

Total and allergen-specific immunoglobulin E in the serum and nasal mucosa of a nonallergic population

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Background: There has been a growing interest in measuring allergen-specific immunoglobulin E (sIgE) directly from the nasal mucosal epithelium of individuals with sinonasal disease. However, there is currently no normative data with which to estimate sensitivity, specificity, and the appropriate positive cutoff level for new testing methods.

Methods: Twenty individuals with no history of sinonasal disease or food allergies underwent serum IgE testing and nasal mucosal brush biopsy (MBB) testing for total and sIgE to 7 common airborne allergens and 1 food allergen. The correlation between sIgE in serum and nasal samples was determined at both the 0.10-kU/L and 0.35-kU/L positive cutoff levels.

Results: sIgE at the 0.35-kU/L cutoff level was detected for at least 1 allergen in 7 of 20 (35%) participants in both the serum and nose. At the 0.10-kU/L cutoff level, 8 of 20 (40%) and 19 of 20 (95%) participants had sIgE to at least 1 allergen in the serum and nose, respectively. At the 0.35-kU/L cutoff level, total serum IgE levels were significantly higher

when at least 1 allergen was detected in the nose ($p = 0.01$). There was a strong association between sIgE in the serum and nasal mucosa ($p < 0.0001$) at both cutoff levels.

Conclusion: The significant association between serum and nasal samples for both total and sIgE suggests that nasal IgE and serum IgE are not independent of one another. Understanding levels of IgE in the nonallergic population will help answer the questions surrounding sensitization as new diagnostic tests for locally-present IgE become available.

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Key Words:

mucosal brush biopsy; sensitization; allergen; serum; nasal mucosa; sIgE

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Immunoglobulin E (IgE) is a 190-kDa protein that plays a key role in many inflammatory conditions, including allergic rhinitis (AR), asthma, eczema, and anaphylaxis.¹ In early human history, IgE-mediated inflammation was beneficial for fighting parasitic infections, a significant cause of infant mortality. However, with the decline in parasitic infections in the developing world, food and airborne substances, which share structural similarities with more

dangerous pathogens, have become important triggers for allergen-specific IgE (sIgE) production.²

In atopic individuals, sIgE is produced when an antigen presenting cell (APC) contacts a sensitizing protein or carbohydrate (allergen) and exposes it to a T helper type 2 (Th2) cell. Under the influence of cytokines, particularly interleukin 4 (IL-4), the Th2 cell stimulates a naive B cell to undergo isotype-switching, or class-switching, and begin production of IgE specific to that allergen.³ sIgE can be produced either in the larger lymphoid structures of the body, such as the spleen and regional lymph nodes, or directly in the mucosal tissues of the aerodigestive tract.⁴ As a result, local sIgE levels may be present even when serum sIgE levels are absent.⁵⁻⁷ Local production of sIgE has been demonstrated in the mucosal epithelium of the sinonasal cavity,^{8,9} bronchial mucosa,¹⁰ esophageal mucosa,¹¹ and adenotonsillar tissue¹² in individuals with clinical disease, regardless of atopic status.

New tests have recently emerged that directly measure local sIgE levels from mucosal epithelium, such as surgical biopsy¹³ and mucosal brush biopsy (MBB).¹⁴ These tests may be particularly useful in cases where the results of

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standard allergy testing are not consistent with the clinical history. Estimates of serum IgE sensitization in the general population range between 40% and 50%, but the prevalence of local IgE sensitization in individuals without allergic disease is currently unknown.¹⁵ This gap in knowledge has limited the determination of sensitivity, specificity, and the appropriate positive cutoff level for local sIgE testing.

The purpose of this study was to measure total IgE and sIgE levels from the nasal mucosal epithelium of individuals without allergic disease, using MBB, and to explore the association between the presence of sIgE in the nose and in the serum, both overall and for individual allergens. The primary hypothesis is that the prevalence of sIgE will be higher in the nose than in the serum, reflecting the region of initial allergen exposure. The secondary hypothesis is that participants with higher levels of total IgE in the serum will be more likely to have sIgE present in the nose.

