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Review

Saliva-Based Biomarkers for Non-Invasive Assessment of Pediatric Gastrointestinal Inflammatory Diseases: Current Evidence and Future Perspectives

Andrea Scavella [1], Silvia Salvatore [2,3], Lorenzo D'Antiga [4,5] and Naire Sansotta [4,*]

1 Department of Medicine and Surgery, University of Insubria, 21100 Varese, Italy
2 Department of Medicine and Technological Innovation, University of Insubria, 21100 Varese, Italy
3 Pediatric Unit, Filippo del Ponte Hospital, 21100 Varese, Italy
4 Department of Paediatric Hepatology Gastroenterology and Transplantation, Papa Giovanni XXIII Hospital, 24127 Bergamo, Italy; ldantiga@asst-pg23.it
5 Department of Medicine and Surgery, University of Milano-Bicocca, 20126 Milan, Italy
* Correspondence: nsansotta@asst-pg23.it

Highlights

What are the main findings?

- Saliva is a painless, low-cost and easily repeatable biological matrix with potential as a complementary source of biomarkers for the non-invasive assessment of paediatric gastrointestinal inflammatory diseases.
- Salivary anti-tTG-IgA has the most substantial paediatric evidence in coeliac disease, although further validation is needed. In contrast, salivary calprotectin in IBD and volatilomic, microbiome and microRNA profiles in EoE remain largely exploratory.
- Pre-analytical variability (collection method, oral health, processing) and lack of standardisation are major barriers to clinical translation.

What are the implication of the main findings?

- Saliva may represent a promising complementary matrix for children, but cannot currently replace endoscopy, faecal calprotectin or serum anti-tTG IgA.
- Future research should prioritise methodological standardisation, prospective multicentre validation and direct comparison with established diagnostic standards.

Abstract

Background/Objectives: Endoscopy with biopsy remains central to the diagnosis and assessment of pediatric inflammatory bowel disease (IBD), eosinophilic esophagitis (EoE), and celiac disease (CD). However, its invasive nature, procedural burden, cost, and limited suitability for repeated assessment highlight the need for non-invasive biomarkers in children. This review critically evaluates the current evidence for saliva-based biomarkers in these disorders. *Methods*: A critical narrative literature search was performed in PubMed/MEDLINE and Scopus to identify studies on salivary biomarkers in pediatric IBD, EoE, and CD. Pediatric evidence was prioritized; adult studies were included only when pediatric data were unavailable or limited, and were explicitly distinguished from pediatric findings. *Results*: Saliva offers major practical advantages in children because collection is painless, inexpensive, repeatable, and feasible without venipuncture or sedation. However, salivary profiles are influenced by oral health, diet, collection procedures, and other pre-analytical factors. In IBD, pediatric evidence remains limited and heterogeneous, with preliminary signals from salivary calprotectin, microbiome, and high-dimensional proteomics. In EoE, salivary microRNAs, volatile organic compounds, and selected microbial taxa show promising but exploratory results, mainly in small cohorts. In CD, salivary anti-tissue transglutaminase

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IgA has the most interesting pediatric evidence, including data from population-based screening studies. *Conclusions*: Saliva cannot currently replace established diagnostic tools. The level of evidence varies substantially across diseases: salivary anti-tTG IgA has the most substantial paediatric evidence and may have a complementary role in population-based screening, whereas findings in IBD and EoE remain largely exploratory. Future studies should prioritise methodological standardisation, prospective multicentre validation and direct comparisons with established diagnostic and monitoring standards.

Keywords: saliva; salivary biomarkers; children; inflammatory bowel disease; eosinophilic esophagitis; celiac disease

1. Introduction

Chronic inflammatory diseases of the pediatric gastrointestinal tract—Crohn's disease (CrD) and ulcerative colitis (UC), collectively IBD, eosinophilic esophagitis (EoE), and celiac disease (CD)—share a common burden: children affected require not a single diagnostic procedure but lifetime monitoring. Esophagogastroduodenoscopy (EGD) with biopsy remains the gold standard for establishing histological diagnosis and for assessing response to dietary or pharmacological treatment [1,2]. However, repeated sedation, procedural risk, cost, and the psychological burden on children and families make it an invasive tool for longitudinal disease surveillance [1–6]. This challenge should prompt the search for accessible biofluids—including blood, urine, stool, breath, and increasingly saliva—that may reflect intestinal inflammatory activity without the need for endoscopy.

Saliva is particularly attractive in pediatric practice because it can be collected rapidly, painlessly, and non-invasively, without the need for venipuncture, even by parents or non-specialized healthcare personnel. Moreover, it contains a wide range of biological components—including cells, nucleic acids, extracellular vesicles, metabolites, and proteins—that may reflect both local oral and systemic inflammatory processes [7]. This review provides a critical synthesis of the available evidence on salivary biomarkers in pediatric IBD, EoE, and CD, evaluating the strength and consistency of current findings while identifying the principal methodological challenges and priorities for future studies.

2. Materials and Methods

A narrative literature review was performed to critically synthesise and appraise the available evidence on saliva-based biomarkers for the diagnosis, characterisation and monitoring of paediatric inflammatory bowel disease (IBD), eosinophilic esophagitis (EoE) and celiac disease (CD).

2.1. Search Strategy

A literature search was conducted in PubMed/MEDLINE and Scopus from database inception to June 2026. Because the evidence base is sparse and highly heterogeneous across diseases and biomarker classes, targeted iterative searches were performed by disease and by biomarker type rather than as a single comprehensive Boolean query. Core search terms included "saliva" OR "salivary biomarkers" OR "salivary calprotectin" OR "salivary antibodies" OR "microRNA" OR "proteomics" OR "metabolomics" OR "volatilomics" OR "volatile organic compounds" OR "oral microbiome", combined with "inflammatory bowel disease" OR "Crohn disease" OR "ulcerative colitis" OR "eosinophilic esophagitis" OR "celiac disease" and with paediatric filters ("children" OR "pediatric" OR "paediatric"). Reference lists of all included papers and relevant reviews were hand-searched to identify

additional studies. Given the iterative, cluster-based nature of the searches, a consolidated total number of unique records retrieved could not be reliably established. Titles and abstracts were screened for relevance; full texts of potentially eligible articles were examined in detail.

