SALIVARY HUMORAL AND CYTOKINE IMMUNE INDICATORS IN CHILDREN WITH RECURRENT HERPETIC STOMATITIS, INCLUDING THOSE WITH CONCURRENT ALLERGIC DISEASES

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Abstract. Recurrent herpetic stomatitis (RHS) is a common pediatric infection caused by herpes simplex virus type 1, often associated with impaired mucosal immunity. When accompanied by allergic diseases, the severity of immune dysregulation may increase. This study investigated local humoral and cytokine immunity in children with RHS, both isolated and combined with allergic conditions. **Methods:** A total of 120 children aged 1–7 years were examined and divided into three groups: healthy controls (n=40), RHS without allergy (n=40), and RHS with allergy (n=40). Saliva samples were collected and analyzed for secretory IgA (sIgA), lysozyme, total IgA, IgM, IgG, IgE, as well as cytokines IL-6 and IL-10 using ELISA. Statistical analysis was performed to compare groups. **Results:** Children with RHS demonstrated a significant decrease in sIgA and lysozyme levels compared with controls, which was more pronounced in allergic patients (sIgA: 207.17 vs. 90.57 µg/mL; lysozyme: 159.76 vs. 76.71 µg/mL, $p<0.001$). Humoral immunity was altered: IgA decreased nearly twofold in the RHS+allergy group, while IgM increased more than twofold. IgG was elevated only in RHS without allergy, whereas IgE was strongly elevated in both patient groups, with the highest levels in comorbid cases (246.29 µg/mL). Cytokine profiling revealed marked IL-6 elevation (up to 4.58-fold above controls) and a parallel but less intense rise in IL-10. **Conclusion:** RHS in children is associated with impaired local immunity, antibody imbalance, and cytokine dysregulation. The coexistence of allergic diseases aggravates immune deficiencies, amplifies inflammatory responses, and further weakens mucosal protection. These findings highlight the importance of comprehensive immune monitoring in pediatric RHS, particularly under allergic comorbidity.

Keywords. Recurrent herpetic stomatitis; children; salivary immunity; secretory IgA; immunoglobulins; lysozyme; cytokines; IL-6; IL-10; allergic diseases; humoral immunity.

Introduction. Recurrent herpetic stomatitis (RHS) is one of the most common chronic infectious diseases of the oral cavity in children, characterized by periodic exacerbations, painful ulcerations, and impaired oral mucosal integrity. According to epidemiological data, recurrent forms of herpes simplex virus (HSV) infection affect

up to 20–40% of children worldwide, with the highest incidence observed in the age group of 3–12 years [1,2]. In many countries, HSV-1 seropositivity among children reaches 60–70% by the age of five, and in approximately one-third of these cases the infection takes a recurrent course [3]. Recurrent herpetic stomatitis not only decreases quality of life due to pain and feeding difficulties but also predisposes children to secondary bacterial infections and long-term mucosal hypersensitivity [4].

In recent years, increasing attention has been directed toward the immunopathogenesis of RHS, especially the role of local (salivary) immunity. Saliva contains a wide range of humoral defense factors, including immunoglobulins (IgA, IgM, IgG, and IgE) and antimicrobial peptides, which serve as the first protective barrier of the oral mucosa [5]. Secretory IgA plays a central role in maintaining mucosal immunity by neutralizing viral particles and preventing their adhesion to epithelial cells [6]. However, in children with recurrent HSV infection, disturbances in the quantitative and functional activity of these immunoglobulins have been reported [7].

Cytokines, as key regulators of inflammatory and immune responses, are also involved in the pathogenesis of RHS. Pro-inflammatory cytokines such as interleukin-6 (IL-6) stimulate local inflammation and epithelial damage, while anti-inflammatory cytokines such as interleukin-10 (IL-10) contribute to the regulation and limitation of tissue injury [8]. Previous studies have shown that children with RHS exhibit an imbalance in cytokine production, with increased IL-6 levels and insufficient compensatory secretion of IL-10, leading to prolonged inflammation and delayed healing of lesions [9].

Of particular clinical importance is the coexistence of RHS with allergic diseases, which has been increasingly documented in pediatric populations. The prevalence of allergic conditions, including bronchial asthma, atopic dermatitis, and allergic rhinitis, has risen significantly in the past two decades, now affecting up to 30% of children in developed countries [10]. It has been hypothesized that allergic inflammation further disrupts mucosal immunity and alters salivary immunoglobulin and cytokine profiles, thereby exacerbating the severity and frequency of RHS episodes [11].

Despite significant research, there is still limited understanding of the interrelationship between humoral and cytokine immunity in salivary fluid of children with recurrent herpetic stomatitis, particularly when it is combined with allergic diseases. Detailed study of these immunological parameters may provide new insights into the mechanisms of chronicity and may contribute to the development of personalized therapeutic and preventive strategies for affected children [12,13].