Subjects and methods

Study population

Approval for this study was obtained from the Institutional Review Board of Weill Cornell Medical College, New York City (Protocol #1206012451). Inclusion criteria included healthy individuals, 18 years of age or older, who were willing to participate in the study. Individuals with a history of chronic rhinitis (allergic or nonallergic), chronic rhinosinusitis (with or without nasal polyps), allergic fungal rhinosinusitis, immediate pruritus or edema (local, distant or systemic) after consuming certain foods, asthma, or eczema were excluded from the study. Individuals who were uncertain about their allergic status on initial interview were also excluded from participating in the study.

Seven common airborne allergens and 1 food allergen were chosen for this study: White oak, Timothy grass, ragweed, cat, *Alternaria*, *Dermatophagoides farinae*, cockroach, and peanut. A food allergen was included because species-specific IgE to food allergens have previously been identified in the nasal cavity using MBB.¹⁶ The actual sensitization of the participants to these allergens, based on either serum IgE testing, skin testing, or local IgE testing, was unknown prior to enrollment. The age and gender of the enrolled participants were recorded. All testing and data collection was performed at a single institution, Weill Cornell Medical College, during the month of August 2014. Timothy grass and ragweed were the most active outdoor allergens during that period.

MBB

Prior to local IgE harvest, the nasal cavity was decongested and anesthetized on both sides with topical 2% lidocaine and 1% phenylephrine. After waiting 5 minutes, a 7.75-inch cytology brush (Aeromed, Glastonbury, CT) was inserted along the medial surface of the anterior third of the inferior turbinate as described.¹⁴ While avoiding direct

pressure on the septum, the brush was rotated and moved back and forth approximately 10 times. After the procedure was repeated on the opposite side with a separate brush, the brushes were rinsed for 2 minutes in phosphate-buffered saline (PBS) to extract mucus and epithelial cells.

Measurement of total IgE, sIgE and total protein (nose)

The suspensions of nasal epithelial cells from each participant were lysed using an ultrasonicator at 45% amplitude for 2 minutes at 15-second intervals (Branson Digital Sonifier, Danbury, CT). Up to 4 additional 15-second pulses were performed until the lysates appeared clear and Trypan Blue exclusion test demonstrated that no viable cells were present. Total protein concentrations for the lysates were determined using a bicinchoninic acid assay (BCA assay; Thermo Fisher Scientific, Rockford, IL). Each lysate was sent frozen to a reference laboratory for immunofluorescent assay to determine total IgE levels as well as sIgE levels for the 8 study allergens (PiRL Laboratories, Portage, MI). Because levels of locally-present sIgE are generally lower than serum sIgE levels, 2 positive cutoff levels were chosen and analyzed separately: 0.10 kU/L and 0.35 kU/L.¹³ Total nasal IgE levels were standardized for total protein (corrected total nasal IgE).

Measurement of total IgE and sIgE (serum)

Venipuncture was performed on all participants, and serum samples were sent frozen to a reference laboratory for immunofluorescent assay to determine total IgE levels as well as sIgE levels for the 8 study allergens (PiRL Laboratories, Portage, MI).

Statistical analysis

Data are presented as mean values $\pm$ standard deviation (SD). Using a linear regression model, the Pearson product-moment correlation coefficient was calculated to test the strength of the correlation between total serum IgE levels and corrected total nasal IgE levels. The nonparametric Mann-Whitney *U* test was used to compare total IgE levels in the serum vs total IgE in the nasal samples and between participants who tested positive for sIgE in the nose vs those who tested negative at each cutoff level. Fisher's exact probability testing was used to test the associations between sIgE in the serum vs sIgE in the nasal samples, outdoor vs indoor sensitization, and gender differences in sensitization at each cutoff level. Two-tailed *p* values were calculated for each comparison using VassarStats online statistical software package¹⁷ and a value of *p* < 0.05 was considered statistically significant.

