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Salivary Biomarkers and Early Childhood Caries

Universidade Fernando Pessoa
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Trabalho apresentado à Universidade Fernando Pessoa
como parte dos requisitos para obtenção
do grau de Mestre em Medicina Dentária

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(Clémentine Alice Juliette Degrève)

ABSTRACT

Background - Dental caries is the most common chronic childhood disease. Early Childhood Caries is a severe and early form of the carious disease that can compromise child's oral and systemic health.

It is essential to make use of all existing preventive methods to avoid and control this serious disease. The identification of caries salivary biomarkers through salivary chair side tests, has been considered a good strategy to identify risky children in order to implement early preventive ECC programs.

Objective: This review will focus on how salivary tests can detect caries related salivary biomarkers, to help identify children at risk of caries development, therefore assisting on ECC prevention strategies.

Methods: A bibliographic search was carried out using the keywords: "early childhood caries", " salivary factors", "salivary biomarkers", "pediatric dentistry", "caries prevention". After applying the search limits and the inclusion/exclusion criteria a total of 22 articles were selected for review.

Keywords: early childhood caries, salivary factors, salivary biomarkers, dental caries, prevention.

RESUMO

Introdução: A cárie dentária é a doença crónica da infância mais comum. A Cárie Precoce da Infância corresponde a uma forma grave e precoce da doença, que compromete a saúde oral e geral da criança.

É essencial usar todos os métodos preventivos existentes para evitar e controlar esta grave doença. A identificação de biomarcadores salivares da cárie dentária avaliados através de testes salivares passíveis de serem efetuados no consultório dentário, tem sido considerada uma boa estratégia para identificar crianças de risco e implementar programas preventivos precocemente.

Objetivo: O objetivo foi fazer uma revisão dos testes salivares que identificam biomarcadores salivares de cárie dentária e que, desta forma, posibilitam a identificação de crianças de risco, ajudando assim na prevenção da CPI.

Metodos: Foi efetuada uma pesquisa bibliográfica usando as palavras-chave: "early childhood caries", " salivary factors", "salivary biomarkers", "pediatric dentistry", "caries prevention". Aplicados os limites da pesquisa e os critérios de inclusão/exclusão foram selecionados para revisão 22 artigos.

Palavras-chave: Cáries precoces da infância, fatores salivares, biomarcadores salivares, cárie dentária , prevenção.

DEDICATED TO:

Tout d'abord à mon Père, dont l'exercice m'a inspiré une vocation.
Avec tact et mesure je souhaite être à la hauteur de prendre ta relève,

À ma Mère, mon Frère et Polo pour leur soutien inconditionnel dans mes aventures,

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INDEX :

TABLE INDEX :

ABBREVIATIONS

ECC: Early Childhood Caries

CPI: Caries precoces da infância

pH: Hydrogen potential

LPO: Lactoperoxidase

AAPD: American Association of Pediatric Dentistry

CTR: Carie test risk

CFU : Colony forming unit

ml : milliliters

DMFT: index of Decayed (D), Missing (M) and Filled tooth (F) teeth

I. INTRODUCTION

Cavities in infants and toddlers have a distinctive pattern, nowadays we call it Early Childhood Caries (ECC). Because of the plurality of definitions, for this literature review, we will use the definition of the American Academy of Pediatric Dentistry: "Early Childhood Carie is defined as 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"(AAPD guidelines 2016).

The first sign of ECC in young children is demineralized areas on the cervical surfaces of anterior maxillary teeth, the disease evolves with the appearing of carious lesions on the occlusal surfaces of second primary molars and distal surfaces of first primary molars (Göran Koch and Sven Poulsen, 2009).

In developing countries ECC is now considered at epidemic proportions, the AAPD epidemiological data (2011-2012 United States national survey) shows that ECC remain specially highly prevalent in poor and near poor United States preschool children (Çolak et al., 2013).

Because of the strong correlation between ECC and later caries development in primary and permanent teeth the early diagnosis and the prevention of ECC is essential to promote a good future oral health (Koch and Poulsen, 2009).

