Review Paper

Cytokines, Minerals, Total Antioxidant Capacity, Nitric Oxide, and Salivary Characteristics as Biomarkers Associated With Early Childhood Caries: A Narrative Review

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Citation Salehabadi N, Moallem Savasari A, Nahvi A. Cytokines, Minerals, Total Antioxidant Capacity, Nitric Oxide, and Salivary Characteristics as Biomarkers Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review. 2023; 11(1):47-58. http://dx.doi.org/10.32598/jpr.11.1.870.4

http://dx.doi.org/10.32598/jpr.11.1.870.4

Article info:
Received: 04 Oct 2022
First Revision: 24 Dec 2022
Accepted: 29 Dec 2022
Published: 01 Jan 2023

Key Words:
Pediatric dentistry, Saliva, Minerals, Antioxidant, Dental caries, Nitric oxide, Cytokines

ABSTRACT

Background: Early childhood caries (ECC) is one of the multifactorial, acute, and progressive types of tooth decay. Some salivary biomarkers are associated with this disease.

Objectives: The data we used in our review were searched from articles published between 1950 to 2021 and using early childhood caries (ECC), children, saliva, salivary biomarkers, salivary characteristics, salivary minerals, cytokines, total antioxidant capacity (TAC) and nitric oxide (NO) as keywords, collected from official web pages (Scopus, PubMed, Embase and Google scholar) and documents published from different international institutions.

Methods: The search was limited to articles published in the English language. After the abstract screening, the full text of 194 relevant studies was reviewed. Finally, 101 relevant studies were selected.

Results: Cytokines with the potential to affect ECC include interleukin (IL)-1 (IL-1β), IL-6, IL-8, IL-10, IL-12, tumor necrosis factor-alpha (TNFα), a soluble cluster of differentiation (sCD)14-cluster of differentiation 14 (CD14), CD63 and vascular endothelial growth factor (VEGF). The minerals associated with the ECC are calcium (Ca), phosphate (PO_4^{3-}), fluoride (F), magnesium (Mg), iron (Fe), and lead (Pb).

Conclusion: Some characteristics of saliva that seem to concern ECC include salivary pH, salivary total protein, salivary total lipid, salivary buffering capacity, and saliva flow rate. Other vital factors observed to have significant effects on the ECC process involve total antioxidant capacity (TAC) and nitric oxide (NO).

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Introduction

In this review article, we discuss the salivary factors associated with early childhood caries (ECC), one of the severe and progressive kinds of dental caries. These factors include minerals, total antioxidant capacity, nitric oxide, cytokines, and other known factors that have been examined in previous articles on the subject [1, 2]. ECC, formerly known as "nursing caries", "baby bottle teeth decay", and "nursing bottle syndrome", is a clinical syndrome that affects infants and young kids and is characterized by rampant caries [2, 3]. Depending on the parents' awareness, the age of the studied children, the study population, and other criteria, the prevalence of caries in children ranged from % to 74% in various papers [1, 4-6]. The rapid progression of caries on the lingual surfaces of the lower molar teeth and labial surfaces of the upper anterior deciduous teeth is a prominent feature of this disease [2, 3].

Streptococcus mutans is a bacterium that has been widely reported in articles as the main causative agent of various caries in children's mouths [1, 4, 7-16]. The socioeconomic status of families, parental education and awareness, extended and incorrect breastfeeding with bottles or breast milk, consumption of sweet foods, and high-sugar diets constitute the majority of the recognized etiologies for this disease, even though some articles also attributed other factors, such as family size, gender, immaturity of children's immune systems and brushing techniques or lack of its use [1, 4-11, 17-20].

Prior studies have also recommended dietary changes, teaching parents to raise their level of awareness, utilizing fluoride in the form of toothpaste or varnish for children, and other methods to treat and prevent ECC [1, 4-6, 8, 9, 11, 17]. In this study, we investigated some biomarkers and salivary factors implicated in past studies more extensively, considering the significance and incidence of this form of progressive caries in children.

Methods

The data we used in our review were searched from articles published between 1950 to 2021 and using ECC, children, saliva, salivary biomarkers, salivary characteristics, salivary minerals, cytokines, total antioxidant capacity (TAC), and nitric oxide (NO) as keywords, collected from official web pages (Scopus, PubMed, Embase and Google scholar) and documents published from different international institutions. The search was limited to articles published in the English language. After the abstract screening, the full text of 194 related studies was reviewed. Finally, 143 relevant studies were selected. The full text of all the articles was found. The following types of studies were excluded from this review: animal studies, studies published in languages other than English, case reports, medical record reviews, meeting abstracts, editorials, letters, and commentaries. The included studies were those in which authors published information on ECC and salivary biomarkers in children.

