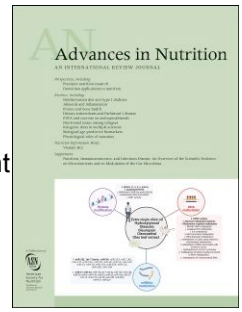
Journal Pre-proof



Saliva as a Matrix for Primary Care: Feasibility and Scoping of its Use for Assessment of Nutrition and Inflammation

Shah Mohammad Fahim, MBBS, Mahesa Miah, Mia Marcotte, Nancy Zoria, Mahdia Ahmed, Samantha L. Huey, PhD, David Erickson, PhD, Julia L. Finkelstein, ScD, Saurabh Mehta, MBBS, ScD

PII: S2161-8313(26)00039-6

DOI: https://doi.org/10.1016/j.advnut.2026.100625

Reference: ADVNUT 100625

To appear in: *Advances in Nutrition*

Received Date: 17 November 2025

Revised Date: 23 March 2026

Accepted Date: 27 March 2026

Please cite this article as: S.M. Fahim, M. Miah, M. Marcotte, N. Zoria, M. Ahmed, S.L Huey, D. Erickson, J.L Finkelstein, S. Mehta, Saliva as a Matrix for Primary Care: Feasibility and Scoping of its Use for Assessment of Nutrition and Inflammation, *Advances in Nutrition*, https://doi.org/10.1016/j.advnut.2026.100625.

Saliva as a Matrix for Primary Care: Feasibility and Scoping of its Use for Assessment of Nutrition and Inflammation

Shah Mohammad Fahim, MBBS [1, 2], Mahesa Miah [2], Mia Marcotte [2], Nancy Zoria [2], Mahdia Ahmed [2], Samantha L Huey, PhD [1, 2], David Erickson, PhD [3, 4], Julia L Finkelstein, ScD [1, 2, 4, 5], Saurabh Mehta, MBBS, ScD [1, 2, 4, 5]*

[1] Cornell Joan Klein Jacobs Center for Precision Nutrition and Health, Cornell University, Ithaca, NY 14853, USA
[2] Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA
[3] Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY 14853, USA
[4] NIH Center for Point-of-Care Diagnostics for Nutrition, Infection, and Cancer for Global Health (PORTENT), Cornell University, Ithaca, NY 14853, USA
[5] Division of Epidemiology, Department of Population Health Sciences, Cornell Weill Medicine, New York City, NY, USA

***** Correspondence: Dr. Saurabh Mehta, The Janet and Gordon Lankton Professor and Founding Director, Cornell Joan Klein Jacobs Center for Precision Nutrition and Health, Cornell University, 3101 Martha Van Rensselaer Hall, 37 Forest Home Drive, Ithaca, NY 14853, USA, Phone: (607) 255-2640, Email: smehta@cornell.edu.

Sources of support: Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development under award number R21HD103974 and the National Institute of Biomedical Imaging and Bioengineering and Fogarty International Center of the National Institutes of Health under Award Number U54EB034654 - PORTENT: A Center for Point of Care Technologies for Nutrition, Infection, and Cancer. PORTENT is a member of the POCTRN+ Network. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations:

AGP: alpha-1 acid glycoprotein; CMIA: chemiluminescence microparticle immunoassay; CP: chronic periodontitis; CRP: C-reactive protein; DBP: vitamin D binding protein; DPP-4: dipeptidyl peptidase-4; ELISA: enzyme-linked immunosorbent assay; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IRMA: immunoradiometric assay; LOD: limit of detection; OL: oral leukoplakia; OLP: oral lichen planus; OSCC: oral squamous cell carcinoma; OSMF: oral submucous fibrosis; PD: periodontitis; PRISMA: preferred reporting items for systematic reviews and meta-analyses; RA: rheumatoid arthritis; RBP: retinol-binding protein; RCT: randomized controlled trial; sTfR: soluble transferrin receptor; T2DM: type 2 diabetes mellitus; WRA: women of reproductive age.

Abstract

Scarce laboratory capacity constrains both primary care and population surveillance for nutritional and inflammation assessment globally. One major contributing factor is the reliance on blood as the primary matrix, and its associated limitations of being invasive, having relatively lower acceptance in apparently healthy individuals and community settings, and being heavily dependent on laboratory and related infrastructure. Saliva offers a non-invasive, inexpensive, and more convenient alternative that causes less discomfort and has the potential to expand the portfolio of point-of-care testing for nutrition and inflammation assessment in primary care. We aimed to synthesize the evidence on the feasibility and scope of using salivary biomarkers for nutrition and inflammation assessment. A literature search was conducted in MEDLINE and CINAHL, without restrictions on language, geographic location, or publication date. Screening and data extraction were done in duplicate on Covidence. A narrative synthesis was done to summarize the results. Seventy-six studies examining salivary biomarkers for nutrition and inflammation were identified across 26 countries, involving 5425 children and 5617 adults. We summarized the results from 33 studies assessing ferritin, iron, zinc, calcium, vitamin A, vitamin B12, vitamin D, vitamin C, and vitamin E in saliva. Additionally, we found 43 studies evaluating salivary inflammatory biomarkers, including C-reactive protein, alpha-1 acid glycoprotein, hepcidin, immunoglobulin A, and cytokines within the context of nutritional impairments. While most biomarker values were detectable in saliva, significant evidence and methodological gaps remain, including the absence of standardized protocols for sample collection, transportation, processing, storage, and laboratory analysis; insufficient information on diagnostic performance, accuracy, and comparability with corresponding blood biomarkers; and challenges in interpreting findings across different population subsets and settings. Future research should focus on improving method reporting and ensuring the clinical relevance of findings to maximize the potential of saliva-based diagnostics for nutrition and inflammation assessment.

Keywords: Saliva; Biomarkers; Nutritional Status; Nutrition Assessment; Cytokines; Point-of-Care.

Statement of Significance: This review synthesizes the current evidence on the feasibility and scope of using salivary biomarkers for nutrition and inflammation assessment. We also identify

research gaps and methodological challenges for the use of saliva as a matrix for primary care, as well as in nutrition and biomedical research.

Introduction

Scarce laboratory capacity constrains both primary care and population surveillance for nutritional and inflammation assessment globally (1, 2). One major contributing factor is the reliance on whole blood or serum as the primary body fluid for assessing nutritional and inflammatory biomarkers, and its associated limitations of being invasive, having relatively lower acceptance in apparently healthy individuals and community settings, and being heavily dependent on laboratory and related infrastructure (3). While blood is considered the standard body fluid to assess most biomarkers, saliva may be a preferable alternative in terms of sample collection (4, 5). Compared to blood, collecting saliva is non-invasive, cheaper to collect and process, and more convenient to collect from anyone in any setting (6, 7). Saliva is an extracellular fluid produced and secreted by major and minor salivary glands (8), containing various biocomponents and metabolites that help maintain oral cavity health (9). Sufficient saliva is necessary to uphold a neutral pH in the oral cavity, protect oral tissues from microorganisms, and assist with swallowing and digestion (10, 11). In children, saliva plays a pivotal role in oral health, contributing to antimicrobial defense and enamel remineralization, while in adults, it supports oral homeostasis, digestion, and immune function, with variations in composition influenced by age, medication use, and systemic health conditions (12). Saliva also plays a crucial role in taste perception and the maintenance of oral hygiene (13). Saliva consists of secretions from both major and minor salivary glands, along with components from the upper respiratory tract and the gingival crevicular fluid (14). Although 99% of saliva is water, the biological composition of salivary fluid is almost similar to that of blood (6, 15). Proteins and other components found in serum also enter saliva from the blood through passive diffusion, active transport, or ultrafiltration (16). Recent evidence on salivary proteomes revealed similarities between salivary proteins and the plasma proteomes (17). Saliva contains various

substances, including electrolytes, enzymes, mucus, and antibodies (13). Additionally, a variety of macro and trace elements, such as inorganic ions, proteins, hormones, and immunological components essential for oral and systemic health, were detected in saliva, and these components can regulate the immune-inflammatory pathways and are involved in the maintenance of oral health homeostasis (18). Several nutritional, inflammatory, and immune response molecules are also abundant in the saliva (19). Advantages of saliva collection also include reduced risk of infectious disease transmission, ease of multiple sampling, and higher acceptance by patients and caregivers (9, 20). However, salivary volume and composition may differ among individuals based on several biological factors, including age, sex, mood, stress, nervous system activity, circadian rhythms, disease status, oral health conditions, and salivary flow rate (21). Salivary biomarker concentrations are also influenced by diet, hydration status, and the time of sample collection (21). Moreover, the lack of standardization in the collection, processing, storage, and laboratory analysis of saliva samples, along with persistent gaps in the diagnostic utility and interpretability of salivary biomarkers, limits their reliability in clinical and research settings (13).

Despite the limitations, salivary analytes hold promise for use as a matrix for primary care for the assessment of health and diseases, particularly in low-resource settings or hard-to-reach remote areas. They offer the potential to evaluate both oral and systemic conditions. In dentistry and periodontology, biomarkers such as matrix metalloproteinases and interleukins can assess gingival inflammation (22) and periodontal disease progression (23), respectively. Some other salivary biomarkers, including various proteins and cytokines, have been found to be elevated in individuals with dental caries, periodontitis, and gingivitis (14, 22, 24). Salivary biomarkers have been used in infectious diseases, for example, for reliably detecting SARS-CoV-2 (via the antibodies to the SARS-CoV-2 spike glycoprotein) in individuals exposed to COVID-19 (25).

Saliva may also be a key indicator of inflammation, as it contains cytokines and other inflammatory mediators that reflect immune responses in conditions such as dental caries, periodontal diseases, and systemic infections (26-28). Studies have also confirmed the use of salivary biomarkers in endocrine disorders (for example, salivary cortisol to identify patients with Cushing's syndrome and Addison's disease), autoimmune diseases (for example, increased concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), lactoferrin, and albumin, and a decreased level of phosphate in saliva of patients with Sjrögen Syndrome), and malignancy (for example, p53 antibody and various cytokines in saliva for early detection and screening of oral squamous cell carcinoma (14, 29, 30). Salivary analytes reflecting nutritional and inflammatory status have been reported across various studies. Inflammation can be assessed in saliva by measuring various cytokines and CRP. Certain immune function biomarkers, such as IgA, are also found in saliva (31). Several nutritional biomarkers can also be quantified in saliva. However, the concentration of these biomarkers, relative to their counterparts in blood, remains understudied. Transferrin and iron are generally at lower concentrations in saliva compared to blood, while zinc and IgA have been found at higher levels (31, 32). Although salivary biomarkers have already been used in some healthcare contexts, their widespread adoption as a matrix for nutrition and inflammation assessment is constrained by the lack of established guidelines (33). Furthermore, evidence on the feasibility and use of salivary biomarkers for nutrition and inflammation assessment is limited, particularly in the context of primary care. This article aims to review the feasibility and scope of using salivary biomarkers for assessing nutrition and inflammation, as well as evaluating nutritional disorders. Additionally, we identify research gaps and suggest priority areas for future investigation in the context of primary care facilities.

Methods

The research question and search strategy were developed in July 2024. A comprehensive literature search was conducted thereafter in MEDLINE (PubMed) and CINAHL (Ebsco), with no restrictions on language, geographic location, or date of publication. The search strategy was specifically developed for MEDLINE and is detailed in Supplementary File 2. This search strategy was translated and refined for CINAHL with assistance from evidence synthesis specialists at the Mann Library, Cornell University. We also used an online search engine to search for other sources, such as manufacturers' websites, to gain more information. The workflow for inclusion of the studies in the review is outlined in Supplementary File 1.

The review included pilot studies, observational studies, randomized controlled trials (RCTs), and quasi-RCTs that met all predefined inclusion criteria. Ecological studies, narrative reviews, systematic reviews, commentaries, letters, expert opinions, book chapters, short communications, and animal studies were excluded. Eligible studies were conducted in diverse settings, including hospitals, healthcare facilities, and community environments, to maximize the inclusion of relevant research. The inclusion criteria for studies were as follows: diagnostic and screening studies utilizing salivary biomarkers for the assessment of nutritional disorders and inflammation, and studies involving cases of malnutrition, nutrient deficiencies, nutrition-related metabolic disorders, and inflammatory conditions.

Participants of any age or sex, with any health condition or lack thereof, were included if their saliva samples were used to measure biomarkers for nutrition or inflammation. Health conditions considered for inclusion encompass malnutrition (both undernutrition and overnutrition), nutrient deficiencies (e.g., vitamin and mineral deficiencies), nutrition-related metabolic disorders, and inflammatory conditions. Studies involving participants with health conditions unrelated to nutritional problems or inflammation (e.g., acute kidney injury, autoimmune liver disease,

squamous cell carcinoma) were excluded. No intervention was required for study inclusion. The primary outcome was the examination of salivary biomarkers for assessing nutritional status and inflammation, as well as the evaluation of nutritional disorders. The secondary outcome involved the profiling and quantification of salivary biomarkers associated with nutritional disorders and inflammatory conditions. Studies were excluded if none of the relevant outcomes were measured, or if they provided no supporting evidence (e.g., contact with authors or access to the original protocol). Table 1 shows the number of articles for each biomarker.

Two review authors independently screened titles and abstracts to identify studies for full-text assessment. Subsequently, full texts were screened by the same two authors. Any disagreements during screening were resolved by consensus or through consultation with a third author. Covidence software was used to facilitate the screening process. The selection process, including the total number of included studies, is illustrated in the PRISMA flowchart (Figure 1) (34). Key information from the included studies was extracted independently by two review authors. Disagreements during data extraction were resolved through discussion or, if necessary, by consulting a third review author. This rigorous process ensured consistency and accuracy in the data collected for the review.

Statistical analysis

We performed random-effects meta-analysis using the "metafor" package in R to estimate the pooled correlation between serum and salivary biomarkers, including ferritin, iron, vitamin D, and C-reactive protein (CRP). Spearman correlation coefficients were converted to Pearson correlation coefficients using established transformation methods before inclusion in the meta-analysis. Correlation coefficients were then transformed to Fisher's *z* values to stabilize variances and normalize their distribution. Pooled estimates were calculated on the Fisher's z

scale and subsequently back-transformed to Pearson's r for interpretation. We reported the pooled correlation coefficients (r) with 95% confidence interval (CI), along with measures of heterogeneity (i.e., I^2 and τ^2). All statistical analyses were performed in R version 4.5.2.

Results

1. Saliva biomarkers for nutrition assessment

a. Ferritin

Iron is an essential mineral crucial for various biological functions in the body, including oxygen transport, blood cell production, amino acid synthesis, energy production, and immune cell regulation (35). Iron deficiency impairs immune function and increases susceptibility to infections (36). It is also necessary for the optimal growth and development of the immune system. Most iron is found in the erythroid bone marrow and mature erythrocytes, within the heme moiety of hemoglobin. The iron needed for red blood cell synthesis (erythropoiesis) is mainly supplied by reticuloendothelial macrophages, which recycle iron from old red blood cells. Stored iron bound to ferritin is primarily found in the hepatocytes (35). Circulating iron is bound to transferrin, which accounts for about 0.1% of total body iron in transit compartments. Transferrin delivers iron to developing erythroid precursors and other tissues (35). Iron distribution changes in response to pregnancy, iron deficiency, and iron overload (35). Biomarkers such as serum ferritin (SF), soluble transferrin receptor (sTfR), and total body iron are used to evaluate iron status. Ferritin was identified in saliva almost four decades ago (37) and has been evaluated in various studies (38-40). Earlier work suggests that changes in salivary ferritin occur even before the hematological ferritin changes (41). Ferritin is highly conserved in saliva, perhaps due to the iron-binding capacity of saliva and the iron-dependent enzymatic functions of the saliva (41). However, the specific biochemical mechanism underlying this conservation and iron homeostasis in the salivary

matrix is unclear. Moreover, research on salivary iron biomarkers in the context of nutrition and inflammation assessment is limited.

We identified seven studies that assessed salivary ferritin concentrations (42-48). The findings regarding salivary ferritin suggest its potential role as a noninvasive biomarker for assessing iron status. The discrepancy in salivary ferritin levels between the studies may be explained by differences in sample size, age of patients, and individual health status. The sample collection, processing, storage, laboratory analysis methods, and instruments used for quantifying salivary ferritin concentrations are reported in Supplementary File 3. The summary of studies with salivary ferritin and results are shown in Table 2 and Supplementary File 4. Two studies reported higher salivary ferritin concentrations than serum ferritin levels, whereas three studies reported higher serum ferritin concentrations than salivary ferritin. Two studies did not report serum ferritin concentrations. Only four studies examined the correlation between serum and salivary ferritin levels (42, 44-46). A random-effects meta-analysis of these four studies demonstrated a moderate positive pooled correlation of 0.47 (95% CI: 0.21 to 0.67) (Figure 2). Although the heterogeneity was substantial (I^2 = 87.7%, τ^2 = 0.08), the direction of association was consistently positive, supporting an overall positive relationship between serum and salivary ferritin concentrations. Overall, the findings suggest that salivary ferritin holds promise as a noninvasive biomarker; however, its clinical utility is limited by inter-study discrepancies likely arising from differences in the sample collection and processing protocols, assay methods, population heterogeneity, oral health status, and underlying disease conditions.

b. **Iron (Fe)**

We identified five studies that assessed salivary iron (Fe) levels (18, 42, 44, 49, 50). The sample collection, processing, storage, laboratory analysis methods, and instruments used for quantifying salivary iron (Fe) concentrations are reported in Supplementary File 3.