It is important to point out that the consequences of ECC and poor oral health can influence children general health and well-being. It can lead to pain, discomfort, acute and chronic infections, risk of hospitalization and emergency dental visits. It may also be responsible for eating difficulties and altered sleeping habits, loss of weight, delayed growth, school absenteeism that can diminish the learning skills and the cost of treatments is usually high (Çolak et al., 2013) (AADP guidelines 2016).

Different factors play a role in the onset and development of this disease: Caries-conducive dietary practices (frequent nighttime bottle-feeding, ad libitum breast-feeding, repeated bottle or no-spill cup feeding with juice or other sugar-added drinks, and frequent in-between meal consumption of sugar-added snacks), inadequate oral hygiene, early oral contamination with

cariogenic bacteria such as *Mutans Streptococci* and *Lactobacilli* and also socio-economics factors. (Koch and Poulsen, 2009) (AADP guidelines 2016).

The carbohydrate rich feeding practices are directly responsible for the production of acids by dental plaque, but the frequency and timing of these cariogenic feeding habits, will boost the caries development process. During sleeping periods, salivation is reduced and can not guarantee its anti-caries role as effectively as during the awake moments, thus repeated night carbohydrates intake will result in a faster enamel demineralization and produce severe tooth destruction (Koch and Poulsen, 2009).

This review will focus on how simple salivary tests applied precociously, can detect caries related salivary biomarkers, which will help identify children at risk of caries development, therefore assisting on ECC prevention strategies applications.

Materials and methods: This thesis aim was to perform a bibliographic review of scientific literature published in the last 10 years on the importance of assessing salivary biomarkers to the prevention of ECC. To this end, during the months of September to February 2017, a bibliographic search was carried out in PubMed, B-on and PMC databases using the following keywords : "early childhood caries", " salivary factors", " salivary biomarkers", "pediatric dentistry", "caries prevention". In the search the following limits were used: articles published in the last 10 years, abstract and full text available, studies in humans and articles in English, French and Portuguese. A total of 98 articles obtain and then selected, firstly by the titles, followed by the reading of the abstracts and, finally, by reading the whole article. Thus obtaining 22 articles for review (Table 1).
Pediatric Dentistry and Oral microbiology books were also considered as a complementary help for this review.

Key words : (Pubmed database, B-on, PMC, Medline)	Number of articles found after the first research	Number of articles found after application exclusion criteria with the filters (Less than 10 year, Studies on humans,Abstract available, full text accessibility, Articles in English,Portuguese and French)
"Salivary **biomarker"** and **"**Early childhood **caries"**	8	5
"Early childhood **caries"**	997	32
"Early childhood **caries"** and **"S**alivary **factors"**	32	5
"Salivary factors" and **"H**igh caries risks **children"**	74	2
"ECC" and **"P**ediatric **dentistry"**	11	3
"Caries prevention" and **"S**alivary **tests"**	398	47
" Salivary **factors"** and **"C**aries **prevention"**	11	4

Board 1 : Materials and Methods

II. THEORICAL FRAME

1. "Whole saliva"

The saliva that we can find in the oral cavity comes mainly from three major salivary glands: sublingual, submandibular and sublingual glands. Minor salivary glands secretions (such as bucal, labial, palatal, palatoglossal and lingual), gingival crevicular fluid, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, epithelial cells, erythrocytes, leukocytes and food debris also contribute to what we call "whole saliva" but in smaller volume (Lazaro, 2015).

Mouth microbiota develop from virtual sterility at birth into one of the most heavily colonized parts of the body. The changes in oral microbiota during the first years of life lead to the development of a stable bacterial ecosystem in the mouth called dental plaque. The first colonizers are facultative anaerobic microorganisms, such as *Streptococci* and *Actinomyces*, followed by more anaerobic genera (Marsh and Martin, 2009).

Studies indicate that early microbial colonization of the oral cavity with early establishment of cariogenic species like *Mutans Streptococci* have been associated with increased risk of dental caries (Holgerson, 2015).

It is also proved that changes in microbial flora during the early variation period, are related to heath issues such as obesity, allergy, intestinal diseases during childhood, obesity and myocardial infarction in adults (Holgerson, 2015).

Therefore prevention of ECC can also assure help to assure a better good general health in the future (Guo and Shi, 2013).

