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# Diagnostic potential of saliva as a biomarker in early childhood caries: A review

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**Abstract**
Early Childhood Caries (ECC) is a severe and early form of the carious disease that can compromise child's oral and systemic health. Despite significant efforts to integrate children's oral health into primary care, identification of children at risk for ECC before the onset of cavitation remains challenging. As teeth are bathed in saliva constantly, the constituents and properties of this oral fluid play an essential role in the occurrence and progression of oral diseases including dental caries. Thus, it can be a promising tool in early identification and thereby preventing the further progression of caries in children. Hence, the aim of this review is to throw light on the current knowledge and utilisation of salivary biomarkers in terms of its predictive potential for ECC. A literature search was done using electronic databases PubMed, Google Scholar, and Cochrane Database for articles in English. Several keywords were used: Early childhood caries, salivary biomarkers, saliva, child. Some of the promising biomarkers include the microbiological species, electrolytes in saliva, antibodies, inflammatory mediators, glucosyltransferase and physical characteristics of saliva. Utilisation of these biomarkers in clinical practice might help the clinicians in improving the oral health status of the child.

**Keywords:** Biomarker, Diagnosis, Early childhood caries, Predictor, Saliva

## Introduction
Early Childhood Caries (ECC) is a serious public health problem with a prevalence ranging from 1-12% in developed and as high as 70% in less developed countries [1]. According to American Academy of Pediatric Dentistry, ECC is "the presence of one or more decayed (non cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of six". It is not just the problem of teeth, also associated with reduced growth and reduced weight gain due to insufficient food consumption to meet the metabolic and growth needs of children*. The early detection can reduce pain and helps in the growth and the overall development of the child [2].
Current clinical practice has a growing impetus on early diagnosis, proper prognostication and screening for a disease in asymptomatic group [3]. Biomarkers are assuming a growing role in all aspects of medicine, starting from screening to follow up after treatment, it may be utilized as a diagnostic tool for the early detection and prompt treatment of this early childhood problem. Biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [4].
Biomarkers in the blood circulation can infiltrate acini of salivary glands and are eventually secreted into the saliva acting as an effective indicator of both local and systemic disorders. These findings have formed the basis for the field of salivary diagnostics and hence sparked investigations that culminated in the identification of saliva-based biomarkers for disorders ranging from cancer to infectious diseases like dental caries [5]. Hence this review was conducted with an objective of identifying various salivary biomarkers for ECC, its accuracy in detecting ECC and application in clinical practice.

## Methodology
A literature search was done that cover the relevant objectives using electronic databases "PubMed," "Google Scholar," and "Cochrane Database," to identify the articles in English language from August 2017 to December 2018. Multiple search keywords were used: ECC,

dental caries, child, saliva, biomarkers. A total of 723 articles were retrieved. After screening for the objectives a total of 78 full text articles were reviewed and compiled.

**Salivary biomarkers in early childhood caries**

It is classified as:

1. Biological constituents of saliva: Micro organisms
2. Physical properties of saliva
3. Chemical constituents of saliva