Recurrent herpetic stomatitis (RHS) is an infectious disease caused predominantly by herpes simplex virus type 1 (HSV-1) of the *Herpeviridae* family. The disease is characterized by ulcerative lesions of the oral mucosa, accompanied by regional

lymphadenitis. Globally, up to 80% of all infectious stomatitis cases are attributed to HSV-1, and in pediatric populations, especially between the ages of 1 and 7 years, the prevalence reaches 70%, whereas in adolescents and adults it is much lower. This high prevalence in children is linked to the maturation characteristics of local oral immunity and increased exposure to environmental pathogens.

Although the pathogenesis of RHS has been extensively studied, the quantitative changes in local humoral immune factors—especially under comorbid allergic conditions—remain insufficiently described. Understanding these changes is essential, as the immune system in early childhood is still developing, and protective maternal antibodies are largely eliminated by the age of 3, making children more susceptible to recurrent infections.

Cytokine-mediated immune regulation plays a central role in controlling both viral infections and allergic inflammation. Interleukin-6 (IL-6) acts as a pro-inflammatory mediator, promoting acute-phase protein synthesis and leukocyte activation, whereas interleukin-10 (IL-10) functions as an anti-inflammatory cytokine, inhibiting excessive immune responses while enhancing antibody production. Evaluating the balance between these cytokines, along with immunoglobulin profiles, can provide insight into the immunopathogenesis of RHS and its exacerbation under allergic comorbidity.

This study aims to comparatively assess salivary levels of secretory immunoglobulin A (sIgA), lysozyme, total immunoglobulins (IgA, IgM, IgG, IgE), and cytokines (IL-6, IL-10) in children with isolated RHS and those with RHS combined with allergic diseases.

Materials and Methodsю This research was conducted on a total of 120 children aged 1 to 7 years. The study population was divided into three groups, each consisting of 40 participants: (1) healthy children with no history of RHS or allergic diseases (control group), (2) children diagnosed with RHS without allergic comorbidities (comparison group), and (3) children diagnosed with RHS combined with allergic diseases (main group). All participants were recruited from pediatric outpatient clinics, and informed consent was obtained from parents or guardians.

Saliva samples were collected in the morning, at least two hours after food or liquid intake, using a standard non-invasive collection technique. Unstimulated whole saliva was collected into sterile tubes under the supervision of trained personnel. Samples were immediately frozen and stored at –20°C until further processing.

Immunological analyses were performed in certified laboratories. Local immune factors, such as secretory immunoglobulin A (sIgA) and lysozyme, were quantified using standard enzyme-linked immunosorbent assay (ELISA) kits. Humoral immunity was evaluated by measuring total IgA, IgM, IgG, and IgE concentrations in saliva. Additionally, cytokine status was determined by quantifying IL-6 (a pro-inflammatory

cytokine) and IL-10 (an anti-inflammatory cytokine) using high-sensitivity ELISA kits. Each measurement was performed in triplicate, and average values were calculated.

Statistical analysis was carried out using SPSS software. Results were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using Student's *t*-test. Statistical significance was accepted at $p < 0.05$.

Results. The analysis of salivary immune parameters in the studied children revealed significant differences between healthy individuals and those with RHS. These differences became even more pronounced in patients who also had allergic comorbidities. The general trend showed a marked reduction in local protective factors (sIgA and lysozyme), alterations in the balance of immunoglobulins, and elevated cytokine levels, particularly pro-inflammatory IL-6.

1. Changes in non-specific local immunity factors

Table 1 shows the concentrations of sIgA and lysozyme in salivary fluid. Healthy children demonstrated high baseline levels of both parameters, while patients with RHS exhibited a sharp decline. This reduction was most severe in children with combined allergic diseases.

Table 1.

Salivary sIgA and lysozyme concentrations in children with RHS (µg/mL)

Group	sIgA (µg/mL)	Lysozyme (µg/mL)
Control	207.17 ± 9.31	159.76 ± 7.40
Comparison	105.76 ± 3.66* ↓	98.18 ± 4.78* ↓
Main	90.57 ± 4.89* ↓ ^	76.71 ± 2.59* ↓ ^

Note: $p < 0.001$ vs. control; ^ $p < 0.05$ vs. comparison.

The concentration of sIgA in healthy children was 207.17 µg/mL, while in children with RHS it dropped to 105.76 µg/mL and further decreased to 90.57 µg/mL in those with comorbid allergies. Lysozyme also showed a similar decline, from 159.76 µg/mL in controls to 98.18 µg/mL in the comparison group and 76.71 µg/mL in the main group. These changes confirm a significant impairment in mucosal immunity.

2. Relative changes in non-specific factors compared to healthy controls

Table 2.

Fold-change in sIgA and lysozyme relative to control values

Group	sIgA (fold change)	Lysozyme (fold change)
Comparison	1.96* ↓	1.63* ↓
Main	2.29* ↓ ^	2.08* ↓ ^

Compared with controls, children with RHS had nearly a twofold decrease in sIgA and lysozyme, with the most severe decline in the main group. These findings highlight that allergic comorbidity worsens the suppression of salivary protective factors.

