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TRABAJO DE FIN DE GRADO

Grado en Odontología

MICROBIOTA AS BIOMARKERS FOR EARLY CHILDHOOD CARIES RISK ASSESSMENT

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Abstract

Introduction: Microbiota is a vast ecosystem made up bacteria, fungi, viruses, etc., that colonizes the human body. More than 700 species of microbiota are found in the oral cavity and among them, *Streptococcus mutans* is the most common bacteria present in the oral cavity and is considered the main cause of the onset of early childhood caries (ECC). ECC refers to any dental caries, missing due to caries or filled teeth in children 6 years of age or younger. Today, saliva biomarkers have become a powerful tool to detect and prevent early caries diseases (especially in children), thanks to the use of salivary flow rate, buffering capacity, microorganisms and proteins (Cathelicidin LL-37, Histatin 1, and inflammatory cytokines) as biomarkers.

Objectives: The aim of this thesis is to describe and review the relationship between the microbiota and dental caries, and to evaluate the potential biomarkers for early childhood caries detection.

Materials and Methods: A total of 54 articles were reviewed by using the electronic database of the Pubmed, library database of selective publication at Universidad Europea de Madrid and Google Scholar.

Conclusions: *Streptococcus mutans* and salivary proteins, including Cathelicidin LL-37, Histatin 1, and inflammatory cytokines can be used as biomarkers to detect early dental caries while salivary flow rate and buffer capacity can’t be used as biomarkers due to the controversy of results found in the articles studied

Key words: microbiota, oral microbiota, biomarkers, saliva, caries, early childhood caries

Resumen

Introducción: La microbiota es un vasto ecosistema de microrganismos, formado por bacterias, hongos, virus, etc., que coloniza diferentes partes del cuerpo humano. Se estima que hay más de 700 especies de bacterias que albergan la cavidad bucal, y, entre ellos, *Streptococcus mutans* es considerado la causa principal de la aparición temprana de caries en niños (CAT). CAT se refiere a cualquier tipo de carie dental, perdida de dientes debido a caries o a empastes en niños hasta los 6 años. Hoy en día, los biomarcadores de saliva se han convertido en una poderosa herramienta para detectar y prevenir las enfermedades de caries tempranas (especialmente en niños). Entre los biomarcadores más utilizados encontramos la tasa de flujo salival, la capacidad amortiguadora de la saliva, la presencia de determinados microrganismos y varias proteínas como la Catelicidina LL-37, la Histatina 1 y las citocinas inflamatorias.

Objetivos: El objetivo de esta tesis es describir y revisar la relación entre la microbiota y las caries dentales, y evaluar los potenciales biomarcadores para detectar la aparición temprana de caries en niños.

Materiales y Métodos: Un total de 54 artículos fueron revisados utilizando la base de datos electrónica Pubmed, varias bases de datos de la biblioteca Crai de la Universidad Europea de Madrid y Google Schoar.

Conclusiones: La conclusión principal de esta tesis es que *Streptococcus mutans* y las proteínas salivales, como la Catelicidina LL-37, la Histatina 1 y las citocinas inflamatorias, pueden ser utilizados como biomarcadores para detectar caries tempranas., La tasa de flujo salival y la capacidad amortiguadora de la saliva no pueden ser utilizados como biomarcadores debido a los resultados controvertidos encontrados en los artículos analizados.

Palabras clave: microbiota, microbiota oral, biomarcadores, saliva, caries, caries de aparición temprana.

Abbreviation in alphabetic order

ADA -- American Dental Association
AMPs -- Antimicrobial peptides
ASM --- American Society of Microbiology
CCS --- Caries classification system
DDE -- Developmental defects of enamel
ECC --- Early childhood caries
EHP -- Enamel hypoplasia
F/B ratio --- Firmicute/Bacteroidetes ratio
GCF --- Gingival cervical fluid
HAS-ECC ------------------- Hypoplasia-associated severe early childhood caries
IBD -- Inflammatory bowel disease
ICDAS --------------------- International Caries Detection and Assessment System
MiC --- Microbial indicator of dental caries
NSAID --- Non-Steroidal anti-inflammatory drug
SCFAs -- Short chain fatty acids
S-ECC -- Severe Early childhood caries
S. mutans --- *Streptococcus mutans*

