergic rhinitis; NAR = nonallergic rhinitis; ECP = eosinophil cationic protein; VAS = visual analog scale.

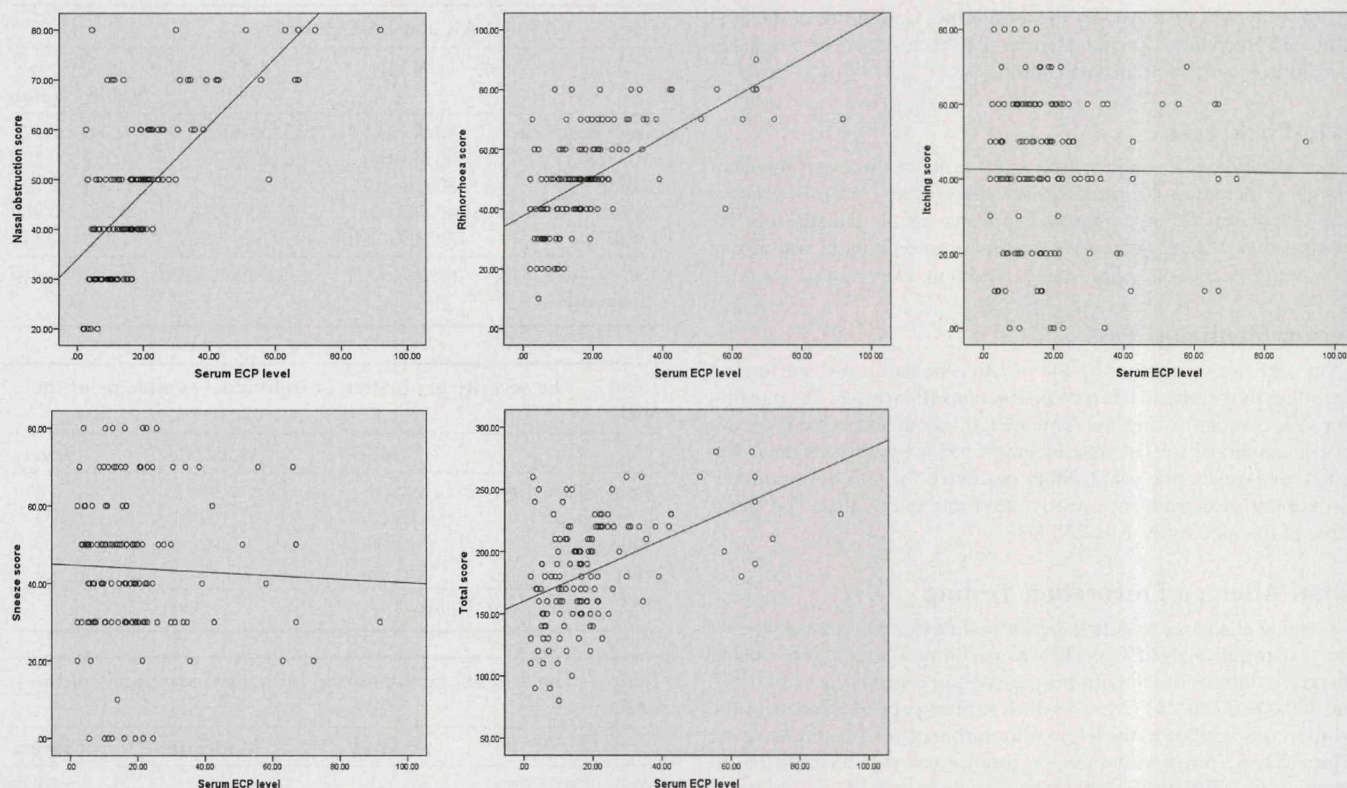


Figure 1. The relationship between visual analog scale (VAS) and serum eosinophil cationic protein (ECP) in the patients with nonallergic rhinitis (NAR). Nasal obstruction, rhinorrhea, and total scores had a statistically significant relationship with serum ECP level. Itching and sneezing scores showed no association with serum ECP level. Statistical analyses were performed by using the Kendall test.

Visual Analog Scale

After the NAPT to DF test, nasal obstruction, rhinorrhea, itching, sneezing, and total scores were recorded in the NAR and LAR groups. Table 1 shows the VAS scores of the NAR and LAR subjects included in the study. There was no statistical difference between the two groups. Tables 2 and 3 show the severity graduation of individual symptoms of the NAR and LAR patients.