2.2. Study Selection and Eligibility

Studies were considered relevant when they evaluated saliva (or closely related oral matrices when saliva-specific data were unavailable) as a biological source for diagnosis, disease activity assessment, treatment monitoring or screening in paediatric IBD, EoE or CD. Paediatric studies were prioritised. Adult studies were included only when paediatric evidence was absent or extremely limited and were used exclusively to provide biological or translational context; such studies are explicitly labelled as adult data throughout the manuscript. Letters to the editor, small pilot studies and one preprint were retained solely when they represented the only available paediatric signal for a given biomarker; their limited evidentiary weight is clearly acknowledged in the text and summary tables. The main reasons for excluding full-text articles were lack of relevance to the target diseases or biomarkers, absence of saliva-specific or relevant oral biomarker data, and lack of applicability to the review objectives. No formal quantitative eligibility thresholds or meta-analysis were applied, consistent with the narrative design of the review.

2.3. Evidence Grading Framework

To allow for transparent appraisal of the strength of the available data, each biomarker or study group was assigned to one of four evidence categories based on study design, sample size, reproducibility, clinical validation and applicability to paediatric populations:

- Strong paediatric evidence: findings from adequately sized paediatric cohorts with independent or external confirmation, a clear clinical comparator (histology, established serology or faecal markers) and predefined diagnostic or monitoring endpoints.
- Moderate paediatric evidence: consistent results from two or more independent paediatric studies, or from a large single screening cohort, but with incomplete external validation, methodological heterogeneity or limited longitudinal data.
- Exploratory/pilot: evidence derived from small single-centre studies, discovery cohorts, letters or preprints; considered hypothesis-generating only.
- Adult-only: adult data reported solely to illustrate biological plausibility when no paediatric data exist.

This qualitative classification was developed by the authors and does not represent a formally validated evidence-grading system.

3. Results

3.1. The Gum–Gut Axis: A Biological Rationale

The rationale for exploring salivary biomarkers in gastrointestinal diseases is grounded in the bidirectional gum–gut axis. Oral microbial dysbiosis may influence the intestinal ecosystem, while intestinal inflammation can be reflected in changes in the oral microbiome and in salivary proteomic and metabolomic profiles [8]. Supporting this concept, oral manifestations are well-recognized extraintestinal features of pediatric IBD [9]. Similarly, oral mucosal abnormalities and alterations in salivary composition have been described in children with celiac disease [10]. Emerging studies have identified disease-associated changes in the oral microbiome in esophageal disorders, including EoE, although their clinical and biological relevance remains uncertain [11]. Collectively, these findings support

the biological plausibility of saliva as a source of biomarkers reflecting gastrointestinal inflammatory processes.

However, the presence of oral or salivary alterations does not necessarily establish their diagnostic value. The biological rationale underlying the oral–gut axis should therefore be distinguished from the clinical validity of individual salivary biomarkers, which is critically evaluated in the following sections.

3.2. Saliva as a Biofluid: Strengths and Structural Limitations

From a practical perspective, saliva offers several advantages for pediatric biomarker research. Collection is non-invasive, requires only a small sample volume, does not need venipuncture or sedation, and is generally well accepted by children and caregivers, making it particularly suitable for longitudinal disease monitoring [7]. However, important analytical challenges remain. Whole saliva is a complex biological matrix comprising glandular secretions, gingival crevicular fluid, desquamated epithelial cells, oral microorganisms, food debris, and nasal and bronchial secretions, all of which can introduce variability unrelated to gastrointestinal disease [7,12]. In addition, saliva composition is affected by multiple factors, including circadian rhythms and physical activity, whereas endogenous proteases can compromise sample stability through protein degradation [7,12]. Other potential confounders—including periodontal disease, dental caries, smoking exposure (including passive smoking in adolescents), and oral hygiene—have been shown to influence salivary composition but are inconsistently assessed in studies of pediatric IBD, EoE, and celiac disease [13]. These methodological sources of variability should be considered when interpreting the available evidence and represent an important challenge for the validation and clinical implementation of salivary biomarkers.

3.3. Salivary Biomarkers in Pediatric Inflammatory Bowel Disease

3.3.1. Salivary Inflammatory Cytokines and Inflammation-Related Molecules

Importantly, no published paediatric studies have specifically evaluated classical salivary inflammatory cytokines (IL-6, TNF-α, IL-1β, IL-10, IL-17A) or matrix metalloproteinases in IBD. Salivary calprotectin and a recent high-dimensional proteomic preprint are discussed separately below. In adults with IBD, interleukin-6 (IL-6) has been associated with both CrD and UC and may correlate with endoscopic disease activity [13,14]. Recent evidence suggests that salivary TNF-α concentrations may be elevated in patients with UC and CrD, particularly in the presence of periodontal inflammation, while other inflammatory mediators, including TNF-α, IL-10, IL-1β, IL-17A, and matrix metalloproteinases, have shown variable associations with disease phenotype and activity [13–16].

Interestingly, a recent paediatric preprint combined high-dimensional salivary proteomics with interpretable machine learning in children with IBD. Using unstimulated saliva and the NULISAseq Inflammation Panel 250, the authors identified a 14-protein signature, achieving 96.2% accuracy in initial-visit samples (CrD, $n = 40$; UC, $n = 12$) and 86.4% in follow-up samples from the same patients (CrD, $n = 16$; UC, $n = 6$). Despite promising results, the single-centre design and lack of independent external validation limit these findings to a hypothesis-generating level [17].