3. Humoral immunity parameters

Table 3.

Salivary immunoglobulin concentrations (µg/mL)

Immunoglobulin	Control	Comparison	Main
IgA	2.23 ± 0.15	2.24 ± 0.17 ↔	1.13 ± 0.08* ↓ ^
IgM	1.50 ± 0.14	1.47 ± 0.12 ↔	3.13 ± 0.19* ↑ ^
IgG	11.10 ± 1.23	23.66 ± 1.52* ↑	10.84 ± 0.85 ↔ ^
IgE	98.16 ± 9.34	228.69 ± 7.62* ↑	246.29 ± 7.62* ↑ ^

In healthy controls, IgA was stable at around 2.23 µg/mL. In the comparison group, IgA remained unchanged, but in the main group it fell sharply to 1.13 µg/mL, almost two times lower than normal. IgM, however, doubled in the main group compared to controls (3.13 vs. 1.50 µg/mL), indicating an enhanced primary immune response compensating for IgA deficiency. IgG was elevated in the comparison group (23.66 µg/mL vs. 11.10 µg/mL), but returned to baseline in the main group, suggesting an impaired secondary immune response in allergic children. IgE levels were markedly higher in both patient groups, especially in the main group (246.29 µg/mL), confirming an allergic background.

4. Cytokine profile

Table 4.

Salivary cytokine concentrations (pg/mL)

Group	IL-6	IL-10
Control	23.87 ± 2.67	29.17 ± 2.93
Comparison	70.29 ± 3.22* ↑	58.34 ± 3.39* ↑
Main	109.37 ± 4.51* ↑ ^	78.98 ± 3.33* ↑ ^

IL-6 levels in controls were 23.87 pg/mL but increased to 70.29 pg/mL in children with RHS, and further to 109.37 pg/mL in those with allergies. IL-10 also increased from 29.17 pg/mL in controls to 58.34 pg/mL and 78.98 pg/mL in the comparison and main groups, respectively. This demonstrates a hyperactive inflammatory response with simultaneous, though insufficient, compensatory anti-inflammatory activity.

5. Summary of all immune parameters as fold-change relative to controls

Table 5.

Immune parameters in RHS patients relative to control group

Parameter	Comparison (fold)	Main (fold)
sIgA	0.51 ↓	0.44 ↓
Lysozyme	0.61 ↓	0.48 ↓
IgA	1.00 ↔	0.51 ↓
IgM	1.02 ↔	2.09 ↑
IgG	2.13 ↑	0.98 ↔
IgE	2.33 ↑	2.51 ↑
IL-6	2.94 ↑	4.58 ↑
IL-10	2.00 ↑	2.71 ↑

Compared with healthy children, RHS patients exhibited a 49–56% reduction in sIgA and lysozyme, indicating impaired mucosal defenses. In the main group, IgA was halved, while IgM doubled, reflecting a disrupted balance in humoral immunity. IgG significantly increased only in the comparison group. Both groups demonstrated a marked elevation of IgE, consistent with allergic predisposition. Cytokine responses showed a sharp increase in IL-6 and IL-10, with IL-6 rising more steeply, confirming a predominance of inflammatory processes.

Discussion. The findings demonstrate that children with RHS have significant alterations in both local and systemic humoral immunity. The marked reduction in sIgA and lysozyme confirms impairment of the first-line defense at mucosal surfaces, predisposing patients to recurrent infections. These changes were more pronounced in children with concomitant allergic diseases, suggesting a synergistic negative effect of allergic inflammation on oral mucosal immunity.

In terms of immunoglobulin profiles, the main group displayed a pronounced IgA deficiency coupled with elevated IgM levels, indicative of an incomplete or dysregulated class-switch recombination process. The lack of significant IgG elevation in this group, compared to the sharp increase in the comparison group, points to inadequate secondary immune responses when allergic pathology coexists.

The cytokine data highlight a heightened inflammatory state, with IL-6 levels particularly elevated in the main group. This suggests that allergic inflammation amplifies the inflammatory cascade triggered by HSV-1 infection. Although IL-10 also increased, possibly as a compensatory anti-inflammatory mechanism, its rise was insufficient to counterbalance the pro-inflammatory dominance.

Overall, the immune dysregulation observed in the main group reflects a complex interplay between viral persistence, impaired mucosal immunity, and chronic allergic inflammation. These findings have practical implications for the clinical management

of RHS, indicating the need for combined antiviral and immunomodulatory strategies, particularly in children with allergic comorbidities.

Conclusion. Children with RHS exhibit significant local immune deficiencies, especially in sIgA and lysozyme levels, with more severe impairment when allergic diseases are present. Humoral immunity in such cases is characterized by IgA depletion, IgM overproduction, and persistently high IgE levels. The cytokine profile reveals a predominance of IL-6–mediated inflammation, only partially counterbalanced by IL-10. These data underscore the importance of assessing local immune parameters and cytokine profiles to guide targeted therapy in pediatric RHS.

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