SALIVARY SPECIFIC IgE TO D1 AND G6 IN PATIENTS WITH RHINITIS WITH OR WITHOUT *S. AUREUS* COLONIZATION

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ABSTRACT

Globally, allergic rhinitis impacts roughly 25% of children and 40% of adults. Immunoglobulin E (IgE) plays a crucial part in allergic inflammation, with two sources: spontaneously produced IgE, and IgE stemming from reactions to environmental allergens. The damaging effects of *S. aureus*, as well as Staphylococcal enterotoxins (Ses), have been proven in numerous airway illnesses. As superantigens, Ses generate intense Th2 inflammation, with 70-80% of IgE being locally synthesized. Our study aimed to determine if any correlation existed between local and systemic specific IgE responses in rhinitis patients – both treated and untreated. Furthermore, we sought to pinpoint significant disparities in serum allergy-specific IgE levels between *S. aureus* positive and negative patients. Results: From 70 patients with a relevant rhinitis history spanning at least two years, we found that in our rhinitis cohort, 36 were sIgE-negative for d1 in blood samples but positive in saliva samples ($\chi2 = 19.76$, $\alpha = 0.181$), while 25 tested negative for g6 in blood samples but positive for g6 in saliva samples ($\chi2 = 6.89$, $\alpha = 0.86$). No significant difference emerged between serum allergy-specific IgE levels in *S. aureus* positive and negative rhinitis patients ($\chi2 = 0.38$). Similar results were noted within the saliva samples. However, mucosal-specific IgE levels were lower among patients receiving active therapy ($p < 0.001$ for both d1 and g6). Conclusion: There is no correlation between mucosal-specific IgE levels and systemic-specific IgE levels or *S. aureus* carriers. We observed that salivary-specific IgE levels were lower in patients undergoing active treatment compared to untreated patients.

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INTRODUCTION

Allergic rhinitis, an increasingly prevalent disease, affects roughly 25% of kids and 40% of adults worldwide. Most cases appear before reaching the age of 20. This illness involves a Th2-mediated immune response, where Th2 lymphocytes trigger the synthesis of cytokine subsets that stimulate IgE antibody production by activated B-cells [1].

Staphylococcus aureus (S. aureus) frequently colonizes the upper respiratory tract, and staphylococcal enterotoxins (Ses) can act as superantigens. These antigens bypass crucial immunological processes like antigen processing and presentation by antigen-presenting cells (APC), binding directly outside the peptide-binding pocket. This may leads to excessive production of T cell cytokines by T cells regardless of specificity of their T cell receptor . Staphylococcal enterotoxin B (SeB) is typical T-cell superantigen. The intensity of such a response can reach a degree known as a cytokine storm [2]. The secreted cytokines IL-4, IL-5, and IL-13 are responsible for isotype switching and the preferential synthesis of IgE. Recent research indicates that IgE to SEs is functional. Additionally, these cytokines activate type 2 innate lymphoid cells (ILC-2) which produce extra IL-5 and IL-13, resulting in eosinophilic inflammation [3].Eosinophils are actively involved in allergic inflammation. They infiltrate tissues in the late phase of allergic inflammation. Locally resident eosinophils are regulated by Il- 33 and epithelial derived innate cytokines thymic stromal lymphopoetin (TSLP). These cytokines secreted by activated epithelial cells directly activate eosinophils. Upon activation they