Although these preliminary findings provide encouraging evidence that saliva may capture complex immune signatures associated with pediatric IBD, they remain hypothesis-generating and require validation in independent cohorts, standardized saliva collection protocols, and peer-reviewed studies before any clinical application can be considered.

3.3.2. Salivary Calprotectin

Salivary calprotectin represents an illustrative example of a biomarker whose performance differs substantially from that of its well-established fecal counterpart, with heterogeneous results across disease phenotypes, anatomical localizations, and study populations, including relevant differences between adults and children. Although initial adult studies suggested a potential role for salivary calprotectin in IBD [13,18], subsequent investigations have failed to demonstrate consistent diagnostic or monitoring utility. Bos et al. found no significant differences in salivary calprotectin levels between adults with IBD and healthy controls, nor any association with fecal calprotectin or disease activity. Similar findings were reported in a larger cohort of 100 adult IBD patients [13,19,20].

Conversely, recent pediatric data have suggested that salivary calprotectin may capture specific inflammatory phenotypes rather than overall intestinal disease burden. In a pediatric cohort, salivary calprotectin was increased in children with early-onset IBD and concomitant oral manifestations, showing correlations with fecal calprotectin concentrations and risk of relapse, whereas these associations were not consistently observed in patients without oral involvement [9,13].

Taken together, these findings suggest that salivary calprotectin may represent a marker of oral–mucosal inflammatory activity rather than a direct surrogate of intestinal inflammation. Therefore, its clinical applicability as a universal non-invasive biomarker for IBD monitoring remains limited, and future studies are needed to evaluate oral inflammatory status, disease phenotype, and age-specific factors when interpreting salivary calprotectin measurements.

3.3.3. Salivary Microbiome and microRNAs

Microbiome-based studies have also reported significant alterations in the oral microbial community of pediatric patients with IBD. Compared with healthy controls, children exhibited reduced microbial diversity and a distinct community structure, characterized by decreased abundance of *Clostridia* and several commensal oral genera, including *Veillonella*, *Prevotella*, and *Fusobacterium*, together with increased *Streptococcus* abundance. Oral dysbiosis was more pronounced in patients with severe disease and was associated with enrichment of potential pathobionts such as *Klebsiella* and *Pseudomonas*. Functional predictions suggested a shift toward a more acidogenic microbiome with reduced vitamin B biosynthesis capacity. Using these microbial signatures, a machine-learning model achieved a mean AUC of 0.76 for distinguishing patients with IBD from healthy controls. Notably, in a subset of patients reassessed after 12 months of therapy, oral dysbiosis partially resolved, with microbial community composition shifting toward that of healthy controls, suggesting that oral microbial alterations may be influenced by disease activity and treatment [21]. Regarding the role of salivary microRNAs (miRNAs), no published studies to date have specifically investigated their potential as biomarkers in pediatric IBD. In adult studies, disease-specific salivary signatures, including miR-101 in Crohn's disease and miR-21, miR-31, and miR-142 family members in ulcerative colitis, have been identified, while salivary exosomal profiling revealed a five-miRNA signature (hsa-miR-1246, hsa-miR-142-3p, hsa-miR-16-5p, hsa-miR-301a-3p, and hsa-miR-4516) that discriminated IBD from healthy controls (AUC 1.00) and active from quiescent disease (AUC 0.86) [22,23]. These findings support the potential of exosome-based salivary biomarkers for disease stratification and monitoring, although validation in pediatric cohorts is still lacking.

In summary, current salivary biomarker evidence in paediatric IBD remains limited. Salivary calprotectin and high-dimensional proteomic signatures are graded as exploratory, with available data derived from small or pilot studies and appearing more closely related to oral mucosal involvement than to overall intestinal disease activity. Oral microbiome

alterations show somewhat stronger signals (moderate paediatric evidence in one larger treatment-naïve cohort) but still lack external validation and standardisation. Classical inflammatory cytokines and microRNAs have been investigated almost exclusively in adults and are therefore classified as adult-only evidence. Overall, no salivary biomarker currently demonstrates sufficient robustness for clinical use in the diagnosis or monitoring of paediatric IBD.

3.4. Salivary Biomarkers in Pediatric Eosinophilic Esophagitis

3.4.1. Salivary Molecular Profiling: microRNAs, Volatilomics, and Metabolomics

Salivary biomarker research in EoE has yielded promising but largely exploratory findings, particularly in microRNA and Volatile Organic Compounds (VOCs). A pediatric study evaluated salivary miRNAs as potential non-invasive biomarkers of EoE in 291 children undergoing endoscopy. Among 56 detectable salivary miRNAs, miR-205-5p showed the greatest differential expression between EoE and non-EoE subjects. A six-miRNA panel (miR-26b-5p, miR-27b-3p, Let-7i-5p, miR-142-5p, miR-30a-5p, and miR-205-5p) discriminated EoE with 70% sensitivity and 68% specificity [24]. Additional exploratory evidence from an adult pilot study identified salivary miR-4668-5p as a potential EoE biomarker, with increased expression in EoE and decreased levels following topical corticosteroid treatment. However, its utility for monitoring treatment response in pediatric EoE remains unvalidated [25]. Overall, current findings suggest convergence around a limited number of candidate miRNAs; however, the diagnostic performance reported to date remains insufficient to replace endoscopic assessment and histological evaluation for diagnosis or detailed monitoring of disease activity.