Index

Introduction

1. Human Microbiota

Microbiota refers to a community of microorganisms, including bacteria, fungi, viruses, protists, etc., that colonizes the human body (1) at four major colonization sites: gut, oral cavity, vagina and skin (2) (Figure 1) (3)

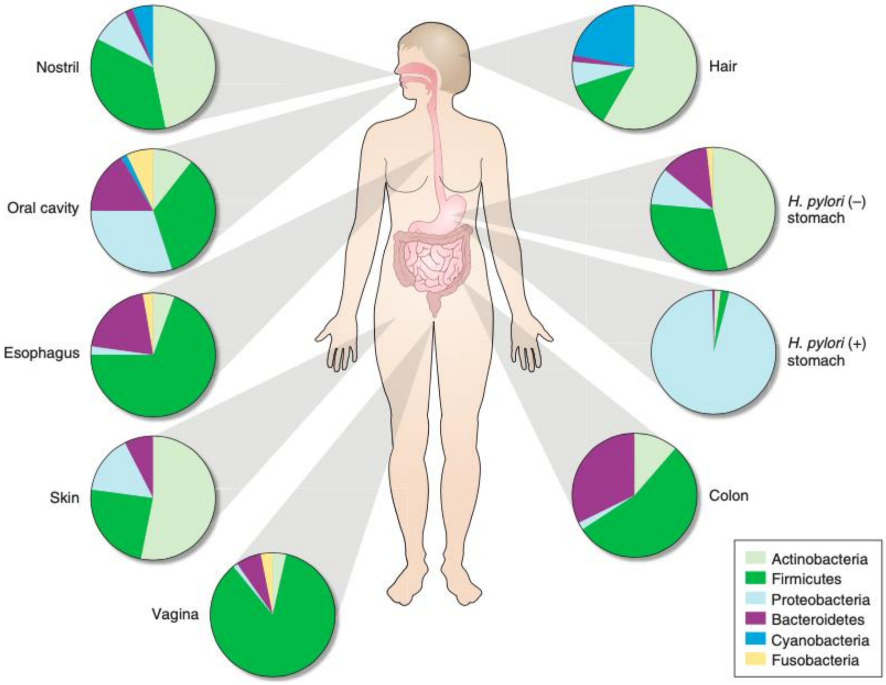


Figure 1: Distribution of the normal human microbiota (Aagaard *et al.* (3))

Microbiota is composed of more than a ten trillion of diverse symbionts including 50 bacterial phyla and about 100-1000 bacterial species (2). It is established right after birth and during life increases its number and diversity and it is influences by the diet, drugs, emotional stress, environment, age, etc. (4)(5). More than 1000 prokaryotic species can be identified in the human intestinal tract, of which 7 major phyla are the most represented: *Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicorbia* and *Cyanobacteria*. Among them, *Firmicutes* and *Bacteroidetes* are dominant, accounting for more or less 90% of the whole population (2) and will change due to aging. The *Firmicutes* and *Bacteroidetes* (F/B) ratio increases in the elder population (6). The alteration of the F/B ratio can also be related to the other diseases, such as obesity and inflammatory bowel disease (Figure 2) (7).

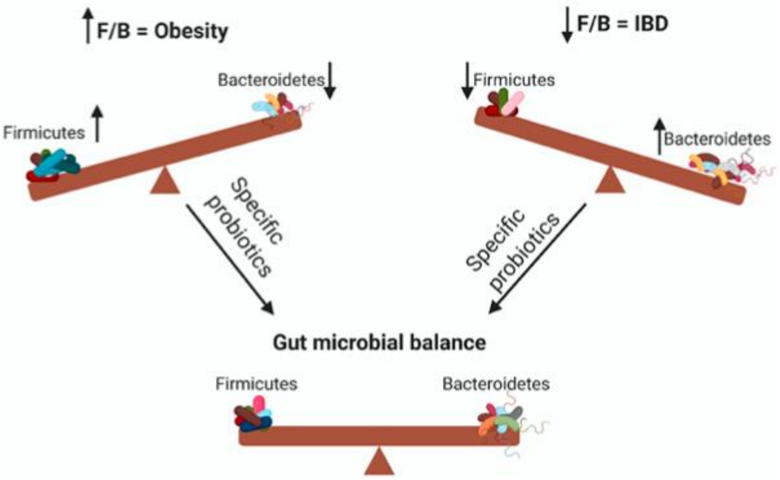


Fig 2: Change in *Firmicute/Bacteroidetes* ratio (Stojanov *et al*. (7))