Results

Study population

All of the participants tolerated the serum and MBB testing well with no adverse events reported. Of the 20

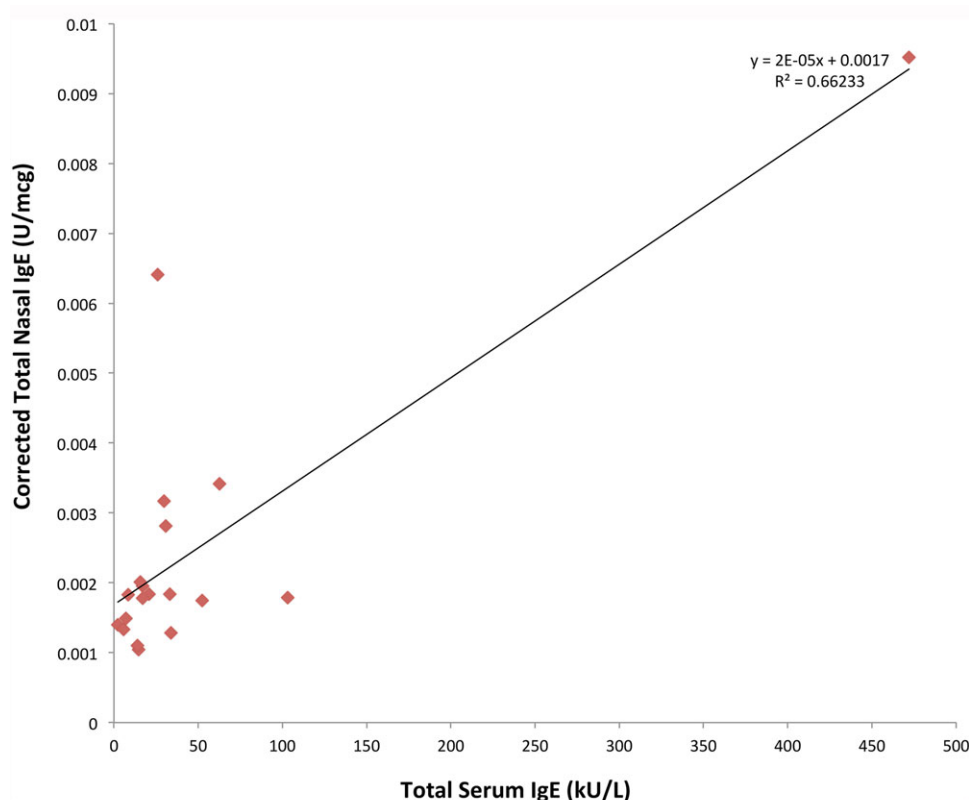


FIGURE 1. Correlation of total IgE in the serum with corrected total IgE in the nasal mucosa. IgE = immunoglobulin E.

participants, 7 (35%) were male and 13 (65%) were female. The mean age was 29.2 years (range, 19–54 years). There was no significant association noted between gender and the rate of sensitization in the serum or nasal mucosa at either the 0.10-kU/L cutoff level ($p = 1.0$) or the 0.35-kU/L cutoff level ($p = 1.0$).

Total IgE

The range of total IgE in serum samples was 2.35 to 472 kU/L, and 2.58 to 26.6 kU/L in nasal samples. One major outlier, with a total serum IgE of 1393 kU/L and total nasal IgE of 72.5 kU/L, was excluded from the total IgE comparisons. The mean serum IgE level (50.87 ± 104.74 kU/L) was significantly higher than the mean nasal IgE level (5.60 ± 5.76 kU/L) ($p < .0001$).