The summary of the results of salivary iron is highlighted in Table 3 and Supplementary File 4. All five studies detected Fe in saliva samples, but the levels varied across studies and among different study populations. Two studies reported lower salivary ferritin concentrations than serum ferritin levels, whereas only one study reported higher salivary ferritin concentrations than serum ferritin (Table 3). Two studies reported the correlations between serum and salivary levels of Fe. A meta-analysis of these two studies using a random-effects model showed a positive pooled correlation of 0.76 (95% CI: -0.12 to 0.97) (Figure 2). Heterogeneity was substantial ($I^2 = 97.7\%$, $\tau^2 = 0.649$), indicating considerable variability in effect sizes across studies. Despite this heterogeneity, each study reported a positive correlation, supporting a consistent positive relationship between serum and salivary Fe levels.

c. Soluble Transferrin Receptor (sTfR)

No studies evaluating sTfR in saliva met our inclusion criteria.

d. Zinc

Zinc is an essential trace element that plays a vital role in maintaining various metabolic functions in the human body (51). Zinc deficiency is associated with impaired metabolism, growth retardation, and an increased risk of infectious diseases (52). This micronutrient is particularly critical for young children, as inadequate zinc levels are linked to stunting, wasting, and increased morbidity and mortality during the early years of life. Zinc plays a key role in physical growth, neurobehavioral development, and reproductive health (51). Additionally, zinc supplementation is effective in managing acute and persistent diarrheal diseases, with evidence suggesting that zinc

supplementation can reduce the duration of acute diarrhea in children. Plasma or serum zinc concentration is commonly used as a biomarker for assessing zinc status (52). However, it is influenced by multiple biological factors, including age, sex, dietary intake, stress, pregnancy, and circadian variations (51). Additionally, plasma or serum zinc levels are tightly regulated through homeostatic mechanisms and typically reflect only a small fraction of total body zinc (53). Thus, severe zinc deficiency is required to elicit measurable changes in plasma or serum zinc concentrations. Moreover, plasma zinc levels do not reliably reflect short-term exposure to zinc supplements or zinc-fortified foods and exhibit significant variability across different populations (51). Therefore, in addition to plasma or serum zinc levels, dietary zinc intake and height-for-age z-scores (or stunting status) are recommended for a more comprehensive assessment of zinc status (51, 52).

Research on salivary zinc as a biomarker remains limited. Some studies suggest that hypogeusia (diminished taste acuity) is a characteristic of zinc deficiency and could serve as a functional indicator of zinc status (51). Consequently, salivary zinc measurement may offer an alternative approach for assessing zinc deficiency, though further investigation is needed to validate its reliability. We identified seven studies that assessed salivary zinc concentrations (49, 54-59). The sample collection, processing, storage, laboratory analysis methods, and instruments used to quantify salivary zinc concentrations are reported in Supplementary File 3. The substantial heterogeneity in sampling protocols and salivary zinc quantification methods across the seven studies assessing salivary zinc concentrations raises concerns regarding the comparability and methodological reliability of their reported findings. The summary of the results is highlighted in Supplementary File 4. The salivary levels of zinc were lower than the serum zinc levels in two studies (54, 55). In one study, salivary zinc concentrations were undetectable for a significant

proportion of the participants (55). Only one study examined the correlations between salivary and plasma zinc concentrations, but it didn't observe a significant relationship (56). These findings indicate that the current evidence regarding salivary zinc is insufficient and inconsistent across populations and study settings, limiting our ability to justify the use of salivary zinc as a reliable alternative. Further research is required to assess its diagnostic performance and to define population-specific reference ranges and cutoff values.

e. Calcium

Calcium is essential for bone growth. Salivary calcium secretions are typically oversaturated, which helps the formation of dental enamel and plays an important role in the protection of teeth (60). Salivary levels of calcium increase physiologically when the salivary flow rate decreases (61). This may not be true for patients with anemia, for whom the salivary calcium level can be lower due to impaired absorption in the gastrointestinal tract. The evidence on salivary calcium concentration is currently limited. Previous work suggests that elevated salivary calcium levels may be associated with reduced bone mineral density and could serve as a functional marker of bone health, particularly in postmenopausal women (62). Therefore, measuring salivary calcium may offer a non-invasive alternative for evaluating calcium deficiency and bone health conditions (12). However, further research is necessary to confirm its accuracy and clinical utility.

In this review, we identified six studies that assessed salivary calcium levels (63-68). The sample collection, processing, storage, laboratory analysis methods, and instruments used for calcium quantification are reported in Supplementary File 3. All six studies investigating salivary calcium levels were cross-sectional in design. A summary of their findings is presented in Supplementary File 4. Salivary calcium levels were detected in all six studies, with reported concentrations ranging from 0.062 mg/dL to 18.79 mg/dL. None of the studies measured calcium levels in blood.

Two studies found significantly lower salivary calcium levels in participants with different nutritional impairments (e.g., patients with Fanconi anemia and children with moderate to severe protein-energy malnutrition) compared to the controls (64, 68). On the other hand, one study reported no significant differences in salivary calcium levels between the children with normal weight, overweight, and obesity (63). One study observed a significantly positive correlation between salivary calcium levels and serum concentrations of vitamin D (66). Another study reported a statistically significant negative correlation between bone mineral density and salivary calcium levels (65). These findings suggest that salivary calcium levels vary across different populations and clinical contexts.

f. Vitamin A

Vitamin A is an essential micronutrient that can be obtained from both plant and animal sources (69). The form found in plants is provitamin A carotenoid, while the form found in animal sources is retinol (70). Vitamin A plays a crucial role in vision, cellular growth, differentiation, and maintaining immune functions (70). Vitamin A deficiency is a global concern, particularly affecting children in low-income settings (71). This deficiency can cause night blindness, xerophthalmia, and an increased risk of infectious diseases, especially in young children (69). Vitamin A is absorbed and stored in the liver as retinol and is released into circulation bound to retinol-binding protein (RBP). Serum or plasma retinol is the most used biomarker for assessing vitamin A status, and serum RBP is used as a proxy for serum retinol in identifying vitamin A deficiency (69). However, there are significant challenges in sample collection and transportation for the assessment of these biomarkers (69). The most accurate tests for vitamin A, including serum or plasma retinol and retinol-binding protein, are invasive, costly, time-consuming, and technically

demanding. Therefore, saliva can be an alternative biofluid for the assessment of vitamin A status (72).

We identified two studies evaluating salivary vitamin A status. The sample collection, processing, storage, laboratory analysis methods, and instruments used to quantify salivary vitamin A concentrations in these studies are reported in Supplementary File 3. Table 4 and Supplementary File 4 summarize the findings from studies that assessed salivary vitamin A. One study reported significantly higher salivary vitamin A levels in males than females ($P = 0.003$) (72). Although the exact biological reason for this difference is unknown, it can be due to hormonal influences, larger salivary gland size, or differences in salivary flow rate between male and female participants. Another study assessed salivary retinol-binding protein (RBP) in healthy individuals (73). The findings suggest that RBP is secreted by all major salivary glands. They observed a higher salivary RBP concentration in resting samples compared to that in stimulated samples; however, it was not statistically significant. Conversely, the salivary output of RBP was significantly higher in stimulated samples compared to resting samples ($p < 0.05$), suggesting an increased secretion rate upon stimulation. In this study, salivary RBP concentrations did not demonstrate significant linear relationships with serum RBP or serum retinol levels (73).

g. Vitamin D

Vitamin D, a fat-soluble vitamin, is essential for calcium absorption and bone formation in children (74). The insufficiency or deficiency of vitamin D is a common problem in the general population (75). Vitamin D deficiency causes hypocalcemia, rickets, and osteomalacia in children and adults. Additionally, the deficiency of vitamin D was found to be associated with obesity, insulin resistance, diabetes mellitus, and cardiovascular diseases (75, 76). Vitamin D is commonly assessed in blood, and the widely accepted biomarker is 25-hydroxyvitamin D (25(OH)D) (77).

However, the optimal level of this biomarker is debated, and there are ongoing challenges in standardizing assay methods for measuring 25-hydroxyvitamin D (25(OH)D) in blood samples (78).

Research on the assessment of vitamin D biomarkers in saliva is still limited. We identified nine studies evaluating salivary vitamin D status (72, 73, 79-85). All but one study assessed either vitamin D2 or D3 in saliva. The methods for sample collection, processing, storage, laboratory analysis, and instruments used for quantifying salivary vitamin D concentrations are reported in Supplementary File 3. The summary of the results is highlighted in Table 5 and Supplementary File 4. Only four studies assessed serum concentrations of vitamin D, and three studies investigated the correlations between serum and salivary levels of vitamin D. A random-effects meta-analysis of these three studies showed a moderate positive pooled correlation (r = 0.51, 95% CI: 0.11 to 0.77) between serum and salivary vitamin D levels (Figure 2). Heterogeneity was substantial (I^2 = 90.9%, τ^2 = 0.15), indicating considerable variability in effect sizes across studies. Despite this heterogeneity, all studies reported positive correlations, supporting an overall positive relationship between serum and salivary vitamin D. Only one study assessed salivary levels of vitamin D-binding protein (DBP) and observed a significant positive correlation between serum and salivary levels of DBP (73). Two studies found that salivary vitamin D levels were lower than serum levels in both healthy controls and in patients with different oral health conditions (Table 5). One study observed statistically significant negative correlations between salivary vitamin D and BMI Z-score, body fat percentage, waist circumference, and weight-to-height ratio (83). However, another study reported no statistically significant differences in salivary vitamin D2 or vitamin D3 levels between the adolescents divided into groups based on their body fat percentage (82). The study

findings underscore the substantial heterogeneity among studies evaluating salivary vitamin D levels and the inconsistencies observed across different population subsets.

h. Vitamin B12

Vitamin B12, also known as cobalamin, is an essential nutrient for humans (86, 87). This water-soluble vitamin helps the formation of red blood cells, the production of DNA, and nerve function in the body (87). The deficiency of vitamin B12 leads to pernicious anemia (87). After consumption, vitamin B12 is transported to the small intestine by binding with salivary haptocorrin, also known as R-binder (86). In the small intestine, the high pH causes haptocorrin to release Vitamin B12, which is then absorbed in the terminal ileum (86). After absorption, the body stores Vitamin B12 in the liver. Transport of B12 in the plasma is facilitated by two proteins: haptocorrin and transcobalamin. Haptocorrin forms are found in various body fluids, including plasma, saliva, bile, gastric juice, and breast milk (87). Transcobalamin is responsible for delivering B12 to all tissues. Genetic deficiency of transcobalamin is associated with severe B12 deficiency, whereas genetic deficiency of haptocorrin appears to have little or no effect on functional B12 status (86). Common biomarkers for Vitamin B12 assessment include serum (or plasma) B12 concentration, serum holo-transcobalamin (holoTC) concentration, serum methylmalonic acid (MMA) concentration, and plasma total homocysteine (tHcy) concentration (86). Each of these biomarkers has limitations. For example, measuring serum (or plasma) vitamin B12 concentrations is not feasible in the field, and samples must be protected from light during collection and handling (86).

Saliva has potential as a biofluid for assessing Vitamin B12 status, but current evidence is very limited regarding the assessment and validation of salivary biomarkers for vitamin B12 status. We identified one study on salivary haptocarrin and three studies evaluating vitamin B12 levels in

saliva samples (44, 72, 73, 88). The sample collection, processing, storage, laboratory analysis methods, and instruments used for quantifying salivary vitamin B12 concentrations are reported in Supplementary File 3. We observed considerable variation across the included studies in terms of sample collection, processing, storage, laboratory analysis, and the instruments used to quantify salivary vitamin B12. Because of this heterogeneity, it was not possible to determine the most reliable methods for collecting and processing saliva samples for vitamin B12 assessment.

Table 6 and Supplementary File 4 summarize the findings of the studies that assessed vitamin B12 in saliva. One study observed a significantly higher concentration of salivary haptocorrin in resting samples compared to the chew-stimulated samples (73). This finding suggests an increased secretion rate of salivary haptorrin upon stimulation. They also observed a moderate correlation between salivary haptocorrin in stimulated and resting samples ($r = 0.57$). In this study, salivary haptocorrin concentrations were not associated with serum haptocorrin ($r^2 = 0.02$, $p > 0.05$) or serum total vitamin B12 levels ($r^2 = 0.08$, $p = 0.05$) (73). One study reported higher salivary vitamin B12 concentrations than serum vitamin B12 levels, whereas another study reported higher serum vitamin B12 concentrations than salivary vitamin B12 (Table 6). Only one study reported the correlation between serum and salivary levels of vitamin B12; however, the strength of correlation was very poor ($r = 0.16$, p-value = 0.035) (44).

i. Folic acid or Folate

Folic acid, also known as folate, is a B vitamin crucial for healthy cellular growth and development, particularly important during early pregnancy to prevent neural tube defects in the newborn baby (89). Serum folate, red blood cell (RBC) folate, and plasma homocysteine concentrations are commonly used as biomarkers of folate status (89). There is limited evidence regarding the levels of folic acid in the saliva. We identified only one study evaluating salivary

folic acid or folate (44). In this study, salivary folic acid concentrations were detected higher than the measurable upper limit of detection (i.e., >24 ng/mL) (44).

j. Vitamin C

We identified only two studies that assessed salivary vitamin C concentrations (72, 90). The sample collection, processing, storage, laboratory analysis methods, and instruments used for quantifying salivary vitamin C concentrations are reported in Supplementary File 3. The summary of the findings is reported in Supplementary File 4. An intervention study showed a significant increase in both serum and salivary vitamin C levels one week after curcumin intake and at the end of the study across all groups. Salivary vitamin C levels were much lower than the serum levels of vitamin C across all groups at all time points. They also observed a strong positive correlation between serum and salivary vitamin C levels across all study groups (90). Another study reported a significantly higher concentration of salivary vitamin C levels among patients with non-erosive Lichen Planus compared to the patients with erosive Lichen Planus (72). They didn't examine the correlation between serum and salivary levels of vitamin C.

k. Vitamin E

We identified only two studies that assessed salivary vitamin E concentrations (72, 90). The methods of saliva collection, processing, storage, laboratory analysis, and instruments used for quantifying salivary vitamin E concentrations are reported in Supplementary File 3. The summary of the findings is reported in Supplementary File 4. An intervention study showed a significant increase ($p<0.05$) in both serum and salivary vitamin E levels one week after curcumin intake and at the end of the study across all groups. The salivary vitamin E levels were much lower compared to the serum levels of vitamin E across all groups at all time points. They observed a strong positive correlation between serum and salivary vitamin C levels across all study groups (90). Another

study observed that salivary vitamin E levels were significantly higher among patients with non-erosive Lichen Planus compared to the patients with erosive Lichen Planus. They didn't examine the correlation between serum and salivary levels of vitamin C (72).