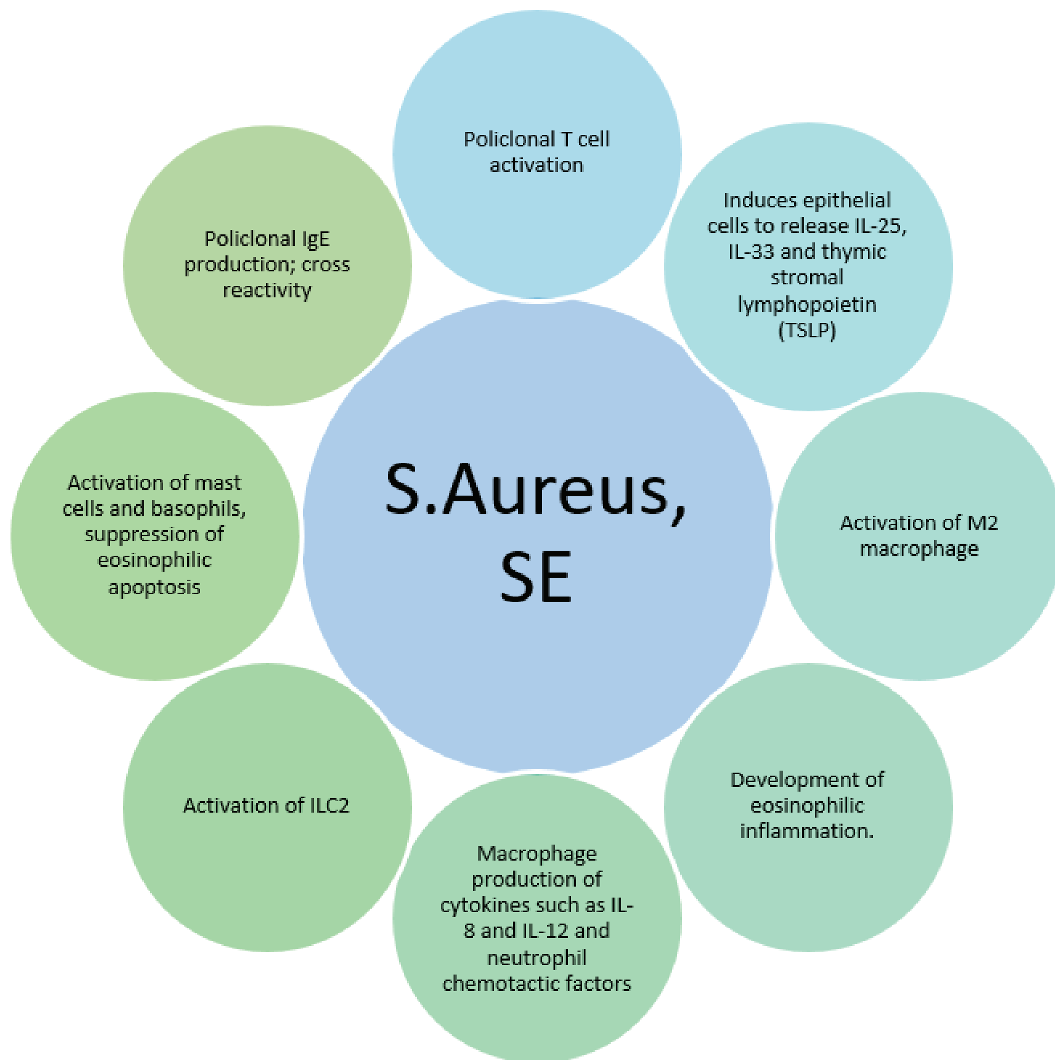

Fig. 1. Role of *S. aureus* and Se in development of inflammation: ***S.****aureus-Staphylococcus aureus* and Se-Staphylococcal enterotoxin- serve as superantigens; Il- 33 and IL25- epithelial derived innate cytokines; TSLP- thymic stromal lymphopoetin epithelial derived during initial stage of allergic inflammation ; M2 macrophage- type 2 macrophages- upon activation release Il8, Il12, neutrophil chemotactic factor and drive the inflammatory cascade; ILC2- Innate lymphoid cells type 2- express cytokine receptors and pathogen-associated receptors of the innate immune response during the early phase of inflammation. Activated ILC 2 secrete IL 4 and IL 13 which potentiate Th 2 inflammatory type.

release eosinophil peroxidase (EPO) and eosinophil derived neurotoxine (EDN). Furthermore, both EPO and EDN stimulate dendritic cells to maturation and antigen presentation [4]. Figure 1 presents the role of *S. aureus* on the development of Th 2 inflammation.

Small doses of antigen (10 micrograms is optimal) adsorbed on mucosal surfaces initiate the IgE response in genetically predisposed individuals [5]. Mature naïve B cells encounter the antigen in the lymph nodes engulfed by mature antigen-presenting cells and presented on their surface for recognition by T cells. After interaction with antigen specific T cells activated B cells migrate to the follicle and extra – follicular region, proliferate, form germinal centers, and differentiate into plasma cells. Moreover, somatic hypermutation (SHM), clonal expansion, and class switching to IgE are realized in musosa [6].

Sensi observed that the production of IgE increased at a faster rate in the nasal mucosa than in the serum of House Dust Mite (HDM) patients following allergen stimulation [7, 8]. Antigen-specific IgE antibodies have been detected in the nasal mucosa 24 hours post Nasal Allergen Provocation Test (NAPT), signifying local antibody synthesis in the nasal mucosa. Since

the 1970s, literature has indicated that 70-80% of IgE is locally produced[9, 10]. In fact, determination of local IgE in the absence of systemic IgE has diagnostic and therapeutic consequences. .

In 2000, Klein Jan and Cameron identified local IgE synthesis in the nasal B cells and plasma cells of individuals suffering from Allergic Rhinitis (AR) [7]. Subsequently, Rondon devised the current definition of Local Allergic Rhinitis (LAR) [7]. Gelardi documented the existence of allergen-specific IgE (asIgE) in the nasal mucosa of patients with allergic rhinitis, non-allergic rhinitis, and healthy controls. The authors reported local IgE detection across all three groups and inferred that local IgE generation could be a normal response to environmental allergens[11].

In a clinical context, both allergic rhinitis (AR) and local allergic rhinitis (LAR) [11] exhibit characteristics common to each type of rhinitis, such as rhinorrhea, sneezing, and nasal itching. LAR refers to patients with a history of allergic disease who have negative skin and blood tests for allergy. These individuals usually display localized signs of atopy, including the presence of sIgE in nasal secretions, positive nasal allergen provocation tests, and responsiveness to allergen-specific immunotherapy. Although multiple endotypes of rhinitis exist, distinctions between them are not consistently well-defined. The precedence of local IgE production over systemic sensitization remains uncertain. Patients with AR may exhibit nasal reactivity to various allergens despite the absence of allergen-specific IgE on the skin or in serum for both allergens, a condition known as "double allergic rhinitis" [1].

Skin prick tests and ssIgE immunoassays are the predominant laboratory tests employed for identifying potential allergens; however, no definitive "gold standard" laboratory test exists for diagnosing AR. The aim of our research was to evaluate the potential relationship between *S. aureus* nasal colonization and local as well as systemic specific IgE production in patients with rhinitis, irrespective of whether they have received treatment.

MATERIALS AND METHODS

Study design

An observational study was carried out utilizing intricate clinical and laboratory methodologies on a cohort of 70 patients possessing a clinically significant history of rhinitis for at least two years. Inclusion criteria consisted of a comprehensive evaluation of atopic status, incorporating specific IgE to Derp1 and g6 in saliva, alongside serum-specific IgE against inhalant allergens and a clinically relevant rhinitis history with or without concomitant

Table 1. Inclusion and exclusion criteria:

Criteria	Inclusion	Exclusion
Age	Over 6 years (cooperation in the collection of sputum samples)	Less than 6 years
Active rhinitis	History of previously confirmed allergic rhinitis or persistent rhinitis complaints for at least 2 months.	History of previous antibiotic therapy (local or systemic) for persistent rhinitis symptoms.
Comorbidities	Asthma and other disorders related to atopic march	Nasal polyposis
Therapy	Application of intranasal corticosteroids and/or immunotherapy is permissible Lack of active therapy (intranasal corticosteroids and/or immunotherapy) is not a contraindication for inclusion.	Biologicals
Other		Pregnancy