Serum ECP

After the NAPT to DF test, serum ECP levels were evaluated in the NAR, LAR, and control groups. Table 4 shows the serum ECP levels in the NAR, LAR, and control groups. Serum ECP was significantly increased in the patients with NAR ($16.3 \mu\text{g/L}$ [$9.8\text{--}22.5 \mu\text{g/L}$]) compared with the controls ($8.8 \mu\text{g/L}$ [$5.4\text{--}20.2 \mu\text{g/L}$]; $p = 0.026$). Serum ECP in the patients with LAR ($11.6 \mu\text{g/L}$ [$7.8\text{--}15.7 \mu\text{g/L}$]) showed no difference compared with the controls ($p = 0.617$). The difference in serum ECP levels had no statistical significance between the NAR and LAR groups.

Relationship between VAS and Serum ECP

Table 5 displays the relationship between VAS and serum ECP in the patients with NAR and LAR. In the NAR group, nasal obstruction, rhinorrhea, and total scores had close and statistically significant relationships with serum ECP levels. In contrast, itching and sneezing scores showed no association with serum ECP levels (Fig. 1). In the LAR group, all scores, including nasal obstruction, rhinorrhea, itching, sneezing, and total scores, were not well associated with serum ECP (Fig. 2).

DISCUSSION

NAR commonly presents in daily life with one of multiple diseases, the incidences of which are similar to that of AR. Molgaard *et al.*

studied 1186 patients and found that NAR was present in 23% of those with PR.¹⁷ Unlike AR, the etiology of NAR in most patients remains unclear; its diagnosis is based on exclusion. Compared with AR, researchers have paid less attention to the pathogenesis, clinical manifestation, and treatment of NAR. LAR is a newly described entity in recent years; it is characterized by local allergic reactions ("entopy") in the nasal mucosa and no evidence of systemic responses of atopy.^{7,9,18,19} It manifests with the same symptoms as those of AR and NAR, including nasal obstruction, rhinorrhea, itching, and sneezing. In this study, the difference in VAS scores between patients with NAR and LAR had no statistical significance. These results agreed well with those of previous studies.

NAPT to common allergens is a major diagnostic method for LAR. In the present study, we used NAPT to DF as a diagnostic approach for LAR, because DF is the most common allergen in the Chinese population. The Phadiatop test is a method of screening for sIgE in serum and is usually used as an atopic screening test in childhood or adolescence.²⁰ It contains >95% of the aeroallergens in CAP and has high specificity and sensitivity.¹⁴ In this study, we used the Phadiatop test to measure sIgE in serum.

However, natural evolution of LAR has not been addressed, some authors have implied that LAR may be a special type of NAR,¹⁹ and others suggested that LAR could be a first step in the development of AR.²¹ Data on LAR remain sparse and controversial. Rondon *et al.*⁷ investigated 50 patients with persistent NAR and found that 54% showed a positive NAPT to DP, with 22% of these patients having nasal sIgE to DP5. Forester *et al.*⁵ concluded that up to 40% of patients with idiopathic rhinitis showed a positive NAPT result and had evidence of locally produced IgE. First, this study proved the existence of LAR in Chinese patients with PR, which is in agreement with the previous articles. Second, our research showed that 8.2% of patients in whom atopy was excluded had a positive NAPT to DF, which was lower than that reported by Forester and Rondon. This