2. **Saliva biomarkers for inflammation assessment**

a. C-reactive protein (CRP)

C-reactive protein (CRP) is a positive acute-phase protein produced in the liver in response to inflammation (91). CRP is released into the bloodstream when microbial invasion occurs and rises quickly within the first 6 hours of infection onset (91). Changes in micronutrient levels during infection and inflammation can mask the accurate assessment of certain micronutrients, including iron and vitamin A biomarkers(92). Therefore, CRP is used as a marker of inflammation or infection to help interpret micronutrient biomarkers (92, 93). An elevated level of CRP has also been found to be associated with cardiometabolic risk factors and chronic diseases (94). CRP is commonly assessed in blood samples; however, recent evidence showed the utility of salivary CRP as a non-invasive alternative method for the assessment of inflammatory conditions. We identified nine studies that measured salivary CRP levels (3, 95-102). The methods of saliva collection, processing, storage, laboratory analysis, and instruments used for quantifying salivary CRP concentrations are reported in Supplementary File 3. The summary of the results is highlighted in Table 7 and Supplementary File 5. Three studies reported elevated levels of salivary CRP among individuals with obesity. One study showed that overweight or obesity is an independent predictor of elevated secretion rate of salivary CRP. Another study found that salivary levels of CRP were positively correlated with BMI Z-score, waist circumference Z-score, and waist-to-height ratio Z-score. The ROC curve analysis showed that salivary CRP has a promising diagnostic value to determine obesity in children (97). Similarly, Tvarijonaviciute et al. 2019 observed a statistically

significant positive correlation between salivary CRP and BMI of the participants (3). However, Wetterö et al. 2021 reported that no significant correlations were observed between BMI and morning and evening values of CRP (98). Two studies didn't report any association of salivary CRP with the BMI of the participants (99, 101). Only three studies examined the correlations between serum and salivary levels of CRP levels (Table 7). The meta-analysis demosnrtaed a moderate positive pooled correlation (Figure 2). Using a random-effects model, the combined Fisher's z-transformed effect corresponded to a pooled correlation of 0.63 (95% CI: 0.29 to 0.83). Heterogeneity was substantial ($I^2 = 96\%$, $\tau^2 = 0.149$), indicating considerable variability in effect sizes across studies. Despite this heterogeneity, all studies reported positive correlations, supporting an overall positive relationship between serum and salivary CRP. These findings suggest that salivary CRP may serve as a promising, noninvasive biomarker for nutrition and inflammation assessment. However, the current evidence remains limited and is weakened by substantial heterogeneity in study design, sample characteristics, and analytical methods.

b. Alpha-1 acid glycoprotein (AGP)

AGP is a positive acute-phase protein whose serum concentration increases in response to infection and inflammatory stimuli (103, 104). Elevated AGP levels have been associated with a wide range of pathological conditions, including infections, tissue injuries, malignancies, cardiovascular diseases, stroke, sepsis, obesity, diabetes, and metabolic syndrome (105). Unlike C-reactive protein (CRP), which exhibits a rapid rise and decline during acute inflammation, AGP increases more gradually and remains elevated for a prolonged period (105). This temporal difference allows AGP and CRP to be used in combination to assess the phase and severity of inflammation, with AGP being particularly relevant as a marker of chronic or prolonged inflammatory states (105). AGP is routinely measured alongside CRP to interpret the time course and intensity of infections

that influence biomarkers of micronutrient status (104). Given its role in inflammation, it is recommended that AGP be measured concurrently with CRP when evaluating and adjusting micronutrient biomarkers, such as serum ferritin, sTfR, serum retinol, RBP, and serum zinc, particularly in research and population-based nutritional surveys (104). While AGP is predominantly assessed in serum samples, its application in noninvasive biofluids, such as saliva, remains largely unexplored. We identified only one study that investigated salivary AGP, highlighting a significant gap in the literature regarding its potential utility in nutrition and inflammation research (105). Salivary alpha-1-acid glycoprotein (AGP) concentrations, as measured by ELISA, exhibited a median (Q1, Q3) of 0.48 (0.41, 0.69) ng/mL, with a range of 0.27–3.64 ng/mL (105). They didn't examine the correlations between serum and salivary levels of AGP.

c. Hepcidin

Hepcidin is a peptide hormone that is crucial in regulating iron homeostasis (106, 107). It is primarily synthesized in the liver and released into circulation by binding to ferroportin, the main iron-exporting protein (107). In response to elevated serum iron levels, hepcidin decreases dietary iron absorption in the intestine and limits iron release from physiological stores (106). Conversely, during conditions of iron deficiency, hepcidin production diminishes, facilitating increased iron absorption and mobilization from storage sites to meet the body's needs (107). Clinical research on hepcidin has largely focused on its involvement in iron-related disorders, including anemia of chronic disease, hereditary hemochromatosis, and iron-loading anemias (106). Hepcidin has been detected in various biological fluids such as serum, urine, saliva, bile, ascitic fluid, and pleural fluid (108, 109). However, the precise concentration, regulatory mechanisms, and physiological significance of hepcidin in these fluids remain incompletely understood, as data on its distribution

and function across different biological compartments are still limited. We identified two studies that assessed salivary hepcidin concentrations (45, 108). The methods of saliva collection, processing, storage, laboratory analysis, and instruments used for quantifying salivary hepcidin concentrations are reported in Supplementary File 3 and the summary of results is highlighted in Supplementary File 5.

One study reported relatively lower hepcidin concentrations in saliva than serum (45). In this study, there was no correlation between salivary ferritin level and salivary hepcidin level (p-value > 0.05). However, they didn't report the correlation coefficient (45). Another study confirmed the presence of hepcidin-25 in saliva, with detected levels (Mean ± SD) of 3.39 ± 2.83 ng/mL and a range of < 0.9-11.8 ng/mL (108). The combined Mean ± SD of hepcidin was 1.85 ± 2.16 ng/mL among 105 adult participants enrolled in both studies on salivary hepcidin (110).

d. Immunoglobulin A

Immunoglobulin A (IgA) is an antibody within the immune system that plays a critical role in the initiation of inflammation, which occurs through the formation of IgA complexes (111). Studies have shown that IgA plays a critical role in maintaining gut homeostasis by regulating the initiation of inflammation at both mucosal and non-mucosal sites (111). IgA deficiency has been linked to autoimmune disorders, skin infections, and asthma (111). Unfortunately, there are currently no treatments for IgA deficiency (111). IgA responses are modulated by dietary nutrients such as fiber, lipids, glutamine, and vitamin A, as well as by microbially derived metabolites such as ATP and short-chain fatty acids; therefore, they are closely related to nutrition (112). In an existing study, a correlation between zinc and vitamin A deficiency in erythrocytes was recorded in patients with IgA deficiency (113). However, further investigation is needed to validate its reliability. IgA is found in the mucous membranes throughout the respiratory, gastrointestinal, and urogenital

tracts (114). It can also be found in your saliva. However, research on salivary or serum IgA as a biomarker for assessing inflammatory/nutritional disorders is limited. Evidence is also scarce on the correlations between serum and salivary IgA levels. Current research gaps for saliva IgA analysis also include missing data and salivary inflammatory levels below detectable limits, and a lack of standardized methodologies for salivary collection and storage. Only a few studies have examined the correlation between salivary IgA and systemic markers of inflammation or nutritional deficiency, making it difficult to assess its diagnostic accuracy (113).

We identified 11 studies that assessed salivary IgA concentrations (68, 115-124). The methods of saliva collection, processing, storage, laboratory analysis, and instruments used for quantifying salivary IgA concentrations are reported in Supplementary File 3. Seven studies were cross-sectional studies, and four studies were randomized trials. Data on serum IgA levels are also scarce in the included studies. Only three studies reported serum IgA concentrations. However, none of the studies examined the correlations between salivary and plasma IgA concentrations. The summary of results is highlighted in Supplementary File 5.

Salivary IgA was not detectable in two studies. Two studies indicated that nutritional interventions (e.g., zinc supplementation or probiotics) might help improve immune response by modulating salivary IgA levels in children with nutritional impairments. One study reported that prebiotic intervention had no effect on salivary sIgA levels in adults with overweight (123). The results from two studies showed no significant difference in salivary IgA between the groups with different nutritional impairments (68, 120). One study found lower salivary IgA levels in undernourished children compared to their well-nourished counterparts (121). In contrast, one study observed that stunted infants had significantly higher salivary IgA levels than those with normal length-for-age Z-scores (115). One study reported significantly higher salivary sIgA levels in adolescents with

obesity (116). Another study found that salivary IgA levels were positively correlated with BMI and fat percentage across all participants. In this study, salivary osmolality was also significantly correlated with salivary IgA in children with overweight or obesity (117). These findings suggest that obesity in children may be linked to increased salivary IgA levels, potentially reflecting altered mucosal immune responses and systemic changes associated with overweight or obesity. Nonetheless, further prospective and longitudinal studies are needed to confirm this explanation.

e. Cytokines

Cytokines are known as signaling molecules of the immune system. They play an important role in the immunological response against pathogens and other antigens by binding to receptors on the surface of target cells. Cytokines are commonly affected by nutritional impairments. Nutrient deficiencies affect the production and activity of cytokines, leading to altered immune response. Therefore, cytokines are evaluated in nutrition studies or clinical settings in order to assess the impact of inflammation on nutritional status and immune function. Cytokines are commonly measured in blood samples; however, recent evidence suggests that saliva can be a potential biological sample for the assessment of cytokines. We have identified 27 studies that assessed salivary cytokines. Studies included in this review mostly reported TNF-alpha and IL-6 in saliva samples. In addition to these two cytokines, the included studies also assessed other cytokines, including IL-1β, IL-1Ra, IL-8, IL-10, IL-1Ra, MCP-1, NGF, sCD40L, MMP-9, MMP-2, PTX-3, PAI-1, VEGF, siCAM-1, sPLA2-IIA, Lp-PLA2, Serpin A12, TNF-R1, and TLR2. Therefore, we report the findings in three subheadings: TNF-alpha, IL-6, and other cytokines.

- **TNF-alpha**

Tumor Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine involved in the regulation of immune responses (125, 126). TNF-α, produced primarily by macrophages as well as adipocytes

and T cells, triggers inflammation and cell death through receptor-mediated signaling (126). Excess TNF-α can lead to chronic inflammation, autoimmune diseases, and metabolic syndrome (126, 127). In obesity, elevated levels of TNF-α contribute to chronic low-grade inflammation and are associated with insulin resistance and metabolic dysregulation (126). TNF-α concentration is commonly measured in the blood to assess the severity of these diseases (128). However, with a short half-life, TNF-α is rapidly eliminated from the blood, resulting in lower serum concentrations (129). There is limited research measuring TNF-α levels in saliva to assess nutrition-related inflammatory conditions. Salivary TNF-α measurement offers a promising non-invasive method for assessing inflammation, but its reliability requires further validation. We identified eleven studies that measured TNF-α concentrations (3, 130-139). The methods for sample collection, processing, storage, laboratory analysis, and instruments used to quantify salivary TNF-α concentrations are reported in Supplementary File 3.

The results of the studies on salivary TNF-α are highlighted in Supplementary File 5. The results from the included studies suggest that TNF-α has the potential to serve as a non-invasive tool to monitor the inflammatory changes in individuals with overweight or obesity. Five studies observed elevated salivary TNF-α levels among participants with overweight or obesity. Two studies did not observe any difference in salivary levels of TNF-α between normal-weight individuals and those with overweight or obesity. One study observed significant positive correlations between body mass index and salivary TNF-α concentrations; however, the sample size was small, limiting the interpretability of the findings (139). Only two studies examined the correlation between salivary and serum levels of TNF-α, and neither observed a significant correlation (3, 131). In the remaining studies, serum TNF-α levels were not directly compared with salivary TNF-α levels,

thereby making it difficult to determine the accuracy of salivary TNF-α in predicting inflammation or disease states.

- **IL-6**

Interleukin-6 (IL-6) is involved in immune regulation, inflammation, and hematopoiesis (140). At the site of inflammation, IL-6 is produced to trigger acute phase protein production and responses, as well as the transition from innate to adaptive immunity (140). Elevated IL-6 levels are implicated in various metabolic conditions, including diet-induced obesity in response to metabolic stress (141). IL-6 is frequently measured in serum to assess inflammatory conditions; however, its involvement in both pro-inflammatory and anti-inflammatory pathways limits the reliability of serum levels alone as definitive indicators of inflammation (142). IL-6 is not routinely measured in the clinical setting since its standardization is low, and assessment is costly compared to other routinely used inflammatory biomarkers (143). Additionally, research on salivary IL-6 as a biomarker of nutrition-related inflammatory conditions is currently limited. Salivary IL-6 measurement offers a promising non-invasive method for assessing inflammation, but its reliability requires further validation. We identified 18 studies that measured IL-6 concentrations (3, 100, 130-133, 136, 144-154). The methods for sample collection, processing, storage, laboratory analysis, and instruments used to quantify salivary IL-6 concentrations are reported in Supplementary File 3. The results of the studies that investigated salivary IL-6 levels are summarized in Supplementary File 5. Only three studies examined the correlation between salivary and serum levels of IL-6; however, none found a significant correlation between salivary and serum IL-6 concentrations (3, 100, 131). Several studies reported elevated salivary IL-6 levels in individuals with overweight or obesity compared to normal-weight controls. The findings underscore the need for more well-designed studies with standardized methods to confirm the

utility of IL-6 as a potential biomarker for the assessment of overweight or obesity. Evidence regarding the effects of nutritional interventions (e.g., zinc supplementation or turmeric consumption) on salivary IL-6 levels was inconsistent across studies. Some studies reported elevated salivary concentrations of IL-6 in individuals with inflammatory conditions, such as PD, gingivitis, and other forms of periodontal inflammation. These findings suggest that salivary IL-6 may reflect the underlying chronic inflammatory condition, irrespective of individual health status. However, most of the included studies were observational in design, and results were not consistent across study populations and clinical contexts. This inconsistency suggests that the reliability of salivary IL-6 as an inflammatory biomarker may depend on the specific disease context and population studied.

- **Other cytokines**

We identified 22 studies assessing salivary cytokines other than TNF-alpha and IL-6. The summary of studies with other cytokines and the findings are reported in Supplementary File 5. Similar to TNF-α and IL-6, findings were heterogeneous across studies and varied among different populations. Only a few studies reported statistically significant differences in cytokine levels between healthy controls and individuals with specific conditions. Similarly, few studies examined correlations between cytokine concentrations in saliva and corresponding serum levels. However, the results were inconsistent, limiting our ability to draw conclusions regarding the diagnostic utility of these salivary cytokines relative to their blood-based counterparts.

Discussion

This review synthesized current evidence on the feasibility and use of salivary biomarkers for the assessment of nutrition (e.g., ferritin, iron, zinc, calcium, vitamin A, vitamin B12, folic acid, vitamin C, vitamin D, and vitamin E) and inflammation (e.g., CRP, AGP, Hepcidin, IgA, and

cytokines), from 76 articles, across 26 countries involving 5425 children and 5617 adults. We cataloged and summarized the results from 33 studies assessing ferritin, iron, zinc, calcium, vitamin A, vitamin B12, vitamin D, vitamin C, and vitamin E in saliva samples. We didn't find any study assessing salivary sTfR. Only one study assessed salivary folic acid; however, the level was above the LOD. Additionally, we found 43 studies evaluating salivary inflammatory biomarkers such as CRP, AGP, hepcidin, IgA, and cytokines within the context of nutritional impairments. While most of the biomarker values were detectable in saliva samples, their application in nutritional surveillance and profiling of inflammatory conditions remains challenging, especially in settings with a high burden of nutritional impairments, inflammation, and infection. We found inconsistencies in the salivary biomarker values across most studies included in this review. For example, in healthy controls, three studies reported higher salivary ferritin levels compared to serum ferritin, whereas two studies showed the opposite trend. In patients with iron deficiency anemia (IDA), three studies found salivary ferritin levels to be consistently higher than serum ferritin levels; however, only one of these studies examined the correlation between the two and reported a strong positive relationship. Similarly, one study conducted in children found higher salivary iron levels than serum iron, while two studies in adults reported higher serum iron concentrations. Four studies assessed vitamin D biomarkers in both saliva and serum. Two of these reported higher salivary levels than serum levels, while the other two observed the opposite pattern. Overall, the results for most biomarkers remain inconsistent, and only a limited number of studies have investigated the correlation between salivary and serum concentrations for each biomarker. The current evidence is also weakened by substantial heterogeneity in study design, sample characteristics, and analytical methods across studies evaluating salivary biomarkers. The variability in the methods for sample collection, processing,

storage, laboratory analysis, and quantification techniques raises concerns regarding the comparability and generalizability of the results. Therefore, more well-designed clinical and longitudinal studies using standardized methodologies are needed to determine the diagnostic accuracy and clinical utility of salivary biomarkers. Additionally, diagnostic test accuracy research is needed to assess the feasibility of saliva-based screening tests, compared to reference standards in blood, for the assessment of nutrition and inflammatory biomarkers, both in primary care settings and population-level studies.

Text Box 1. The criteria required for a biomarker to be measured in saliva

- It should be present at a detectable level in saliva
- The concentration should be stable within the salivary matrix
- It should reflect the body's physiological and pathological states
- Sample collection should be easy, safe, and comfortable for the participants
- Sample collection, processing, transportation, storage, and laboratory analysis should be performed using standardized protocols and techniques
- The laboratory assay methods should be validated and optimized to minimize measurement errors
- The detection method needs to be sensitive, specific, and reproducible across individuals and different populations
- The concentration should be within the sensitivity range of available detection methods
- The measurements should show low intra- and inter-assay variability
- The biomarker should correlate with the corresponding blood biomarker
- The findings should be reliable and clinically meaningful, with optimal diagnostic accuracy
- The assessment should be cost-effective to adopt in routine clinical and primary care practice

The criteria required for a biomarker to be measured in saliva are listed in Text Box 1. Ideally, for a biomarker to be considered measurable in saliva, its concentration should be stable within the salivary matrix (155). The oral microbiome and endogenous salivary components, including enzymes, hormones, and epithelial cells, may influence saliva-based diagnostic assays (155). For example, proteolytic enzymes may influence the degradation of targeting proteins, and microbial activity may alter the concentrations of certain metabolites, leading to inaccurate measurements of salivary analytes (155, 156). Therefore, the biomarker to be measured in saliva must resist degradation by microbial and enzymatic activity (155). Additionally, the biomarker should be

present at a detectable level in saliva for assessing nutrition or inflammation status and should reflect the body's physiological and pathological states to ensure clinical relevance (157). It should also correlate with corresponding blood biomarkers (155). The detection method needs to be sensitive, specific, and reproducible, accounting for the variability in salivary pH, flow rates, oral health conditions, underlying diseases, and other biological determinants (155, 158, 159). Moreover, the sample collection protocols, processing and storage techniques, and laboratory analysis methods should be standardized to account for the intra- and inter-individual variability in salivary biomarker concentrations (155, 158). Appropriate statistical tests with adjustment for all potential confounders are also needed to address the issue of intra- and inter-individual variability. Additionally, multiple other challenges need to be addressed before salivary tests can be implemented routinely in clinics, schools, or communities. Figure 3 highlights several methodological gaps in the current understanding of salivary biomarkers for assessing nutrition and inflammation, particularly in low-resource settings.