An Italian pilot study investigated the salivary volatilome using gas chromatography–ion mobility spectrometry (GC–IMS) in children with active EoE ($n = 13$), inactive EoE ($n = 22$), other gastrointestinal conditions ($n = 19$), and healthy controls ($n = 46$). Among 63 detected VOC signal areas, 16 VOCs showed significantly different abundance across comparisons between EoE and healthy controls, EoE and other gastrointestinal diseases, and active versus inactive EoE. Within this VOC signature, cis-3-hexen-1-ol and 2-phenylethanol showed consistent discriminatory capacity across different groups. A linear discriminant analysis based on the 16-VOC panel achieved accuracies of 83.3% for distinguishing EoE from healthy controls, 81.2% for EoE versus gastrointestinal controls, and 80.0% for active versus inactive disease, suggesting that salivary volatilomic profiling may capture disease-associated metabolic signatures and activity status [26]. In contrast, a pediatric study evaluating 20 candidate biomarkers in serum and saliva identified a stronger discriminatory signal in blood-based markers rather than salivary analytes [27]. Exploratory evidence comes from a Letter to the Editor by Hiremath et al., who performed untargeted salivary metabolomics in 28 children (19 EoE, 9 controls). Several metabolites showed significant fold changes between EoE and controls, including increased 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol and N-heptanoylglycine, and decreased deoxyadenosine, deoxycytidine, and adenosine. Two additional metabolites differed between active and inactive EoE, with increased urea and decreased serylarginine in active disease [28]. Although these findings support the potential of salivary VOC and metabolomic profiling in EoE, larger longitudinal pediatric studies are required to determine their clinical utility.

3.4.2. Salivary Microbiome in EoE

Salivary microbiome alterations have been explored in pediatric EoE. In a 16S rRNA sequencing study including 26 children with EoE and 19 controls, overall microbial community composition was largely comparable between groups, although lower richness and alpha diversity were observed in EoE. Notably, *Haemophilus* abundance was significantly

increased in active EoE compared with inactive disease and positively correlated with histologic severity, suggesting that specific microbial taxa may reflect disease activity rather than global dysbiosis. In addition, *Streptococcus* showed a trend toward increased abundance in children with active EoE compared with non-EoE controls ($q = 0.06$), while four broad salivary microbial communities correlated with histologic severity [29]. A further longitudinal study investigated both oral and esophageal microbial communities using oral swabs and esophageal samples from 33 children with EoE and 35 non-EoE controls. No significant differences were identified in the oral microbiota between EoE and control subjects, whereas more pronounced alterations were detected in esophageal samples, with enrichment of *Proteobacteria* (including *Neisseria*) and of *Corynebacterium* (*Actinobacteria*) in active EoE subjects and predominance of *Firmicutes* in controls [30]. It is important to note that this study considered data from both oral and esophageal samples, and these data are not interchangeable. These findings suggest that microbial alterations in EoE may vary according to the anatomical compartment analyzed and may be more pronounced at the site of inflammation.

Taken together, salivary biomarker research in paediatric EoE is still at an early stage. MicroRNA panels, volatilomic signatures and selected microbial taxa have shown promising preliminary discriminatory capacity; however, the available evidence remains exploratory or pilot in nature. Most studies are single-centre, are limited in sample size, and lack external validation. Differences between active and inactive disease have been reported, yet robust longitudinal data linking salivary markers to treatment response or relapse prediction remain scarce. Thus, their potential role in disease monitoring has yet to be established. At present, saliva cannot replace endoscopic and histological assessment in EoE.

3.5. Salivary Biomarkers in Pediatric Celiac Disease

3.5.1. Salivary Transglutaminase IgA Antibodies (tTG-IgA)

The potential use of saliva as a non-invasive biological matrix for CD screening has been explored mainly by an Italian research group through the detection of tissue transglutaminase IgA antibodies (tTG-IgA). In 2004, the feasibility of detecting salivary tTG-IgA using a fluid-phase radioimmunoassay was demonstrated with positivity in 97.4% of children with CD, as well as a strong correlation between salivary and serum antibody titers ($r = 0.826$, $p = 0.0014$), supporting the potential value of saliva-based testing as a surrogate marker of systemic autoimmunity [31]. This approach was subsequently assessed in large pediatric screening cohorts. In a population-based study including 4048 school-aged children, salivary tTG-IgA testing identified 32 positive subjects, of whom 31 were confirmed by serum testing and 28 showed villous atrophy at duodenal biopsy, resulting in a CD prevalence of 1.16% [32]. These findings were further confirmed in a larger cohort of 5733 screened children, in which 46 subjects were salivary tTG-IgA-positive, 45 were serologically confirmed, and 42 had histological evidence of CD, with an overall prevalence of 1.2% [33]. An independent Letter to the Editor by Ocmant et al. published in 2007 further supported the feasibility of this approach by evaluating a commercial ELISA-based assay for salivary anti-tTG-IgA detection, reporting 90% sensitivity and 96.7% specificity for distinguishing untreated CD patients from controls [34]. Collectively, these studies provided early evidence that salivary tTG-IgA assessment may represent a feasible and minimally invasive strategy for CD screening, although confirmation according to established serological and histological criteria remains required. Additional evidence from an Italian pediatric cohort investigated the diagnostic performance and longitudinal behavior of salivary anti-tTG antibodies. In a retrospective study including 105 children evaluated for suspected CD, salivary anti-tTG antibodies, analyzed using an Enzyme-Linked Immuno Magnetic Electrochemical method (ELIME), were detected in affected children and showed complete concordance with serum

positivity among confirmed cases (sensitivity 100%, specificity 71.4%). Notably, salivary anti-tTG antibodies remained detectable after six months of gluten-free diet, suggesting that salivary antibody levels may persist beyond initial diagnosis and could potentially provide additional information during follow-up [35]. However, the limited sample size and retrospective design require further validation before clinical application.