Children and adults with clinically significant history of rhinitis for at least two years .Blood and saliva samples were gathered during polen season when when active production of a specific IgE is expected. All patients must have active symptoms. Previous infectious disease or administration of antibiotics or biological therapy were exclusion criteria. Nasal polyposis as a different clinical entity was also an exclusion criteria. Local steroid therapy and application of antihistamines were permissible as not disease-modifying drugs. Immunotherapy was not an exclusion criteria Local biomarkers investigated in this study might serve as valuable biomarker throughout the course.

asthma. A thorough medical history was gathered for all patients, encompassing the type of therapy administered during sample acquisition and the presence of comorbidities. All participants were guided on the proper technique for procuring unstimulated saliva samples to facilitate subsequent scrutiny of local IgE production. Additionally, nasal swabs were obtained from every individual involved in the study. Full list of inclusion and exclusion criteria is presented in Table 1.

Collection of saliva and blood samples was performed during polen season. Specific IgE in saliva and serum samples is measured at only one time point. Another limitation is lack of healthy controls. Study design is presented on Fig2.

Immunological assessment

Serological evaluations were conducted utilizing the Euroline Allergy Profile Inhalation and Enzyme Allergo Sorbent Test (EAST) by Euroimmun® (Medizinische Labordiagnostica, AG, 2014, Luebeck, Germany), encompassing prevalent aero-allergens. The EUROLINE method yields semi-quantitative results demonstrated via the EAST system in seven categories, ranging from class 0 to 6 (<0.35 kU/L EAST class 0 to >100 kU/L EAST class 6). Analysis of specific IgE antibodies pertaining to d1-Dpt allergen and g6-timothy allergen in saliva specimens was performed using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). All serological examinations were completed within an accredited Laboratory of Clinical Immunology.

These two methods are categorized in the subsequent data evaluation by EAST classes from 0 to 6 class. Such an approach would allow a comparative analysis between blood and saliva samples. In fact, the ImmunoCAP system detected

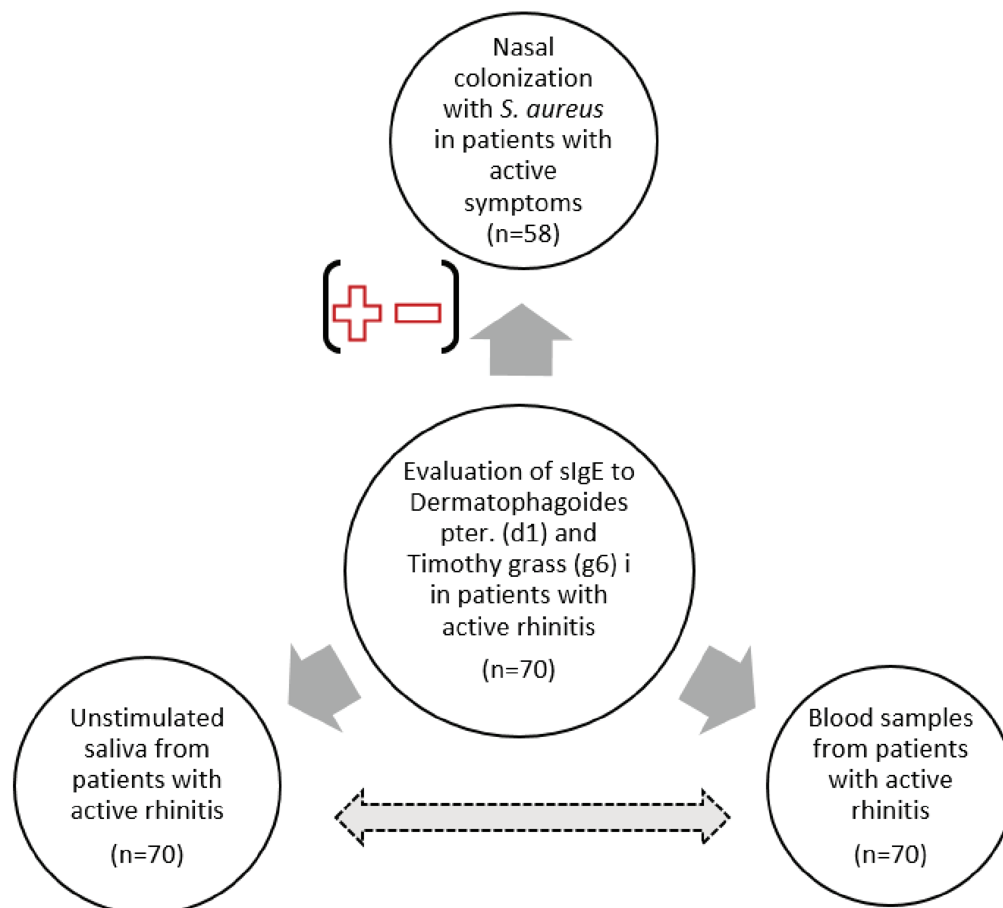

Fig. 2. Study desing: Unstimulated saliva (n=70), serum from blood samples (n=70) and nasal swabs of patients (n=58) with clinical features of active rhinitis were collected and analyzed. Saliva and serum samples (n=70) were analyzed using ImmunoCAP system (quantitative method) and Euroline Allergy Profile Inhalation (semi-quantitative method), respectively, and results compared. Nasal swabs (n=58) were cultured and subsecuently analyzed for *S. aureus* using matrix-assisted laser desorption ionization-time of flight mass spectrometry.

much lower concentrations of the specific IgE to d1 and d6 lower than 0.35 KU/L. Such quantities are a practically imperceptible for Euroline system. It is speculative to claim that such a low concentrations of specific IgE in saliva samples have prominent clinical significance considering the study included patients with active rhinitis symptoms. In fact, it is necessary to emphasize that all participants in this study have clinically confirmed symptoms . On the other hand active treatment is typical for most included patients. According to literature in majority of studies in this field local sIgE response have been measured in nasal swabs. However, we should note that the collection of saliva is less traumatic for the patients than collection of nasal swabs. Saliva could be a suitable material in evaluation of atopy most probably among children.

