Keywords
Rampant caries; secretory IgA; histatin; Streptococcus mutans; salivary flow rate; children; Iraq

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Received-17-05-2026
Revised-21-06-2026
Accepted-26-06-2026
Doi:10.1922/ejprd.v34i4s.1486

European Journal of Prosthodontics and Restorative Dentistry (2026) 34(4S), 500-505

Salivary Immunological Defence in Children with Rampant Dental Caries: Comparative Assessment of Secretory Immunoglobulin A, Histatin, and Streptococcus mutans in Babylon Governorate, Iraq

Abstract

Objective: This study compared salivary secretory immunoglobulin A (s-IgA) and histatin concentrations, alongside salivary flow rate, resting pH, buffering capacity, and Streptococcus mutans presence, between children with rampant dental caries and a control group of lower caries burden in Al-Hilla, Babylon Governorate, Iraq.

Material and Methods: A cross-sectional comparative study enrolled 100 children aged 5–12 years: 55 in a control group and 45 with rampant dental caries. Caries experience was recorded using the WHO DMFT/dmft index. Salivary flow rate, resting pH, and buffering capacity were assessed chairside. Salivary s-IgA and histatin were quantified by ELISA, and S. mutans was assessed by salivary culture. Group comparisons used independent t-tests and chi-square tests, with Pearson correlation analysis performed across the full sample.

Results: Mean DMFT/dmft was significantly higher in the rampant caries group than in controls (15.60 vs. 5.82; $p<0.001$). Salivary flow rate differed significantly between groups ($p=0.032$), with low flow more frequent among rampant caries children. Salivary pH, buffering capacity, s-IgA, histatin, and S. mutans presence did not differ significantly between groups. A significant positive correlation was found between DMFT and buffering capacity ($r=0.271$; $p=0.006$), and between histatin and s-IgA ($r=0.220$; $p=0.028$).

Conclusion: Salivary immune markers did not independently distinguish children with rampant caries from controls, despite a clear and significant clinical separation in caries burden. Salivary flow rate emerged as the most clinically relevant discriminator. These findings support incorporating chairside salivary flow assessment into paediatric caries risk evaluation, and contribute evidence from an Iraqi population that remains underrepresented in the literature.

Introduction

Rampant dental caries describes a pattern of aggressive, multi-surface decay that progresses faster than ordinary caries and frequently involves teeth not typically prone to early breakdown. In children, it carries consequences well beyond the mouth itself — pain, infection, disrupted sleep, and interference with normal eating and growth are all common outcomes when the disease is left unaddressed (American Academy of Pediatric Dentistry, 2025; Purohit & Priya, 2022).

Iraq carries a heavy burden of childhood caries, yet very little biological data exist describing what actually differs, at a salivary level, between children who develop rampant disease and those who do not. Most of what clinicians rely on locally is extrapolated from studies conducted elsewhere — Europe, East Asia, or wealthier Gulf states — despite meaningful differences in diet,

water composition, and access to preventive dental care across these settings (Kotha, 2022; Fatani et al., 2022).

Saliva is not a passive fluid. It actively shapes the oral environment through several interconnected mechanisms: mechanical washing of food debris and sugars from the tooth surface, chemical buffering of the acids produced by cariogenic bacteria, delivery of calcium and phosphate that support enamel repair, and distribution of antimicrobial proteins that limit bacterial colonisation (Gupta et al., 2024; Scannapieco & Gershovich, 2022). Among these proteins, secretory immunoglobulin A (s-IgA) and histatin have drawn particular research interest because of their direct antibacterial actions against Streptococcus mutans, widely regarded as the principal organism driving caries initiation (Gornowicz et al., 2014; Lemos et al., 2019).

s-IgA works by binding to bacterial surface antigens, which interferes with the ability of S. mutans to adhere to the tooth pellicle — a necessary first step in plaque formation. Histatin, a smaller histidine-rich peptide secreted mainly by the parotid and submandibular glands, has both antibacterial and antifungal properties and has been proposed as a marker that might fall in children with more severe disease (Usman et al., 2025; Jurczak et al., 2015). Despite the biological plausibility of both markers, published findings on whether they actually differ between caries-active and caries-resistant children remain mixed, with some studies reporting clear differences and others finding none at all (Ahmad et al., 2022; Gorbatova et al., 2025).