3.5.2. Salivary Microbiome in CD

Salivary alterations in CD have also been investigated through microbiome-based approaches. A cross-sectional 16S rRNA sequencing study including children with active CD ($n = 12$), CD on a gluten-free diet (GFD) ($n = 14$), and healthy controls ($n = 10$) identified distinct salivary microbial profiles in CD. Both untreated and GFD-treated patients showed increased *Bacteroidetes* and reduced *Actinobacteria* compared with controls, together with decreased abundance of *Rothia* species and lower levels of *Rothia mucilaginosa* compared with healthy subjects, suggesting persistent oral microbial alterations despite dietary treatment [36]. These findings are partially consistent with previous observations summarized by De Angelis et al., who reported that children with treated CD display differences in salivary microbial composition compared with healthy controls, including altered abundance of several oral-associated taxa such as *Streptococcus thermophilus*, *Veillonella parvula*, *Actinomyces*, *Atopobium*, *Corynebacterium durum*, and *Porphyromonas* spp. In addition, dietary patterns appeared to influence salivary microbial profiles, as demonstrated in Saharawi children with CD, in whom transition from an African-style to an Italian-style GFD modified the abundance of genera including *Granulicatella*, *Capnocytophaga*, *Porphyromonas*, and *Neisseria* [37]. Overall, available evidence suggests that salivary microbial composition may differ in children with CD and may be influenced by both disease status and dietary exposure. However, current studies remain limited by small sample sizes, heterogeneity in patient populations and dietary conditions, precluding definitive conclusions.

Among the salivary biomarkers investigated in paediatric gastrointestinal diseases, anti-tTG IgA represents the most extensively studied and clinically promising candidate, particularly for coeliac disease screening. Paediatric studies have reported encouraging diagnostic performance and correlations between salivary and serum antibody levels. However, these findings should be interpreted cautiously, as most studies are relatively old, derive largely from related Italian research groups, and have employed different analytical platforms. Moreover, independent validation in contemporary, multicentre paediatric cohorts remains limited. Therefore, although salivary anti-tTG IgA currently represents the strongest paediatric evidence among the biomarkers reviewed, its clinical utility, particularly for population-based screening, requires further standardisation and external validation.

The results for all gastrointestinal diseases investigated are summarized in Table 1.

Table 1. Salivary biomarkers in paediatric gastrointestinal diseases.

Disease	Biomarker	Key Findings	Evidence Grading	Key Limitations
IBD	Salivary calprotectin	Heterogeneous findings; possible increase in early-onset paediatric IBD [9,13,18–20].	Exploratory/pilot	Limited paediatric data; oral inflammation as a potential confounder.
	IL-6, TNF-α, IL-10, IL-1β, IL-17A, MMP-10	Altered cytokine profiles reported in adults, correlates with activity in some studies [13–16].	Adult-only	No paediatric validation; uncertain disease specificity.
	Microbiome	Reduced diversity and altered microbial composition in paediatric IBD [22,23].	Exploratory/pilot	Small cohorts; heterogeneous microbial signatures.

Table 1. *Cont.*

Disease	Biomarker	Key Findings	Evidence Grading	Key Limitations
	microRNAs/ exosomal profiling	Adult miRNA signatures associated with IBD and disease activity [22,23].	Adult-only	No paediatric validation; limited external validation.
	High-dimensional salivary proteomics	Preliminary paediatric proteomic signature [17].	Exploratory/pilot	Preprint; discovery-stage evidence.
EoE	microRNAs	Six-miRNA panel: 70% sensitivity and 68% specificity [24,25].	Exploratory/pilot	Small cohorts; no external validation.
	Volatilome (VOCs)	16-VOC panel: 80–83% accuracy across disease comparisons [26].	Exploratory/pilot	Pilot evidence; no independent validation.
	Microbiome	Altered oral taxa in active EoE, with inconsistent findings [29,30].	Exploratory/pilot	Small studies; inconsistent results.
CD	Salivary anti-tTG IgA	Paediatric studies report sensitivity of 90–100% and specificity of 71.4–96.7%, depending on the assay [31,35].	Moderate paediatric	Older studies; heterogeneous assays; limited multicentre validation.
	Microbiome	Altered microbial profiles in active and GFD-treated paediatric CD [36,37].	Exploratory/pilot	Small cohorts; high heterogeneity; dietary confounding; no independent validation.

Abbreviations: CD, coeliac disease; EoE, eosinophilic oesophagitis; GFD, gluten-free diet; IBD, inflammatory bowel disease; VOC, volatile organic compound.

4. Discussion

The evidence supporting salivary biomarkers varies substantially among the three diseases. Salivary anti-tTG IgA in celiac disease represents the most extensively investigated biomarker in the paediatric population and is supported by moderate paediatric evidence, although independent contemporary validation remains limited. In contrast, the majority of findings in paediatric IBD and EoE remain exploratory, deriving mainly from small cohorts, discovery-based approaches or single-centre studies, often without independent external validation.

Several common themes emerge. First, the clinical relevance of salivary biomarkers depends on their ability to reflect the established biological pathways of validated biomarkers. Salivary anti-tTG antibodies in CD illustrate this principle as an extension of an established serological approach, whereas salivary calprotectin in IBD has shown limited and heterogeneous clinical utility, particularly given the established role of fecal calprotectin. Second, microbiome-based signatures may display disease-specific patterns, but their reproducibility across cohorts remains limited. Taxonomic findings are often inconsistent and inadequately adjusted for oral health factors, particularly periodontal status, which may substantially influence salivary profiles. Third, microRNA and proteomic approaches are promising, particularly in EoE. However, most evidence derives from small, single-center discovery cohorts, and reported diagnostic performance remains insufficient to replace histological assessment or established diagnostic pathways.

Therefore, although some studies have reported high discriminatory performance, these results should be interpreted in the context of the discovery or pilot nature of the

investigations and, in a few instances, the preliminary status of the work, including preprint publications, pending independent validation.

A further important consideration is how salivary biomarkers compare with currently established diagnostic and monitoring tools.

In IBD, faecal calprotectin remains the reference non-invasive marker of intestinal inflammation. Available data on salivary calprotectin are limited and suggest that its levels may be influenced by oral mucosal inflammation, rather than reliably reflecting overall intestinal disease activity. Therefore, no clear advantage over faecal calprotectin has been demonstrated to date.