Gut microbiota helps the human body in the metabolism of daily diet, such as carbohydrates, protein, lipids, fiber fermentation and absorption of nutrition such as vitamins. Second, it establishes the mucosal firewall with intestinal epithelium, cooperating with the immunological components, thereby preventing tissue inflammation and disease (8). Gut microbiota produces short chain fatty acids (SCFAs): butyrate, acetate, and propionate (9), which provide the energy to the intestinal tissues and enhance the integrity of the epithelial barrier providing the protection for the gut health (4).

Dysbiosis is a process characterized by a microbial imbalance in the host. In gut, high diversity refers to the healthy intestinal microflora and reduced bacterial diversity and change of *Firmicute/Bacteroidetes* ratio can be related to dysbiosis. Many factors can cause gut dysbiosis, including genetic factors, medications, age, radiation or chemotherapy, alteration of immune system and malnutrition (10). Recently, more studies have suggested that the dysbiosis is highly related to the inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn’s disease. Both ulcerative colitis and Crohn’s disease are the risk factors of colitis-associated colorectal cancer (10)(11). Other diseases such as diabetes type II, allergies and obesity can also associated with dysbiosis (12) (Figure 3).

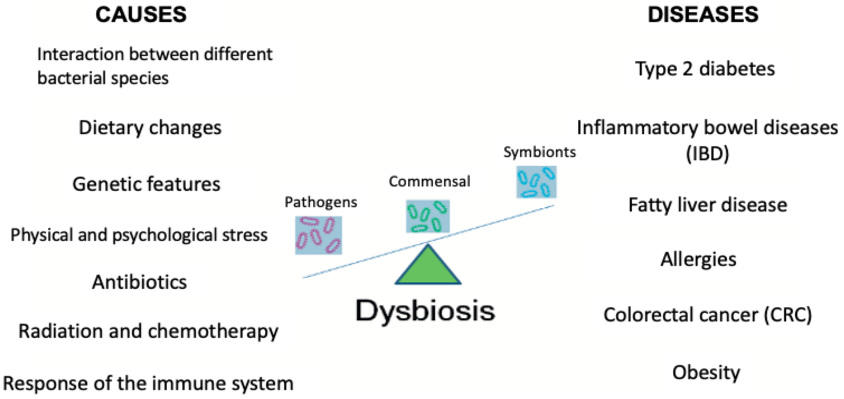


Figure 3: Dysbiosis and its relative factors and diseases. (Tomasello *et al*. (12)**)**

Medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics lead to dysbiosis (9). In fact long-term use of NSAIDs can lead to gastric ulcers and increase both *Bacteroidetes* and *Enterobacteriaceae* bacteria, leading to high risk of diarrhea and intestinal inflammation (10). In addition, long-term use of antibiotics will also affect the reduction of *Firmicute* and increase of *Bacteroidetes*. However, certain antibiotics can also be used as inhibitors to increase the microbial diversity and to improve the symptom of Inflammatory bowel disease (IBD) (10).