A. Discovery-based gaps

To realize the potential of salivary biomarkers for nutrition and inflammation, we have identified several discovery-based gaps in this review that need to be addressed. First, the source of analytes measured in saliva samples is unclear. Salivary biomarkers were measured in unstimulated whole saliva in most of the included studies. Perhaps, the analytes enter saliva by diffusing through the cells of the salivary glands or by ultrafiltration through the tight junctions (160). However, further investigation is required to confirm the source of the different nutritional and inflammatory biomarkers assessed in saliva. Furthermore, many biomarkers are present at substantially lower concentrations in saliva than in blood (161, 162). These physiologically lower levels can challenge accurate quantification, may compromise detectability, and complicate interpretation of the

findings (162). Even minor variations in sample collection, handling, storage, or analytic procedures can introduce measurement error and limit reproducibility (162). Hence, standardization of collection and processing protocols, together with optimization of assay methodologies using highly sensitive platforms, is essential to ensure reliable and clinically meaningful results. It is also essential to determine whether the concentrations of these biomarkers are influenced by salivary pH, production, and flow rates. Salivary pH can alter the assay results (162). On the other hand, a high salivary flow rate dilutes the sample, leading to an inaccurate assessment of the biomarkers. When feasible, salivary pH and flow rate data can be used to adjust the concentrations of salivary biomarkers. Second, nutrition biomarkers indicate intake, status, or function. While blood and some urinary biomarkers are well established for assessing these aspects, there are still gaps in categorizing salivary biomarkers as indicators of intake, status, or different functions (33). Some salivary biomarkers for nutrition assessment may reflect micronutrient status, but further research is needed to determine how to use them for assessing intake and various functions. Third, there is limited evidence on the correlation between salivary and blood biomarkers for nutritional and inflammatory assessments. Establishing these correlations is essential to validate saliva as a reliable substitute for blood, especially in settings where blood collection is logistically challenging or culturally sensitive. Without this comparative assessment and validation, the clinical and public health relevance of salivary biomarkers remains uncertain. Fourth, our findings underscore the lack of data on salivary biomarkers among children, adolescents, and women of reproductive age (WRAs) in low-resource settings. Most available studies were conducted in adult populations or clinical environments, limiting the generalizability of the findings in different geographic settings. This is particularly concerning given the high burden of malnutrition and inflammation among children in low- and middle-income countries.

Fifth, the diagnostic accuracy of salivary biomarkers has not been systematically evaluated in populations with a high burden of infections and chronic inflammation. In such contexts, inflammation can alter the concentration and detectability of nutritional biomarkers, potentially leading to misinterpretation and diagnostic errors. Understanding how these markers behave in saliva under conditions of concurrent infection and malnutrition is essential for developing reliable screening tools. Finally, another important gap is the lack of evidence regarding the ratio-based indices in salivary biomarker research. In blood, indices such as the sTfR:ferritin ratio have proven valuable in distinguishing iron deficiency from inflammation-induced changes. The potential to develop similar composite indices using salivary biomarkers remains unexplored. These tools could enhance diagnostic precision, particularly in settings where both malnutrition and infection are prevalent.

B. Sample collection, processing, and storage gaps

This review identified several methodological issues in the aspects of sample collection, processing, and storage that may compromise the reliability and comparability of salivary biomarkers in nutrition and inflammation assessment. One major challenge is the variation in saliva collection methods. The variability in collection methods (e.g., stimulated vs. unstimulated, or drooling vs. spitting) and duration of sample collection can affect the levels and determination of salivary biomarkers of interest (21). Nevertheless, there are no established methods and standardized protocols for collecting saliva samples. Additionally, most current methods have not been optimized or validated for participants with different age groups and individuals with disease-related physiological alterations, which may influence the volume, flow rate, and concentrations of the collected samples (12). We found only a few studies that have standardized their collection protocols. Most studies included in this review collected unstimulated whole saliva samples from

the participants. This is the most acceptable and convenient way for clinical settings and research studies. The whole saliva is a complex mixture of various biocomponents secreted by different salivary glands (12). The secretions from these glands differ in composition, and thus, the detectable range and diagnostic values can be variable (12). However, some studies collected stimulated saliva samples from the participants. Saliva origin, production rate, and composition may vary between unstimulated and stimulated samples (21). However, it is unclear whether unstimulated or stimulated saliva offers more reliable and reproducible measurements of nutrition and inflammatory biomarkers. Further investigation is required to compare unstimulated and stimulated saliva samples for the measurement of nutritional and inflammatory biomarkers.

The passive drool method is considered a standard practice and can be easily performed for adults, with minimal limitations for children aged over 5 years (21, 163, 164). However, this method may not be suitable for children under three years old (165). It is because the passive drool technique requires patience and active cooperation from the children. It may also cause discomfort in younger children. Therefore, some studies suggest using passive drool methods for saliva collection in children younger than three years under the supervision of study personnel (166). Other studies recommend applying the suction method or collecting an oral swab instead in young children (165, 166). However, some analytes, including salivary CRP, IgA, and cytokines, may not be detectable if analyzed in oral swabs collected from children. We found various methods and durations for collecting saliva samples from participants of different age groups. The spitting method and passive drool technique were the most commonly used methods in the included studies. These methods may provide undiluted saliva and are less likely to underestimate salivary analytes. However, alternative techniques, such as the use of cotton pads or swabs, were also found to be used in some studies. These methods have limitations as they can alter biomarker concentrations

due to the position of the swab in the oral cavity and dilution effects (21, 163). Absorbing the saliva specimen on cotton may also interfere with substances in cotton, leading to over- or underestimation of the analytes measured in saliva (160). It is important to standardize the swab placement protocol for studies that intend to collect saliva samples using cotton swabs. Salivary production and composition are also influenced by the time of day when the specimen is collected (163). Diurnal variation may also affect the interpretation of the saliva assay results (21). While most studies collected saliva samples in the morning, some studies collected at daytime before or after lunch, or in the evening. One approach can be collecting saliva samples early in the morning to avoid variation in the salivary analytes caused by diurnal effects. It is important to maintain the same time every day for all the participants of the study. If time and resources allow, saliva samples can be collected at two time points in a day (e.g., morning and evening) to account for diurnal variation.

We also observed variability in salivary sample collection regarding the collection site (e.g., home, school, or clinic), duration of food and drink restrictions (e.g., 30 minutes, 1 hour, 2 hours, or overnight for 12 hours), participant position during collection, and collection device across studies, which may influence sample quality and volume. If samples are collected at home, participants and/or caregivers should be instructed on the collection protocol and properly counseled to ensure standardized collection and timely transportation of the samples to the laboratory. The same protocol and collection device should be used throughout the study for all participants to avoid variability. At least 1 mL of saliva should be collected from each participant, regardless of the collection site, age, or disease status. This minimum volume is required to ensure the detection of low-abundance biomarker materials in the saliva sample. Also, a sufficient volume is needed to fulfill the requirements of sample processing and laboratory analysis methods. Furthermore, an

adequate volume of saliva sample is needed to calculate the saliva flow rate and ensure the accuracy and reliability of the findings (13). Another challenge of saliva samples is standardizing the sample processing and storage protocols (160, 167). We found substantial variation in sample processing in the studies included in this review. It is recommended to process the saliva samples as soon as possible after collection. Processing of saliva samples within 1 hour may prevent significant sample degradation (168). However, most studies did not report the duration from saliva sample collection to processing. Only a few studies reported that they processed the samples within 2 hours of collection. It is important to maintain a window of less than 2 hours from sample collection, transportation to the laboratory, and processing of samples to maintain sample integrity. The use of preservatives or additives such as protease and dipeptidyl peptidase-4 (DPP4) inhibitors during or after collection was inconsistent across studies included in this review. These inhibitors may help to prevent the enzymatic degradation of proteins and peptides, yet only a few studies have used them. This inconsistency poses a risk to the accuracy of measurements, particularly for protein-based biomarkers. Another concern is the reduced salivary flow observed in patients with certain conditions, such as asthma and autoimmune diseases. Lower saliva volumes can hinder sample collection and reduce the concentrations of the biomarker of interest, further complicating interpretation. Despite this, only a few studies adapted their protocols to account for patient-specific limitations. Centrifugation of the saliva sample is a critical step to remove food materials, blood, cellular debris, bacteria, and other contaminants (164). Although centrifugation was performed in most studies, the parameters such as speed, temperature, and duration varied significantly across the included studies. Some studies centrifuged samples immediately after collection before storage, while others stored samples first and centrifuged on the day of laboratory analysis. The lack of standardized sample processing and centrifugation methods may affect

777 sample quality and biomarker stability, which can further influence the downstream analysis of
778 saliva samples. Maintenance of optimal temperature during sample collection, transportation,
779 processing, and storage is crucial for the integrity and stability of salivary analytes. We observed
780 variable practices regarding the transportation temperature (e.g., on dry ice, ice box, liquid
781 nitrogen, and 4°C). Additionally, the protocol for sample storage also varied widely across studies.
782 While lower temperatures (e.g., −80 °C) are generally recommended to preserve biomarker
783 integrity (164), some studies stored samples at higher (i.e., 2-8 °C, -20 °C, -30 °C) or unspecified
784 temperatures. Temperature issues and inconsistent storage practices may lead to biomarker
785 degradation and variability in results and interpretation (164). Therefore, it is essential to
786 standardize protocols for sample handling, preparation, and storage. The standard practice for
787 saliva collection, processing, and storage is highlighted in Text Box 2.

Text Box 2. Standard Practice for Saliva Collection, Processing, and Storage

- Participants should not eat or drink anything for at least 30 minutes before saliva collection
- Participants should refrain from caffeine intake and tooth-brushing at least 60 minutes before saliva collection
- Study staff should document any consumption of caffeine, alcohol, nicotine, medications, and nutritional supplements before the sample collection
- Study staff should also document the presence of any oral or systemic diseases and any acute symptoms of diseases or inflammation before the sample collection
- Participants should rinse out their mouths with water to remove residual food particles
- After rinsing, the participants should wait at least 10 minutes before saliva collection to avoid dilution of salivary analytes
- Study staff and participants should wash their hands with soap and water and put on gloves before saliva collection
- For whole saliva collection, participants should be instructed to pool saliva naturally in the mouth and collect a minimum of 1 mL of saliva in each collection tube
- For the passive drool method, the participants should tilt their head forward and gently drool down the saliva into the collection tubes, preferably using a straw
- Participants should be careful so that the froth does not get aspirated
- Participants should not collect or produce saliva samples by clearing their throats
- Study staff should document the sample volume and the duration of sample collection to calculate the salivary flow rate
- Study staff should conduct a visual assessment of the samples immediately after collection to ensure the saliva sample is not contaminated with blood, mucus, food particles, or other substances
- Samples should be stored on ice or at -20°C and transported to the laboratory for processing, maintaining the cold chain, and using appropriate transport methods

- Upon arrival at the laboratory, samples should be processed immediately and stored in a freezer without delay
  - If the analysis is to be performed immediately, samples can be stored at room temperature for 30-90 minutes and at 4°C for 3-6 hours
  - If the analysis is to be performed within a month, freeze the samples at or below -20 ºC
  - For long-term storage (> 3 months), freeze the samples at or below -80 ºC

C. Methodological challenges for laboratory analyses

The existing evidence revealed that several methodological challenges persist in the laboratory analysis of nutrition- and inflammation-related saliva biomarkers. Evidence supporting the analytical validity of many salivary biomarkers remains limited, with insufficient standardization and incomplete reporting of key assay performance characteristics. These challenges limit the reproducibility, comparability, and overall reliability of findings across studies. One major issue is the variability in analytical techniques used to measure salivary biomarkers. Furthermore, there remains limited information regarding analysis methods, instruments, reagents, and the best practices for handling and preparation of saliva samples required to implement salivary biomarker analysis in both clinical and community settings (167, 169).

One methodological challenge is the analysis of salivary zinc and calcium biomarkers together. While atomic absorption spectrophotometry has been commonly employed due to its accessibility and simplicity, this method is susceptible to interference from calcium-rich precipitates. This interference can lead to inaccurate estimations of total calcium levels in saliva, which in turn affects the interpretation of results. The lack of a standardized and validated method for salivary calcium assessment remains a key barrier to reliable biomarker development and application (63). Another methodological gap lies in the analysis of salivary vitamins. For example, one study attempted to detect vitamins A, B12, and D in saliva but was unsuccessful (73). However, the study did not report sufficient information about the analytical methods used, nor did it discuss potential sources of error or limitations in detection. This lack of reporting limited our

understanding of whether the failure was due to low salivary concentrations, inadequate assay sensitivity, or other technical constraints. Without such details, it is difficult to draw meaningful conclusions or to guide future methodological improvements. These examples underscore the need for rigorous validation of laboratory methods and more detailed reporting of analytical protocols and limitations. Establishing standardized procedures and improving assay sensitivity are critical steps toward enhancing the utility of salivary and other non-invasive biomarkers in nutrition and inflammation research.

D. Diagnosis and interpretability-related challenges

It is challenging to use salivary biomarkers for the diagnosis of nutrient deficiencies and inflammatory conditions linked to growth impairments. The quantification of biomarkers or analytes in saliva does not indicate clinical significance or their utility in clinical practice. Understanding the distinction between diagnostic tests and surveillance tests in saliva biomarkers is important in the context of clinical decision-making and public health programs. There are no age- and sex-specific reference values for salivary biomarkers, which restricts the ability to interpret results meaningfully in clinical or community settings. Another challenge of using saliva for diagnostic purposes is identifying disease-specific markers.

One of the main limitations of the studies included in this review is their reliance on cross-sectional designs and the lack of longitudinal data, which may restrict our understanding of the temporal relationships between salivary biomarker levels and various health outcomes. Evidence is also limited regarding the sensitivity and specificity of tests conducted on saliva samples. Most of the studies included in this review had small sample sizes, limiting the statistical power to detect meaningful associations or differences across population subgroups. Larger, well-designed studies are needed to establish normative data, to determine the reference ranges, and to assess the

sensitivity and specificity of salivary biomarkers for identifying nutritional deficiencies and inflammatory states.

The use of saliva-based diagnostics may increase the risk of false-positive or false-negative results due to methodological heterogeneity and biological variability. However, this limitation can be addressed by standardizing sample collection and analytical procedures, validating assays against established serum markers, and implementing appropriate quality control measures. Another significant limitation is the relatively low R^2 values comparing salivary to serum biomarkers. This indicates that the independent variables cannot adequately explain the proportion of variance in the dependent variable, which, in most cases, is salivary biomarkers. It is crucial to gather information on other variables that may play a critical role in explaining the outcomes. Factors such as oral health conditions, tobacco or alcohol use, and diet can change the composition of salivary analytes (170-172). A diet containing high sugar and/or high caffeine may affect salivary pH and alter the microbial composition of the oral cavity (21). Some salivary biomarkers, such as CRP, AGP, hepcidin, IgA, and cytokines, can be influenced by age, sex, hormones, mood, stress, infection, and disease states. Furthermore, the interpretation of salivary biomarker concentrations is complicated by physiological factors such as production, flow rate, and dilution (160). Variability in saliva production, affected by pH, hydration status, time of day, and whether saliva is collected at rest or under stimulation, can introduce significant measurement issues (160). Some medications, for example, anti-hypertensive drugs, may cause dry mouth by preventing salivary glands from producing adequate saliva (173, 174). Additionally, certain diseases and medical conditions, such as high blood sugar and elevated blood pressure, may alter the oral microbiome profiles and disrupt saliva production (173-175). Therefore, it is important to control for all potential confounding factors (e.g., age, sex, time of the day, pH, hydration status, diet,

medications, and health issues) that might affect the salivary composition while interpreting the findings of salivary nutritional and inflammatory biomarkers (160). However, data on clinical history, oral health condition, dietary habits, nutrient intake, smoking, and alcohol consumption were limited in most of the studies included in this review. The lack of data on these important confounding variables, as well as the inability to adjust for them, limited the comparability of findings across studies.

Nevertheless, the findings suggest the potential of saliva as a biological specimen to assess several nutritional and inflammatory biomarkers. However, most studies included in this review have only presented preliminary findings on the biomarkers of nutrition and inflammation. There remains a gap in validating the salivary biomarkers in large cohorts and diverse population subgroups. Additionally, evidence is limited regarding the comparability of salivary analytes to conventional blood biomarkers. Therefore, it is unclear whether saliva samples can be used for the evaluation and monitoring of micronutrient status and inflammatory conditions in different population subsets. Further well-designed research with standardized methodologies is needed to identify and validate salivary biomarkers of nutrition and inflammation for clinical, primary care, and programmatic applications. We recommend large-scale, multicenter clinical trials and independent validation studies to provide evidence for the clinical utility of salivary diagnostics in diagnosing and/or monitoring nutritional status and inflammatory conditions. The studies should also assess the diagnostic performance and accuracy of salivary biomarkers compared to conventional blood biomarkers. Additionally, the integration of artificial intelligence (AI) and machine learning (ML) approaches with salivary biomarker assessment may improve the accuracy, reliability, and interpretability of these measures.

E. Future directions

Despite the gaps we discussed above, this review highlights the potential of salivary biomarkers for assessing nutrition and inflammation in different settings. Although the current evidence base is limited and findings remain inconsistent for many biomarkers, our review suggests that several salivary biomarkers could be assessed in research studies alongside their corresponding blood biomarkers. For instance, salivary ferritin can be measured using the chemiluminescent microparticle immunoassay (CMIA), a widely used and standardized method for quantifying ferritin concentrations in clinical and field settings. The studies included in this review indicate that this technique can also be applied to saliva samples. However, important questions remain regarding analytical sensitivity, assay optimization for saliva matrices, and reproducibility across laboratories. Addressing these gaps will be essential before salivary ferritin can be reliably integrated into broader research or clinical applications.