In celiac disease, serum anti-tTG IgA (with endomysial antibodies when required) is the cornerstone of both diagnosis and the no-biopsy pathway recommended by ESPGHAN. Salivary anti-tTG IgA has shown encouraging concordance with serum antibody levels in screening studies but still requires confirmation through established diagnostic criteria and currently lacks robust, contemporary multicentre external validation. Its potential added value lies mainly in settings where blood sampling is particularly burdensome or poorly accepted (young children, needle-phobic patients, or large-scale screening settings), because saliva collection is completely non-invasive and can be performed without venipuncture. However, these potential applications remain to be validated in prospective studies.

In EoE, endoscopic and histological assessment remains essential for diagnosis and evaluation of treatment response, as no salivary or other non-invasive biomarker has yet been validated as a substitute. Blood eosinophil-related parameters and symptom scores provide complementary but limited information, and the salivary miRNA, volatilomic and microbial signals reported to date remain exploratory. Their diagnostic and monitoring performance has not yet been adequately established or compared directly with histological assessment.

Overall, prospective paediatric studies directly comparing salivary candidates with established disease-specific reference standards are needed to determine whether these biomarkers provide clinically meaningful additional information and, ultimately, a measurable advantage over current diagnostic and monitoring strategies.

An additional limitation is the heterogeneity of saliva collection and processing protocols. Remarkably, factors such as stimulated versus unstimulated saliva, whole saliva versus extracellular vesicle fractions, fasting status, collection timing, oral inflammatory conditions, smoking, medications, diet, and processing delays may affect analyte measurements, but are inconsistently reported.

In addition, differences in sampling methods should be considered when interpreting the available evidence. Some microbiome studies have used oral swabs or combined oral and oesophageal sampling rather than whole saliva, and these matrices are not interchangeable. Future paediatric studies should therefore report collection procedures (stimulated or unstimulated saliva), sampling conditions (time of day and fasting status), sample processing and storage, oral health status, relevant medications and dietary factors, and the analytical matrix used. Such standardisation would improve inter-study comparability and facilitate the validation of candidate salivary biomarkers.

4.1. Future Perspectives

Several priorities may facilitate the translation of salivary biomarker research into clinical practice. Integrated multi-analyte and multi-omic approaches, combined with interpretable machine-learning strategies, may provide greater diagnostic and prognostic value than single biomarkers and should be evaluated in adequately powered, well-characterized cohorts.

Methodological improvements are also needed to enhance analyte stability and reproducibility, including exosome-based approaches and standardized protocols for saliva collection, processing, and storage.

Most importantly, prospective multicenter pediatric studies should use predefined diagnostic accuracy endpoints, compare salivary biomarkers with established non-invasive and invasive standards, and incorporate longitudinal sampling linked to treatment response, histological remission, and relapse.

4.2. Conclusions

Saliva represents a promising, non-invasive source of biomarkers for paediatric gastrointestinal diseases, but its clinical application remains limited. Salivary anti-tTG IgA in coeliac disease has the most substantial paediatric evidence, although further independent validation is needed. In paediatric IBD and EoE, available findings remain largely exploratory and require confirmation in larger prospective studies. Methodological standardisation and multicentre validation are essential to establish the diagnostic and monitoring value of salivary biomarkers and determine their potential complementary role in clinical practice.

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Abbreviations

The following abbreviations are used in this manuscript: AUC, Area under the curve; CD, Celiac disease; CrD, Crohn's disease; EGD, Esophagogastroduodenoscopy; ELIME, Enzyme-Linked Immuno Magnetic Electrochemical; ELISA, Enzyme-linked immunosorbent assay; EoE, Eosinophilic esophagitis; GFD, Gluten-free diet; GC–IMS, Gas chromatography–ion mobility spectrometry; IBD, Inflammatory bowel disease; IgA, Immunoglobulin A; IL, Interleukin; miRNA/miRNAs, MicroRNA/microRNAs; MMP, Matrix metalloproteinase; tTG-IgA, Tissue transglutaminase immunoglobulin A; UC, Ulcerative colitis; VOC/VOCs, Volatile organic compounds.

References

1. Zhang, G.; Zhao, G.; Li, P.; Zhang, S. The Application of Gastrointestinal Endoscopy in Children: A Narrative Review. *Front. Pediatr.* **2025**, *13*, 1691692. [CrossRef] [PubMed]
2. Hathagoda, W.; Rajindrajith, S.; Devanarayana, N.M. Upper Gastrointestinal Endoscopy in Pediatric Gastroenterology: Diagnostic and Therapeutic Applications. *World J. Gastrointest. Endosc.* **2025**, *17*, 111386. [CrossRef] [PubMed]
3. Tringali, A.; Balassone, V.; De Angelis, P.; Landi, R. Complications in Pediatric Endoscopy. *Best Pract. Res. Clin. Gastroenterol.* **2016**, *30*, 825–839. [CrossRef] [PubMed]
4. Abou-Zamzam, A.A.; Markovitz, B.P. Is It Safe? Are There Limits with Procedural Sedation for Endoscopy in Children? *Pediatr. Crit. Care Med.* **2015**, *16*, 783–784. [CrossRef] [PubMed]