Sample processing and identification.

Nasal samples were streaked onto Columbia Agar containing 5% sheep blood (BD™ Becton Dickinson GmbH, Heidelberg, Germany) and incubated for 18-20 hours at 35°C. Additionally, the nasal swabs were cultivated in tryptic soy broth (Soybean-Casein Digest Medium, BD™ Becton Dickinson GmbH, Heidelberg, Germany) for enrichment purposes. Broth cultures that emerged were subsequently plated on Columbia agar plates only if the initial swab culture remained sterile. Single colonies exhibiting morphological characteristics similar to *S. aureus* were restreaked to obtain pure cultures and identified utilizing matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Billerica, MA, USA). In summary, a single colony from these pure cultures was placed on a polished steel MSP 96 target (Bruker Daltonics, Billerica, MA, USA) and covered with 1 µL of saturated-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics). Strains that remained unidentified

Table 2 Demographic and clinical characteristics of subjects:

Disease		Gender		Age
		Male	Female	
Allergic rhinitis	Count	16	26	
	%	66.7%	56.5%	
	Mean			26.21
	Maximum			53.00
	Minimum			6.00
	Standard Deviation			12.63
Asthma and allergic rhinitis	Count	0	3	
	%	.0%	6.5%	
	Mean			37.67
	Maximum			41.00
	Minimum			34.00
	Standard Deviation			3.51
Rhinitis	Count	8	17	
	%	33.3%	37.0%	
	Mean			34.84
	Maximum			52.00
	Minimum			19.00
	Standard Deviation			9.56
Total	Count	24	46	
	%	100.0%	100.0%	
	Mean			29.79
	Maximum			53.00
	Minimum			6.00
	Standard Deviation			12.11

From a total of 70 patients analyzed, 42 had allergic rhinitis (M/F: 16/26; mean ± SD age: 26.21 ± 12.63 yr) and 25 were with persistent rhinitis sumptoms and negative serum samples for any inhalant allergen (M/F: 8/17; mean ± SD age: 34.84 ± 9.56 yr).

underwent resubmission using the extended protocol involving 1 µL of 70% formic acid. The acquired mass spectra were analyzed employing a microflex LT mass spectrometer (Bruker Daltonics), utilizing the research-use-only (RUO) software workflow and reference library MBT v. 4.1.100. All isolates were successfully identified with scores exceeding 2.0.

Statistical methods

Statistical analyses were conducted utilizing SPSS®, IBM 2009 version 19 and Graph Pad Prism version 9.0.0 . Descriptive statistics were employed to delineate the clinical and demographic features of patients, treatment status, and immunological parameters. One-way ANOVA analysis and correlation analysis were calculated between category characteristic. A significance threshold of $\alpha = 0.05$ was established. If $p < \alpha$, the null hypothesis is rejected.

RESULTS

Demographic and clinical characteristics

Seventy patients were included in the study. Demographic and clinical characeristics of the study group are shown on Table 2

As per the research methodology, all participants had a documented history of rhinitis persisting for a minimum of two years. Among them, 42 subjects (60%) were previously diagnosed with allergic rhinitis. Concurrent diagnoses of rhinitis and asthma were reported by three patients, while 25 participants (37,5%) exhibited rhinitis symptoms of unidentified etiology. In group with LAR (n=20) were not receiving therapy at the time of inclusion in the study and n=40 were receiving intranasal corticosteroids or had started sublingual immunotherapy. In addition, we tested 12 volunteers. Specific IgE for any inhalant allergen in saliva samples was detected in all of them. Such a result guided our subsequent analysis on the effect of therapy on the amount of IgE in saliva samples.

S. aureus colonization in patients with rhinitis

Nasal samples were evaluate for *S. aureus* in order to to assess its influence on the production of a specific IgE.A total of 58 nasal swabs were obtained and analyzed following the procedure outlined by the manufacturer. *S. aureus* was identified in 23 rhinitis-afflicted participants. Meanwhile, 39 serum samples tested positive for at least one specific aeroallergen. Observing the relationship between *S. aureus* carriage and specific IgE presence revealed no significant distinction between the serum allergen-specific IgE levels in *S. aureus*-positive and *S. aureus*-negative rhinitis patients ($\chi2 = 0.38$) A comparable analysis of the saliva samples yielded analogous outcomes, as delineated in figure 2 . We performed a one-way analysis of variance ANOVA (F= 0.40 , p= 0.749) and did not found any statistically significant differences between groups with *S. aureus* colonization in the nasal cavity and without colonization. Locally produced specific IgE to Dermatophagoides pter. (d1) and Timothy grass (g6) seem to be independent processes. No significant differences were found between groups as shown in figure 3

Local and systemic specific IgE distribution

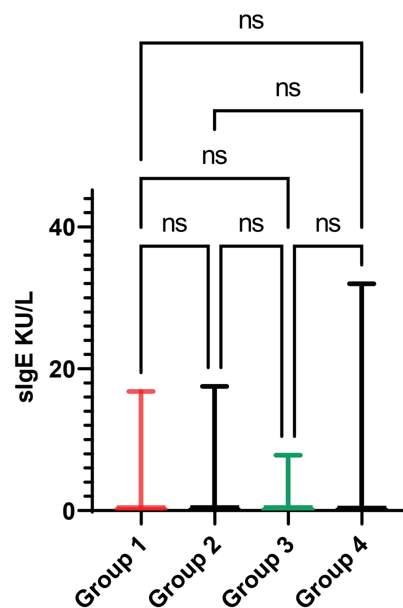

Fig. 3. *S. aureus* colonization and S IgE in saliva samples: Patients were divided into four groups according to two criteria: colonization with *S. aureus* and detection of d1 or g6 specific IgE in saliva samples**. Group 1**: ***S. aureus*** positive nasal swab/ d1 sIgE detected in saliva; **Group 2**: *S. aureus* negatve nasal swab/ d1 sIgE detected in saliva; **Group 3**: *S. aureus* positive nasal swab/ g6 sIgE detected in saliva; **Group 4**: *S. aureus* negative nasal swab/ g6 sIgE detected in saliva. No significant differences were found between groups.