1.1 Oral Microbiota

According to the American Society of Microbiology (ASM), there are more than 700 species of microbiota found in the oral cavity, distributing in 11 areas, including the teeth (or denture), tongue dorsum, buccal mucosa, both hard and

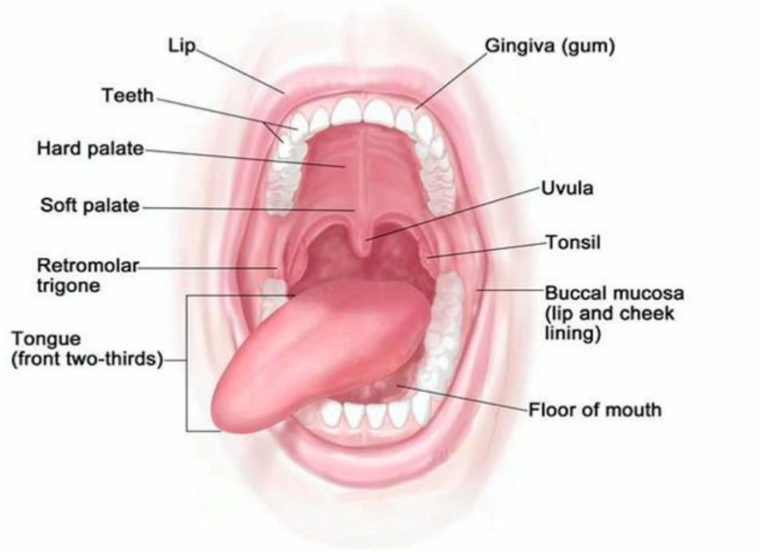


Figure 4: Anatomy of the oral cavity (Ahsan *et al*. (28))

soft palate, keratinized gingiva, supragingival plaque, subgingival plaque, saliva, lips and throat (Figure 4) having their own positive or negative effect on oral cavity and overall health (13).

Most of them maintain commensal relationship with the host, protecting the oral cavity and preventing the development of the disease, but when there is an unbalanced relationship between microbial community and the host, a variety of oral infections may be produced, including caries, periapical diseases, pulp diseases, among them. Any alteration of the structure or function of oral microbiota in the oral cavity is an important consideration leading to various infectious diseases, thus serving as a biomarker for early diagnosis or prognosis and development of diseases (14).

1.2 Development of biofilm

Dental biofilm is an aggregation of the microorganism that could increase the resistance of bacteria to antibiotics or disinfectants (15). The first phase of biofilm formation is characterized by the accumulation of acquired pellicle that mainly formed by saliva, thereby forming a “conditioning film” on the tooth surface (16). Once the coherence is established between the bacteria and acquired pellicle, the primary colonizers *Streptococci* and *Actinomyces* play an important role on creating the first layer of supragingival plaque, attracting more gram positive and gram-negative bacteria to adhere on the first layer biofilm (16). Maturation occurs in the third stage of development, interacting with more bacteria in the oral cavity and gathering together with the biofilm that has formed before. The most important species at this phase is *Fusobacterium*, which helps accumulate the following bacteria, especially Gram-negative bacteria, on the initial biofilm (17). Once the supragingival plaque is completely formed, bacteria start to produce an acidic environment generating demineralization on the tooth structure or even result in gingival inflammation. Furthermore, when the supragingival plaque is not well cleaned, it can merge under the gums forming subgingival plaque, leading to periodontitis (18).

1.3 Classification of bacteria found in oral cavity

The common bacteria that are found in healthy oral cavities can be basically classified in two groups depending on their composition and reaction to the gram stain test (19).

Bacteria in saliva mainly come from the surface of dental biofilm or the tongue. More than 90 bacterial groups can be identified, among which *Streptococcus* is the most predominant gram-positive bacteria in saliva (20)(21).

Streptococcus mutans is an acid-producing bacteria that produces an acidic pH environment, and continue to survive in a lower pH (below 4.5) environment. (22). At the same time, due to the multi-layer film on the tongue surface, it can create a highly diverse and stable environment to harbor various bacteria, including gram-negative bacteria, such as *Veillonella* and *Prevotella* (18)(20).

On the surface of the hard tissues, dental biofilms/plaque are form by the oral microbiota, which can be divided into two types: supragingival and subgingival plaque. Supragingival plaque is associated with more gram-positive bacteria, including *Streptococcus*, *Actinomyces* and *Lactobacilli*, while in the subgingival plaque have been found more gram-negative bacteria such as *Fusobacterium*, *Treponema* and *Neisseria* (18)(20)(21).