2. Salivary pH

The pH regulation in the oral cavity and particularly on tooth surface is accomplished by saliva and its buffer ability. Normal salivary non-stimulated pH varies between 6.75 and 7.25. Food and drinks ingestion can produce changes in salivary pH, as well as medication (eg. antiacids) and systemic conditions (eg. Gastroesiphageal reflux). (figure 1) (Marsh, and Martin, 2009).

At a 5.5 or lower saliva pH enamel hydroxyapatit*e* crystals begin to demineralize. Several studies have showed that salivary pH from ECC children group was statistically lower than that in caries-free children. (Singh, 2010 and Animireddy, 2014).

3. The Buffer capacity

The oral cavity is quite often exposed to pH variations, which can damage teeth (erosion) or mucosal surfaces. Saliva contains buffering agents that promote pH recovery to the normal range as fast as possible. In resting saliva the most important buffering agent is inorganic phosphate, in stimulated saliva it is carbonic acid/bicarbonate system (Prabhakar, 2009).

Clinical studies have shown a negative association between salivary buffering capacity and dental caries but salivary buffer capacity tests alone have only a weak association with caries activity or future caries outcome (Prabhakar, 2009).

Another study by Animireddy (2014) showed that saliva buffering ability from ECC children group was statistically lower than that in caries-free children.

In spite of these results it's important to stress that the major caries attack occurs within the dental plaque and on the subsurface enamel, were saliva buffer capacity has only a minimal effect. Therefore the buffer capacity measured in the whole saliva is more accurate as a predictor of dental erosion than of caries risk (Prabhakar, 2009).

4. The Salivary Flow rate

Saliva flushing and neutralizing effects, also called saliva clearance are probably the most important factors in saliva to help prevent dental caries. In general, the higher the flow rate, the faster the clearance, and the higher the buffer capacity. So without any doubt salivary flow is the most important salivary factor to help control the development of caries (Hart et al., 2011 and Prabhakar, 2009).

Thus the whole saliva flow rate will be a relevant factor to evaluate the susceptibility to caries development. However, there isn't a linear association between the flow rate and caries activity, instead there is a so called "threshold effect". This means that when the salivary flow rate falls down the "threshold level" caries activity increases rapidly. In practice, the unstimulated salivary flow needs to be higher than 0,7 ml/min, otherwise it is considerate as a risk for caries development (Guo and Shi, 2013).

Salivary flow rate is easily evaluated: resting saliva flow rate it assessed by simple observation of lower lip labial secretion and counting the time needed for droplets of saliva to form at the gland orifices; stimulated saliva is measured by chewing a piece of wax and spitting into a cup for a delimited time and measuring the milliliters of saliva obtained. (Navazesh and Kumar, 2008).

5. Proteins and Glycoproteins

Saliva contains protective factors such as mucins, immunoglobulins (IgA, IgG, and IgM), and some enzymes: Lactoperoxydase (LPO), lysozyme. These proteins play an important role in the maintenance of oral health, they are known to have antibacterial, antifungal or antiviral properties (Gornowicz, 2014).

i. Proteins:

Among salivary proteins mucins, histatins, defensins, proline and lactoferrin have anti-bacterial properties by making the bacterial wall porous. Mucins are also responsible for saliva's viscosity and lubricity. Protection against desiccation, oral mucosa lesions and bacterial aggressions is ensured by the viscous barrier formed on the surface of mucous membranes by mucins. Histatins accumulate an antifungal and bactericidal activity. (Hart, 2011).

ii. Immunoglobulin A (IgA's):

IgA's plays the main role as secretory immunoglobulin, it prevents dental caries by inhibiting bacterial adherence and inactivating bacterial enzymes and toxins (Gornowicz, 2014).

iii. Enzymes:

Lysozyme and LPO have an antiseptic action by lysing the bacterial walls (Gornowicz, 2014).

Unlike other salivary biomarkers, the assessment of these particular protective factors can not be easily accomplished using a chair side test, during our daily dental practice. When considered important for the patient's adequate oral diagnosis and treatment, laboratory tests, such as Laboratory enzyme-linked immunosorbent assay tests (ELISA), can de requested, for chair side tests are not specific enough to mesure possible alterations relating salivary proteins and glycoproteins (Bhalla, 2010).