Salivary flow rate is a separate but related consideration. Children who produce less saliva clear sugars and acids more slowly, and every other protective mechanism — buffering, remineralisation, antimicrobial delivery — becomes less effective simply because there is less fluid to carry it. Reduced flow has been linked to higher caries prevalence in several international cohorts, though Iraqi-specific data on this relationship are essentially absent (Ravikumar et al., 2023; Ansari et al., 2025).

This study set out to compare salivary s-IgA and histatin between Iraqi children with rampant dental caries and a lower-caries comparison group, while also examining flow rate, resting pH, buffering capacity, and S. mutans presence in the same children. By assessing all of these parameters together rather than in isolation, we aimed to understand not just whether any single marker differs by group, but how flow rate, chemistry, and immunity interact to shape caries risk in this specific population.

Material and Methods

Study Design and Participants

A cross-sectional comparative study was conducted at the Specialized Dental Center, Al-Hilla, Babylon Governorate, Iraq, between November 2024 and January 2026. One hundred children aged 5 to 12 years were recruited: 55 children with lower caries experience formed the control group, and 45 children diagnosed with rampant dental caries formed the comparison group. Rampant caries was defined as active decay affecting more than five tooth surfaces across multiple quadrants. Children with systemic conditions known to affect salivary gland function or immune status were excluded.

Caries Assessment

Caries experience was recorded by a single calibrated examiner using the WHO DMFT/dmft index under standard clinical lighting (World Health Organization, 2013). Decayed, missing (due to caries), and filled surfaces were summed across both dentitions to give each child a combined score.

Saliva Collection

Unstimulated whole saliva was collected by the passive drooling method between 10:00 and 17:00 (Navazesh, 1993). Children avoided food, drink, and oral hygiene procedures for at least one hour prior to collection and rinsed with plain water immediately beforehand. Approximately 2–3 mL of saliva was collected into sterile tubes, placed on ice, and transported promptly to the laboratory.

Salivary Flow Rate, pH, and Buffering Capacity

Resting salivary flow rate was assessed chairside and categorised as low or normal based on the time required for saliva to reappear on dried labial mucosa. Resting pH was measured with a colour-indicator strip read against a reference chart. Buffering capacity was scored using a multi-pad colorimetric strip, with total scores reflecting the degree of acid-neutralising capacity present in stimulated saliva.

Quantification of s-IgA and Histatin

Salivary s-IgA was quantified using the Human sIgA Sandwich ELISA Kit (Elabscience Biotechnology Co., Wuhan, China; Catalog No. E-EL-H1275; detection range 0.31–20 ng/mL). Salivary histatin was quantified using a validated Human Histatin 5 ELISA kit (detection range 12.5–800 ng/mL). Both assays followed the manufacturers' sandwich ELISA protocols, with optical density read at 450 nm and concentrations calculated against standard curves generated for each assay run.

Detection of Streptococcus mutans

Saliva samples were inoculated onto Mitis Salivarius Bacitracin (MSB) agar, a selective medium for S. mutans, and incubated at 37°C under 5% CO_2 for 48 hours. Colonies showing the characteristic raised, rough, frosted-glass morphology typical of S. mutans were identified by colony morphology and Gram staining. Each sample was scored as either positive (organism detected) or negative (no growth).

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the scientific committee under Protocol Number 189, dated 13 January 2025. Verbal informed consent was obtained from the parent or guardian of each participating child.

Statistical Analysis

Data were analysed in SPSS version 26. Normality of continuous variables was assessed using the Shapiro–Wilk test prior to selecting parametric comparison methods. Continuous variables were compared between

groups using the independent-samples t-test; categorical variables were compared using the chi-square test. Pearson correlation coefficients were calculated to examine relationships among DMFT, salivary flow rate, pH, buffering capacity, s-IgA, and histatin across the full sample. Statistical significance was set at $p \leq 0.05$.

Results

A total of 100 children completed the study: 55 in the control group and 45 with rampant dental caries. The mean DMFT/dmft score was 5.82 ± 1.07 in the control group compared with 15.60 ± 1.71 in the rampant caries group, a difference that was both large and highly significant ($p<0.001$), confirming clear clinical separation between the two groups. Resting salivary pH did not differ meaningfully between groups (6.99 vs. 6.92; $p=0.425$), nor did buffering capacity (6.51 vs. 7.67; $p=0.062$), though the rampant caries group trended numerically higher on the latter. Salivary s-IgA concentrations were essentially identical between groups (13.22 vs. 12.35 ng/mL; $p=0.826$), as were histatin concentrations (578.84 vs. 600.50 ng/mL; $p=0.739$). None of these four parameters reached statistical significance, as summarised in Table 1.