Studies have found that different bacterial species in the oral cavity may interfere with the others. For example, *Streptococcus gordonii* can kill many bacteria in the mouth due to the production of hydrogen peroxide, and it can also inhibit the growth of *A. Naeslundii*.

1.4 Dental disease and microbiota

The environment of oral cavity is very unstable, might change due to various factors, including diet, habits like smoking, alcohol, temperature, pH, redox potential or saliva flow. Dental caries is a continuous pathological process that proceeds in the destruction of hard dental structure with cariogenic microorganisms, mainly with *Streptococcus mutans*, correlated as an initial process of caries evolution (23).

Streptococcus mutans usually identified as a dominant bacterium for dental caries. It is an acid-producing bacteria that can cause acidic environments and lead to demineralization of the tooth structure. Both *S. mutans* and *Lactobacillus* are key pathogen in dental caries (20). *Veillonella, Actinomycetes, Granules, Ciliates, Thiomonas, Bifidobacterium, Prevotella* and other bacteria are also closely related to dental caries (23). Other articles used the model of "microbial indicators of dental caries" (MiC) to study the structure and function of the dental caries microbiota located in different parts of children’s oral cavity, and study which microbiota can be used as an indicator of early dental caries risk prediction (24). By comparing the results of the MiC of the caries-active group and the caries-free group, it was indicating that the microbiota related to caries is mainly caused by *S. mutans*, *Actinomycin* and *Lactobacillus*, especially *S. mutans* (25) (Figure 5).

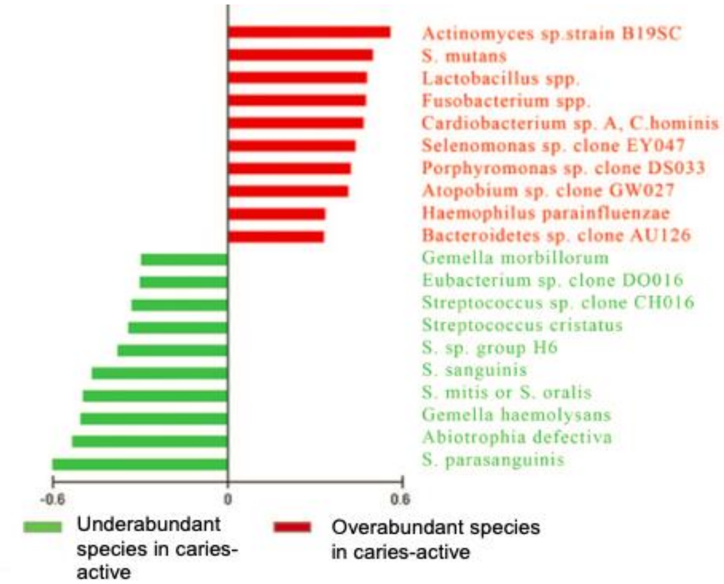


Figure 5: Bacteria species in caries-active and caries-free subject. (Corby *et al*. (25))

Gingivitis is the reversible periodontal inflammatory disease caused by the accumulation of bacterial plaque in the gingival sulcus. Once the ecological niche in gingival sulcus is broken, it causes the destruction of connective tissue surrounding by the tooth, creating an anaerobic environment and thus resulting in the irreversible periodontitis. The three main bacteria *Prophyromomas gingivalis (P. gingivalis), Treponema denticola (T. denticola) and Tannerella forsythia (T. forsythia)* are defined as “*red complex*” that are closely associated with periodontitis, although they are present in healthy individuals (20). Study revealed that the number of red complex microorganisms are reduced with the disappearance of inflammation after the periodontal treatment, hence the red complex can also be a biomarker for detecting the periodontal disease (20)(26).