In an immunological examination, the local and systemic detection of specific IgE antibodies to allergens Dermatophagoides pteronissinus (d1) and Timothy grass (g6) allowed for the categorization of patients into distinct groups. We utilized the quantification of specific IgE in saliva and serum samples as a distinguishing factor for these classifications: No specific antibodies detected (IgE < 0.35 KU/L for serum samples, or even lower for saliva samples); Weak antibody detection (0.7 KU/L < IgE < 3.5 KU/L); Defined antibody detection (3.5 KU/L < IgE < 17.5 KU/L); Strong antibody detection (17.5 KU/L < IgE < 50 KU/L); High antibody titer (17.5 KU/L < IgE < 50 KU/L); Very high antibody titer (50 KU/L < IgE < 100 KU/L). In our study, we identified d1-specific IgE in the saliva samples from 60 patients. Among these individuals, 36 demonstrated no detectable d1-specific antibodies in their serum samples. I cases of timothy grass sensitization (g6) 44 participants revealed the presence of IgE in saliva samples. However, 20 of these individuals exhibited no specific antibodies against g6 in their serum samples. Relationships between serum and salivary IgE to the two allergens are presented in figure 4.

Analysis of variance of the variance among serum and saliva specific Ig E to Dermatophagoider pter. and Timothy grass showed a statistically significant dependence between saliva d1 sIgE vs. Serum g6 sIgE and serum g6 sIgE vs. Saliva g6 sIgE (F 7.380, p<0.0001). Comparison of all other variables showed no statistical significance. Such a result may be due to the time of sample collection. During polen season active production of a specific IgE is expected in genetically susceptible individuals.It is scientifically acknowledged that IgE can constitute a normal immune response to various antigens without

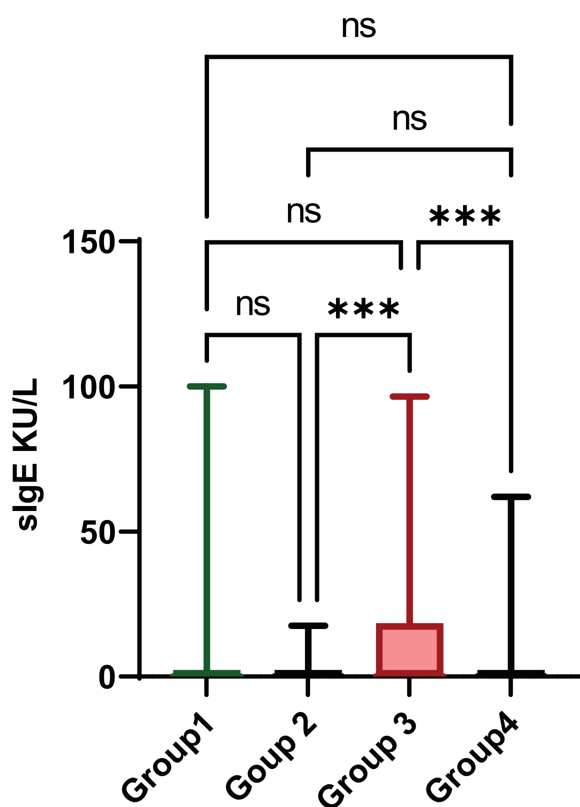

Fig. 4. Serum and saliva sIgE levels: Patients were divided into four groups according to the detection of specific IgE in saliva and blood samples. **Group 1:** Patients with positive serum samples to Dermatophagoides pter. (d1); **Group 2:** Patients with positive saliva samples to d1; **Group 3:** Patients with positive serum samples to Timothy grass (g6); **Group 4:** Patients with positive saliva samples to g6

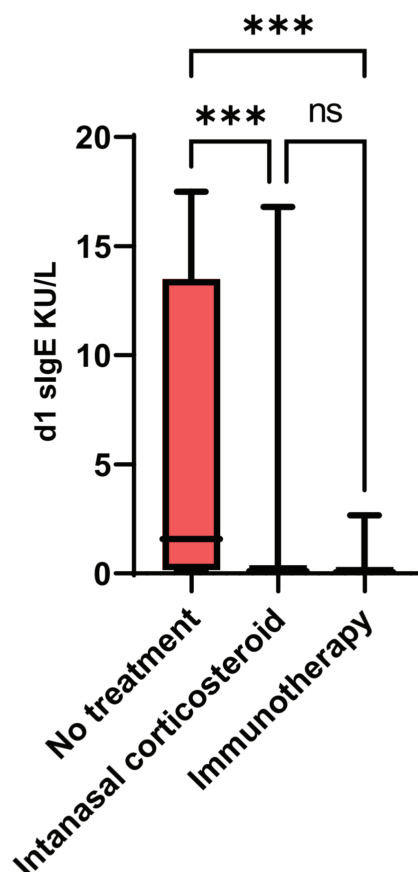

Fig. 5. Influence of therapy: Higher concentration of specific IgE in saliva samples in untreated patients was detected. No superiority of either therapy was found.

being linked to clinical manifestations. However, it is important to emphasize that our study's inclusion criterion is the presence of persistent rhinitis symptoms for at least two months. This fact directs our focus towards whether patients are undergoing treatment or not. A significant percentage of the participants in our research received nasal steroid treatment or immunotherapy, which may be attributed to the inclusion criteria for clinically relevant rhinitis. Among them, 47.5% (n = 32) reported using intranasal steroids, 25.7% (n = 18) were undergoing immunotherapy, and 28.6% (n = 20) were not receiving any treatment.