**Biological constituents of saliva: Micro organisms**

The most important microbial species associated with ECC are *Streptococcus, Lactobacillus* and *Actinomyces*. Organisms like *Atopobium* genus, *Aggregatibacter* sp., *Streptococcus oralis*, *S.mitis, S.infantis, S. parasanguinis, A. defectiva, G. haemolysans, Selemonas* sp., and *Porphyromonas* sp. were also found to be involved in the development of caries [6, 7]. Species such as *Bergeyella* sp., *Campylobacter concisus*, *Granulicatella adiacens, Kingella denitrificans, Kingella oralis, Leptotrichia hofstadii/ Leptricihia sp., Streptococcus anginosus / S. gordonii,* were found to be associated with healthy tooth surfaces [6]. The contribution of *C.albicans* to total microbial acid formation appears to be relevant for caries progression but relevant conclusive evidence are lacking for its use as a predictor of ECC [8, 9]. Commercial kits for the detection of salivary *streptococcus* and *lactobacilli* can be used to predict ECC and $10^5$ *mutans streptococci* per milliliter of saliva could be considered a high value.[10] Two commercially available salivary bacterial cultural tests are Dentocult Strip Mutans (Orion Diagnostica, Espoo, Finland) and Ivoclar CRT (Ivoclar Vivadent, Amherst, N.Y.). Both tests can detect levels of SM and LB, but each test requires a 48-hour incubation period and a follow-up appointment for discussing the results with the patients. Another test, Saliva-Check Mutans (GC America), resolves the recall issue as the test can detect salivary SM levels chairside in 15 minutes which cost around Rs 600 per patient (around Rs 6000 that can be used for 10 patients). CariScreen is another test which is commercially available for testing the biofilm activity. Lactic acid production by these cariogenic micro organism can be assessed using ClinproTM Cario L-PopTM (3M ESPE) and biofilm activity using Caries Screen SM (Orion Diagnostica, Espoo, Finland).

**Physical properties of saliva as possible markers**

**Salivary flow, pH, Buffer system and viscosity**

The dental caries process is controlled to a large extent by a natural protective mechanism inherent within the saliva. The most important caries protective functions of saliva are the flushing and neutralizing effects which is dependent on the flow rate (FR) and buffering capacity (BC) and pH of saliva which can be assessed using commercial kits. Reduction in the salivary FR in children with caries as reported with unstimulated salivary flow rate < 0.29±0.08ml/min can be considered as a warning sign before the occurrence of caries in young children [11, 12]. The stimulated flow rate can be easily tested in the dental office or clinic by having the patient chew on paraffin wax and expectorate into a graduated cup, measuring mLs. Inadequate saliva is measured as less than 0.7 ml of stimulated saliva per minute. If the flow rate remains low over time, this can place a patient at high or extreme risk for caries.

BC is based on the phosphate system, the carbonic acid, and bicarbonate system. A clear inverse relationship between salivary BC and caries susceptibility exists, with BC reduced to 0.4 units in caries active children [11]. Carbonic Anhydrase VI (CA VI) from the acinar cells, catalyses the conversion of salivary bicarbonate and microbe-delivered hydrogen ions to carbon dioxide and water, thereby accelerates the removal of acid from the mouth. A negative correlation of CAVI with caries can be used as a predictor in ECC [13]. Saliva check Buffer(GC America Inc) is a test kit available to check the buffering capacity and pH of saliva which are cost effective (Rs 200-300 per patient- around Rs 4000 that can be used for 20 patients) and simple methods that can be used in clinical practice. Ivoclar CRT Buffer and Dentobuff strips are other commercially available strips used to measure salivary buffering capacity which are also budget friendly.

The concentration of hydrogen ions (pH) in the saliva maintains the integrity of oral cavity. Increase in pH results in remineralization of the tooth surface due to the increased degree of super saturation. Components of saliva and plaque are more or less similar as it is bathed in saliva. The acidic plaque pH contributing to dental caries is a known fact. pH > 7 in caries free children suggests that a reduction in pH can be used as a biomarker to predict the caries activity in young children [11, 12, 14].

Fresh mixed human saliva is viscoelastic fluid with distinct surface activity. Glycoproteins particularly mucin are responsible for the viscoelastic character of saliva giving a lubricating film that enables free movement of oral tissues. Measuring salivary viscosity is of paramount importance since an elevated salivary viscosity was found to be associated with an increased occurrence of oral diseases particularly dental caries and periodontal diseases.[15] Animreddy *et al* found a significant difference in salivary viscosity among children with and without caries measured using Ostwald viscometer wherein salivary viscosity was less among caries free children compared to children with caries [12]. Salimat device can be also used to measure viscosity of saliva.

**Chemical constituents of saliva as markers**

**Electrolytes in saliva**

The balance between demineralization and remineralization depends on the salivary calcium and phosphate concentration. Calcium and phosphate contents of saliva decreased in individuals with active carious lesions suggests its relationship to dental caries [16, 17]. Calcium and phosphorus measured colorimetrically have shown their values as less as 2 units in caries active children [5].