These specific tests have been found to be helpful in identifying caries risk populations. (Hart, 2010).

These specific tests have been found to be helpful in identifying caries risk populations. (Hart, 2010). A study lead by Agnieszka Gornowicz, in 2014 showed that high caries activity in adolescents was associated with increased levels of some salivary components such as sIgA, histatin, and LPO, which possess strong bactericidal or bacteriostatic effects, resulting in aggregation of oral bacteria and their clearance from the oral cavity. (Gornowicz, 2014)
In the case of ECC study results are not conclusive, because of the complexity of the disease, it is actually impossible to link ECC with a single risk factor, but more information on the molecular epidemiology of salivary biomarkers can also justify the use of this methodology as a diagnostic tool in ECC (Bhalla, 2010).

6. Saliva Check buffer Test

The Saliva-Check Buffer (figure 2) is a chair side test that measures and assesses the patient's saliva condition, helping to determine the patients' caries risk. It evaluates hydration, salivary consistency, resting saliva pH, stimulated saliva flow, stimulated saliva pH and saliva buffering capacity. (Singh, 2015)

Resting Flow Rate Assessment:
Count the time needed for droplets of saliva to form at the orifices on the lower lip minor glands. More than 60 seconds corresponds to a low resting flow rate (red section), between 30-60 seconds is considerate a normal flow rate (yellow section) and less than 30 seconds corresponds to a high flow rate (green section) (GC® dental corp. America, 2017).

Salivary Consistency Visual Assessment:
-Sticky frothy (only residues) (red section)
-Frothy bubbly: increased viscosity (yellow section)
-Watery clear saliva: normal viscosity (green section) (GC® dental corp. America, 2017).

Resting Saliva pH Test:

Patients' saliva is collected by spitting into a collection cup and pH is measured with a pH indicator strip dipped for 10 seconds. The results will be found by comparing the color revealed on the pH indicator strip with the table in figure 3:

-Highly acidic saliva will be in the red section, pH 5.0 - 5.8.
-Moderately acidic saliva will be found in the yellow section, pH 6.0 - 6.6.
-Healthy saliva will be in the green section pH 6.8 - 7.8. (GC® dental corp. America, 2017)

Stimulated Saliva Flow Rate Assessment:

The patient chews a piece of paraffin for 30 seconds and spits into the collection cup and continues to chew during five minutes, expectorating every 15-20 seconds into the cup.
If the volume is inferior to 3.5 ml the stimulated saliva flow rate is very low (red section), between 3.5-5 ml is low (yellow section) and superior to 5 ml it is considerate normal (green section) (GC® dental corp. America, 2017).

Stimulated Saliva pH Test:

A pH indicator strip is inserted into the stimulated saliva previously collected. The result is compared with the pH obtained in resting saliva pH test. A normal result should give the same values obtained in the resting saliva pH test (figure 3) (GC® dental corp. America, 2017).

Buffer Capacity Test:

Using the pipette in the Saliva Check Buffer Test kit some of the stimulated saliva previously collected is drawn up and a drop is placed onto each of the 3 test pads represented in figure 4A. Wait two minutes and compare the color of each pad with the color table below.
Some up the points scored for each color pads and record the results. Saliva buffering ability will differ according to the results in figure 4B (GC® dental corp. America, 2017).

When all the tests are finished, the dental professional can analyze the global results:

- High Risk, a majority of red points: alert on an existing problem;
- Moderate risk, a majority of yellow points: the patient needs careful monitoring;
- Low risk, the majority of points in the green section: no problem (GC® dental corp. America, 2017).

Gathering these results and evaluating them together, will point out the at risk patients and allow the dental professional to adapt a plan for a better caries prevention. A plan that fits the patients' special needs and that includes bringing their saliva back into balance. Understanding each individuals' saliva characteristics can give valuable informations to determine treatment choices and strategies for a better oral health (Animireddy, 2014).