Our review also suggests that larger clinical studies are needed to collect and analyze saliva samples from diverse population subsets across different geographic locations, following standardized protocols to estimate key nutritional and inflammatory biomarkers. These studies should be used to address the gaps mentioned above, enhance the diagnostic performance and accuracy of salivary biomarkers, and compare their concentrations with traditional blood biomarkers to confirm the utility of salivary diagnostics compared to current methods. Advances in machine learning analysis can also help identify the most effective salivary biomarkers or clusters of salivary biomarkers capable of predicting nutritional and inflammatory disorders. Machine learning algorithms may also guide how to adjust for inflammatory biomarkers while evaluating micronutrient biomarkers that are often affected by infection and inflammation, such as serum ferritin and retinol-binding protein. All these efforts would facilitate the use of salivary diagnostics for nutrition and inflammation assessment in various settings.

Figure 4 illustrates the applications of saliva-based diagnostics for evaluating nutritional status and inflammatory conditions across different settings. Due to their non-invasiveness, affordability, and higher acceptance, salivary diagnostics can be easily implemented in community, school, and/or primary care clinical settings. In the community, saliva-based diagnostics can be used for surveillance or programmatic assessment of micronutrient status, as well as for evaluating interventions aimed at improving micronutrient levels. In schools, saliva diagnostics can be employed for screening micronutrient status or monitoring children at risk of micronutrient malnutrition, and for evaluating targeted interventions or school meal programs designed to improve nutritional status in school-going children. In primary care or clinical settings, saliva diagnostics can be used for triage or rapid assessment of micronutrient levels, and continuous monitoring of nutritional status in hospitalized patients. Saliva biomarkers can also be useful for assessing treatment responses in clinical practice. If larger studies demonstrate sufficient diagnostic performance, biosensor or lateral flow-based point-of-care devices can be developed for salivary diagnostics. These innovations would enhance public health programs in primary care, community, and school settings and improve clinical care.

Conclusions

In summary, saliva contains many nutritional and inflammatory biomarkers and has potential utility as a noninvasive matrix for primary care in the context of nutrition and inflammation assessment. However, evidence comparing the biomarkers in saliva to their counterparts in currently accepted biomatrices, including blood and urine, remains scant. The methodological inconsistencies underscore the need for standardized, validated protocols for saliva sample collection, processing, and storage. Harmonization of these procedures is essential to ensure the reproducibility and clinical utility of salivary biomarkers in assessing nutritional and inflammatory status. Additionally, analytical performance, such as sensitivity and specificity of

924 the tests, is a key issue that might affect the decision to determine whether saliva-based
925 approaches are sufficient to achieve clinical benefits. Larger studies with adequate sample size,
926 both in clinical and community settings, are crucial for guiding not only implementation
927 decisions but also the development of the saliva-based assays and point-of-care diagnostic tools.
928 Future research should also prioritize studies in children, adolescents, and WRAs both in the
929 USA and in international settings, standardize saliva sample collection, processing, and storage
930 methods, emphasize in-depth methodological reporting, and examine the correlation between
931 salivary and serum biomarker concentrations to further understand whether salivary biomarkers
932 can be a reliable, inexpensive, and non-invasive alternative for assessing nutritional and/or
933 inflammatory disorders. Addressing the gaps discussed in this review is essential to harness the
934 full potential of saliva-based diagnostics in the assessment of nutritional status and inflammatory
935 conditions in different population subsets and various settings.

936 **Acknowledgements**

We acknowledge the support of Matthew Kibbee, Natasha Bishop, and Elizabeth DiGiovine, who reviewed and provided feedback on the search strategy. We also acknowledge the support of Brooke Overholt, Beatrice Juskeviciute, and Lilly Smith, who helped with screening and data extraction. Finally, we acknowledge funding support from the Eunice Kennedy Shriver National Institute of Child Health and Human Development under award number R21HD103974, the National Institute of Biomedical Imaging and Bioengineering and Fogarty International Center of the National Institutes of Health under Award Number U54EB034654.

Author contributions

The authors' responsibilities were as follows: SMF, DE, JLF, SM: conceptualized and designed this research; SMF, MM, MM, NZ, MA: performed the relevant literature search of the current

evidence and screening of articles, acquisition of study reports, study selection, data extraction, synthesis of results; and all authors were responsible for writing the original draft and reviewing and editing the manuscript. All authors read and approved the final manuscript.

Data Availability:

No datasets were generated or analyzed during the current study.

Funding: Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development under award number R21HD103974 and the National Institute of Biomedical Imaging and Bioengineering and Fogarty International Center of the National Institutes of Health under Award Number U54EB034654 - PORTENT: A Center for Point of Care Technologies for Nutrition, Infection, and Cancer. PORTENT is a member of the POCTRN+ Network. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Disclosures: Corresponding authors will be queried for their own and their co-authors' conflict of interest disclosures during the manuscript submission process using the Declaration of Interests tool (https://declarations.elsevier.com/). The tool will generate a Word file for upload with your manuscript submission.

Declaration of Generative AI and AI-assisted technologies in the writing process:

Not applicable

References

1. Wilson ML, Fleming KA, Kuti MA, Looi LM, Lago N, Ru K. Access to pathology and laboratory medicine services: a crucial gap. *The Lancet*. 2018;391(10133):1927-38.
2. Madimenos FC, Gildner TE, Eick GN, Sugiyama LS, Snodgrass JJ. Bringing the lab bench to the field: Point-of-care testing for enhancing health research and stakeholder engagement in rural/remote, indigenous, and resource-limited contexts. *American Journal of Human Biology*. 2022;34(11):e23808.

3. Tvarijonaviciute A, Martinez-Lozano N, Rios R, de Teruel MCM, Garaulet M, Cerón JJ. Saliva as a non-invasive tool for assessment of metabolic and inflammatory biomarkers in children. *Clinical Nutrition*. 2020;39(8):2471-8.
4. Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. *World journal of diabetes*. 2014;5(6):730.
5. Kessler K, Hornemann S, Rudovich N, Weber D, Grune T, Kramer A et al. Saliva samples as a tool to study the effect of meal timing on metabolic and inflammatory biomarkers. *Nutrients*. 2020;12(2):340.
6. Williamson S, Munro C, Pickler R, Grap MJ, Elswick R. Comparison of biomarkers in blood and saliva in healthy adults. *Nursing research and practice*. 2012;2012.
7. Tiwari M. Science behind human saliva. *Journal of natural science, biology, and medicine*. 2011;2(1):53.
8. Ilea A, Andrei V, Feurdean CN, Băbțan A-M, Petrescu NB, Câmpian RS et al. Saliva, a magic biofluid available for multilevel assessment and a mirror of general health—A systematic review. *Biosensors*. 2019;9(1):27.
9. Genco RJ. Salivary diagnostic tests. *The Journal of the American Dental Association*. 2012;143:3S-5S.
10. Chojnowska S, Baran T, Wilińska I, Sienicka P, Cabaj-Wiater I, Knaś M. Human saliva as a diagnostic material. *Advances in medical sciences*. 2018;63(1):185-91.
11. Dawes C, Pedersen AL, Villa A, Ekström J, Proctor GB, Vissink A et al. The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. *Archives of oral biology*. 2015;60(6):863-74.
12. Aljerf L, Mashlah A. Characterization and validation of candidate reference methods for the determination of calcium and magnesium in biological fluids. *Microchemical Journal*. 2017;132:411-21.
13. Liao C, Chen X, Fu Y. Salivary analysis: An emerging paradigm for non-invasive healthcare diagnosis and monitoring. *Interdisciplinary Medicine*. 2023;1(3):e20230009.
14. Zhou Y, Liu Z. Saliva biomarkers in oral disease. *Clinica Chimica Acta*. 2023;548:117503.
15. Bel'skaya L, Sarf E, Kosenok V. Correlation interrelations between the composition of saliva and blood plasmain norm. *Klinicheskaia Laboratornaia Diagnostika*. 2018;63(8):477-82.
16. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. *J Oral Biol Craniofac Res*. 2016;6(1):66-75.
17. Loo JA, Yan W, Ramachandran P, Wong DT. Comparative human salivary and plasma proteomes. *J Dent Res*. 2010 Oct;89(10):1016-23.
18. Romano F, Castiblanco A, Spadotto F, Di Scipio F, Malandrino M, Berta GN et al. ICP-mass-spectrometry ionic profile of whole saliva in patients with untreated and treated periodontitis. *Biomedicines*. 2020;8(9):354.
19. Martina E, Campanati A, Diotallevi F, Offidani A. Saliva and oral diseases. *Journal of clinical medicine*. 2020;9(2):466.
20. Malamud D. Saliva as a diagnostic fluid. *BMJ: British Medical Journal*. 1992;305(6847):207.
21. Bhattarai KR, Kim H-R, Chae H-J. Compliance with saliva collection protocol in healthy volunteers: strategies for managing risk and errors. *International journal of medical sciences*. 2018;15(8):823.
22. Paqué PN, Hjerppe J, Zuercher AN, Jung RE, Joda T. Salivary biomarkers as key to monitor personalized oral healthcare and precision dentistry: A scoping review. *Frontiers in Oral Health*. 2022;3:1003679.
23. Rocha ACSD, Orlandini RK, Motta ACF, Pinheiro JB, Silva GAe, Oliveira VdC et al. Variability of salivary analytes under daily conditions and their implications for periodontitis biomarkers. *Frontiers in Dental Medicine*. 2024;5:1369186.
24. Alamoudi A, Alamoudi R, Gazzaz Y, Alqahtani AM. Role of salivary biomarkers in diagnosis and detection of dental caries: a systematic review. *Diagnostics*. 2022;12(12):3080.
25. Fernandes L, Pacheco V, Borges L, Athwal H, de Paula Eduardo F, Bezinelli L et al. Saliva in the diagnosis of COVID-19: a review and new research directions. *Journal of Dental Research*. 2020;99(13):1435-43.
26. Hegde MN, Attavar SH, Shetty N, Hegde ND, Hegde NN. Saliva as a biomarker for dental caries: A systematic review. *Journal of conservative dentistry: JCD*. 2019;22(1):2.
27. Arias-Bujanda N, Regueira-Iglesias A, Balsa-Castro C, Nibali L, Donos N, Tomás I. Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: A systematic review and meta-analysis. *Journal of clinical periodontology*. 2020;47(1):2-18.
28. Nijakowski K, Surdacka A. Salivary biomarkers for diagnosis of inflammatory bowel diseases: A systematic review. *International journal of molecular sciences*. 2020;21(20):7477.
29. Bastías D, Maturana A, Marin C, Martínez R, Niklander SE. Salivary biomarkers for oral cancer detection: an exploratory systematic review. *International Journal of Molecular Sciences*. 2024;25(5):2634.

30. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. *Critical Reviews in oral biology & medicine*. 2002;13(2):197-212.
31. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. *Clinica chimica acta*. 2007;383(1-2):30-40.
32. Nagler RM, Hershkovich O, Lischinsky S, Diamond E, Reznick AZ. Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. *Journal of investigative medicine*. 2002;50(3):214-25.
33. Aljerf L, Alhaffar I. Salivary distinctiveness and modifications in males with diabetes and Behçet's disease. *Biochemistry research international*. 2017;2017(1):9596202.
34. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *bmj*. 2021;372.
35. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF et al. Biomarkers of Nutrition for Development (BOND)—iron review. *The Journal of nutrition*. 2018;148:1001S-67S.
36. Katona P, Katona-Apte J. The interaction between nutrition and infection. *Clinical Infectious Diseases*. 2008;46(10):1582-8.
37. Agarwal PK, Agarwal KN, Agarwal DK. Biochemical changes in saliva of malnourished children. *Am J Clin Nutr*. 1984 Feb;39(2):181-4.
38. Franco-Martinez L, Tvarijonaviciute A, Martinez-Subiela S, Marquez G, Martinez Diaz N, Cugat R et al. Changes in lactate, ferritin, and uric acid in saliva after repeated explosive effort sequences. *The Journal of sports medicine and physical fitness*. 2019 Jun;59(6):902-9.
39. Canatan D, Akdeniz SK. Iron and ferritin levels in saliva of patients with thalassemia and iron deficiency anemia. *Mediterranean journal of hematology and infectious diseases*. 2012;4(1):e2012051.
40. Venkatesh Babu NS, Bhanushali PV. Evaluation and association of serum iron and ferritin levels in children with dental caries. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*. 2017 Apr-Jun;35(2):106-9.
41. Jagannathan N, Neelakantan P, Ramani P, Premkumar P, Natesan A, Sherlin HJ. Salivary ferritin–A concise update on current concepts. *Let the Science be your passion*. 2012.
42. Canatan D, Akdeniz SK. Iron and ferritin levels in saliva of patients with thalassemia and iron deficiency anemia. *Mediterranean journal of hematology and infectious diseases*. 2012;4(1).
43. Jagannathan N, Thiruvengadam C, Ramani P, Premkumar P, Natesan A, Sherlin H. Salivary ferritin as a predictive marker of iron deficiency anemia in children. *Journal of Clinical Pediatric Dentistry*. 2012;37(1):25-30.
44. Alpdemir M, Eryilmaz M, Alpdemir MF, Topçu G, Azak A, Yücel D. Comparison of widely used biochemical analytes in the serum and saliva samples of dialysis patients. *Journal of medical biochemistry*. 2018;37(3):346.
45. Guo L-N, Yang Y-Z, Feng Y-Z. Serum and salivary ferritin and Hepcidin levels in patients with chronic periodontitis and type 2 diabetes mellitus. *BMC Oral Health*. 2018;18:1-9.
46. Haji-Sattari F, Zarabadipour M, Hajmanoochehri F, Mirzadeh M. Salivary and Serum Ferritin Levels: Is There a Correlation? *Journal of Iranian Dental Association*. 2019;31(4):214-7.
47. Rajkumaar J, Mathew MG. Association of severe early childhood caries with salivary ferritin. *Journal of Family Medicine and Primary Care*. 2020;9(8):3991-3.
48. B LS, Rathnavelu V, Sabesan M, Ganesh A, Anandan S. A Study to Assess the Levels of Salivary Ferritin in Iron Deficiency Anemia Subjects and Healthy Subjects. *Cureus*. 2021 Aug;13(8):e17241.
49. Rezazadeh F, Salehi S, Rezaee M. Salivary level of trace element in oral lichen planus, a premalignant condition. *Asian Pacific Journal of Cancer Prevention: APJCP*. 2019;20(7):2009.
50. Costa EM, de Azevedo JAP, Martins RFM, Rodrigues VP, Alves CMC, Ribeiro CCC et al. Salivary iron (Fe) ion levels, serum markers of anemia and caries activity in pregnant women. *Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecology and Obstetrics*. 2017;39(03):094-101.
51. King JC, Brown KH, Gibson RS, Krebs NF, Lowe NM, Siekmann JH et al. Biomarkers of Nutrition for Development (BOND)—zinc review. *The Journal of nutrition*. 2016;146(4):858S-85S.
52. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. 2004.
53. Wan Y, Zhang B. The impact of zinc and zinc homeostasis on the intestinal mucosal barrier and intestinal diseases. *Biomolecules*. 2022;12(7):900.
54. Luca E, Crivoi F, Galeş C, Popescu E, Ciubotariu D, Nechifor M. Research on serum and saliva concentration of some bivalent cations in patient with suppurations of the oro-maxillo-facial area. *Revista Medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi*. 2009;113(3):899-903.