5. Biber, J.L.; Allareddy, V.; Allareddy, V.; Gallagher, S.M.; Couloures, K.G.; Speicher, D.G.; Cravero, J.P.; Stormorken, A.G. Prevalence and Predictors of Adverse Events during Procedural Sedation Anesthesia-Outside the Operating Room for Esophagogastroduodenoscopy and Colonoscopy in Children: Age Is an Independent Predictor of Outcomes. *Pediatr. Crit. Care Med.* **2015**, *16*, e251–e259. [CrossRef] [PubMed]
6. Lightdale, J.R.; Liu, Q.Y.; Sahn, B.; Troendle, D.M.; Thomson, M.; Fishman, D.S. Pediatric Endoscopy and High-Risk Patients: A Clinical Report from the NASPGHAN Endoscopy Committee. *J. Pediatr. Gastroenterol. Nutr.* **2019**, *68*, 595–606. [CrossRef] [PubMed]
7. Dongiovanni, P.; Meroni, M.; Casati, S.; Goldoni, R.; Thomaz, D.V.; Kehr, N.S.; Galimberti, D.; Del Fabbro, M.; Tartaglia, G.M. Salivary Biomarkers: Novel Noninvasive Tools to Diagnose Chronic Inflammation. *Int. J. Oral Sci.* **2023**, *15*, 27. [CrossRef] [PubMed]
8. Byrd, K.M.; Gulati, A.S. The "Gum-Gut" Axis in Inflammatory Bowel Diseases: A Hypothesis-Driven Review of Associations and Advances. *Front. Immunol.* **2021**, *12*, 620124. [CrossRef] [PubMed]
9. Liguori, S.; Musella, G.; Adamo, D.; Miele, E.; Coppola, N.; Canfora, F.; Del Giudice, C.; Spagnuolo, G.; Rengo, S.; Mignogna, M.D.; et al. Salivary Calprotectin as a Biomarker in Early Onset Inflammatory Bowel Disease: A Pilot Study. *J. Clin. Med.* **2025**, *14*, 4232. [CrossRef] [PubMed]
10. Macho, V.M.P.; Coelho, A.S.; Veloso E Silva, D.M.; de Andrade, D.J.C. Oral Manifestations in Pediatric Patients with Coeliac Disease—A Review Article. *Open Dent. J.* **2017**, *11*, 539–545. [CrossRef] [PubMed]
11. Bernard, R.; Fazili, I.; Rajagopala, S.V.; Das, S.R.; Hiremath, G. Association between Oral Microbiome and Esophageal Diseases: A State-of-the-Art Review. *Dig. Dis.* **2022**, *40*, 345–354. [CrossRef] [PubMed]
12. Esser, D.; Alvarez-Llamas, G.; de Vries, M.P.; Weening, D.; Vonk, R.J.; Roelofsen, H. Sample Stability and Protein Composition of Saliva: Implications for Its Use as a Diagnostic Fluid. *Biomark. Insights* **2008**, *3*, 25–27. [CrossRef] [PubMed]
13. Chrabie, A.; Kazzi, C.A.; Berjass, D.; Geagea, A.G.; Dosh, L.; Leone, L.G.; Kattar, S.A.; Ouaini, N.; Cappello, F.; Leone, A.; et al. Salivary Biomarkers in Inflammatory Bowel Disease. *Explor. Dig. Dis.* **2026**, *5*, 1005117. [CrossRef]
14. Majster, M.; Lira-Junior, R.; Höög, C.M.; Almer, S.; Boström, E.A. Salivary and Serum Inflammatory Profiles Reflect Different Aspects of Inflammatory Bowel Disease Activity. *Inflamm. Bowel Dis.* **2020**, *26*, 1588–1596. [CrossRef] [PubMed]
15. Enver, A.; Ozmeric, N.; Isler, S.C.; Toruner, M.; Fidan, C.; Demirci, G.; Elgun, S.; Silva, A.P.B. da Evaluation of Periodontal Status and Cytokine Levels in Saliva and Gingival Crevicular Fluid of Patients with Inflammatory Bowel Diseases. *J. Periodontol.* **2022**, *93*, 1649–1660. [CrossRef] [PubMed]
16. Gürsoy, U.K.; Gürsoy, M.; Loimaranta, V.; Rautava, J. Salivary Th17 Cytokine, Human β-Defensin 1-3, and Salivary Scavenger and Agglutinin Levels in Crohn's Disease. *Clin. Oral Investig.* **2024**, *28*, 108. [CrossRef] [PubMed]
17. Rupp, B.T.; Reyna, J.; Giunta, A.; Weaver, T.; Chason, K.; Liu, J.; Gulati, A.S.; Byrd, K.M. Interpretable Machine Learning Applied to High-Dimensional Salivary Proteomics in Pediatric Inflammatory Bowel Diseases. *Inflamm. Bowel Dis.* **2026**, izag181. [CrossRef] [PubMed]
18. Nijakowski, K.; Surdacka, A. Salivary Biomarkers for Diagnosis of Inflammatory Bowel Diseases: A Systematic Review. *Int. J. Mol. Sci.* **2020**, *21*, 7477. [CrossRef] [PubMed]
19. Rodrigues, C.; Gomes, A.T.P.C.; Leal, J.; Pereira, P.; Lopes, P.C.; Mendes, K.; Correia, M.J.; Veiga, N.; Rosa, N.; Soares, C.; et al. Oral Health in Inflammatory Bowel Disease: The Overlooked Impact and the Potential Role of Salivary Calprotectin. *BMC Oral Health* **2025**, *25*, 729. [CrossRef] [PubMed]
20. Bos, V.; Crouwel, F.; Waaijenberg, P.; Bouma, G.; Duijvestein, M.; Buiter, H.J.; Brand, H.S.; Hamer, H.M.; De Boer, N.K. Salivary Calprotectin Is Not a Useful Biomarker to Monitor Disease Activity in Patients with Inflammatory Bowel Disease. *J. Gastrointest. Liver Dis. JGLD* **2022**, *31*, 283–289. [CrossRef] [PubMed]
21. Elmaghrawy, K.; Fleming, P.; Fitzgerald, K.; Cooper, S.; Dominik, A.; Hussey, S.; Moran, G.P. The Oral Microbiome in Treatment-Naïve Paediatric IBD Patients Exhibits Dysbiosis Related to Disease Severity That Resolves Following Therapy. *J. Crohn's Colitis* **2023**, *17*, 553–564. [CrossRef] [PubMed]
22. Schaefer, J.S.; Attumi, T.; Opekun, A.R.; Abraham, B.; Hou, J.; Shelby, H.; Graham, D.Y.; Streckfus, C.; Klein, J.R. MicroRNA Signatures Differentiate Crohn's Disease from Ulcerative Colitis. *BMC Immunol.* **2015**, *16*, 5. [CrossRef] [PubMed]
23. Yang, C.; Chen, J.; Zhao, Y.; Xu, Y.; Wu, J.; Xu, J.; Chen, F.; Chen, N. Identification of Salivary Exosome-Derived miRNAs as Potential Biomarkers for Non-Invasive Diagnosis and Proactive Monitoring of Inflammatory Bowel Disease. *Int. J. Mol. Sci.* **2025**, *26*, 7750. [CrossRef] [PubMed]
24. Jhaveri, P.B.; Lambert, K.A.; Bogale, K.; Lehman, E.; Alexander, C.; Ishmael, F.; Jhaveri, P.N.; Hicks, S.D. Salivary microRNAs in Pediatric Eosinophilic Esophagitis. *Allergy Asthma Proc.* **2023**, *44*, 145–152. [CrossRef] [PubMed]
25. Bhardwaj, N.; Sena, M.; Ghaffari, G.; Ishmael, F. MiR-4668 as a Novel Potential Biomarker for Eosinophilic Esophagitis. *Allergy Rhinol.* **2020**, *11*, 32923026. [CrossRef] [PubMed]