Discussion

I) Cytokines

Cytokines are low molecular weight and biologically active proteins that affect several endocrine and metabolic functions and are known products of the immune system and inflammation [23]. Many cytokines are secreted by adipose tissue and help regulate the body's inflammatory system [21, 79]. Some of these regulatory cytokines are directly involved in obesity and glucose metabolism [24, 26]. Some adipokines may also regulate endothelial function [23, 26]. Here we review studies conducted on ECC and related cytokines individually (Table 1):

1- Interleukin (IL) 6 (IL-6): IL-6 is an adipokine associated with obesity and hyperinsulinemia [23, 24]. Reducing adipose tissue mass through weight loss (by exercise) reduces IL-6 and is associated with improved insulin sensitivity and endothelial function [23]. IL-6 is a known inflammatory factor associated with ECC and is elevated in ECC cases [21, 24, 26, 27]. In one study, the rate of this factor in children with caries was reported twice as high as that without caries [26]. This factor is one of the cytokines involved in the cytokine cascade in ECC children [21]. However, studies have shown that maintaining good oral hygiene, the inflammation level reduces, and the IL-6 level reduces [25]. It is known as a diagnostic biomarker in the saliva of ECC children [27].

2- Tumor necrosis factor alpha (TNFα): Like IL6, it is associated with obesity and hyperinsulinemia [23, 24] and regulates endothelial function [23, 26]. High levels of this factor have been found in children with caries [26, 27]. This factor is also one of the cytokines involved in the cytokine cascade in ECC children [21]. This factor also decreases with treatment and is known as a diagnostic biomarker in ECC saliva [27].

3- IL-8: Like the two mentioned above, this factor is one of the first responses of the host to inflammation and plays a role in immunity [26]. IL-8 is one of the cy-

tokines involved in the cytokine cascade in ECC children [21]. Interleukin 8 is secreted by macrophages and endothelial cells as a neutrophil-attracting chemical, leading to neutrophil infiltration. IL-8 promotes neutrophil attraction and increases the release of lysosomal enzymes against bacteria [22]. In one study, no significant difference was observed in the amount of this factor in the three groups of caries-free (CF), ECC, and severe early childhood caries (s-ECC), and it was concluded that the development of dental caries may be associated with neutrophil chemotaxis deficiency [22]. The increase (IL8) was significant in the presence of dental caries, and a significant positive relationship was observed between this factor and TNFα [27].

4- Soluble cluster of differentiation (sCD)14: A cluster of differentiation 14 (CD14) is a 55 kDa cell membrane glycoprotein that mainly detects bacterial products such as LPS, endotoxins, and peptidoglycans [29, 80]. In addition to being attached to the surface of monocytes, macrophages, and neutrophils, it can also be found as a soluble circulating component called sCD14 [81]. sCD14 is secreted by the main salivary glands in saliva and mediates the function of CD14-lacking cells [29, 80]. CD14 is present in the whole human saliva, and the accumulation of CD14 in parotid saliva is ten times higher than the whole saliva, and its concentration is comparable to the serum CD14 concentration [29]. The function of salivary CD14 is essential in maintaining oral health and even in intestinal homeostasis [29, 30]. sCD14 is a regulator regulating humoral and cellular immune responses through interaction with B and T cells [28]. Therefore, it plays a vital role in the natural immunity of the oral cavity [29, 80]. A significant increase in this factor in ECC children has resulted in studies introducing this factor as an inflammatory biomarker and innate immune response in these children [29].

5- CD63: CD63 is a membrane protein found mainly in azurophilic granules and is involved in the quality control of antimicrobial proteins [32]. The result of neutrophil activation is characterized by the secretion of HNP 1-3, leading to the excretion of azurophilic granules. This action can be detected with the help of the CD63 marker on the neutrophils' surface [31]. In ECC children, HNP 1-3 increases in saliva, but CD63 decreases on salivary neutrophils' surface [31].