55. Jayaram L, Chunilal S, Pickering S, Ruffin RE, Zalewski PD. Sputum zinc concentration and clinical outcome in older asthmatics. *Respirology*. 2011;16(3):459-66.
56. De Carvalho FG, Rosa FT, Suen VMM, Freitas EC, Padovan GJ, Marchini JS. Evidence of zinc deficiency in competitive swimmers. *Nutrition*. 2012;28(11-12):1127-31.
57. Marín Martínez L, Molino Pagán D, López Jornet P. Trace elements in saliva as markers of type 2 diabetes mellitus. *Biological trace element research*. 2018;186(2):354-60.
58. Sharma A, Subramaniam P. Association of salivary zinc levels to dental caries and body mass index. A comparative study. *Journal of Clinical Pediatric Dentistry*. 2021;45(4):265-8.
59. Aziz J, Vaithilingam RD, Radzi Z, Rahman MT. Inflammatory Responses in Periodontitis with or Without Rheumatoid Arthritis Alter Salivary Metallothionein and Zinc. *Biological Trace Element Research*. 2023;201(7):3162-74.
60. Hay D, Smith D, Schluckebier S, Moreno E. Relationship between concentration of human salivary statherin and inhibition of calcium phosphate precipitation in stimulated human parotid saliva. *Journal of Dental Research*. 1984;63(6).
61. Shannon IL, Feller R. Parotid saliva flow rate, calcium, phosphorus, and magnesium concentrations in relation to dental caries experience in children. *Pediatr Dent*. 1979;1(1):16-20.
62. Logan D, Wallace S, Woodside J, McKenna G. The potential of salivary biomarkers of nutritional status and dietary intake: a systematic review. *Journal of Dentistry*. 2021;115:103840.
63. Pannunzio E, Amancio OMS, Vitalle MSdS, Souza DNd, Mendes FM, Nicolau J. Analysis of the stimulated whole saliva in overweight and obese school children. *Revista da Associação Médica Brasileira*. 2010;56:32-6.
64. Mattioli TMF, Koubik AC, de Oliveira Ribas M, França BHS, Brancher JA, de Lima AAS. Salivary flow rate, calcium, urea, total protein, and amylase levels in fanconi anemia. *Journal of Pediatric Hematology/Oncology*. 2010;32(2):e46-e9.
65. Kumbhojkar SV, Kale AD, Kumbhojkar VR, Desai KM. Salivary calcium as a diagnostic tool for screening of osteoporosis in postmenopausal women. *Journal of Oral and Maxillofacial Pathology*. 2019;23(2):192-7.
66. Pratyusha N, Vinay C, Uloopi K, RojaRamya KS, Ahalya P, Devi C. Association of serum Vitamin D and salivary calcium and phosphorus levels in 3-11-year-old schoolchildren with dental caries. *Journal of Indian Society of Pedodontics and Preventive Dentistry*. 2021;39(3):240-5.
67. Çevik-Aras H, Musa S, Olofsson R, Almståhl A, Almhöjd U. Patients with oral lichen planus display lower levels of salivary acidic glycoproteins than individuals without oral mucosal disease. *Clinical Oral Investigations*. 2023;28(1):2.
68. Johansson I, Lenander-Lumikari M, Saellström A-K. Saliva composition in Indian children with chronic protein-energy malnutrition. *Journal of dental research*. 1994;73(1):11-9.
69. Tanumihardjo SA, Russell RM, Stephensen CB, Gannon BM, Craft NE, Haskell MJ et al. Biomarkers of Nutrition for Development (BOND)—vitamin A review. *The Journal of nutrition*. 2016;146(9):1816S-48S.
70. Gilbert C. What is vitamin A and why do we need it? *Community Eye Health*. 2013;26(84):65.
71. Maad AH, Aljerf L, AlJerf A, Dayoob CR, Sukkar AJ, Alajlani M. A cross-sectional study on demographic characteristics, nutritional knowledge, and supplement use patterns. *International Journal of Kinesiology & Sports Science*. 2024;12(4):21.
72. Rezazadeh F, Abedi F, Ranjbar Z, Khorami ET, Tabesh A. Evaluating the Salivary Levels of Vitamins A, B12, C, D3, E in Oral Lichen Planus Patients in Southwest of Iran. 2023.
73. Blakeley M, Sobczyńska-Malefora A, Carpenter G. The origins of salivary vitamin a, vitamin B12 and vitamin D-binding proteins. *Nutrients*. 2020;12(12):3838.
74. Lee JY, So T-Y, Thackray J. A review on vitamin d deficiency treatment in pediatric patients. *The Journal of Pediatric Pharmacology and Therapeutics*. 2013;18(4):277-91.
75. Rebelos E, Tentolouris N, Jude E. The role of vitamin D in health and disease: a narrative review on the mechanisms linking vitamin D with disease and the effects of supplementation. *Drugs*. 2023;83(8):665-85.
76. Park JE, Pichiah PT, Cha Y-S. Vitamin D and metabolic diseases: growing roles of vitamin D. *Journal of obesity & metabolic syndrome*. 2018;27(4):223.
77. Aljerf L, Aljerf N. Food products quality and nutrition in relation to public. Balancing health and disease. *Prog Nutr*. 2023;25(1):10-23751.
78. Giustina A, Bilezikian JP, Adler RA, Banfi G, Bikle DD, Binkley NC et al. Consensus statement on vitamin D status assessment and supplementation: whys, whens, and hows. *Endocrine reviews*. 2024;45(5):625-54.

79. Bahramian A, Falsafi P, Abbasi T, Ghanizadeh M, Abedini M, Kavoosi F et al. Comparing serum and salivary levels of vitamin D in patients with recurrent aphthous stomatitis and healthy individuals. *Journal of Dentistry*. 2018;19(4):295.

80. Costantini E, Sinjari B, Piscopo F, Porreca A, Reale M, Caputi S et al. Evaluation of salivary cytokines and vitamin D levels in periodontopathic patients. *International journal of molecular sciences*. 2020;21(8):2669.

81. Pruszkowska-Przybylska P, Sitek A, Rosset I, Sobalska-Kwapis M, Słomka M, Strapagiel D et al. Association of saliva 25 (OH) D concentration with body composition and proportion among pre-pubertal and pubertal Polish children. *American Journal of Human Biology*. 2020;32(5):e23397.

82. Araujo DS, de Oliveira Scudine KG, Pedroni-Pereira A, Gavião MBD, Pereira EC, Fonseca FLA et al. Salivary uric acid is a predictive marker of body fat percentage in adolescents. *Nutrition research*. 2020;74:62-70.

83. Usman M, Woloshynowych M, Britto JC, Bilkevic I, Glassar B, Chapman S et al. Obesity, oxidative DNA damage and vitamin D as predictors of genomic instability in children and adolescents. *International Journal of Obesity*. 2021;45(9):2095-107.

84. Samanta I, Patil DJ, More CB. Assessment of vitamin D levels in patients with oral potentially malignant disorders and oral squamous cell carcinoma-A cross-sectional study. *Journal of Oral Biology and Craniofacial Research*. 2024;14(1):27-32.

85. Gholizadeh N, Pirzadeh F, Mirzaii-Dizgah I, Sheykhbahaei N. Relationship between salivary vitamin D deficiency and oral lichen planus. *Photodermatology, Photoimmunology & Photomedicine*. 2020;36(5):384-6.

86. Allen LH, Miller JW, De Groot L, Rosenberg IH, Smith AD, Refsum H et al. Biomarkers of Nutrition for Development (BOND): vitamin B-12 review. *The Journal of nutrition*. 2018;148:1995S-2027S.

87. Rizzo G, Laganà AS. A review of vitamin B12. *Molecular nutrition*. 2020:105-29.

88. Al-hamdani GM, Yas LS. Serum and Salivary Vitamin B12 Levels among Iron Deficiency Anemia Patient with Recurrent Aphthous Stomatitis: An Analytical Cross-Sectional Study. *Dental Hypotheses*. 2023;14(2):55-8.

89. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory III JF, Mills JL et al. Biomarkers of nutrition for development—folate review. *The Journal of nutrition*. 2015;145(7):1636S-80S.

90. Rai B, Kaur J, Jacobs R, Singh J. Possible action mechanism for curcumin in pre-cancerous lesions based on serum and salivary markers of oxidative stress. *Journal of oral science*. 2010;52(2):251-6.

91. Thurnham DI. Interactions between nutrition and immune function: using inflammation biomarkers to interpret micronutrient status. *Proceedings of the Nutrition Society*. 2014;73(1):1-8.

92. Mei Z, Namaste SM, Serdula M, Suchdev PS, Rohner F, Flores-Ayala R et al. Adjusting total body iron for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *The American journal of clinical nutrition*. 2017;106:383S-9S.

93. Tuuminen T, Sorsa M, Tornudd M, Poussa T, Antila E, Jaakkola K. The association between high sensitivity C-reactive protein and micronutrient levels: A cross-sectional analysis based on a laboratory database. *Clinical Nutrition ESPEN*. 2019;33:283-9.

94. Pesqueda-Cendejas K, Parra-Rojas I, Mora-García PE, Montoya-Buelna M, Ruiz-Ballesteros AI, Meza-Meza MR et al. CRP serum levels are associated with high cardiometabolic risk and clinical disease activity in systemic lupus erythematosus patients. *Journal of Clinical Medicine*. 2022;11(7):1849.

95. Naidoo T, Konkol K, Biccard B, McKune A, Dubose K. Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children: cardiovascular topic. *Cardiovascular Journal of Africa*. 2012;23(9):501-6.

96. Ebersole JL, Kryscio RJ, Campbell C, Kinane D, McDevitt J, Christodoulides N et al. Salivary and serum adiponectin and C-reactive protein levels in acute myocardial infarction related to body mass index and oral health. *Journal of periodontal research*. 2017;52(3):419-27.

97. Selvaraju V, Babu JR, Geetha T. Association of salivary C-reactive protein with the obesity measures and markers in children. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2019:1239-47.

98. Wetterö J, von Löhneysen S, Cobar F, Kristenson M, Garvin P, Sjöwall C. Pronounced diurnal pattern of salivary C-reactive protein (CRP) with modest associations to circulating CRP levels. *Frontiers in immunology*. 2021;11:607166.

99. Agho ET, Owotade FJ, Kolawole BA, Oyetola EO, Adedeji TA. Salivary inflammatory biomarkers and glycated haemoglobin among patients with type 2 diabetic mellitus. *BMC Oral Health*. 2021;21:1-8.

100. Alqaderi H, Hegazi F, Al-Mulla F, Chiu C-J, Kantarci A, Al-Ozairi E et al. Salivary biomarkers as predictors of obesity and intermediate hyperglycemia in adolescents. *Frontiers in Public Health*. 2022;10:800373.

101. Bizjak DA, Ammerpohl O, Schulz SV, Wendt J, Steinacker JM, Flechtner-Mors M. Pro-inflammatory and (Epi-) genetic markers in saliva for disease risk in childhood obesity. *Nutrition, Metabolism and Cardiovascular Diseases*. 2022;32(6):1502-10.

102. Bachtiar E, Bachtiar BM, Kusumaningrum A, Sunarto H, Soeroso Y, Sulijaya B et al. The utility of salivary CRP and IL-6 as a non-invasive measurement evaluated in patients with COVID-19 with and without diabetes. *F1000Research*. 2024;12:419.

103. Luo Z, Lei H, Sun Y, Liu X, Su D-F. Orosomucoid, an acute response protein with multiple modulating activities. *Journal of physiology and biochemistry*. 2015;71:329-40.

104. Luo H, Geng J, Zeiler M, Nieckula E, Sandalinas F, Williams A et al. A practical guide to adjust micronutrient biomarkers for inflammation using the BRINDA method. *The Journal of nutrition*. 2023;153(4):1265-72.

105. Gannon BM, Glesby MJ, Finkelstein JL, Raj T, Erickson D, Mehta S. A point-of-care assay for alpha-1-acid glycoprotein as a diagnostic tool for rapid, mobile-based determination of inflammation. *Current research in biotechnology*. 2019;1:41-8.

106. Rauf A, Shariati MA, Khalil AA, Bawazeer S, Heydari M, Plygun S et al. Hepcidin, an overview of biochemical and clinical properties. *Steroids*. 2020;160:108661.

107. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2012;1823(9):1434-43.

108. Arnold J, Sangwaiya A, Manglam V, Geoghegan F, Thursz M, Busbridge M. Presence of hepcidin-25 in biological fluids: bile, ascitic and pleural fluids. *World journal of gastroenterology: WJG*. 2010;16(17):2129.

109. Dewan P, Dixit A, Gomber S, Kotru M, Banerjee BD, Tyagi V et al. Serum and urinary hepcidin for diagnosing iron-deficiency anemia in under-5 children. *Journal of pediatric hematology/oncology*. 2019;41(4):e216-e20.

110. Busbridge M, Griffiths C, Ashby D, Gale D, Jayantha A, Sanwaiya A et al. Development of a novel immunoassay for the iron regulatory peptide hepcidin. *British journal of biomedical science*. 2009;66(3):150-7.

111. Hansen IS, Baeten DL, den Dunnen J. The inflammatory function of human IgA. *Cellular and molecular life sciences*. 2019;76(6):1041-55.

112. Huus KE, Petersen C, Finlay BB. Diversity and dynamism of IgA− microbiota interactions. *Nature Reviews Immunology*. 2021;21(8):514-25.

113. Abu Jamra SR, Komatsu CG, Barbosa Jr F, Roxo-Junior P, Navarro AM. Proposal to screen for zinc and selenium in patients with IgA deficiency. *Nutrients*. 2023;15(9):2145.

114. Patel A, Jialal I. Biochemistry, immunoglobulin A. StatPearls [Internet]: StatPearls Publishing; 2023.

115. Miller EM, McConnell DS. Brief communication: chronic undernutrition is associated with higher mucosal antibody levels among Ariaal infants of northern Kenya. *American journal of physical anthropology*. 2012;149(1):136-41.

116. Fadel HT, Pliaki A, Gronowitz E, Mårild S, Ramberg P, Dahlèn G et al. Clinical and biological indicators of dental caries and periodontal disease in adolescents with or without obesity. *Clinical oral investigations*. 2014;18:359-68.

117. Perez MM, Pessoa JS, Ciamponi AL, Diniz MB, Santos MTBR, Alves HHdO et al. Correlation of salivary immunoglobulin A with Body Mass Index and fat percentage in overweight/obese children. *Journal of applied oral science*. 2018;27:e20180088.

118. Schlesinger L, Arevalo M, Arredondo S, Diaz M, Lönnerdal B, Stekel A. Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. *The American journal of clinical nutrition*. 1992;56(3):491-8.

119. de Souza DC, Matos VA, Dos Santos VO, Medeiros IF, Marinho CS, Nascimento PR et al. Effects of high-intensity interval and moderate-intensity continuous exercise on inflammatory, leptin, IgA, and lipid peroxidation responses in obese males. *Frontiers in physiology*. 2018;9:567.

120. van Heerden C, Oosthuizen, R., van Wyk, Prinsloo, P., Anderson R. Evaluation of neutrophil and lymphocyte function in subjects with iron deficiency. *South African Medical Journal*. 1981;59(4):111-3.

121. Cripps A, Otczyk DC, Barker J, Lehmann D, Alpers MP. The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea. *Papua New Guinea Medical Journal*. 2008;51(3/4):120-30.

122. Surono IS, Koestomo FP, Novitasari N, Zakaria FR. Novel probiotic Enterococcus faecium IS-27526 supplementation increased total salivary sIgA level and bodyweight of pre-school children: a pilot study. *Anaerobe*. 2011;17(6):496-500.

123. Vulevic J, Juric A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *The Journal of nutrition*. 2013;143(3):324-31.
124. Solé D ZM, Leser PG, et al. Levels of IgA in saliva of normal and atopic individuals, determined by secretory anti-IgA antibodies and serum antigen. *Rev Bras Alerg Imunol*. 1987;10:120-5.
125. Silva LB, dos Santos Neto AP, Maia SM, dos Santos Guimarães C, Quidute IL, Carvalho AdA et al. The role of TNF-α as a proinflammatory cytokine in pathological processes. *The Open Dentistry Journal*. 2019;13(1).
126. Popa C, Netea MG, Van Riel PL, Van Der Meer JW, Stalenhoef AF. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *Journal of lipid research*. 2007;48(4):751-62.
127. Jang D-i, Lee A-H, Shin H-Y, Song H-R, Park J-H, Kang T-B et al. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *International journal of molecular sciences*. 2021;22(5):2719.
128. Valaperti A, Li Z, Vonow-Eisenring M, Probst-Müller E. Diagnostic methods for the measurement of human TNF-alpha in clinical laboratory. *Journal of pharmaceutical and biomedical analysis*. 2020;179:113010.
129. Berkhout LC, l'Ami MJ, Ruwaard J, Hart MH, Heer PO-d, Bloem K et al. Dynamics of circulating TNF during adalimumab treatment using a drug-tolerant TNF assay. *Science translational medicine*. 2019;11(477):eaat3356.
130. Martínez-Lozano N, Tvarijonaviciute A, Ríos R, Barón I, Scheer FA, Garaulet M. Late eating is associated with obesity, inflammatory markers and circadian-related disturbances in school-aged children. *Nutrients*. 2020;12(9):2881.
131. Soto-Méndez MJ, Romero-Abal ME, Aguilera CM, Rico MC, Solomons NW, Schümann K et al. Associations among inflammatory biomarkers in the circulating, plasmatic, salivary and intraluminal anatomical compartments in apparently healthy preschool children from the western highlands of Guatemala. *PLoS One*. 2015;10(6):e0129158.
132. de Vargas LdS, Jantsch J, Fontoura JR, Dorneles GP, Peres A, Guedes RP. Effects of zinc supplementation on inflammatory and cognitive parameters in middle-aged women with overweight or obesity. *Nutrients*. 2023;15(20):4396.
133. Alasqah MN. Influence of adjunctive non-surgical peri-implant therapy on clinical and salivary cytokine profile in obese patients. *Photodiagnosis and Photodynamic Therapy*. 2022;37:102721.
134. Alkan B, Guzeldemir-Akcakanat E. Evaluation of adipokine levels in obese women with periodontitis: A cohort study. *American Journal of Dentistry*. 2021;34(5):267-72.
135. Attlee A, Hasan H, AlQattan A, Sarhan N, Alshammari R, Ali S et al. Relationship of salivary adipocytokines, diet quality, physical activity, and nutrition status in adult Emirati females in United Arab Emirates. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2019;13(1):40-6.
136. Syrjäläinen S, Gursoy UK, Gursoy M, Pussinen P, Pietiäinen M, Jula A et al. Salivary cytokine biomarker concentrations in relation to obesity and periodontitis. *Journal of clinical medicine*. 2019;8(12):2152.
137. Foratori-Junior GA, Mosquim V, Buzalaf MAR, de Carvalho Sales-Peres SH. Salivary cytokines levels, maternal periodontitis and infants' weight at birth: A cohort study in pregnant women with obesity. *Placenta*. 2021;115:151-7.
138. Lehmann-Kalata A, Miechowicz I, Korybalska K, Swora-Cwynar E, Czepulis N, Łuczak J et al. Salivary fingerprint of simple obesity. *Cytokine*. 2018;110:174-80.
139. Ostrowska L, Gornowicz A, Pietraszewska B, Bielawski K, Bielawska A. Which salivary components can differentiate metabolic obesity? *PloS one*. 2020;15(6):e0235358.
140. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor perspectives in biology*. 2014;6(10):a016295.
141. Covarrubias AJ, Horng T. IL-6 strikes a balance in metabolic inflammation. *Cell metabolism*. 2014;19(6):898-9.
142. McElvaney OJ, Curley GF, Rose-John S, McElvaney NG. Interleukin-6: obstacles to targeting a complex cytokine in critical illness. *The Lancet Respiratory Medicine*. 2021;9(6):643-54.
143. Cioffi I, Scialò F, Di Vincenzo O, Gelzo M, Marra M, Testa A et al. Serum interleukin 6, controlling nutritional status (CONUT) score and phase angle in patients with Crohn's disease. *Nutrients*. 2023;15(8):1953.
144. Doğan GE, Toraman A, Şebin SÖ, Doğan Ç, Güngör A, Aksoy H et al. Salivary IL-6 and IL-10 levels in subjects with obesity and gingivitis. *American Journal of Dentistry*. 2016;29(5):261-5.