When fluoride is present in oral fluids, fluorapatite forms during the remineralization process which is less soluble than hydroxyapatite. Ion specific electrode and spectrophotometric values of F ranged from 0-1ppm in children with active caries [18, 19]. A drop in the values to these levels can be used as an indicator for the caries risk.

In the oral cavity, salivary nitrate comes in contact with bacteria that are capable of rapidly reducing nitrate to nitrite which when encounters the acid environment, leads to the formation of the complex mixture of nitrous oxide and nitrous acid. This nitrous acid is unstable and will spontaneously decompose to produce nitric oxide (NO). NO has a strong antibacterial effect [19]. Concentration of NO >50µM and values <40µM in caries active and caries free children are seen when measured using Griess method [20, 21]. The increased concentration of NO suggests its defense role towards caries [22].

Copper has a significant impact on the acid solubility of enamel. The dissolution of enamel is reduced in the presence

of copper ions due to its ability to precipitate a protective copper phosphate phase on the tooth surface. Furthermore, copper exerts a cariostatic effect through inhibition of bacterial growth and bacterial metabolic enzymes [23]. Spectrophotometric measurements of Cu shows values of 0.2-0.3 units in children with caries. This inverse relationship between copper and dental caries gives its predictive role for ECC [19].

**Salivary proteins**

**Salivary Antimicrobial peptides**

Major antibody classes that operates in saliva: secretory IgA and IgG. IgA's plays the main role as secretory immunoglobulin. It prevents dental caries by inhibiting bacterial adherence and inactivating bacterial enzymes and toxins.[24] Elisa kit for Ig A & G have shown concentration of 196.14±100.07 mg/dl; 9.78±3.26 mg/dl respectively in caries active children which were significantly higher compared to caries free [24]. Although such increased concentration were seen in many studies controversial results were also noted in others when methods of concentration assessment was different [24-27, 8, 24, 28, 29] SIg A ELISA Kits which are commercially available for around Rs 6000 that can be used for almost 24 patients.

**Defensins**

Alpha-defensins (HNP 1,2,3) are found in neutrophils, macrophages whereas β-defensins (HBD-1,2) undergo a specific expression in salivary duct cells. They protect salivary glands against bacterial (Gram-negative: *P. aeruginosa, E. coli;* Gram-positive: *S. aureus, S. pneumoniae, S. faecalis*), viruses (HIV-1, HSV-1, HAdV), and fungi invasion (*C. albicans*). A conclusive role of alpha defensins in ECC could not be established [30]. β-Defensins participates in specific immune response as chemotactic factors [31]. Beta defensins measured using ELISA have shown a significant increase in ECC [32].

**Histatins**

HST-1,3,5 constitute components of the acquired enamel pellicle (AEP). They have a high affinity for hydroxyapatite and are involved in the maintenance of tooth integrity by favouring a suitable calcium phosphate environment. HST destabilize cellular membrane of bacteria by assimilating with its surface leading to cell damage. Inhibition of collagenases activity which increases under pathological conditions is related to inflammatory and degenerative diseases and carcinogenic processes [31]. HST levels in saliva of children with severe ECC, as high as 50ng/ml measured using ELISA can be used as a diagnostic tool in those individuals [32].

**Free amino acids**

Human saliva contains appreciable quantities of amino acids which are assessed using amino acid analysers. These molecules are exchangeable with plaque fluid, being not only metabolic substrate for plaque microflora, but also products of these micro organic metabolic pathways. Proline was most frequently absent in caries free group whereas absence of glycine was observed in children experiencing caries [33].

**Glucosyltransferase**

Glucosyltransferase (GTF) is an extracellular or cell-associated enzyme synthesized by microbial groups like the Mutans streptococci, and is responsible for the biosynthesis of extracellular polysaccharides. GTF has been proven to be an effective antigen in eliciting caries-protective secretory IgA antibodies in rodent models [34]. S. mutans produces 3 types of Gtf: GtfB, GtfC, GtfD. GtfB plays an important role in the adherence and accumulation of mutans streptococci in the dental plaque of young children. Sensitivity and specificity of GtfB for caries was 84 and 68 respectively when measured using ELISA kits [35]. The level of GtfB enzyme showed a significant increase with the increase of caries experience [27].