In order to evaluate the influence of therapy on the local production of specific IgE, we applied a one-factor analysis. Results are shown on fig 5.

Tukey's multiple comparisons test showed a higher concentration of specific IgE in saliva samples in untreated patients. No superiority of either therapy approaches was found (F=11.43, p <0.0001)

DISCUSSION

Immunoglobulin E (IgE) is widely recognized as a crucial mediator in allergic inflammation and helminth infections. There is a significant overlap in serum levels of total IgE between allergic and non-allergic individuals, which may be attributed to geographical variations in the prevalence of exposure to helminth infections, as the concentration of IgE in human serum rapidly escalates during such an infection. Moreover, "growth curves" of total IgE levels are established during childhood in children without atopy by determining total IgE levels at various time points during growth. Indeed, the trajectory of total IgE during different time points in childhood is strikingly similar in never-allergic and allergic children, and this process is independent of the absolute concentration of total IgE. These total IgE levels are derived from two distinct sources: spontaneously produced IgE ("normal," baseline IgE) and specific IgE generated in response to environmental allergens ("abnormal" or atopic IgE). Seasonal exposure to allergens can trigger a rapid increase in allergen-specific IgE levels, which can consequently elevate total IgE levels. As a result, higher IgE levels can be sustained through repeated contact with allergens. Repeated exposure to inhalant or food allergens serves as a prime example of maintaining this process. Furthermore, populations of IgE-secreting plasma cells are significantly higher in the nasal mucosa compared to peripheral cell populations. This fact highlights the local production of secretions as an especially intriguing focus for research on key components in the pathogenesis of diseases with primarily organ-localized manifestations, such as allergic rhinoconjunctivitis. Comparative analyses of specific IgE immunoglobulin levels in serum and saliva have been performed for various food allergens, such as shrimp, eggs, soy, wheat, chestnut, peanuts, kiwi, banana, tomato, and cocoa. In the majority of foods, no significant difference was observed between serum and saliva levels. However, the mean concentration of IgE antibodies against cow's milk and papaya in serum was markedly lower than that in saliva (p <0.05), whereas the mean concentration of IgE antibodies against fish and corn in serum was notably higher than that in saliva (p <0.05) [12-15].

Another potential mechanism for maintaining the total IgE pool is polyclonal T cell activation through microbial stimulation. Gram-positive microorganisms has been identified in the role of superantigens. As superantigens may also served: coagulase-negative staphylococci, beta-hemolytic streptococci (groups B, C, and G), *Mycoplasma arthritidis, Yersinia enterocolitica, Yersinia pseudotuberculosis, Plasmodium falciparum, Clostridium perfringens, Candida albicans, and Toxoplasma gondii. S.aureus* strains secreted up to 24 different superantigens. In case of group A streptococcal strains 11 types are reported [16].

S. aureus superantigens (SAgs) stimulate large proportion of T cells by cross-linking their T cell receptor[17].

The pathogenic role of *S. aureus* and its Ses has been recognized in various airway diseases such as chronic rhinosinusitis with nasal polyposis and severe asthma [3]. Furthermore, scientific literature indicates that toxic shock syndrome toxin (TSST-1) amplifies the allergen-specific IgE production in vitro. This effect was regulated by IFNγ and IL-4 concentrations. During the pollen season restimulation of in vivo-primed peripheral blood mononuclear cells with TSST-1 induced the allergen-specific IgE production in vitro. Outside the polen season additional exogeneous IL4

is needed to induce allergen- specific IgE production in vitro[17].

S. aureus might exacerbate local eosinophilic inflammation, thereby intensifying the overall nasal symptom score. Some studies have suggested that nasal S. aureus or specific IgE in serum against Ses are correlated with an increased risk of asthma and heightened symptom severity [18]. By functioning as superantigens, SEs can provoke intense Th2 inflammation. Nobusuke Hohchi documented the impact of *S. aureus* colonization in a mouse model of allergic rhinitis wherein the Ovalbumin-sensitized *S. aureus*-inoculated (AR-SA) group exhibited elevated eosinophil and neutrophil counts, IgE and IgG1 levels, and expressions of IL-4 mRNA and IL-5 mRNA compared to control groups. Furthermore, higher *S. aureus* counts were detected in the nasal mucosa of patients in the same group [19]. It is fascinating to explore the possibility that *S. aureus* contributes to the increased production of serum-specific IgE in response to various inhalant allergens by amplifying the Th2 immune response. Our findings suggest that there is no association between *S. aureus* colonization and serum-specific IgE levels, as well as saliva-specific IgE levels, in patients with rhinitis ($\chi2 = 0.38$ for serum samples and $\chi2 = 0.113$ for Dermatophagoides pter d1 and $\chi2 = 0.474$ for thymothy grass g6 in saliva samples, $F= 0.40$, $p= 0.749$).

The majority of serum IgE is derived from mucosal surfaces [9]. During the initial development of allergic rhinitis, allergen provocation triggers IgE production at the mucosal level, with a minor fraction eventually entering the blood stream . According to manufacturers' instructions, specific IgE concentrations of 0.35 KU/L or higher become detectable. The process of entering circulation is likely influenced by multiple factors such as allergen exposure frequency, age, and presence or absence of modifying therapy. Conversely, various studies in this field have followed patients for different periods; long-term studies remain scarce.