145. Pîrsean C, Neguț C, Stefan-van Staden R-I, Dinu-Pirvu CE, Armean P, Udeanu DI. The salivary levels of leptin and interleukin-6 as potential inflammatory markers in children obesity. *PLoS One*. 2019;14(1):e0210288.
146. Deng Q, Wong HM, Peng S. Alterations in salivary biomarkers in relation to periodontal health and obesity among Hong Kong adolescents. *Journal of Dentistry*. 2024;146:105055.
147. Potempa-Jeziorowska M, Jonczyk P, Świętochowska E, Kucharzewski M. Analysis of ghrelin, leptin, and interleukin-6 salivary concentration among children aged 7–10 years and its relationship with nutritional status and some anthropometric data. *Pediatric Endocrinology Diabetes and Metabolism*. 2022;28(4):263-73.
148. Baskaradoss JK, Tavares M, Al-Mulla F, Al-Ozairi E, Abu-Farha M, Bin-Hasan S et al. Association between frequency of Toothbrushing and metabolic syndrome among adolescents: a 5-Year Follow-Up study. *International journal of environmental research and public health*. 2022;19(1):508.
149. Ostrowska L, Smarkusz-Zarzecka J, Gornowicz A, Lendzion K, Zyśk B, Pogodziński D. Analysis of selected salivary adipokines and cytokines in patients with obesity—A pilot study. *International Journal of Molecular Sciences*. 2023;24(4):4145.
150. Abduljabbar T, Al-Sahaly F, Kellesarian SV, Kellesarian TV, Al-Anazi M, Al-Khathami M et al. Comparison of peri-implant clinical and radiographic inflammatory parameters and whole salivary destructive inflammatory cytokine profile among obese and non-obese men. *Cytokine*. 2016;88:51-6.
151. Safabakhsh D, Jazaeri M, Abdolsamadi H, Abassi E, Farhadian M. Comparison of salivary interleukin-6, interleukin-8, C-reactive protein levels and total antioxidants capacity of obese individuals with normal-weight ones. *Romanian journal of internal medicine= Revue roumaine de medecine interne*. 2022;60(4):215-21.
152. Zyśk B, Ostrowska L, Smarkusz-Zarzecka J, Witczak-Sawczuk K, Gornowicz A, Bielawska A. Pro-inflammatory adipokine and cytokine profiles in the saliva of obese patients with non-alcoholic fatty liver disease (NAFLD)—a pilot study. *International journal of molecular sciences*. 2023;24(3):2891.
153. Acevedo S, M. Andrade J. Effects of a spice-blended honey muffin on salivary inflammation markers in adults with obesity: a feasibility trial. *Annals of Medicine*. 2023;55(2):2245325.
154. Al-Hamoudi N, Abduljabbar T, Mirza S, Al-Sowygh ZH, Vohra F, Javed F et al. Non-surgical periodontal therapy reduces salivary adipocytokines in chronic periodontitis patients with and without obesity. *Journal of investigative and clinical dentistry*. 2018;9(2):e12314.
155. Li Y, Ou Y, Fan K, Liu G. Salivary diagnostics: opportunities and challenges. *Theranostics*. 2024;14(18):6969.
156. Kashyap B, Kullaa A. Salivary metabolites produced by oral microbes in oral diseases and oral squamous cell carcinoma: a review. *Metabolites*. 2024;14(5):277.
157. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics–Current views and directions. *Experimental Biology and Medicine*. 2017;242(5):459-72.
158. Ebersole JL, Hasturk H, Huber M, Gellibolian R, Markaryan A, Zhang XD et al. Realizing the clinical utility of saliva for monitoring oral diseases. *Periodontology 2000*. 2024;95(1):203-19.
159. Spielmann N, Wong DT. Saliva: diagnostics and therapeutic perspectives. *Oral diseases*. 2011;17(4):345-54.
160. Hofman LF. Human saliva as a diagnostic specimen. *The Journal of nutrition*. 2001;131(5):1621S-5S.
161. Koh DS-Q, Koh GC-H. The use of salivary biomarkers in occupational and environmental medicine. *Occupational and environmental medicine*. 2007;64(3):202-10.
162. Xu S, Mumuni AN, Tuason RTS, Maki KA. Methodological considerations in saliva-based biomarker research: Addressing patient-specific variability in translational research protocols. *Current Protocols*. 2025;5(10):e70235.
163. Granger DA, Johnson SB, Szanton SL, Out D, Schumann LL. Incorporating salivary biomarkers into nursing research: an overview and review of best practices. *Biological research for nursing*. 2012;14(4):347-56.
164. Mortazavi H, Yousefi-Koma A-A, Yousefi-Koma H. Extensive comparison of salivary collection, transportation, preparation, and storage methods: a systematic review. *BMC Oral Health*. 2024;24(1):168.
165. Fey JM, Bikker FJ, Hesse D. Saliva collection methods among children and adolescents: a scoping review. *Molecular Diagnosis & Therapy*. 2024;28(1):15-26.
166. Martínez AD, Ruelas L, Granger DA. Household fear of deportation in Mexican-origin families: Relation to body mass index percentiles and salivary uric acid. *American journal of human biology*. 2017;29(6):e23044.
167. Khurshid Z, Zohaib S, Najeeb S, Zafar MS, Slowey PD, Almas K. Human saliva collection devices for proteomics: an update. *International journal of molecular sciences*. 2016;17(6):846.

168. Henson BS, Wong DT. Collection, storage, and processing of saliva samples for downstream molecular applications. Oral biology: molecular techniques and applications: Springer; 2010. p. 21-30.
169. Barranco T, P Rubio C, Tvarijonaviciute A, Rubio M, Damia E, Lamy E et al. Changes of salivary biomarkers under different storage conditions: effects of temperature and length of storage. *Biochemia medica*. 2019;29(1):94-111.
170. Kivlighan KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shirtcliff EA. Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and behavior*. 2004;46(1):39-46.
171. Kivlighan KT, Granger DA, Schwartz EB. Blood contamination and the measurement of salivary progesterone and estradiol. *Hormones and Behavior*. 2005;47(3):367-70.
172. Padilla GA, Calvi JL, Taylor MK, Granger DA. Saliva collection, handling, transport, and storage: Special considerations and best practices for interdisciplinary salivary bioscience research. *Salivary bioscience: Foundations of interdisciplinary saliva research and applications*. 2020:21-47.
173. Nonzee V, Manopatanakul S, Khovidhunkit S-oP. Xerostomia, hyposalivation and oral microbiota in patients using antihypertensive medications. *Journal of the Medical Association of Thailand*. 2012;95(1):96.
174. Dodds MW, Yeh CK, Johnson DA. Salivary alterations in type 2 (non-insulin-dependent) diabetes mellitus and hypertension. *Community dentistry and oral epidemiology*. 2000;28(5):373-81.
175. von Bültzingslöwen I, Sollecito TP, Fox PC, Daniels T, Jonsson R, Lockhart PB et al. Salivary dysfunction associated with systemic diseases: systematic review and clinical management recommendations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2007;103:S57. e1-S. e15.

Tables

Table 1. Number of articles for each biomarker

Salivary Biomarkers	Number of Studies
Minerals	
Iron	5
Ferritin	7
sTfR	0
Zinc	7
Selenium	0
Calcium	6
Vitamins	
Vitamin A	2
Vitamin B12	4
Folic acid	1
Vitamin C	2
Vitamin D	9
Vitamin E	2
Inflammatory biomarkers	
AGP	1
CRP	9
Hepcidin	2
IgA	11
Cytokines	27
- TNF-α	11
- IL-6	18
- Other cytokines	22

1337

1338 **Table 2.** Summary of studies with salivary ferritin and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of ferritin (Mean ± SD)	Serum levels of ferritin (Mean ± SD)	Correlation coefficients between serum and salivary levels of ferritin	Reference
(a) Turkey (b) University hospital (c) Cross-sectional study	(a) patients with Transfusion-dependent β-thalassemia major (n = 71) (b) patients with thalassemia intermedia (n = 10) (c) patients with thalassemia trait (n = 15) (d) patients with IDA (n = 30) (e) age- and sex-matched healthy controls (n = 34)	(a) 6-23 (11.6±4.45) years (b) 6-25 (12.4±6.0) years (c) 15-18 (16.0 ±1.9) years (d) 7-14 (9.1±2.12) years (e) 7-15 (10±2.22) years	(a) Transfusion-dependent β-thalassemia major: 2529.6 ± 1081.3 ng/dl (b) thalassemia intermedia: 1166.0 ± 951.3 ng/dl (c) thalassemia trait: 112.2 ± 145.9 ng/dl (d) IDA: 18.6 ± 8.53 ng/dl (e) healthy controls: 42.5 ± 42.25 ng/dl	(a) thalassemia major: 2826.35 ±1711 ng/dl (b) thalassemia intermedia: 1492.1 ± 1445.1 ng/dl (c) thalassemia trait: 126 ± 150 ng/dl (d) IDA: 6.7 ± 3.49 ng/dl (e) healthy controls: 29.67 ± 16.78 ng/dl	(a) thalassemia major: r =0.364 (p=0.034) (b) thalassemia intermedia: rho =0.891 (p=0.001) (c) thalassemia trait: rho =0.831 (p<0.001) (d) IDA: r =0.880 (p<0.001) (e) healthy controls: r =0.842 (p<0.001)	Canatan et al. 2012
(a) India (b) Dental college	(a) patients with IDA (n = 30)	8-14 years	(a) patients with IDA: 153.24 ± 23.58 ng/dl	(a) patients with IDA: 31.69 ± 6.28 ng/dl	Not done	Jagannathan et al. 2012

(c) case-control study	(b) healthy controls (n = 30)		(b) healthy controls: 93.87 ± 26.15 ng/dl	(b) healthy controls: 90.45 ± 8.23 ng/dl		
(a) Turkey (b) Hospital (c) Cross-sectional study	(a) healthy control (n = 40) (b) peritoneal dialysis (n = 44) (c) hemodialysis patients (n = 44)	(a) 43.9±8.5 years (b) 45.8 ±13.3 years (c) 48.2±12.3 years	(a) healthy controls: 27.5 ± 52.9 ng/mL (b) peritoneal dialysis: 10.8 ± 7.57 ng/mL (c) before hemodialysis: 34.9 ± 22.2 ng/mL (d) after hemodialysis: 61.1 ± 58.0 ng/mL	(a) healthy controls: 55.5 ± 55.9 ng/mL (b) peritoneal dialysis: 501 ± 192 ng/mL (c) before hemodialysis: 869 ± 1070 ng/mL (d) after hemodialysis: 1255 ± 612 ng/mL	(a) healthy controls: r = 0.55 (p<0.001) (b) peritoneal dialysis: not reported (c) before hemodialysis: not reported (d) after hemodialysis: not reported	Alpdemir et al. 2018
(a) China (b) University hospital (c) Cross-sectional study	(a) participants with T2DM (n = 22) (b) participants with CP (n = 22) (c) participants with both CP and T2DM (n = 22) (d) healthy controls (n = 22)	(a) 56.45 ± 11.80 years (b) 58.09 ± 9.97 years (c) 62.82 ± 10.72 years (d) 52.45 ± 10.01 years	(a) T2DM: 12.61 ± 6.4 ng/mL (b) CP: 16.74 ± 11.5 ng/mL (c) CP + T2DM: 12.81 ± 7.9 (d) healthy controls: 6.50 ± 3.0 ng/mL	(a) T2DM: 196.3 ± 208.0 ng/mL (b) CP: 265.1 ± 142.5 ng/mL (c) CP + T2DM: 197.7 ± 176.1 ng/mL (d) healthy controls: 196.2 ± 100.0 ng/mL	Overall: R^2 = 0.064 (p = 0.017)	Guo et al. 2018
(a) Iran (b) Clinic/medical laboratory	Volunteers with no obvious systemic disorders	35.2±11.4 years	Not reported	Not reported	rho=0.27 (p = 0.004)	Haji-Sattari et al. 2019

(c) cross-sectional study	and no history of smoking or medication intake (n =107)					
(a) India (b) Dental College (c) Case-control study	(a) children with caries (n = 60) (b) age- and sex-matched controls (n = 60)	(a) 4.2 ± 0.9 years (b) 4.1 ± 0.7 years	(a) children with caries: 1595.30±186.50 ng/mL (b) controls: 921.60±129.10 ng/mL	Not done	Not done	Rajkumaar et al. 2020
(a) India (b) Hospital (c) Comparative study	(a) female patients with IDA (n = 15) (b) non-anemic female control (n = 15)	(a) 36.26±7.28 years (b) 31.32±3.51 years	(a) female patients with IDA: 1393.70±479.00 ng/mL (b) non-anemic female controls: 941.80±629.00 ng/mL	(a) female patients with IDA: 3.63±2.31 ng/mL (b) non-anemic female controls: 45.65±7.97 ng/mL	Not done	Lokesh Sundaram B et al. 2021

1339 SD, standard deviation; IDA, iron deficiency anemia; CP, chronic periodontitis; T2DM, type 2

1340 diabetes mellitus.

1341

1342 **Table 3.** Summary of studies with salivary iron and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of iron (Mean ± SD)	Serum levels of iron (Mean ± SD)	Correlation coefficients between serum and salivary levels of iron	Reference
(a) Turkey (b) University hospital (c) Cross-sectional study	(a) patients with Transfusion-dependent β-thalassemia major (n = 71) (b) patients with thalassemia intermedia (n = 10) (c) patients with thalassemia trait (n = 15) (d) patients with IDA (n = 30) (e) age- and sex-matched healthy controls (n = 34)	(a) 6-23 (11.6±4.45) years (b) 6-25 (12.4±6.0) years (c) 15-18 (16.0 ±1.9) years (d) 7-14 (9.1±2.12) years (e) 7-15 (10±2.22) years	a) Transfusion-dependent β-thalassemia major: 253.64 ± 91.0 mg/dL (b) thalassemia intermedia: 150.1 ± 61.1 mg/dL (c) thalassemia trait: 101.1 ± 41.16 mg/dL (d) IDA: 24.6 ± 10.0 mg/dL (e) healthy controls: 74.20 ± 40.7 mg/dL	(a) thalassemia major: 192.25 ± 76.87 mg/dL (b) thalassemia intermedia: 132.20 ± 55.7 mg/dL (c) thalassemia trait: 93.40 ± 39.14 mg/dL (d) IDA: 13.80 ± 24.70 mg/dL (e) healthy controls: 62.37 ± 33.0 mg/dL	(a) thalassemia major: r =0.972, p<0.001 (b) thalassemia intermedia: rho =0.720, p=0.019 (c) thalassemia trait: rho =0.955, p<0.001 (d) IDA: r =0.368, p=0.045 (e) healthy controls: r =0.885, p<0.001	Canatan et al. 2012
(a) Brazil (b) Community (c) Cohort study	Pregnant women (n = 42)	Not reported	(a) First trimester: 57.8 ± 77.3 mg/dL (b) Third trimester: 93.1 ± 175.7 mg/dL (c) Post-	(a) First trimester: 106.4 ± 27.5 mg/dL (b) Third trimester: 90.0 ± 40.5 mg/dL (c) Post-partum:	(a) significant positive correlation between serum Fe levels in the first trimester and	Costa et al. 2017

			partum: 42.3 ± 124.3 mg/dL	86.3 ± 26.3 mg/dL	salivary Fe levels in the second trimester (r = 0.99, p = 0.045). (b) no other statistically significant correlations	
(a) Turkey (b) Hospital (c) Cross-sectional study	(a) healthy control (n = 40) (b) peritoneal dialysis (n = 44) (c) hemodialysis patients (n = 44)	(a) 43.9±8.5 years (b) 45.8 ±13.3 years (c) 48.2±12.3 years	(a) healthy controls: 0.65 ± 0.51 µmol/L (b) peritoneal dialysis: 1.16 ± 1.42 µmol/L (c) before hemodialysis: 0.84 ± 1.00 µmol/L (d) after hemodialysis: 0.57 ± 0.74 µmol/L	(a) healthy controls: 12.96 ± 6.98 µmol/L (b) peritoneal dialysis: 11.29 ± 3.62 µmol/L (c) before hemodialysis: 11.99 ± 7.05 µmol/L (d) after hemodialysis: 13.75 ± 11.01 µmol/L	(a) healthy controls: not reported (b) peritoneal dialysis: not reported (c) before hemodialysis: not reported (d) after hemodialysis: r = 0.40, p = 0.007	Alpdemir et al. 2018
(a) Iran (b) Hospital (c) Case-control study	(a) patients with OLP (n = 40) - non-erosive group (n = 22) - erosive group (n = 18) (b) age- and sex-matched controls (n = 40)	(a) 46.6±11.85 years (b) 46.06±12.13 years	(a) patients with OLP: 0.16 ± 0.24 mmol/L - non-erosive group: 0.13 ± 0.23 mmol/L - erosive group: 0.19 ± 0.25 mmol/L (b) age-	Not done	Not done	Rezazadeh et al. 2019

			and sex-matched controls: 0.11 ± 0.10 mmol/L			
(a) Italy (b) Hospital (c) Cross-sectional study	(a) healthy control group (n = 20) (b) untreated periodontitis group (n = 24) (c) treated periodontitis group (n = 22)	(a) 44.3±10.8 years (b) 55.4 ± 13.8 years (c) 53.1 ± 14.6 years	(a) healthy control group: 9.74 ± 6.57 µg/L (b) untreated periodontitis group: 12.56 ± 13.88 µg/L (c) treated periodontitis group: 26.66 ± 27.53 µg/L	Not done	Not done	Romano et al. 2020