**Salivary amylase**

Salivary enzyme amylase, found in abundance in the AEP, is capable of modulating bacterial colonization and to provide additional glucose for the biofilm formation process.[36] Moreover, it also binds to the membrane of bacteria such as *S.mutans* and Lactobacillus, promoting their removal from the oral cavity by the salivary clearance and lowering the risk of dental caries. Controvertible evidence are reported between amylase and dental caries in young children when assessed using ELISA kits [16, 37].

**Salivary glycoproteins**

**Mucins**

Mucins found in human saliva protect teeth from demineralization induced by the acid produced from microbial metabolism. The AEP is composed primarily of $MG_1$, which serves as an attachment site for bacteria, as well as a permeable barrier for organic acid challenge and may aid bacterial colonization on oral surfaces. $MG_2$ can interact with oral microorganisms by promoting their agglutination. Role of mucins in ECC still remains doubtful [38].

**Proline-rich proteins**

The acidic PRPs (aPRP) play a role in the formation of the AEP and promote the adhesion of S. mutans on HAP surfaces. aPRPs are important in maintaining a supersaturation of calcium ions in relation to ionic phosphate in saliva. Furthermore, aPRPs play a significant role in the protection of tooth surfaces. Mass spectrometric analysis have revealed an elevated PRP peaks [32], whereas gel electrophoresis have shown reduction in ECC [39].

**Agglutinin**

Salivary agglutinin (SAG) was originally characterized as a S. mutans agglutinating glycoprotein. SAG is highly glycosylated and extremely sticky, potentially binding to the pellicle and interacting with unattached bacteria, resulting in the aggregation of bacteria that are more easily swallowed or flushed away. A correlation between increased levels of agglutinin in saliva and increased numbers of S. mutans in dental plaque and susceptibility to dental caries are seen. Caries-resistant individuals show a two-fold enhancement of saliva-mediated aggregation compared with that of the caries susceptible group [38]. However, owing to the lack of convincing evidence in the literature, SAG cannot yet be used as predictor for ECC incidence.

**Lactoferrin**

Lactoferrin is a non-enzymatic antibacterial protein which is widely spread in body fluids. In its iron free state it is known as apolactoferrin, that helps in binding of bacteria thus allowing ease of removal of these bacteria from the oral cavity via mechanical action of saliva or swallowing. Lower concentrations of salivary lactoferrin estimated using ELISA kits may be a risk factor for dental caries in children. [40] Further evidences are required for the conclusive role of

lactoferrin as a marker for ECC.

**Lysozyme**

Lysozymes are salivary proteins which play an important role in innate defense mechanisms. It is an antibacterial enzyme found in high amounts in body fluids including saliva. It can activate bacterial autolysins and destroy the cell walls. It promotes bacterial clearance through aggregation and adherence. Controversial evidence that supports the relationship between lysozyme and ECC obtained using western blotting and ELISA kits suggests its role in as a risk factor for ECC [40, 41].

**Soluble CD14**

Soluble CD14 is a glycoprotein involved in innate immunity which functions in Lipopolysaccharide/cell-wall products signaling. Role of sCD14 as a biomarker in dental caries in adult was proved in many studies [42]. Since specific immune responses have not yet been developed completely in 3-5 year old children, the increase in sCD14 level in saliva may be a reparative response of immune system to the inadequacy of such immunoglobulins as IgA and IgG, covering the gap between innate and specific immune responses. It seems that this inflammatory protein is developed in response to the bacterial stimulus of ECC but sCD14 assessed using sCD14 kits have found controvertible findings in children [43, 44].