6- Vascular endothelial growth factor (VEGF): This cytokine is affected in obesity and diabetes [26, 33, 35]. Increased VEGF levels in saliva are observed in disorders of glucose metabolism [26, 34, 36]. This factor is 63% higher in children with caries [26].

7- IL-1: It is one of the cytokines involved in the cytokine cascade in ECC children [21]. IL-1β is the active and systemic form of IL-1 and is involved in the production of IL-8 by macrophages and vascular endothelial cells. No significant difference in IL-1β levels was observed between the three groups of CF, ECC, and severe early childhood caries (s-ECC), and it was concluded that the development of dental caries might be associated with neutrophil chemotaxis deficiency [22].

8- IL-10: It is one of the cytokines involved in the cytokine cascade in ECC children [21].

9- IL-12: It is one of the cytokines involved in the cytokine cascade in ECC children [21].

II) Minerals

Salivary remineralization helps maintain the physical and chemical integrity of tooth minerals and thus protects teeth against decay [44]. Here we examine minerals associated with caries in previous studies.

1- Calcium: Saliva contains phosphate, protein, and bicarbonate buffer and is saturated with minerals of the teeth, such as calcium and phosphate [39]. Concentrations of glucose, calcium, and magnesium are the three primary parameters examined in the clinic due to their essential role in a wide range of biochemical reactions and, consequently, many health diseases [38]. Calcium and magnesium are closely related to oral health due to buffering activity [38, 40, 41]. The exact balance between demineralization and remineralization, which affects hard tooth tissues, depends on salivary calcium, phosphate, and alkaline phosphatase [37]. Pellicle proteins delay demineralization in the simultaneous presence of calcium and phosphate in saliva and plaque [43]. Genetic changes in genes involved in enamel growth are associated with calcium and phosphorus levels in saliva. As the presence of calcium in saliva increases, the pH of saliva becomes more acidic [82]. A significant positive correlation is observed between salivary calcium and the number of healthy teeth. Therefore, as calcium increases in saliva, DMFT decreases. Also, a significant positive correlation is observed between salivary calcium and periodontitis. Therefore, with calcium increasing in saliva, bleeding on probing (BOP) increases [40]. An association has been found between tooth decay with buffering capacity and buccal calcium and phosphate [44]. An article reported higher levels of salivary calcium in the CF group than in the group of children with ECC [37, 42]. It seems that saliva should be saturated with calcium and phosphorus to ensure the avail-

ability of sufficient amounts of products for remineralization, and the consumption of milk and cheese can be useful for this purpose [37]. Since a study among children has shown that the concentration of calcium in saliva increases with age, there seems to be a significant relationship between calcium and dental caries activity [42]. However, another study found no significant difference between the calcium levels of the two groups of ECC and CF children [62].

2- Phosphate: Saliva contains phosphate, protein, and bicarbonate buffer and is saturated with dental minerals, such as calcium and phosphate [39]. Demineralization is delayed by pellicle proteins in the simultaneous presence of phosphate and calcium in saliva and plaque [43]. The amount of phosphate in the saliva is significantly related to hydroxyapatite's insolubility on the enamel surface (inhibition of demineralization); of course, phosphate's protective effect in combination with fluoride will be much more effective than the presence of each of them alone [39]. Studies have also shown that in the presence of phosphorus compounds, such as sodium tripolyphosphate and sodium pyrophosphate tetrabasic, the dissolution of hydroxyapatite (HA) in tooth enamel (part of the demineralization process) is reduced [45, 46]. Premature loss of deciduous teeth with intact roots has been reported in children with hypophosphatasia who have low bone mineral density and abnormal fractures [83]. Genetic changes in genes involved in enamel growth are associated with calcium and phosphorus levels in saliva. As the presence of phosphate in saliva increases, saliva flow increases, and, like the presence of calcium, the pH of saliva becomes more acidic [82]. A significant positive relationship was observed between the rate of active caries and phosphate concentration in the gingival crevice fluid of carious teeth [44]. In a study using sodium fluoride (NaF) mouthwash, a significant reduction in phosphate ions in plaque was observed [84]. A study reported that the amount of phosphate was lower in the CF group than in the ECC [37]. Almost contradictory to the previous study, in another study, the results show that phosphate levels increase, as caries activity decreases [41]. Nevertheless, another study found no significant difference between the phosphate levels of the two groups of ECC and CF children [62].