7. Saliva based Carie Activity Tests:

An individual evaluation of salivary factors responsible for caries risk, will help the dentist to follow the best preventive and treatment strategy in order to fulfill each patients' needs.
The use of salivary chair side tests have demonstrated several advantages in this context: they improve patient-doctor communication, help in motivation and increase patients´ oral heath awareness by early detection of problems. Caries activity tests have been used in dental research for many years and some tests have been adapted for chair side usage. Saliva is the major component of most caries activity tests. Presently, there is no ideal test, but caries activity tests are considerate a valuable aid to motivate patients in a plaque control program and help categorize patients in high, medium and low caries activity (Prabhakar, 2009).

i. Salivary *Mutans Streptococci* and *Lactobacilli* Test:

Microbiological studies have shown that *Mutans streptococci* is the chief pathogen associated with childhood dental caries onset and that *Lactobacilli* are associated with dental caries progression. (Hart et al., 2011)

These species are capable of metabolizing dietary carbohydrates to acid, leading to enamel demineralization, and creating a lower plaque and saliva pH to help colonies grow even more. High counts of *Mutans streptococci* and *Lactobacilli* in saliva indicate a high caries risk status. If protective factors cannot take effect, carious lesions will develop. These facts explain the importance of assessing salivary concentration of *Lactobacilli* and *Mutans streptococci* in children to prevent EEC and its implications (Marsh, 2006).

CTR Bacteria – Caries Risk Test

This chair side test intends to evaluate patients' caries risk by assessing *Mutant Streptococci* and *Lactobacilli* counts in saliva (Ivoclar vivadent®, 2017)(Bhayat, 2013).

Step by Step Procedure : (Ivoclar vivadent®, 2017) (figure 5)

1. Stimulate the patient's salivary secretion by chewing the enclosed paraffin tablet
2. Collect saliva in a graduated cup.
3. Remove the agar carrier from the test tube.
4. Place a NaHCO3 seal on the base of the test.
5. Gently remove the protective sheets from the two agar surfaces without touching the agar.
6. Wet both agar surfaces with saliva using a pipette, without touching the agar.
7. Allow excess saliva to drain.
8. Return the agar carrier to the test tube and close it
9. Using an indelible pen, indicate the name of the patient and the date of sampling on the test tube.
10. Keep the test tube in a vertical position in the incubator, for 48 hours at 37 ° C.
11. After removing the test tube from the incubator compare the density of Mutans Streptococci and lactobacilli colonies with the corresponding evaluation pictures in the enclosed model chart (figure 6) (Ivoclar vivadent®, 2017).

Findings of 10^5 CFU or more of lactobacilli and mutans streptococci per ml saliva indicate a high caries risk (Ivoclar vivadent®, 2017).

CRT bacteria provides fundamental informations on *Mutans streptococci* and/or *Lactobacilli* salivary counts. As a result, you have the possibility of introducing adequate counter measures at an early stage, even before any changes in the tooth structure can be detected or when carious lesions are still in initial state of development. (Ivoclar vivadent®, 2017).

After a 48 hour incubation period these test results are ready and their interpretation is easily understood. So, parents and children, can evaluate themselves the number of cariogenic bacteria present and caries risk becomes visible. This visual evidence reveald by the salivary test will help them to verify the existing problem, and allow a stronger adherence to the preventive and therapeutic stategies proposed by the dentist. (Bhayat, 2013).

ii. Saliva-Check Mutans:

This test by GC Dental Products Corp ® is a simple *Mutans Streptococci* detection kit that does not require specific equipment such as an incubator, only a timer and the results take only15 minutes. The *Mutans Streptococci* is detected by two monoclonal antibodies and there is no contamination by any other bacteria. (Singh, 2015)

Instructions of use:

1. The patient chews the gum provided for 1 minute to stimulate saliva secretion,
2. A sample of stimulated saliva is collected in the mixing container.
3. Add one drop of reagent 1 (figure7) to the saliva and tap the mixing container 15 times for 10 seconds with a finger to mix them. (Reagent 1 is an alkaline solution which will break down and dissolve viscous components of saliva).
4. Add 4 drops of reagent 2 (yellow) to the mixing container and shake the device for several seconds. Check for a color change in the sample to light green (alkaline to neutral pH) (Figure 7).
5. Using the graduated pipette, take saliva from the mixing container and dispense it into the sample window at the end of the test device (figure 8).
6. Leave it for 15 minutes at room temperature. A red thick line should be observed in the control (C) window of the test, indicating that the test is working properly.
7. At the same time, check the test (T) window: the result is positive if a thin red line appears in the T window, indicating that *Mutans Streptococci* salivary level is high (>5 x105 CFU/mL of saliva). Thus the patient has a potential high caries risk. If no line can be observed after 15 minutes, a low *Mutans Streptococci* salivary level is present in the sample, and the patients´ potential caries risk is low at the time (Figure 9).(GC® dental corp. America, 2017).

iii. Lactobacillus Colony Count Test:

This tests´ purpose is to estimate the number of acidogenic and aciduric bacteria in saliva by counting the number of colonies on tomato peptone agar plates (pH 5.0) inoculated with a sample of stimulated saliva. This is one of the oldest tests to detect the individual risk of carie activity (Prabhakar, 2009).

A strong relationship between a high Decayed Missing and Filled Tooth Index (DMFT) and

high counts of ***Lactobacilli*** has been found but these species are more associated with the progression of carious lesions than with its initiation (Bhayat, 2013).
The *lactobacilli* count can be used for planning recall intervals, as an educational aid and monitoring strategy in dietary couseling for treatment planning (Prabhakar, 2009).

iv. Snyder Test:

This test mesures the speed of acid formation on a sample of stimulated saliva inoculated into a glucose agar medium adjusted to a 4.7 to 5 pH, using an indicator dye (bromocresol green). After 24-48 hours the rate of color change from green to yellow indicates the patients' caries activity: limited, definite or marked caries activity (Prabhakar, 2009).

III. DISCUSSION:

Caries risk assessment allows the estimation of the probability of caries incidence. An accurate caries risk evaluation can identify patients at high caries risk and allow preventive therapies precocious implementation and improving treatment effectiveness. (Guo and Shi, 2013).
Saliva plays an essential role in maintaining oral health and preventing caries. Several salivary biomarkers have been identified and evaluated for its potential in assessing dental caries risk. According to this literature review the most useful for its diagnosis value, result accuracy and ease of execution in the dental office are tests that evaluate:

Salivary flow rate, pH and buffer ability:

The studies by Animireddy, (2014), Singh, (2015) and Prabhakar, (2009) were unanimous in attesting that the tests that evaluate Salivary flow rate, pH and buffer ability are a valid tool for determining the risk of dental caries, especially when analyzed together in the Saliva Check Buffer Test.

These studies have found an inverse relationship between DMFS and salivary pH. The pH values for caries-free groups were in the range of 6.9-7.2 and for caries-active groups were 5.8 - 6.2. (Animireddy, 2014; Singh, 2015; Prabhakar, 2009).

When testing the buffering capacity the mean buffering capacity for caries-free groups was higher when compared to caries-active groups and also an inversely proportional relationship was found between salivary buffering ability and DMFS. According to the authors individuals with a high salivary buffer capacity are often caries-resistant (Animireddy, 2014; Singh, 2015; Prabhakar, 2009).

The salivary flow rate was regarded as an important risk factor for caries incidence. A reduced secretion was considered a potential risk factor when the flow rate was lower than 0.30 ml/ min for unstimulated saliva and lower than 0.7 ml/min for stimulated salivary flow (Animireddy, 2014; Singh, 2015; Prabhakar, 2009).

Salivary *Mutans Streptococci* and *Lactobacilli*:

Studies such as Bhayat (2013), Guo and Shi (2013) and Singh (2015) unequivocally associated a higher salivary concentration of *Mutans Streptococci* and *Lactobacilli* with increased caries risk (Bhayat, 2013; Guo and Shi, 2013; and Singh, 2015).

Mutans streptococci are essentially related to dental caries initiation process and *Lactobacilli* are more associated with acid production and dental caries progression. Thus, there is a significant relation between high salivary counts of *Mutans Streptococci* and *Lactobacilli* and dental caries (Bhayat, 2013; Guo and Shi, 2013; and Singh, 2015).