2. Biomarkers

According to the definition of the WHO, a biomarker is “a substance or a process that can be measured in the body and can be used to predict the incidence or outcome of disease.” (27). Nowadays, we are not only concerned about the outcome of disease, but also on the impact of treatment, intervention or environmental exposure. The basic biomarkers include pulse, blood pressure, or even complex chemical methods that can be tested repeatedly, such as blood tests or tissue tests (27). Biomarkers can be classified into five categories depending on the stages of diseases:

1. Antecedent biomarkers, according to the risk factor and its relationship with disease.

2. Screening biomarkers, based on the screening of clinical aspects.

3. Diagnostic biomarkers, by recognizing the disease through the symptoms.

4. Staging biomarkers, depending on the severity of the disease.

5. Prognostic biomarkers, predicting the prognosis and its related treatments and complications (28).

2.1 Saliva biomarkers

Saliva is a complex body fluid that plays an essential role in processing and digesting the food in oral cavity and initial digestive system. Due to its easy collection and duplication of samples, saliva is commonly used for oral biomarker. As mentioned before, saliva not only works as the lubrication, but also regulates the pH between 6.6-7.1 due to its buffer capacity and prevents bacterial infection based on its compositions (29).

Low saliva flow rate is a risk factor for dental caries. The use of the medications, alteration of salivary glands or age can affect the saliva flow rate. Studies have shown that unstimulated saliva flow rates below 0.3ml/min or stimulated saliva flow rates below 0.7ml/min can be considered as high risk of dental caries (29, 30). Saliva pH and buffering capacity is also highly correlated with dental caries. There is evidence that the salivary buffering capacity can protect tooth structure from caries. In the case of low buffering capacity, it cannot compensate for the acidic environment of dental biofilm, thereby reducing the remineralization process of early enamel lesions (30).

Saliva is composed of 99.5% of water with 0.3% proteins and 0.2% of inorganic substances (29). The most common inorganic substances include sodium, potassium, calcium, etc. Meanwhile the organic components compose mucins, amylases, lysozymes, lactoferrin, cystatins, etc. Some of them can be used for the indicators of caries or other diseases, including periodontal disease, oral cancer or carcinomas (31).

The benefit of saliva as a biomarker is that it is fast, non-invasive, and can be collected repeatedly. Moreover, it is easy to obtain and does not require professional training (29). For these reasons, saliva diagnostic technique is now commonly used to detect or to discover human pathological diseases.

Gingival cervical fluid (GCF) can be used as a biomarker. Due to its location and origin, it is particularly used to detect periodontal disease (32). GCF is an inflammatory exudate that can be found in inflamed periodontal tissues, and it contains molecules from the blood, cells and tissues of the periodontium. Among them, eight potential markers have been identified, including alkaline phosphatase, β-glucuronidase, cathepsin B, collagenase 2 (matrix metalloproteinase, MMP-8), gelatinase (MMP-9), dipeptidyl peptidase (DPP) II and III and elastase (32). The sample can be easily collected by inserting a paper strip in the gingival pocket, and also can be collected repeatedly as saliva.

There are several antimicrobial components in the saliva that can be potential indicators for caries biomarkers, especially for early childhood caries (ECC), including antimicrobial peptides (AMP), major salivary glycoproteins and minor salivary glycoproteins. (Table 1)

Antimicrobial peptides (AMPs), also called cationic peptides, are the most important role in immunity. They participate in the first line of defense and help resist caries, acting on bacteria, fungi and viruses (29). It contains four components: *Cathelicidin LL3, Defensin, Histatins and Statherin*. Among them*, LL37, Histatin 1 and Statherin* were found to be highly associated with dental caries and can be used as biomarkers.

Among Salivary glycoproteins, mucins and immunoglobulin participate in the formation of acquired enamel pellicle and establishes the first layer of dental biofilm (30), represent the first line of defense in the oral cavity (29)

Salivary proteins		**Function**	**Association with ECC**
Antimicrobial peptides			
Cathelicidin LL37		Antimicrobial activity	High relation with ECC.
Histatin	Histatin 1	Reduce bacterial colonization. Maintain teeth integrity.	High relation with ECC
	Histatin 3		Weak evidence
	Histatin 5		Weak evidence
Defensin	Alpha-defensin	Antimicrobial, antivirus and antifungal activity.	Positive correlation with ELISA. More evidence needed.
	Beta-defensin		Weak evidence
Statherin		Reduce bacterial colonization. Maintain teeth integrity.	High relation with ECC
Major glycoprotein			
Mucin	MUC5	Promote the aggregation with microorganism to facilitate removal.	Weak evidence
	MUC7		High relation with ECC
Proline-rich proteins	Acidic PRP	Maintain the supersaturation of calcium ions in saliva	High relation with ECC
Immunoglobulins	IgA	Immune response	High relation with ECC
	IgM & IgG		More evidence needed.
Minor glycoprotein			
Agglutinin		Aggregate with microorganism for easily removal.	Weak evidence
Lactoferrin		Antimicrobial activity	More evidence needed.
Lysozyme		Antibacterial enzyme	More evidence needed.
Cystatins		Antimicrobial activity, Help remineralize.	More evidence needed.