26. Capuano, R.; Petrella, C.; Barbato, C.; D'Arcangelo, G.; Russo, G.; Catini, A.; Minni, A.; Di Natale, C.; Oliva, S. Salivary Volatilome Profiling in Pediatric Eosinophilic Esophagitis: A Pilot Study on a Non-Invasive Approach in Clinical Practice. *Life Med.* **2026**, *5*, lnag012. [CrossRef] [PubMed]
27. Thulin, H.; Mansouri, L.; Altman, M.; Merid, S.K.; Lundahl, J.; Nilsson, C.; Säfholm, J. Biomarkers for a Less Invasive Strategy to Predict Children with Eosinophilic Esophagitis. *Allergy* **2024**, *79*, 3464–3474. [CrossRef] [PubMed]
28. Hiremath, G.; Codreanu, S.G.; Sherrod, S.D.; Tyree, R.; Correa, H.; Choksi, Y.; McLean, J.A.; Locke, A. Salivary Metabolomic Signatures in Pediatric Eosinophilic Esophagitis. *Allergy* **2025**, *80*, 354–358. [CrossRef] [PubMed]
29. Hiremath, G.; Shilts, M.H.; Boone, H.H.; Correa, H.; Acra, S.; Tovchigrechko, A.; Rajagopala, S.V.; Das, S.R. The Salivary Microbiome Is Altered in Children with Eosinophilic Esophagitis and Correlates with Disease Activity. *Clin. Transl. Gastroenterol.* **2019**, *10*, e00039. [CrossRef] [PubMed]
30. Benitez, A.J.; Hoffmann, C.; Muir, A.B.; Dods, K.K.; Spergel, J.M.; Bushman, F.D.; Wang, M.-L. Inflammation-Associated Microbiota in Pediatric Eosinophilic Esophagitis. *Microbiome* **2015**, *3*, 23. [CrossRef] [PubMed]
31. Bonamico, M.; Ferri, M.; Nenna, R.; Verrienti, A.; Di Mario, U.; Tiberti, C. Tissue Transglutaminase Autoantibody Detection in Human Saliva: A Powerful Method for Celiac Disease Screening. *J. Pediatr.* **2004**, *144*, 632–636. [CrossRef] [PubMed]
32. Bonamico, M.; Nenna, R.; Montuori, M.; Luparia, R.P.L.; Turchetti, A.; Mennini, M.; Lucantoni, F.; Masotti, D.; Magliocca, F.M.; Culasso, F.; et al. First Salivary Screening of Celiac Disease by Detection of Anti-Transglutaminase Autoantibody Radioimmunoassay in 5000 Italian Primary Schoolchildren. *J. Pediatr. Gastroenterol. Nutr.* **2011**, *52*, 17–20. [CrossRef] [PubMed]
33. Nenna, R.; Tiberti, C.; Petrarca, L.; Lucantoni, F.; Mennini, M.; Luparia, R.P.L.; Panimolle, F.; Mastrogiorgio, G.; Pietropaoli, N.; Magliocca, F.M.; et al. The Celiac Iceberg: Characterization of the Disease in Primary Schoolchildren. *J. Pediatr. Gastroenterol. Nutr.* **2013**, *56*, 416–421. [CrossRef] [PubMed]
34. Ocmant, A.; Mascart, F. Effective Detection of Celiac Disease Using Salivary Anti-Transglutaminase. *Am. J. Med.* **2007**, *120*, e15; author reply e17. [CrossRef] [PubMed]
35. Condò, R.; Costacurta, M.; Docimo, R. The Anti-Transglutaminase Auto-Antibodies in Children's Saliva with a Suspect Coeliac Disease: Clinical Study. *ORAL Implantol.* **2013**, *6*, 48–54.
36. Noruzpour, A.; Gholam-Mostafaei, F.S.; Looha, M.A.; Dabiri, H.; Ahmadipour, S.; Rouhani, P.; Ciacci, C.; Rostami-Nejad, M. Assessment of Salivary Microbiota Profile as a Potential Diagnostic Tool for Pediatric Celiac Disease. *Sci. Rep.* **2024**, *14*, 16712. [CrossRef] [PubMed]
37. De Angelis, M.; Vannini, L.; Di Cagno, R.; Cavallo, N.; Minervini, F.; Francavilla, R.; Ercolini, D.; Gobbetti, M. Salivary and Fecal Microbiota and Metabolome of Celiac Children under Gluten-Free Diet. *Int. J. Food Microbiol.* **2016**, *239*, 125–132. [CrossRef] [PubMed]

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