Table 1. Comparison of clinical and salivary parameters between control and rampant caries groups.

Parameter	Control (n=55)	Rampant Caries (n=45)	p-value
DMFT/dmft	5.82 ± 1.07	15.60 ± 1.71	<0.001*
Salivary pH	6.99 ± 0.36	6.92 ± 0.40	0.425
Buffering capacity	6.51 ± 3.01	7.67 ± 3.10	0.062
s-IgA (ng/mL)	13.22 ± 18.98	12.35 ± 20.45	0.826
Histatin (ng/mL)	578.84 ± 330.17	600.50 ± 311.76	0.739

Salivary flow rate was the one parameter that did distinguish the groups. Low resting flow was present in only 4 of 55 control children (7.3%) but in 10 of 45 rampant caries children (22.2%) — roughly three times the rate — and this difference was statistically significant ($p=0.032$), as shown in Table 2.

Table 2. Salivary flow rate distribution between control and rampant caries groups.

Flow Rate	Control No. (%)	Rampant Caries No. (%)	p-value
Low	4 (7.3%)	10 (22.2%)	
Normal	51 (92.7%)	35 (77.8%)	0.032*
Total	55 (100%)	45 (100%)	

S. mutans culture results showed no significant difference in presence between groups. The organism was detected in 28 of 55 control children (50.9%) and 29 of 45 rampant caries children (64.4%), a numerically higher rate in the caries group that did not reach significance ($p=0.174$), as presented in Table 3.

Table 3. Streptococcus mutans culture results between control and rampant caries groups.

S. mutans	Control No. (%)	Rampant Caries No. (%)	p-value
Nil	27 (49.1%)	16 (35.6%)	
Present	28 (50.9%)	29 (64.4%)	0.174
Total	55 (100%)	45 (100%)	

Sex-stratified analysis found no significant differences in DMFT (males 10.72 vs. females 9.77; $p=0.354$) or flow rate distribution (low flow in 19.1% of males vs. 9.4% of females; $p=0.162$), indicating that the group-level findings were not confounded by sex, as detailed in Table 4.

Table 4. Comparison of DMFT and salivary flow rate by sex.

Parameter	Male (n=47)	Female (n=53)	p-value
DMFT/dmft	10.72 ± 5.17	9.77 ± 5.01	0.354
Low flow rate, No. (%)	9 (19.1%)	5 (9.4%)	0.162

Correlation analysis across the full sample revealed a significant positive relationship between DMFT and buffering capacity ($r=0.271$; $p=0.006$) and a significant negative relationship between DMFT and salivary flow rate ($r=-0.214$; $p=0.032$). DMFT did not correlate significantly with s-IgA ($r=-0.103$; $p=0.306$), histatin ($r=-0.009$; $p=0.926$), or pH

(r=−0.081; p=0.422). Histatin and s-IgA showed a significant positive correlation with each other (r=0.220; p=0.028), independent of caries status. The full correlation matrix is presented in Table 5.

Table 5. Pearson correlation coefficients among DMFT and salivary parameters (full sample, n=100).

Variable Pair	r	p-value
DMFT — Buffering capacity	0.271	0.006**
DMFT — Salivary flow rate	−0.214	0.032*
DMFT — s-IgA	−0.103	0.306
DMFT — Histatin	−0.009	0.926
DMFT — Salivary pH	−0.081	0.422
Histatin — s-IgA	0.220	0.028*

Discussion

The clearest finding in this study is also the simplest one: children with rampant caries produced less saliva. Low resting flow was three times more common among caries-affected children than among controls, and this was the only parameter — out of six measured — that reached statistical significance. Everything else we measured, including the two immune proteins we set out to investigate, showed no meaningful difference between groups.

That result deserves to be taken seriously rather than explained away. s-IgA and histatin are biologically plausible candidates for caries protection, and the literature on both is genuinely split — some studies report lower concentrations in caries-active children, consistent with a protective role, while others, like ours, find no difference at all (Ahmad et al., 2022; Gorbatova et al., 2025; Sharma et al., 2024). One explanation is that whole-saliva ELISA measurement captures total protein concentration but says nothing about local concentration at the tooth surface, where protection would actually need to occur. A child with normal total s-IgA in pooled saliva could still have inadequate antibody delivery to a specific high-risk tooth if their overall salivary volume is low.