Table 1: Chemical compositions in saliva (Abdullah et al (29))

3. Definition of caries

Dental caries is one of the most prevalent chronic diseases in the world. It results from an ecological imbalance between tooth structures and oral biofilms (33). It begins with the colonization of biofilms on the tooth surfaces. Most of the biofilms are occupied mainly by streptococci, which has been shown to be closely related to dental caries. *Streptococcus mutans* produce a weak acid environment, causing the pH dropping below the critical level (pH 5.5), and leading to mineral loss and destruction of hard tissue which is known as demineralization (33). The demineralization process can be reserved in the early stage of caries formation with enough exposure of fluoride. Fluoride acts as a catalyst of calcium and phosphate ions, helping to bond with tooth structures and to remineralize. Study showed that the use of fluoridated water can reduce the caries rate population, also the application of fluoride gel or fluoride varnish every three months are recommended for high-risk individuals (34).

Dental caries is also a multifactorial disease, involving many risk factors, including bacteria, personal behavior and environmental factors. Individual behavior factors can be defined as changeable or unchangeable according to their characteristics. Excessive sugar intake or frequent snacking is the main risk for the early childhood caries. It can be improved by reducing the frequency of intake, substituting low-sugar foods (such as yogurt or fruit or replacing xylitol), or through appropriate oral hygiene instructions (brushing technique and flossing) (34).

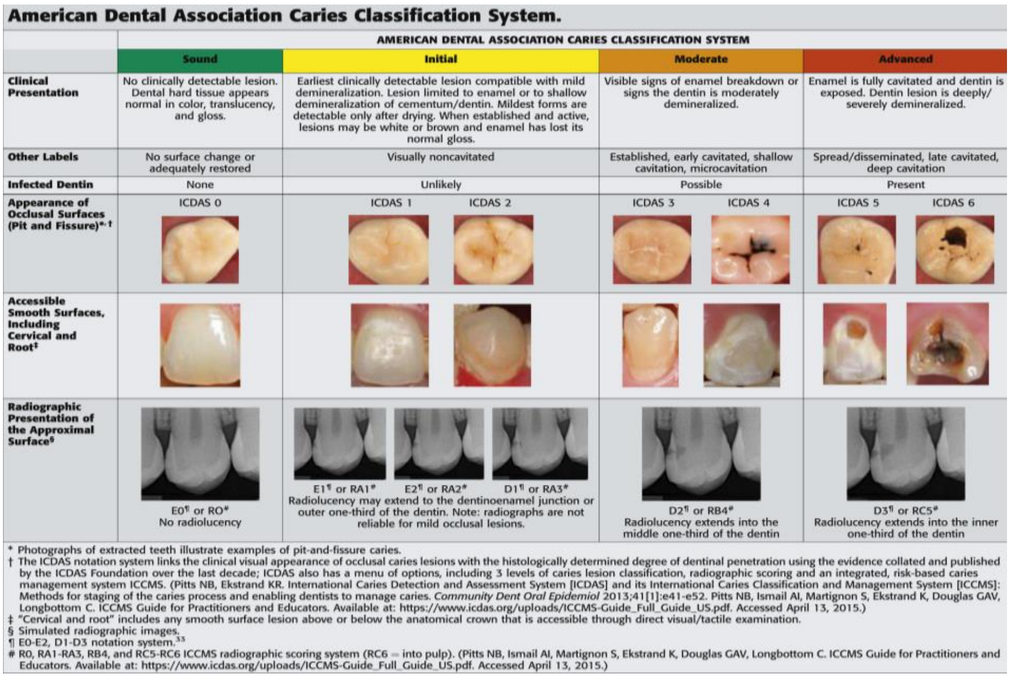
Unchangeable factors include medication-induced hyposalivation, aging and individuals with special needs (34). In elder populations, the risk of dental caries and periodontal diseases is greater due to the limited movement, change of immune function and impaired wound healing and repair, although there is insufficient evidence to show that there is a correlation between the immune system and dental caries (35). In the elderly, the root caries lesions are easy to develop due to gingival recession, where the cementum is much softer than enamel. Once the caries is formed, it is easier to penetrate and reach to the pulp, causing toothache and even necrosis (33). Medication-induced hyposalivation in patients suffering from oral cancer, receiving radiotherapy or cytostatic drugs may develop sialo-adenitis can cause irreversible secretion damage, which is considered high-risk groups, and may develop new caries within 1 year (34). The moisturizing mouthwash or gels are recommended to relieve discomfort, the use of xylitol or other sugarless chewing gum is also recommended to stimulate saliva secretion (36).