If patients with high counts of *Mutans Streptococci* and *Lactobacilli* could be detected before appearing the signs of disease, dental caries prevention would be most successful (Bhayat, 2013; Guo and Shi, 2013; and Singh, 2015).

According to the studies conducted by Bhayat (2013), Singh, (2015) salivary tests like Saliva-Check-Mutans (by GC® dental corp.) and CTR Bacteria (by Ivoclar vivadent®), that assess the concentration of *Mutans Streptococci, Lactobacilli* or both are a valid and useful tool to detect caries risk children (Bhayat, 2013; Singh, 2015).

There are some limitations to this review:
- Most existing studies on salivary tests have been used and validated to determine general caries risk in children, there are few studies available that assess the risk of ECC.
- It would not be feasible in this kind of work to exhaustively explain all the caries salivary biomarkers and all existing tests to evaluate them, so it was decided to develop essentially the chair tests, for being the most useful on daily dental visits. Others, due to the need for complex and time-consuming laboratory tests, sophisticated and expensive equipment, specialized training for its execution (eg Fosdick Calcium Dissolution Test) or because they lack a consensual researchers opinion on their diagnostic value, have also not been addressed in this review (for example, Reduction Tests or Salivary *Candida*, *Actinomyces* or non-*mutant streptococci* tests).

It is important to emphasize that, even chair side tests are expensive (between 60 and 150 euros), time consuming and difficult to apply to young children.

Among the chair side tests presented, it is believed that the CTR Bacteria - Caries Risk Test is the easiest to apply in young children, to assess the risk of ECC, because it has a simpler protocol of use and is less dependent on child's cooperation. In addition, the fact that it assesses *Mutans Streptococci* and *Lactobacilli* salivary counts simultaneously makes it quite complete and reliable.
Despite the thesis limitations, it can be said that in an era in which Modern Dentistry seeks to maximize prevention and minimize invasive restorative approaches, the use of chair side caries risk assessment tests like Saliva Check Buffer Test and CTR Bacteria - Caries Risk Test in children, will be a plus for dentists, children and their caregivers:
- allows the identification of children at risk of developing ECC,
- allows implementation of early preventive and therapeutic strategies for these children
- helps children and parent motivation and education for oral health and hygiene, by making it easy to understand the results and putting to evidence a problem that, otherwise, would be more easily neglected until obvious signs or symptoms occurred.

These tests are useful for visualizing a problem and communicating it to parents and children. The disclosure of an oral problem at an early stage, before the disease is installed, and its effects can be seen, helps to take conscience that there is a fine line between health and

sickness, and will serve as an alert, for preventive and control measures must be taken and oral health must not be neglected. (Pavlov and Naulin-lfi, 1999).

IV. CONCLUSION

EEC is a severe and early form of caries disease that can lead to serious complications in the child's future health. Early Diagnose is the key to a better oral health, the ability to monitor caries disease patient status, disease onset, progression, and treatment outcome through noninvasive means, such as salivary tests, is the most desirable goal in oral health-care promotion and delivery. There are three prerequisites for this goal to be achieved: specific biomarkers associated with caries EEC, a noninvasive approach to detect and monitor these biomarkers, and the technologies to discriminate between and among biomarkers.

Further studies are needed to reveal more specific biomarkers to assess ECC risk. It will also be important to develop salivary tests easier to apply in young children, so that they can measure their EEC risk, thus aiding in ECC prevention and caries activity monitoring in children.

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VI. APPENDICES :

-Figures:

Figure 1: Scale of pH - https://coopersdigest.com/wellnessblog/2017/4/11/acid-vs-alkaline.

Figure 2: Example of Saliva-Check buffer test by GC® dental corp. - www.gcamerica.com/products/preventive/Saliva_Check_BUFFER/Saliva-CheckBUFFER_8IFU.pdf.