**Antioxidants in saliva as markers**

The primary antioxidants include uric acid, albumin, ascorbic acid, glutathione and antioxidant enzymes (peroxidase) [45]. Salivary peroxidase brings about the control of oral bacteria that form dental plaque, to imbalances in the ecology, and which lead to dental caries. Salivary peroxidase catalyzes the peroxidation of thiocyanate ion ($SCN^-$) to generate oxidation products; this inhibits the growth and metabolism of many micro-organisms thereby inhibiting caries or slowing down the progress of caries [46]. The imbalances in levels of free radicals, reactive oxygen species, and antioxidants in saliva may play an important role in the onset and development of dental caries. A significant increase in the total antioxidants of saliva in ECC obtained using spectrophotometric method and antioxidant assay kits gives a clue to detect the disease in an early stage [47-49]. Commercial kits for antioxidant assessment per patient cost around 300-350 Indian Rupees.

**Cortisol in saliva as markers**

Cortisol is the major glucocorticoid hormone produced in the adrenal cortex. Corticosteroids cause atrophic changes in the major salivary glands, which may affect the total volume of saliva (quantity) and its composition (quality) [50]. Corticosteroids released during stress inhibit the immune response. This causes an inhibition of salivary immunoglobulins (especially IgA), as well as other antimicrobial proteins present in the saliva, such as, lactoferrin, lysozyme, and lactoperoxidase. This allows the cariogenic bacteria to multiply. Changes in the quality and quantity of the saliva concomitantly, may lead to the increased adherence and generation of a cariogenic biofilm on the tooth surfaces and increase caries susceptibility. Most of the children with ECC have shown an increase in the level of salivary cortisol. [50-52]. Cost for assessing salivary cortisol using ELISA kits varies from 150-250 Indian Rupees.

**Inflammatory markers**

**Cytokines**

Cytokines are the guiding factors of inflammation and its progression to tissue necrosis. Pulpitis is a sequelae of dental caries characterised by inflammatory mediators. Children with ECC were found to have increased IL-6 which has a strong correlation with severity and extent of carious lesions in the oral cavity, and therefore, minimizing its levels will help in improving the inflammatory condition in the oral cavity. Ratios of IL-6/IL-10 and IL-8/IL-10 cytokines may prove to be useful markers of inflammation because they provide information about the overall cytokine balance in the pulp. Significant elevation of IL-6, IL-8 and TNF-α with optimum sensitivity and specificity were also found that might imply their involvement as potential non-invasive diagnostic/prognostic markers in ECC [53, 54].

**End products of lipid peroxidation**

Lipid peroxidation is initiated by oxidative stress, which causes decrease in the antioxidant defense mechanism. Alteration of free radicals reactive oxygen species and antioxidants initiates thereby leads to progression of dental caries. The cellular damage caused by free radicals leads to peroxidation of lipids producing malondialdehyde (MDA). ECC is a multifactorial inflammatory disease that initiates lipid peroxidation reaction leading to the production of MDA which in turn alters the immunological mediators such as salivary peroxidase system and modifies the bacterial metabolism leading to dental caries. An increased level of MDA in saliva of children with active dental caries can be used as a diagnostic tool to predict the initiation and progression of the carious process [55].

**Discussion**

Many salivary biomarkers are studied in relation to children's caries experience. Salivary characteristics associated with increased caries experience includes: S mutans, S oralis, S mitis, Lactobacilli, Actinomyces, pH, Buffering Capacity, IgA, Histatins, Glucosyltransferase, Cortisol, Antioxidants, Inflammatory mediators, Electrolytes: Calcium, Phosphorus, Fluoride, Nitric Oxide. An accurate caries risk evaluation helps in identification of children at high caries risk and thereby allows to provide preventive therapies and improving treatment effectiveness. These salivary biomarkers should be utilized in clinical practice especially to identify the high risk groups. Salivary diagnostic testing not only presents the clinician with the ability to provide a higher standard of care for patients; it also can help increase the patient's understanding of the overall value of comprehensive care and subsequently facilitate positive behavioral changes.