1343 SD, standard deviation, IDA, iron deficiency anemia; OLP, oral lichen planus
1344

1345 **Table 4.** Summary of studies with salivary vitamin A and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of vitamin A (Mean ± SD)	Serum levels of vitamin A (Mean ± SD)	Correlation coefficients between serum and salivary levels of vitamin A	Reference
(a) United Kingdom (b) Hospital (c) Cross-sectional study	Healthy participants (n = 14)	Not reported	(a) RBP at resting stage: 2100.00 ± 2150.00 ng/mL (b) RBP at stimulated stage: 1600.00 ± 1700.00 ng/mL	(a) Serum retinol: 2.08 ± 0.39 µmol/L (b) Serum RBP: Not reported	(a) Salivary RBP concentration vs. serum RBP concentration: $r^2 = 0.09$, $p > 0.05$ (b) Salivary RBP vs. serum retinol concentration: $r^2 = 0.04$, $p = 0.05$	Blakeley et al. 2020
(a) Iran (b) Dental college (c) Cross-sectional study	(a) patients with OLP (n = 28) - non-erosive group (n = 17) - erosive group (n = 11) (b) healthy controls (n = 30)	44.96 ± 10.32 years	(a) Vitamin A in patients with OLP: 10.45 ng/mL - non-erosive group: 10.81 ± 0.95 ng/mL - erosive group: 9.91 ± 0.68 ng/mL (b) Vitamin A in healthy controls: 12.97 ng/mL	Not done	Not done	Rezazadeh et al. 2023

1346 SD, standard deviation; RBP, retinol binding protein; OLP, oral lichen planus
1347

1348 **Table 5.** Summary of studies with salivary vitamin D and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of vitamin D (Mean ± SD)	Serum levels of vitamin D (Mean ± SD)	Correlation coefficients between serum and salivary levels of vitamin D	Reference
(a) Iran (b) University hospital (c) Cross-sectional study	(a) patients with RAS (n = 26) (b) healthy individuals (n = 26)	(a) 38.80 ± 12.02 years (b) 40.80 ± 11.60 years	(a) RAS Patients: 17.36 ± 8.01 ng/dL (b) healthy individuals: 20.79 ± 6.31 ng/dL	(a) RAS Patients: 33.07 ± 12.41 ng/dL (b) healthy Individuals: 50.89 ± 9.30 ng/dL	r = 0.56 (p < 0.001)	Bahramian et al. 2018
(a) Italy (b) Clinic/hospital (c) Cross-sectional study	(a) patients with PD (n = 21) (b) healthy controls (n = 21)	(a) 56.9 ± 5.4 years (b) 54.3 ± 5.0 years	(a) PD: 3.7 ± 2.4 ng/mL (b) healthy controls: 4.6 ± 3.6 ng/mL	Not done	Not done	Costantini et al. 2020
(a) Poland (b) Schools (c) Cross-sectional study	(a) pre-pubertal children (n = 100) (b) pubertal children (n = 76)	6 to 13 years	(a) pre-pubertal children: 33.03 ± 18.36 ng/mL (b) pubertal children: 23.82 ± 14.72 ng/mL (c) overall: 29.05 ± 17.45 ng/mL	Not done	Not done	Pruszkowska-Przybylska et al. 2020
(a) Brazil (b) Schools (c) Cross-sectional study	(a) below %BF cutoff (n = 172) (b)	15.66 ± 0.9 years	(a) below %BF cutoff (n = 111) - 25-	Not done	Not done	Araujo et al. 2020

	above %BF cutoff (n = 67)		Hydroxyvitamin-D2: 1.67 ± 0.71 ng/mL - 25-Hydroxyvitamin-D3: 4.08 ± 3.15 ng/mL (b) above %BF cutoff (n = 138) - 25-Hydroxyvitamin-D2: 1.74 ± 0.58 ng/mL - 25-Hydroxyvitamin-D3: 4.00 ± 2.56 ng/mL			
(a) United Kingdom (b) Hospital (c) Cross-sectional study	Healthy participants (n = 14)	Not reported	(a) Resting salivary DBP concentrations: 65.0 ± 55.6 ng/mL (b) Stimulated salivary DBP concentrations: 55.0 ± 51.9 ng/mL (c) Resting salivary DBP output: 40 ± 25.9 ng/min (d) Stimulated DBP output: 80 ± 44.5 ng/min	(a) Serum 25-OH-D: 40 ± 22 nmol/L (b) Serum DBP: Not reported	(a) Salivary DBP concentration vs. serum DBP concentration: r^2 = 0.33 ($p<0.05$) (b) Salivary DBP output vs. serum DBP concentration: r^2 = 0.45 ($p<0.01$) (c) Salivary DBP concentration vs. Serum 25-OH-D concentration: r^2 = 0.11 ($p>0.05$)	Blakeley et al. 2020

					(d) Salivary DBP output vs. Serum 25-OH-D concentration: r^2 = 0.15 ($p>0.05$)	
(a) Iran (b) University hospital (c) Case-control study	(a) OLP (n = 64), (b) age- and sex-matched healthy controls (n = 45)	Not reported	Unstimulated saliva 25(OH)D concentration (ng/mL): (a) Healthy controls: 0.62 ± 0.07 (b) OLP patients: 0.45 ± 0.05 Stimulated saliva 25(OH)D concentration (ng/mL): (a) Healthy controls: 0.63 ± 0.07 (b) OLP patients: 0.43 ± 0.05 Unstimulated saliva 25(OH)D output (ng/min): (a) Healthy controls: 0.43 ± 0.06 (b) OLP patients: 0.21 ± 0.04 Stimulated saliva	(a) Healthy controls: 36.69 ± 3.79 ng/mL (b) OLP patients: 36.18 ± 3.18 ng/mL	(a) Serum 25(OH)D vs. unstimulated saliva 25(OH)D: r = 0.148 (p = 0.152) (b) Serum 25(OH)D vs. stimulated saliva 25(OH)D: r = 0.014 (p = 0.895) (c) Serum 25(OH)D vs. unstimulated saliva flow rate: r = 0.013 (p = 0.911) (d) Serum 25(OH)D vs. stimulated saliva flow rate: r = 0.009 (p = 0.935)	Gholizadeh et al. 2020

			25(OH)D output (ng/min): (a) Healthy controls: 0.60 ± 0.11 (b) OLP patients: 0.25 ± 0.04			
(a) United Kingdom (b) Schools and clinics (c) Cross-sectional study	(a) participants with normal BMI (n = 59) (b) participants with obesity (n = 53)	(a) 13.63 ± 2.36 years (b) 14.60 ± 2.05 years	(a) participants with normal BMI: 7.64 ± 3.65 ng/mL (b) participants with obesity: 6.29 ± 5.12 ng/mL	Not done	Not done	Usman et al. 2021
(a) India (b) Dental college (c) Cross-sectional study	(a) OL (n = 15) (b) OSMF (n = 15) (c) OSCC (n = 15) (d) healthy controls (n = 15)	(a) OL: 46.67 ± 18.63 years (b) OSMF: 40.13 ± 11.02 years (c) OSCC: 53.33 ± 8.76 years (d) healthy controls: 42.13 ± 16.90 years (e) overall: 45.57 ± 14.94 years	(a) OL: 31.39 ± 1.72 ng/mL (b) OSMF: 22.37 ± 1.31 ng/mL (c) OSCC: 24.06 ± 1.92 ng/mL (d) healthy controls: 33.61 ± 2.25 ng/mL	(a) OL: 24.19 ± 2.04 ng/mL (b) OSMF: 20.12 ± 1.85 ng/mL (c) OSCC: 18.15 ± 1.71 ng/mL (d) healthy controls: 36.29 ± 3.12 ng/mL	r = 0.737 (p < 0.001)	Samanta et al. 2023
(a) Iran (b) Dental college	(a) patients with OLP	44.96 ± 10.32 years	(a) patients with OLP: 8.96 ± 6.62	Not done	Not done	Rezazadeh et al. 2023

(c) Cross-sectional study	(n = 28) - non-erosive group (n = 17) - erosive group (n = 11) (b) healthy controls (n = 30)		ng/mL - non-erosive group: 8.88 ± 6.98 ng/mL - erosive group: 8.95 ± 6.01 ng/mL (b) healthy controls: 14.22 ng/mL			

SD, standard deviation; RAS, Recurrent Aphthous Stomatitis; PD, periodontitis; %BF, body fat percentage; DBP, vitamin D binding protein; BMI, body mass index; OL, oral leukoplakia; OSMF, oral submucous fibrosis; OSCC, oral squamous cell carcinoma; OLP, oral lichen planus.

1353 **Table 6.** Summary of studies with salivary vitamin B12 and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of vitamin B12 (Mean ± SD)	Serum levels of vitamin B12 (Mean ± SD)	Correlation coefficients between serum and salivary levels of vitamin B12	Reference
(a) Turkey (b) Hospital (c) Cross-sectional study	(a) healthy control (n = 40) (b) peritoneal dialysis (n = 44) (c) hemodialysis patients (n = 44)	(a) 43.9±8.5 years (b) 45.8 ±13.3 years (c) 48.2±12.3 years	(a) healthy controls: 27500.00 ± 52900.00 pg/mL (b) peritoneal dialysis: 10800.00 ± 7570.00 pg/mL (c) before hemodialysis: 34900.00 ± 22200.00 pg/mL (d) after hemodialysis: 61100.00 ± 58000.00 pg/mL	(a) healthy controls: 247.0 ± 120.0 pg/mL (b) peritoneal dialysis: 500.0 ± 303.0 pg/mL (c) before hemodialysis: 368.0 ± 58.6 pg/mL (d) after hemodialysis: 286.0 ± 96.6 pg/mL	(a) healthy controls: not reported (b) peritoneal dialysis: not reported (c) before hemodialysis: not reported (d) after hemodialysis: not reported (e) all groups: r = 0.16, p = 0.035	Alpdemir et al. 2018
(a) Iraq (b) Hospital (c) Cross-sectional study	(a) IDA with RAS (n = 25) (b) IDA without RAS (n = 25) (c) healthy controls (n = 25)	38.3 ± 13.3 years	(a) IDA with RAS: 100.0 ± 14.8 pg/mL (b) IDA without RAS: 120.0 ± 111.1 pg/mL (c) healthy controls:	(a) IDA with RAS: 111.0 ± 15.4 pg/mL (b) IDA without RAS: 145.0 ± 57.2 pg/mL (c) healthy controls:	Not done	Al-hamdani et al. 2023

			330.0 ± 30.0 pg/mL	396.0 ±18.2 pg/mL		
(a) United Kingdom (b) Hospital (c) Cross-sectional study	Healthy participants (n = 14)	Not reported	(a) Resting: 48.00± 16.30 pg/mL (b) Stimulated: 30.00 ± 17.78 pg/mL	(a) Serum total vitamin B12: 196 ± 92 pmol/L (b) Serum haptocorrin: not reported	(a) Salivary vs. serum haptocorrin concentration: $r^2 = 0.02$, $p > 0.05$ (b) Salivary haptocorrin vs. serum total vitamin B12 concentration: $r^2 = 0.08$, $p = 0.05$	Blakeley et al. 2020
(a) Iran (b) Dental college (c) Cross-sectional study	(a) patients with OLP (n = 28) - non-erosive group (n = 17) - erosive group (n = 11) (b) healthy controls (n = 30)	44.96 ± 10.32 years	(a) patients with OLP: 910000 pg/mL - non-erosive group: 940000.00 ± 760000.00 pg/mL - erosive group: 870000.00 ± 510000.00 pg/mL (b) healthy controls: 980000.00 pg/mL	Not done	Not done	Rezazadeh et al. 2023

SD, standard deviation; IDA, iron deficiency anemia; RAS, recurring aphthous ulcers; OLP, oral lichen planus.

1357 **Table 7.** Summary of studies with salivary CRP and results

(a) Country (b) Settings (c) Design	Study participants with sample size	Age of the participants	Salivary levels of CRP (Mean ± SD)	Serum levels of CRP (Mean ± SD)	Correlation coefficients between serum and salivary levels of CRP	Reference
(a) South Africa (b) School (c) Cross-sectional	(a) Children with normal weight children (n = 93) (b) Children with overweight (n = 24) (c) Children with obesity (n = 53)	(a) 9.71 ± 1.84 years (b) 9.25 ± 1.67 years (c) 9.55 ± 1.63 years	(a) 0.00001 ± 0.000001 µg/mL (b) 0.00001 ± 0.000001 µg/mL (c) 0.00001 ± 0.000001 µg/mL	Not done	Not done	Naidoo et al. 2012
(a) USA (b) Hospital (c) Cross-sectional	(a) Acute myocardial infraction (n = 92) (b) Controls (n = 111)	(a) 54.2 ± 11.9 years (b) 48.6 ± 8.9 years	(a) 0.003 ± 0.006 µg/mL (b) 0.0001 ± 0.001 µg/mL	(a) 0.005 ± 0.005 µg/mL (b) 0.001 ± 0.001 µg/mL	Not done	Ebersole et al. 2017

(a) USA (b) Community (c) Cross-sectional	(a) Children with normal weight (n = 40) (b) Children with overweight or obesity (n = 36)	(a) 8.38 ± 0.23 years (b) 8.49 ± 0.23 years	(a) 0.00004 µg/mL (b) 0.0002 µg/mL	Not done	Not done	Selvaraju et al. 2019
(a) Sweeden (b) Community (c) Cross-sectional	Healthy participants (n = 107)	Median age: 57, Age range: 45–69 years	(a) Morning level: 0.06 ± 0.22 µg/ml (b) Evening level: 0.01 ± 0.02 µg/ml	2.4 ± 4.68 µg/mL	a) Morning salivary CRP vs. serum CRP: no significant correlation (b) Evening salivary CRP vs. serum CRP: rho = 0.27 (p<0.01)	Wetterö et al. 2020

(a) Spain (b) School (c) Observational study (Cohort study)	Healthy children (n = 129)	11 ± 0.74 years	0.002 ± 0.01 µg/ml	1.20 ± 3.12 ug/mL	Overall: rho = 0.77 (p < 0.001) Boys: rho = 0.81 (p < 0.001) Girls: rho = 0.78 (p < 0.001)	Tvarijonaviciute et al. 2020
(a) Nigeria (b) Hospital (c) Case-control	(a) Patients with diabetes (n = 39) (b) Controls (n = 36)	(a) 57.0 ± 12.0 years (b) 34.5 ± 8.6 years	(a) 0.05 ± 0.04 µg/ml (b) 0.02 ± 0.02 µg/ml	Not done	Not done	Agho et al. 2021
(a) Kuwait (b) School (c) Longitudinal cohort study	(a) Children with normal weight (n = 232) (b) Children with overweight or obesity (n = 121)	(a) 17.1 years (b) 17.0 years	Visit 1 (n = 815): 0.000002 ± 0.000001 µg/mL Visit 2 (n = 42): 0.000003 ± 0.000001 µg/mL Visit 3 (n = 352): 0.00003 ± 0.000001 µg/mL	Visit 1: Not done Visit 2: Not done Visit 3 (n = 352): 0.00001 ± 0.000001 µg/mL	Visit 1: NA Visit 2: NA Visit 3: r = 0.7 (p < 0.0001)	Alqaderi et al. 2022

(a) Germany (b) Clinic (c) Explorative pilot study	(a) Children with normal weight (n = 19) (b) Children with overweight or obesity (n = 19)	(a) 9.7 ± 2.5 years (b) 10.1 ± 1.9 years	(a) 0.0002 ± 0.001 µg/mL (b) 0.001 ± 0.001 µg/mL	Not done	Not done	Bizjak et al. 2022
(a) Indonesia (b) Hospital (c) Cross-sectional	(a) Patients with diabetes (n = 10) (b) Control (n = 13)	Not reported	(a) 0.242 ± 0.094 µg/ ml (b) 0.248 ± 0.091 µg/ ml	Not done	Not done	Bachtiar et al. 2023

1358 SD, standard deviation; CRP, C-reactive protein.

1359

Figure Legend

Figure 1. PRISMA diagram showing the study selection process in this review

Figure 2. Forest plot of pooled correlation coefficients between serum and salivary biomarkers

Figure 3. Methodological Challenges and Considerations for the Analysis of Saliva Samples in Nutrition and Biomedical Research.

Figure 4. Future applications of saliva-based diagnostics for the assessment of nutritional status and inflammatory conditions in different settings