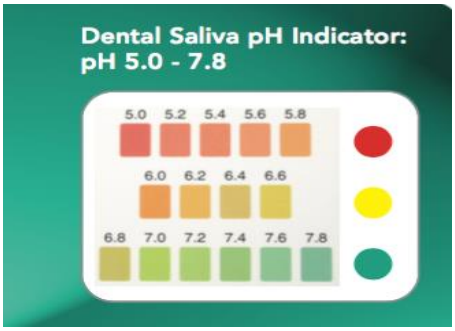

Figure 3: Scale of salivary pH. (GC ® dental Corp.) - http://www.gcamerica.com/products/preventive/Saliva_Check_BUFFER/Saliva-CheckBUFFER_8IFU.pdf

Figure 4 A: Test pads (GC® Dental Products Corp.) - www.gcamerica.com/products/preventive/Saliva_Check_BUFFER/Saliva-CheckBUFFER_8IFU.pdf

Figure 4 B: Classification of the buffer ability (GC Dental Products Corp®). - www.gcamerica.com/products/preventive/Saliva_Check_BUFFER/Saliva-CheckBUFFER_8IFU.pdf

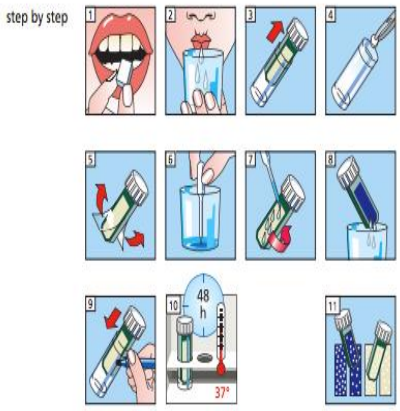

Figure 5: Step by step procedure CTR bacteria by Ivoclar Vivadent ® - http://www.ivoclarvivadent.fr/fr/p/tous/produits/prophylaxie/evaluation-risque-carieux/crt-bacteria

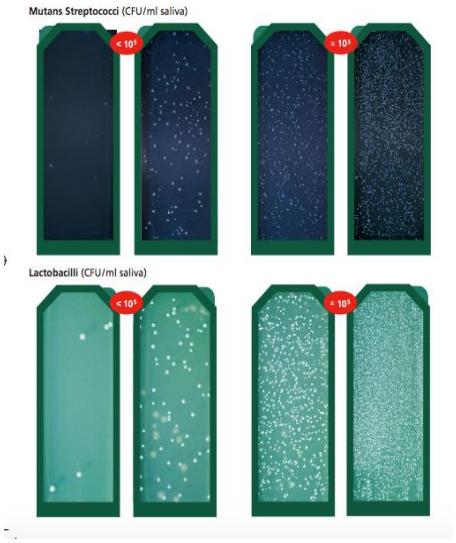

Figure 6: Interpretation picture of the number of S.Mutans and Lactobacilli by Ivoclar Vivadent ® -

http://www.ivoclarvivadent.fr/fr/p/tous/produits/prophylaxie/evaluation-risque-carieux/crt-bacteria

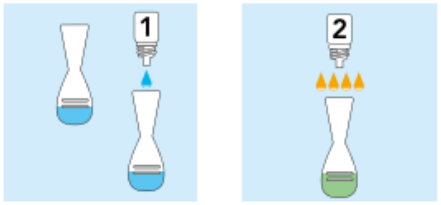

Figure 7: Add of the reagents 1 and 2 (by GC dental corp ®.) -

http://cdn.gceurope.com/v1/PID/salivacheckmutans/leaflet/LFL_Saliva-Check_Mutans_fr.pdf

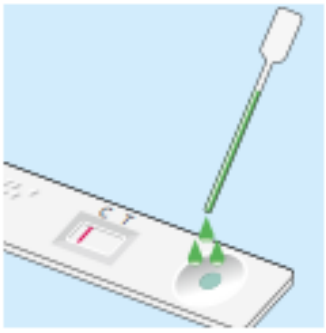

Figure 8 : Putting saliva mix in the testing window (by GC® dental corp.) - http://cdn.gceurope.com/v1/PID/salivacheckmutans/leaflet/LFL_Saliva-Check_Mutans_fr.pdf

Figure 9 : Positive result (top) and negative result (low) (by GC® dental corp.) - http://cdn.gceurope.com/v1/PID/salivacheckmutans/leaflet/LFL_Saliva-Check_Mutans_fr.pdf