Ramadani et al. demonstrated that local IgE production rates are sufficient to saturate the entire IgE receptor system on mast cells present at the site before ultimately entering the bloodstream . In cases of allergic rhinitis, symptoms may manifest only when specific local IgEs are present. "Local allergic rhinitis" (LAR) is a recognized endotype of rhinitis involving nasal allergen provocation tests (NAPT) in patients exhibiting typical allergic rhinitis symptoms but testing negative for skin prick tests (SPT) or serum-specific IgE against any allergen [7, 12]. The exact prevalence of LAR remains uncertain; however, it ranges from approximately 25% to 45% among patients with chronic, non-infectious rhinitis [7, 12]. Several research studies have suggested that local allergic rhinitis (LAR) does not progress to allergic rhinitis (AR) accompanied by systemic atopy over time. Conversely, other investigations have posited that "local IgE" levels may be a risk factor for systemic IgE-dependent reactions [7, 8]. Mortada et al. in their review [8] provide data from a 10-year follow-up of LAR patients, indicating that only small percentage of them developed systemic sensitization. On the other hand LAR is considered as a significant risk factor in the emergence of asthma. Another systemic review describes a high frequency of LAR among children [15] Terada et al described that nasal allergen reactivity was present in 16.1% of children under 16 years old with non-allergic rhinitis (NAR) and frequency of LAR may increase with age [9, 15]. There is potential for the initial stage of allergic rhinitis to exhibit serum concentrations of free IgE too low for detection by current IgE tests [12]. Indeed, immune responses to allergens in healthy individuals necessitate disparate antibody production, consisting of low IgG1, IgG4, and secretory IgA (sIgA) levels, with or without a minimal amount of IgE present [20].

It is hypothesized that local IgE production, with initial negative Skin Prick Test (SPT) and IgE test, represents a stage of allergic rhinitis. This stage may persist for an extended duration, particularly among individuals with very low IgE production. Treatment using monoclonal anti-IgE antibodies, which bind to allergen-specific IgE present in the circulation, might lead to elevated serum IgE levels. Consequently, low producers may become detectable. In our study, patients receiving omalizumab were not included.

In our cohort of patients diagnosed with rhinitis, 36 individuals demonstrated specific IgE-negativity for Dermatophagoides preronissinus (d1) in blood samples and positivity in saliva samples ($\chi2 = 19.76$, $\alpha = 0.181$), whereas 25 patients were negative for g6 in blood samples and positive for g6 in

saliva samples ($\chi2$ = 6.89, α = 0.86). In accordance with previous research, our study verified the independent manifestation of a local IgE-specific response and systemic sensitization to the same or another allergen. In addition we performed analyses of variance and found statistically significant dependence between saliva dermatophagoides pter. (d1) sIgE vs. Serum timothy grass (g6) sIgE and serum thymothy grass sIgE vs. Saliva timothy grass sIgE (F 7.380, p<0.0001). Such a result may be due to the point of sample collection. All samples are gathered during polen season. Earlier investigations confirmed Local Allergic Rhinitis (LAR) among children exhibiting significantly higher nasal sIgE levels (nasal sIgE > 0.35 kU/L according to EAST classification system) compared to control subjects and positive results in the Nasal Allergen Provocation Test (NAPT). Dust mites represent the most prevalent allergens in LAR. In our study population of sixty patients, we identified IgE specific to dermatophagoides pter. (d1) in saliva samples. Among these individuals, thirty-six did not display specific antibodies to Dermatophagoides pter. (d1) in their serum samples. As for timothy grass (g6) sensitization, forty-four participants exhibited salivary IgE specificity toward timothy grass (g6). Within the same group, twenty individuals lacked specific antibodies against timothy grass in their serum samples. In contrast, Miranda reported undetectable levels of specific IgE in saliva samples among atopic and non-atopic children [21]. However, follow-up individuals at least in two time points would contribute to clarifying the dynamic of local IgE response in atopics and non-atopics.

When the constituents of saliva originate from the bloodstream, the concentrations of biochemical and immunological components present in the saliva can mirror their levels in the blood. There is a growing interest in substituting blood samples with salivary samples for analyzing various biomarkers, owing to the non-invasive nature of saliva collection and the elimination of risks linked to blood sample acquisition [13]. Saliva can be gathered and assessed as either unstimulated or stimulated from specific glandular pairs. Unstimulated whole saliva refers to the initial saliva found in the oral cavity for a majority of a 24-hour period [13]. Identifying diverse biomarkers in saliva can potentially serve as an invaluable technique in monitoring the management of chronic diseases.

The ImmunoCAP system is recognized as the optimal approach for quantifying specific IgE in nasal swabs, with 0.12 kUA/L established as the threshold value [22].

In our study, we observed reduced levels below 0.12 kUA/L in the patient group (0.04 KU/L was the lowest level of detection). It is crucial to note that these findings were primarily conducted within the context of implemented therapy for most participants. Specific IgE levels were assessed at a single time point during the pollen season, underlining the importance of continuously monitoring local-specific IgE production in patients with rhinitis. The non-invasive nature of this test renders it an appropriate biomarker for therapy evaluation, disease management, and diagnostic precision.

Egger [23] stated that fluticasone propionate application did not significantly influence the increase in systemic allergen-specific IgE production following allergen exposure. Wilson [24] suggested that grass pollen immunotherapy could hinder the seasonal rise of basophils and eosinophils in the nasal epithelium among individuals with allergic rhinitis. It has also been reported that immunotherapy can increase the ratio of IFN-γ to IL-5 micro RNA cells within nasal mucosa [25].