3- Fluoride: Fluoride has been introduced as a key to preventing tooth decay. It reduces the prevalence and severity of caries in three general ways: I) Reduction of enamel demineralization caused by fermentation of carbohydrates in dental plaque in the presence of cariogenic bacteria. II) Remineralization of primary enamel caries. III) Preventing the activity of bacteria in dental plaque [54]. Fluoride, like phosphate, aids in the remineralization process by reducing hydroxyapatite (HA) dissolution in tooth enamel. When phosphate is present, it has a double effect [39]. The American Academy of Pediatric Dentistry (AAPD) recommends fluoride intake for children, including community water fluoridation or, in its absence, following a diet containing fluoride and fluoride varnish on a scheduled basis [54]. Use of fluoride as a varnish [47, 52-55], silver diamine fluoride (SDF) [48-50, 53], mouthwash [47, 54, 56], water fluoridation [47, 48, 51, 54], milk fluoridation [54], toothpaste [47, 54] and fluoride foams [54, 56] among others have shown the effect of preventing demineralization in children. In a study using sodium fluoride (NaF) mouthwash treatment, a significant increase in F_2 ion in plaque was observed [84].

4- Magnesium: Concentrations of glucose, calcium, and magnesium are the three main parameters examined in the clinic due to their essential role in a wide range of biochemical reactions and, consequently, many health diseases. The use of saliva has been suggested as a safer, stress-free, and non-invasive alternative for rapid screening of glucose, calcium, and magnesium in clinical settings, and these three parameters have been identified as disease biomarkers [38].

Magnesium with calcium is closely related to oral health due to its buffering activity [38, 40, 41]. It can also play a role as a normalizing factor because it is very stable during the day [38].

5- Iron: Recent evidence suggests that salivary gland function is affected by maternal iron deficiency and lead exposure during pregnancy. Children's low-iron nutritional formulas have pathogenic effects on caries; decreased salivary flow is seen in children with iron deficiency anemia. Iron has a local effect on preventing decay [7]. A study states that since nutritional iron deficiency is prevalent in developing countries, iron supplements are used to compensate for this problem and due to the discoloration of the teeth after iron intake, parents believed that iron drops are a cause of tooth decay in children. This study disproves this belief and states that ferrous sulfate reduces tooth decay progression in pathogenic diets [57]. Elevated blood lead levels are common in economically disadvantaged children. A relationship exists between high enamel lead and the prevalence of tooth decay [58-60]. Exposure to lead also increases caries and decreases saliva flow (Table 2) [7, 60].

Table 1. ECC-related cytokines and their function

Cytokine	Function	References
IL-1 (IL-1β)	Involved in cytokine cascade, involved in IL-8 production by macrophages and endothelial cells	[21, 22]
IL-6	Associated with obesity and hyperinsulinemia, regulation of endothelial function, ECC-related inflammatory factor, involved in the cytokine cascade, one of the first host responses to inflammation, a diagnostic biomarker in ECC saliva in children	[21, 23-27]
IL-8	Involvement in cytokine cascade, neutrophil uptake, and increased release of lysosomal enzymes, among the first host responses to inflammation, a diagnostic biomarker in ECC saliva in children	[21, 22, 26, 27]
IL-10	Involved in the cytokine cascade	[21]
IL-12	Involved in the cytokine cascade	[21]
(sCD14)CD14	A potent regulator of humoral and cellular immunity, an important role in the natural immunity of the oral cavity, inflammatory biomarker, and innate immune response	[28-30]
CD63	Marker on the neutrophils' surfaces, quality control of antimicrobial proteins	[31, 32]
TNFα	One of the first host responses to inflammation, a diagnostic biomarker in the saliva of ECC children, associated with obesity and hyperinsulinemia, involved in cytokine cascade	[21, 23, 24, 26, 27]
VEGF	Related to obesity and diabetes, saliva associated with impaired glucose metabolism	[26, 33-36]

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Abbreviations: ECC: Early childhood caries; IL: Interleukin; sCD: Soluble cluster of differentiation; CD: Cluster of differentiation; VEGF: Vascular endothelial growth factor.