Another contributing factor is simply the degree of biological variability that exists between individual children in mucosal immune output. s-IgA and histatin secretion rates are shaped by genetics, prior antigenic exposure, salivary gland maturity, and even circadian timing of collection, and this variability can be large enough to obscure a true but modest group-level difference, particularly in a sample of this size. It is entirely possible that a real protective signal exists but is masked by the wide individual spread in immune protein output we observed in both groups — a possibility that larger, more tightly controlled cohorts would be better positioned to detect (Ahmad et al., 2022; Sharma et al., 2024).

This is where flow rate becomes more than just one variable among several — it functions as the delivery mechanism for everything else saliva does. Buffering capacity depends on enough fluid reaching the tooth surface to neutralise acid. Remineralising calcium and phosphate need a continuous flow to be deposited. Antimicrobial proteins, however potent in concentration, are only useful if they physically reach the bacteria they are meant to inhibit. A child producing too little saliva is disadvantaged across every one of these mechanisms simultaneously, which may explain why flow rate outperformed any single protein as a discriminator in our data (Ravikumar et al., 2023; Ansari et al., 2025; Marruganti et al., 2023).

The positive correlation between DMFT and buffering capacity is, admittedly, counterintuitive at first glance — one might expect children with more caries experience to have weaker, not stronger, buffering. A plausible explanation is that chronic exposure to an acidic, cariogenic oral environment can drive compensatory salivary gland stimulation over time, elevating measured buffering capacity even as disease accumulates. This finding should be interpreted cautiously, however, because the cross-sectional design cannot establish the direction of this relationship, and confirmation in longitudinal studies would be needed before drawing firm conclusions about a compensatory mechanism.

The histatin–s-IgA correlation, while modest, fits a coherent biological narrative: both proteins are part of the same broad mucosal immune response, and children who produce more of one tend to produce more of the other, largely independent of their caries status. This reinforces the idea that what we are observing in these two markers reflects general immune activity rather than a caries-specific signal.

S. mutans presence followed the same pattern as the immune markers — numerically higher in the rampant caries group but not statistically significant. This is broadly consistent with a growing recognition that caries is rarely attributable to a single organism; complex polymicrobial communities, rather than S. mutans alone, are now understood to drive lesion progression in many children (Hajishengallis et al., 2022; Manchanda et al., 2023). A simple presence/absence culture result may simply be too blunt an instrument to capture meaningful differences in microbial burden between groups that both carry the organism at substantial rates.

From a practical standpoint, the clinical message here is straightforward and immediately usable. Salivary flow rate can be assessed in any dental clinic in under a minute, requires no laboratory equipment, and clearly distinguishes risk in this population. A child identified with low resting flow should prompt a conversation about hydration, possible mouth breathing, and more frequent recall — interventions that cost nothing and require no specialised diagnostic infrastructure, which matters

considerably in a setting like Iraq where access to laboratory-based salivary testing is limited (Twetman, 2024).

Several limitations should be acknowledged. The cross-sectional design cannot establish whether low salivary flow precedes caries development or results from it. The control group, while lower in caries experience than the rampant group, was not entirely caries-free, which may have narrowed the biological contrast available for detection. Sample size, while adequate to detect the large DMFT difference, may have been underpowered to detect smaller differences in the immune markers given their wide variability. S. mutans was assessed qualitatively as presence or absence on culture rather than quantitatively, meaning that differences in bacterial load between groups — as opposed to simple detection — could not be captured; future work using qPCR or colony-forming unit counts would provide a more sensitive measure of microbial burden. Future work should also follow children longitudinally and examine functional salivary protein activity rather than total concentration alone.

Conclusion

Children with rampant dental caries in this Iraqi cohort did not differ from controls in salivary s-IgA, histatin, pH, or S. mutans presence, despite a large and highly significant difference in caries experience. Salivary flow rate was the sole parameter to distinguish the groups, with low flow nearly three times more common among caries-affected children. These findings point toward salivary volume — rather than any single immune protein — as the more clinically meaningful target for caries risk assessment in this population, and support the routine use of simple, low-cost chairside flow assessment in paediatric dental practice in Iraq.

Acknowledgements

We thank the staff of the Specialized Dental Center, Al-Hilla, Babylon Governorate, for their assistance with patient recruitment and sample collection.

Conflict of Interest Statement

None to declare.

Funding: *The authors declare that no funds, grants, or other financial support were received during the preparation of this study.*

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