As shown in Figure 1, there was a strong correlation between total serum IgE and corrected total nasal IgE ($r = 0.81$; $p < .0001$). At the 0.35-kU/L cutoff level, the mean total serum IgE in participants who tested positive for at least 1 of the test allergens in the nose was 120.65 ± 174.62 kU/L, compared to 18.67 ± 13.81 kU/L in participants who tested negative for sIgE to all allergens in the nose ($p = 0.01$). Because only 1 participant tested negative to all allergens in the nose at the 0.10-kU/L cutoff level, a comparison between the mean total serum IgE levels could not be made.

sIgE

The numbers and percentages of participants who tested positive to each individual allergen, or positive to at least 1 allergen overall, at both cutoff points, are presented in Table 1. At the 0.35-kU/L cutoff level, 7 of 20 (35%) participants tested positive for IgE to at least 1 allergen in both the serum and nose. However, at the 0.10-kU/L cutoff level, 8 of 20 (40%) participants tested positive to at least 1 allergen in the serum, while 19 of 20 (95%) participants tested positive in the nose. The grade distribution for all 320 tests is shown in Figure 2, and the prevalence of positive tests in serum and the nose for each cutoff level is shown in Table 2.

The associations between the presence of serum sIgE and nasal sIgE for individual allergens and all allergens taken together, at both cutoff levels, are also presented in Table 1. At the 0.10-kU/L cutoff level, 25% of participants were sensitized to outdoor allergens in the serum, whereas 90% of participants were sensitized to outdoor allergens in the nose ($p < .0001$). At the 0.35-kU/L cutoff level, 25% of participants were sensitized to outdoor allergens in the serum, whereas only 10% of participants were sensitized to outdoor allergens in the nose ($p = 0.41$). For indoor allergens, at the 0.10-kU/L cutoff level, 30% of participants were sensitized to allergens in the serum, whereas 40% of participants were sensitized to allergens in the nose ($p = 0.74$). At the 0.35-kU/L cutoff level, 30%

TABLE 1. Participants who tested positive for each study allergen and overall for at least 1 study allergen, at both positive cutoff levels, and the associations between serum sIgE and nasal sIgE*

Allergen	0.10-kU/L cutoff			0.35-kU/L cutoff		
	+ Serum (n = 20) n (%)	+ Nose (n = 20) n (%)	p	+ Serum (n = 20) n (%)	+ Nose (n = 20) n (%)	p
Outdoor						
White oak	3 (15)	18 (90)	1.0	2 (10)	0 (0)	1.0
Timothy grass	4 (20)	4 (20)	0.01	4 (20)	2 (10)	0.03
Ragweed	4 (20)	7 (35)	0.007	3 (15)	1 (5)	0.15
<i>Alternaria</i>	0 (0)	2 (10)	1.0	0 (0)	0 (0)	1.0
Indoor						
<i>D. farinae</i>	7 (35)	8 (40)	0.004	6 (30)	6 (30)	<0.0001
Cat	3 (15)	4 (20)	0.004	3 (15)	2 (10)	0.02
Cockroach	2 (10)	2 (10)	0.005	2 (10)	2 (10)	0.005
Peanut	1 (5)	2 (10)	0.10	1 (5)	0 (0)	1.0
Overall	8 (40)	19 (95)	<0.0001	7 (35)	7 (35)	<0.0001

*Values of p are based on Fisher's exact testing. Statistically significant values are in bold.

sIgE = allergen-specific immunoglobulin E.

of participants were sensitized to indoor allergens in both the nose and the serum ($p = 1.0$).

Discussion

This is the first study to estimate the prevalence of local sIgE sensitization in individuals without allergic disease. Using

the 0.35-kU/L cutoff level, the prevalence of sensitization in this study population in both the serum and nasal mucosal epithelium was 35%. An estimate of serum sensitization in the general U.S. population over the age of 6 years, as reported by the National Health and Nutritional Examination Survey (NHANES), which also uses the 0.35-kU/L cutoff level, is 44.6%.¹⁵ Similarly, for people living in a metropolitan area, outdoor allergen sensitization was 25% in the current study vs 37.8% in the NHANES data. It is possible that the sensitization prevalence was lower compared to the NHANES data because the population in the current study only included adults with no allergic history.