The primary treatment for individuals with asthma and allergic rhinitis involves the local administration of corticosteroids. In a study conducted by Jerome Kerzerho, the impact of systemic and local corticosteroid administration on mucosal tolerance development in patients with bronchial asthma was examined . The results demonstrated that inhaled corticosteroids enhanced the development of Treg cells [26]. Our research further revealed that specific IgE levels in saliva were significantly associated with the course of treatment for Dermatophagoides pter. (d1) and Tymothy grass (g6) saliva samples, as indicated by single factor analysis: $F(2,57) = 21.85$, $P < .001$ for Dermatophagoides pter. (d1), and $F(2,41) = 12.42$, $P < .001$ for Timothy grass (g6), with Eta Squared values of 0.411 for d1 and 0.377 for g6. Tukey's multiple comparisons test showed a higher concentration of specific IgE in saliva samples in untreated patients (F=11.43, p <0.0001).

CONCLUSION

In the examined cohort, localized IgE production to allergens d1 and g6 plays a significant role in the progression of rhinitis. It is important to note that mucosal-specific IgE concentrations do not exhibit a correlation with systemic specific IgE levels or *S. aureus* colonization. Our findings reveal a reduced presence of salivary-specific IgE in patients undergoing active treatment compared to those without treatment. This observation highlights the potential utility of local specific IgE as a reliable biomarker for individuals suffering from rhinitis within an academic context.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by ETHICS COMMITTEE IRB 00006384 (protocol code PROTOCOL № 3/2024 and May 09, 2024)."

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REFERENCES

1. Nur Husna, S.M., et al., *Allergic Rhinitis: A Clinical and Pathophysiological Overview.* Front Med (Lausanne), 2022. **9**: p. 874114.
2. Deacy, A.M., S.K. Gan, and J.P. Derrick, *Superantigen Recognition and Interactions: Functions, Mechanisms and Applications.* Front Immunol, 2021. **12**: p. 731845.
3. Flora, M., et al., *Staphylococcus Aureus in chronic airway diseases: An overview.* Respir Med, 2019. **155**: p. 66-71.
4. ravers, J. and M.E. Rothenberg, *Eosinophils in mucosal immune responses.* Mucosal Immunol, 2015. **8**(3): p. 464-75.
5. Huby, R.D., R.J. Dearman, and I. Kimber, *Why are some proteins allergens?* Toxicol Sci, 2000. **55**(2): p. 235-46.
6. De Schryver, E., et al., *Local immunoglobulin e in the nasal mucosa: clinical implications.* Allergy Asthma Immunol Res, 2015. **7**(4): p. 321-31.
7. Cantone, E., A. Detoraki, and E. De Corso, *Local Allergic Rhinitis: A Different Rhinitis Endotype? Literature Overview.* Applied Sciences, 2022. **12**(21).
8. Mortada, M.M. and M. Kurowski, *Challenges in Local Allergic Rhinitis Diagnosis, Management, and Research: Current Concepts and Future Perspectives.* Medicina (Kaunas), 2023. **59**(5).
9. Rondon, C., et al., *IgE Test in Secretions of Patients with Respiratory Allergy.* Curr Allergy Asthma Rep, 2018. **18**(12): p. 67.
10. KleinJan, A., et al., *Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients.* Eur Respir J, 2000. **15**(3): p. 491-7.
11. Bousquet, J., et al., *Rhinitis associated with asthma is distinct from rhinitis alone: The ARIA-MeDALL hypothesis.* Allergy, 2023. **78**(5): p. 1169-1203.
12. Matricardi, P.M., *The Very Low IgE Producer: Allergology, Genetics, Immunodeficiencies, and Oncology.* Biomedicines, 2023. **11**(5).
13. Williamson, S., et al., *Comparison of biomarkers in blood and saliva in healthy adults.* Nurs Res Pract, 2012. **2012**: p. 246178.
14. Eckl-Dorna, J., et al., *Tracing IgE-Producing Cells in Allergic Patients.* Cells, 2019. **8**(9).
15. Terada, T. and R. Kawata, *Diagnosis and Treatment of Local Allergic Rhinitis.* Pathogens, 2022. **11**(1).
16. Spaulding, A.R., et al., *Staphylococcal and streptococcal superantigen exotoxins.* Clin Microbiol Rev, 2013. **26**(3): p. 422-47.
17. Abdurrahman, G., et al., *Allergy-A New Role for T Cell Superantigens of Staphylococcus aureus?* Toxins (Basel), 2020. **12**(3).
18. Shaghayegh, G., et al., *S. aureus biofilm metabolic activity correlates positively with patients' eosinophil frequencies and disease severity in chronic rhinosinusitis.* Microbes Infect, 2023. **25**(8): p. 105213.
19. Hohchi, N., et al., *Synergism of Staphylococcus aureus colonization and allergic reaction in the nasal cavity in mice.* Int Arch Allergy Immunol, 2012. **159**(1): p. 33-40.
20. Gloudemans, A.K., B.N. Lambrecht, and H.H. Smits, *Potential of immunoglobulin A to prevent allergic asthma.* Clin Dev Immunol, 2013. **2013**: p. 542091.
21. Miranda, D.O., et al., *Serum and salivary IgE, IgA, and IgG4 antibodies to Dermatophagoides pteronyssinus and its major allergens, Der p1 and Der p2, in allergic and nonallergic children.* Clin Dev Immunol, 2011. **2011**: p. 302739.
22. Santamaria, L., et al., *Nasal specific IgE to Der p is not an acceptable screening test to predict the outcome of the nasal challenge test in patients with non-allergic rhinitis.* World Allergy Organ J, 2020. **13**(9): p. 100461.
23. Egger, C., et al., *Effects of nasal corticosteroids on boosts of systemic allergen-specific IgE production induced by nasal allergen exposure.* PLoS One, 2015. **10**(2): p. e0114991.
24. Wilson, D.R., et al., *Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium.* Clin Exp Allergy, 2001. **31**(11): p. 1705-13.
25. Wachholz, P.A., et al., *Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios.* Immunology, 2002. **105**(1): p. 56-62.
26. Kerzerho, J., et al., *Effects of systemic versus local administration of corticosteroids on mucosal tolerance.* J Immunol, 2012. **188**(1): p. 470-6.