According to this literature review the most useful biomarkers based on its diagnosis value, result accuracy and ease of execution in the dental office are summarised in table 1. Commercially available chairside kits for assessing various salivary biomarkers is summarised in table 2.

**Saliva as a Biomarker: Scope and Limitations**

Salivary composition is influenced by the method of collection and degree of stimulation of salivary flow. Changes in salivary flow rate may affect the concentration of salivary biomarkers and also their availability. Variability in salivary flow rate is expected between individuals and in the same individual under different conditions. Low concentration of some analytes compared to blood and serum (lipophilic molecules diffuse more easily than lipophobic molecules).

Hence Sensitive detection devices are required.

Collection of saliva is undemanding and noninvasive. It is cheaper to store and transport. Many of the hazards associated with blood collection do not apply to saliva. Saliva levels are a more accurate reflection of the active hormone in the body. HIV and hepatitis infections are much less of a danger from saliva than from blood.

No salivary parameter identified thus far is able to select caries-susceptible patients with high sensitivity and specificity on a single test basis [42]. Estimation of microbial load and physical properties of saliva are the only chairside estimation done in clinical setting. ELISA kits to assess antibodies and for assessing physical properties of saliva like FR, BC, pH and microbiological level are accurate, cost effective and simple methods. Kits for estimation of salivary cortisol, antioxidants are expensive compared to the other chairside kits for salivary biomarkers. Spectroscopy Methods used for the assessment of peptides are time consuming in day to day clinical practice.

## Conclusion

Salivary biomarkers have many potential roles in the diagnosis and management of ECC. Some of the biological and physical biomarkers have showed substantial evidence in predicting the risk for ECC. Nevertheless, the association between salivary factors and dental caries has yet to be established. Hence, clinicians are advised to integrate various salivary parameters with sociodemographic, behavioral and clinical risk in predicting and preventing ECC in their practice.

**Table 1:** Salivary biomarkers in ECC based on the evidence

| Salivary biomarkers | Substantive evidence as a biomarker | Controvertible evidence as a biomarker |
|---|---|---|
| Biological constituents | • S mutans, S oralis, S mitis<br>• Lactobacilli,<br>• Actinomyces | • Candida albicans |
| Physical properties | • pH,<br>• Buffering Capacity | • Salivary Flow,<br>• CAVI |
| Chemical constituents | • IgA,<br>• Histatins,<br>• Glucosyltransferase,<br>• Cortisol,<br>• Antioxidants,<br>• Inflammatory mediators,<br>• Electrolytes: Calcium, Phosphorus, Fluoride, Nitric Oxide | • Ig G, Ig M,<br>• Defensins,<br>• Proline rich proteins,<br>• Amylase,<br>• Lactoferrin,<br>• Lysozyme,<br>• End products of lipid peroxidation,<br>• sCD14,<br>• Agglutinins,<br>• Mucins,<br>• Electrolytes: Copper |

**Table 2:** Commercially available chairside kits for assessing salivary biomarkers

| Salivary biomarkers | Commercial kits |
|---|---|
| Biological constituents<br>• Streptococcus mutans and Lactobacillus<br>• Lactic acid production by cariogenic bacterias | • Saliva-Check Mutans (GC America)- Rs 6000- 6500 for 10 tests kit as on 29/03/19<br>• Ivoclar CRT Bacteria- Rs 8500- 9000 for 6 tests as on 29/03/19<br>• Dentocult Strip Test<br>• Caries Screen SM (Orion Diagnostica, Espoo, Finland)<br>• ClinproTM Cario L-PopTM (3M ESPE) |
| Physical properties<br>• pH<br>• Buffer capacity<br>• Viscosity | • Test strips- Rs 1170- 1800 for 100 strips as on 29/03/19<br>• Dentobuff Strip System (Orion Diagnostica, Espoo, Finland)<br>• Saliva-Check (GC America, Alsip, Ill.)- Rs 4000-4500 for 20 tests as on 29/03/19<br>• CRT Buffer<br>• Checkbuf<br>• Oral Tester Buffer<br>• SALIMAT device |

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