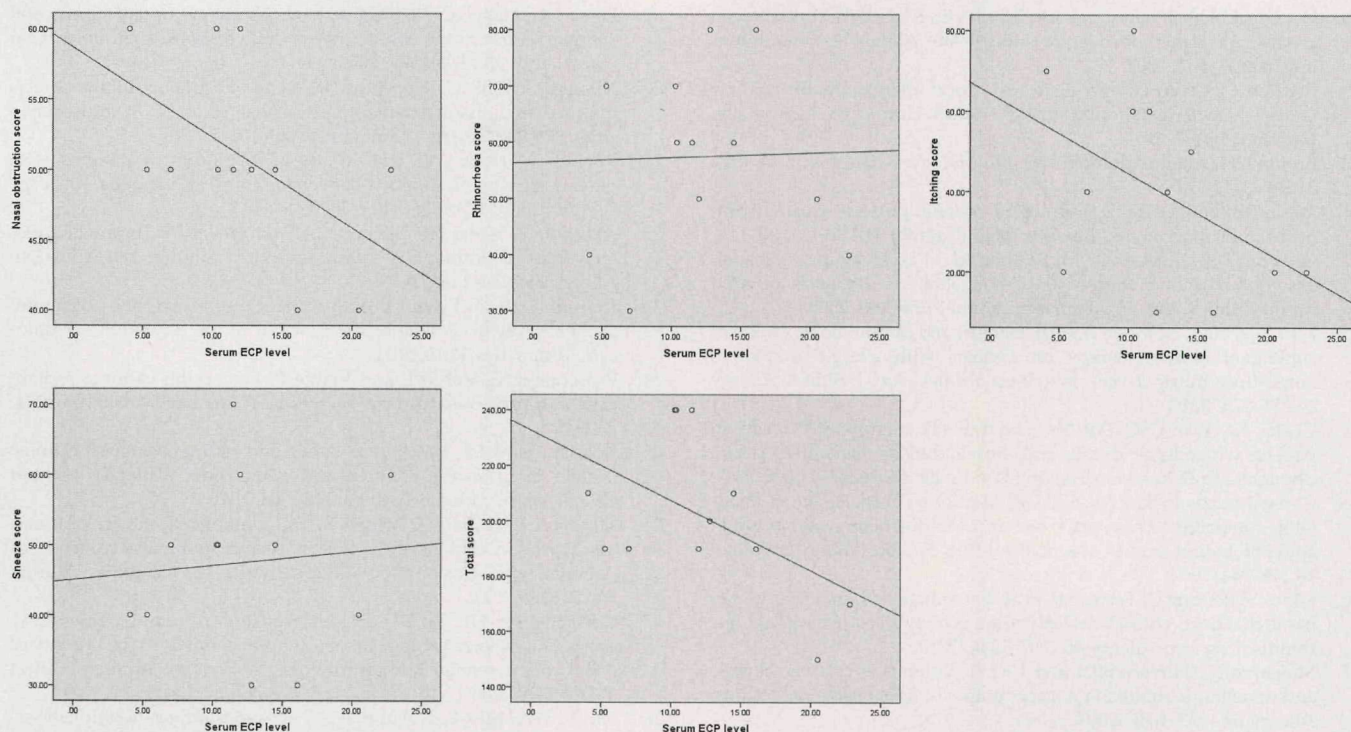


Figure 2. The relationship between visual analog scale (VAS) and serum eosinophil cationic protein (ECP) in the patients with local allergic rhinitis (LAR). All scores, including nasal obstruction, rhinorrhea, itching, sneezing, and total scores, were not well associated with serum ECP. Statistical analyses were performed by using the Kendall test.

may be caused by the single type of allergen used for NAPT in our study. It should be noted that this study only involved NAPT, but detection of local sIgE may be performed in future work.

Studies have reported that patients with LAR have reported a good response to topical nasal corticosteroids and oral antihistamines and may also benefit from immunotherapy. In our experience, patients with LAR had a good response to the topical medications (>2 weeks), including nasal corticosteroids and antihistamines. Their nasal symptoms could be improved by these treatments.

Eosinophils are important inflammatory cells, containing granular proteins with physiological activity.²² The main contents of these granules are four proteins: ECP, major basic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin.²³ ECP is a well-known protein and can be found in body fluids as a sign of eosinophil activation,²⁴ which is assessed as a marker in many inflammatory diseases, especially asthma.

The relationship between asthma severity and serum ECP level has been well described,²⁵ but the relationship between serum ECP and PR is still uncertain. Marcucci *et al.*²⁶ and Chen *et al.*²⁷ stated that the serum ECP level does not increase in patients with AR. In contrast, Sin *et al.* believed that serum ECP increases in seasonal AR disease.²⁸ In our previous work, we confirmed an increased serum ECP concentration in patients with AR, which has a relationship with allergic symptoms.

The present study evaluated the serum ECP level in NAR and LAR subjects and its relationship to nasal symptoms severity. The results indicate that serum ECP in the NAR group was obviously elevated compared with that in the healthy controls. Furthermore, it was closely related to nasal obstruction, rhinorrhea, and total scores. We infer that eosinophils and ECP may play important roles in NAR and can act as markers of NAR severity. Unlike in the NAR group, there was no up-regulation of serum ECP in the LAR group, and no correlation of ECP with the symptom scores was shown. This result agrees well with the findings of Rondon *et al.*⁷ and implies that the

ECP level in the circulation has no relationship to the pathogenesis of LAR. Detection of ECP in nasal lavage fluid or secretions may be appropriate for exploration of the pathogenesis of LAR. Unfortunately, we did not investigate these parameters. This should be performed as part of future research.

In conclusion, the present study provides the first evidence that LAR exists in Chinese subjects. We also suggest that eosinophils and ECP take part in the pathogenesis of NAR. We found no relationship between serum ECP level and LAR. A local examination for ECP and sIgE in nasal lavage fluid or secretions in patients with LAR should be performed in a future study.

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