Table 2. ECC-related minerals and their effect on caries

Minerals	Chemical Symbol	Effect on Caries	References
Calcium	Ca	One of the components of saliva and minerals of teeth, buffering activity, related to oral health, one of the factors affecting the balance between demineralization and remineralization, helping to prevent demineralization, helping remineralization, a significant relationship with the number of healthy teeth, related to active caries	[37-44]
Phosphate	$P(PO_4^{3-})$	One of the components of saliva and minerals of teeth, one of the effective factors in the balance between demineralization and remineralization, helping to prevent demineralization, reducing the dissolution of enamel hydroxyapatite, related to buffering capacity, related to the rate of active caries	[39, 43-46]
Fluoride	F	A key to prevent tooth decay, reduce demineralization, increase remineralization of primary enamel caries, prevent bacterial activity, reduce the dissolution of enamel hydroxyapatite	[39, 47-56]
Magnesium	Mg	Buffering activity, related to oral health, as a normalizing factor in saliva	[38]
Iron	Fe	Preventing caries	[7, 57]
Lead	Pb	Increasing caries, decreasing salivary flow	[7, 58-60]

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Table 3. Saliva characteristics and their function

Saliva Characteristics	Function	References
pH	Effect on enamel demineralization, caries progression, related to DMFT and black stains on enamel surfaces	[41, 42, 61-70]
Total protein	Effective in oral health, aids in personalization, associated with dental caries	[25, 42, 65, 71, 72]
Flow rate	Preventing tooth decay and demineralization, reducing saliva pH	[7, 61, 65, 73, 74]
Buffering capacity	Neutralizing acidity caused by bacterial activity, preventing tooth decay	[41, 62, 65
Total lipid	Associated with caries	[74-78]

DMFT: Decayed, missing, and filled teeth.

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III) Characteristics of saliva

1- Salivary pH: It seems that the effects of lowering the saliva's pH on the incidence of caries are such that acids and acidic saliva cause demineralization activity [63, 65]. The critical absolute pH value for enamel demineralization is between 5.2 and 5.5. Enamel demineralization can convert primary caries into cavities [63]. Changes in pH are inversely related to DMFT, meaning that people with lower pH have higher DMFT and caries growth rates [61, 69]. A study also stated that the pH of children's saliva is the best predictor of DMF [63]. Changes in salivary pH are decreased in children with ECC, and the oral environment becomes more acidic [62-65, 68, 69]. Conversely, in children without caries or decreased caries activity, the saliva's pH increases and the oral environment becomes more alkaline [41, 62]. A study found that the decreased salivary pH in ECC children was due to a diminution in saliva flow, which slowed the transfer of caries-protective substances (such as bicarbonate) at the caries site [65]. Studies have also shown an association between the pH level of children's saliva and black spots on tooth enamel. These black spots, specific manifestations of insoluble plaques, were significantly associated with high pH in one study, but another study found no significant association between salivary pH and these manifestations [61, 66]. Despite all the studies showing a strong relationship between pH decrease and caries incidence, some studies show no relationship between salivary pH and buffering capacity and caries [42, 67].

2- Total salivary protein: Many of the components that make up the mouth and are effective in host defense are protein in nature. The concentration of proteins and polypeptides in saliva is essential to maintain oral health because the extent and severity of oral diseases are often associated with quantitative and qualitative changes in the salivary proteomes [25]. Salivary protein profiles can help personalize age-related changes that seem to be related to dietary habits, the environmental and geographical factors [71]. Total salivary protein also helps teeth grow in children [71]. The average salivary protein level has been measured at 2-4 mg/mL [71]. Very high and very low molecular weight proteins are present in the saliva of children and adolescents more than in adults [71]. The total salivary protein level is elevated with increasing caries, and in children with ECC [42, 65, 72]. One study found that total saliva protein was inversely related to salivary flow [73]; however, another study showed no significant relationship between total salivary protein and caries [42].

3- Saliva flow rate: Increasing the speed of saliva flow prevents demineralization and decreases saliva pH by increasing the contact of salivary anti-caries factors with teeth [65]. Decreased salivary flow has a catastrophic effect on dental health; taking medications, such as antihistamines, or exposure to certain factors, such as lead can significantly affect the occurrence of this process [7]. Salivary flow is decreased in children with ECC [65, 74]. Saliva flow rate is inversely related to saliva's total protein [73]. In contrast to other studies, a study shows no association between salivary flow and caries [42].

4- Salivary buffering capacity: Bicarbonate, phosphate, and the buffering system of salivary proteins neutralize the acidity resulting from bacterial activity following the consumption of beverages and foods, thereby preventing the colonization of pathogenic microorganisms [65]. Buffering capacity is higher in children without caries, and decreasing caries activity increases buffering capacity [41, 62]. Therefore, it is predictable that the buffering capacity is decreased in children with ECC [65]. However, in contrast to the above studies, a study stated that no relationship was found between buffering capacity and decay [42].