It is unknown whether atopy is present in all humans, but it seems likely that this primitive feature of the adaptive immune system would exist at some level. The finding that 95% of participants had sIgE in their nasal mucosa when using the 0.10-kU/L cutoff level suggests that sIgE is universal in the aerodigestive tract, but perhaps not always in a sufficient amount to collect and measure. The current study showed almost a 10-fold lower level of total IgE in the nasal mucosa than in the serum. Although the possibility of false-positive detection must be considered when results are at the lower limit of the assay, the cluster of grade 0/1 sensitizations in the nasal mucosa (between the 0.10-kU/L and 0.35-kU/L cutoff levels) was not seen in the serum, using the same assay.

Of the 12 participants with at least 1 grade 0/1 sensitization in the nose, 11 of them were sensitized only to outdoor allergens, notably White oak and ragweed. It has also been reported that individuals with nonallergic rhinitis (NAR) were more likely to test positive for oak and ragweed IgE in the nasal cavity using MBB if the epithelium was sampled during the outdoor pollen season.⁷ In a study of Japanese cedar pollen, sIgE in tears peaked sooner than serum IgE during the pollen season (March-June) and decreased during the rest of the year, while serum levels remained high.¹⁸

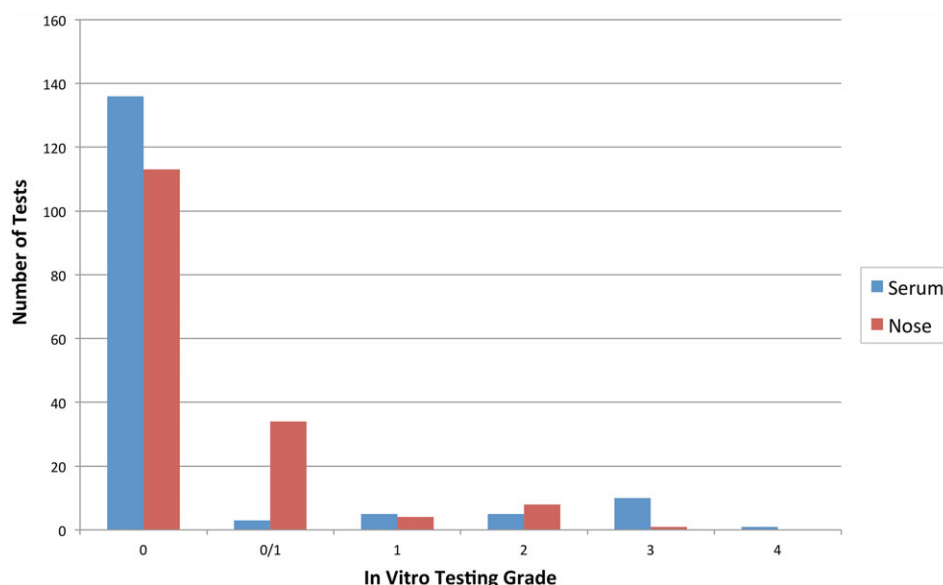


FIGURE 2. Grade distribution of allergen-specific IgE levels in both the serum and nasal mucosa. IgE = immunoglobulin E.

TABLE 2. Prevalence and percentage of positive tests from both the nasal mucosa and serum, at both positive cutoff levels

	0.10 kU/L (n = 160) n (%)	0.35 kU/L (n = 160) n (%)
Nasal MBB	47/160 (29.4%)	13/160 (8.1%)
Serum	24/160 (15%)	21/160 (13.1%)

MBB = mucosal brush biopsy.

Considering that the participants in the current study were all tested during the outdoor pollen season, these observations suggest that local sIgE levels might reflect current or recent allergen exposure more so than serum sIgE levels.