5- Salivary total lipid: Salivary lipids can play a vital role in tooth decay due to their constituent glycoproteins [77]. Common lipids in saliva include neutral lipids, glycolipids, and phospholipids. Lipids are obtained from three sources, serum elements, cell extraction, and glandular origin [74]. The role of salivary lipids in causing tooth decay is not well understood [74]. However, few studies confirm a positive association between salivary triglycerides and total lipids and caries occurrence, while a disagreement exists about salivary cholesterol levels [75, 76, 78]. Triglycerides and lipids are elevated in ECC, but cholesterol levels do not differ (Table 3) [74].

IV) Total antioxidant capacity (TAC)

The reaction of reducing the molecule of oxygen to water produces too much energy, which can lead to the formation of free radicals or reactive oxygen species (ROS) [85]. Oxidative stress is attributed to an imbalance between the production of free radicals, such as reactive oxygen species (ROS), and the activity of enzymatic and non-enzymatic antioxidant systems. Antioxidant systems provide the body with a strong defense against free radicals [86, 87]. Biomarkers known for oxidative degradation in saliva are 8-hydroxy-deoxyguanosine (8-OHdG) and malondialdehyde (MDA). Enzymatic antioxidants (such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) and non-enzymatic (such as uric acid (UA) and glutathione (GSH)) can be measured in saliva and together form total antioxidant capacity (TAC) [86, 88]. A significant increase in antioxidant levels in the saliva of people with caries has been observed in all age groups of children, adolescents, and adults [36, 42, 85, 89-92]. As expected, TAC levels in children with ECC are significantly higher than in CF children therefore TAC has been identified as a crucial biomarker in saliva that can help diagnose ECC [42, 64, 65, 72, 85, 86, 91-93]. In children with ECC, an increase in TAC levels results in a decrease in oxidative degradation at the caries level, and an increase in TAC levels (increased UA and SOD) is a defense mechanism to protect oral tissues from the damaging effects of endogenous or exogenous reactive oxygen and nitrogen to reduce the level of oxidative degradation [86]. MDA, which is a type of TAC, is known as an indicator of oxidative stress. One of the activities of free radicals that disrupts cell integrity is lipid peroxidation; MDA is a stable end product of cell membrane peroxidation [94]. MDA levels are increased in children with ECC [65].

V) Nitric oxide (NO)

NO is a salivary biomarker that is chemically or enzymatically synthesized in the body, chemically by dietary nitrate metabolism, and enzymatically by the breakdown of L-arginine by the nitric oxide synthases secreted by the salivary glands and other tissues [95, 96]. The specific nature of nitric oxide is critical. Nitric oxide is essentially a free radical that can increase oxidative stress; on the other hand, it has been shown that it prevents the progression of dental caries and plays a role in immunity with an antibacterial effect and induction of macrophage-mediated toxicity [72, 97-99]. In children with ECC, NO levels are higher than in CF children [98-101]. The mean nitrite level was reported to be more than 50 µM in children with dental caries, but less than 40 µM in children without caries [98-101]. In contrast to the above studies, some studies have shown that NO levels in the saliva of children with caries are lower than others, and a significant difference is observed [18, 72, 97, 98].

Conclusion

Cytokines with the potential to affect ECC include IL-1-IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα, sCD14)CD14), CD63 and VEGF. The minerals associated with the ECC are calcium (Ca), phosphate (PO_4^{3-}), fluoride (F), magnesium (Mg), iron (Fe), and lead (Pb). Some Characteristics of saliva that seem to be related to ECC are salivary pH, salivary total protein, salivary total lipid, salivary buffering capacity, and saliva flow rate. Other crucial factors observed to have significant effects on the ECC process involve TAC and NO.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors

Authors contributions

Conceptualization: Negareh Salehabadi and Azam Nahvi; Literature search and data analysis: Negareh Salehabadi, Aaryousha Moallem Savasari; Initial draft peparation, editing & review: Azam Nahvi, Negareh Salehabadi and Aaryousha Moallem Savasari.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgements

We sincerely thank the experts of the research center of Mazandaran University of Medical Sciences and all those who helped us in finalizing this research.

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