The ability to determine which allergens are most responsible for producing symptoms in a particular region of the body, at a particular time, is 1 of the potential benefits of local sIgE testing. This might provide the clinician with guidance concerning which allergens their patient should avoid or which extracts to include in the immunotherapy treatment vial. Another clinical use for this testing is to gain supplemental information when conventional skin or serum testing does not correlate with the clinical history, as is often the case with NAR. In 1 study, local sIgE in the nasal mucosa was found in 100% of patients with the diagnosis of NAR, based on negative skin and serum testing.⁷ Other studies have confirmed that sIgE may be present in locally-symptomatic regions with no evidence of serum or skin sensitization.^{19–21} This preferential sensitization in symptomatic individuals is not well understood, but may be related to the location and extent of the initial exposure.

Questions still remain concerning the relationship between local and serum sIgE. This study demonstrated that, similar to individuals with AR, local sIgE is more likely to be detected in individuals without allergic disease when total serum IgE levels are higher.²² However, although there was a strong correlation in this study between the presence of sIgE in the nasal mucosa and in the serum, at both cutoff levels, there was a weak association noted between nasal sIgE from MBB and serum sIgE in individuals with symptomatic AR and positive skin-prick testing.¹⁴ This suggests that a shift in the production or distribution of sIgE occurs as an individual begins to develop clinical symptoms related to their sensitivity.

Along with the presence of clinical symptoms and serum sensitization status, the positive cutoff level used also has an impact on the prevalence of local sIgE sensitization. Combining the data from this study with prior studies measuring nasal sIgE levels in individuals with symptomatic chronic rhinitis, both with and without serum or skin sensitization, 100% of symptomatic individuals had local sIgE present at the 0.10-kU/L cutoff level, regardless of serum or skin testing results. In contrast, only about one-half of the asymptomatic participants in this study were positive

at this cutoff level, regardless of serum testing results (55% with negative serum sIgE; 40% with positive serum sIgE). However, using the 0.35-kU/L cutoff level, nasal sIgE was only detected in individuals when the serum or skin testing was positive, whether they were symptomatic or not (35% in asymptomatic individuals; 75% in asymptomatic individuals).^{7,14}

This study was limited by the small number of participants. Although the majority of participants lived in the same geographic region, New York City contains a very heterogeneous population that comes from many other areas of the country and the world. Another limitation of this study was that only local sIgE from the nasal mucosa was examined. Allergen sensitization and sIgE fluctuation may differ significantly based on the region of the aerodigestive tract and the clinical disease being studied, such as food allergy or eosinophilic esophagitis. Similarly, only 2 positive cutoff levels were chosen, while the most appropriate level may exist between the 2 and also depend on the region and clinical scenario.

It is clear that for allergic inflammation to take place in a locally-symptomatic region, such as the nose, sIgE must be present. These antibodies can either be produced in that tissue or transported there from the blood or regional lymph tissue. Where initial sensitization occurs, when it occurs, whether or not it occurs in all humans and at what level does it produce clinical disease will be the subject of future investigations. As our understanding of the importance of local sIgE continues to evolve, along with the development of more sensitive and specific assays and improved tissue sampling techniques, the next steps involve careful analysis of positive cutoff levels and expanding the investigation into other age categories and different regions of the aerodigestive tract.

Conclusion

In individuals with no history of allergic disease, the prevalence of sIgE in the nasal mucosa was similar to the prevalence in serum when using a 0.35-kU/L positive cutoff level. At a 0.10-kU/L positive cutoff level, the prevalence of sIgE was notably higher compared to serum, suggesting that low levels of sensitization may be more common than previously considered. Individuals with higher levels of total IgE in the serum were more likely to have sIgE in the nasal mucosa, and there was a strong association between sIgE in both regions at both cutoff levels. Further work will be necessary to help clarify the relationship between sIgE in local regions of the aerodigestive tract, sIgE in the serum, and the development of clinical disease. 

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