Today, many studies have shown that the risk of dental caries is not only due to eating habits or poor oral hygiene, but also genetic factors and socioeconomic problems (37). Poverty may lead to a lack of education and reduce the demand for dental care or reduce access to healthcare services, unable to afford oral hygiene products such as fluoride toothpaste, mouthwash, or dental floss (37). Although research showed that the prevalence of dental caries in developed countries is decreasing, many children and adults still suffer from caries (33).

Regardless of how these factors affect the possibility of lesion formation, the accumulation of plaque and prolonged low pH are still the main reasons for demineralization, which ultimately leads to dental caries. Effective cleaning can minimize the incidence of caries, hence the education and maintenance of good oral hygiene are essential for everyone.

3.1 Classification of Dental Caries

Based on the clinical manifestations of caries lesion, the American Dental Association (ADA) has created a caries classification system (CCS) to make corresponding treatment decisions (38). In CCS, it defines four sites where caries lesions can be found: pit and fissure, interproximal, cervical and smooth surface and root, with four types of clinical appearances: sound, initial, moderate and advanced (Figure 6)



American Dental Association Caries Classification System.

AMERICAN DENTAL ASSOCIATION CARIES CLASSIFICATION SYSTEM

	Sound	Initial	Moderate	Advanced
Clinical Presentation	No clinically detectable lesion. Dental hard tissue appears normal in color, translucency, and gloss.	Earliest clinically detectable lesion compatible with mild demineralization. Lesion limited to enamel or to shallow demineralization of cementum/dentin. Mildest forms are detectable only after drying. When established and active, lesions may be white or brown and enamel has lost its normal gloss.	Visible signs of enamel breakdown or signs the dentin is moderately demineralized.	Enamel is fully cavitated and dentin is exposed. Dentin lesion is deeply/ severely demineralized.
Other Labels	No surface change or adequately restored	Visually noncavitated	Established, early cavitated, shallow cavitation, microcavitation	Spread/disseminated, late cavitated, deep cavitation
Infected Dentin	None	Unlikely	Possible	Present
Appearance of Occlusal Surfaces (Pit and Fissure)*,†	ICDAS 0	ICDAS 1 ICDAS 2	ICDAS 3 ICDAS 4	ICDAS 5 ICDAS 6
Accessible Smooth Surfaces, Including Cervical and Root‡				
Radiographic Presentation of the Approximal Surface§	E0¶ or R0# No radiolucency	E1¶ or RA1# E2¶ or RA2# D1¶ or RA3# Radiolucency may extend to the dentinoenamel junction or outer one-third of the dentin. Note: radiographs are not reliable for mild occlusal lesions.	D2¶ or RB4# Radiolucency extends into the middle one-third of the dentin	D3¶ or RC5# Radiolucency extends into the inner one-third of the dentin

* Photographs of extracted teeth illustrate examples of pit-and-fissure caries.
† The ICDAS notation system links the clinical visual appearance of occlusal caries lesions with the histologically determined degree of dentinal penetration using the evidence collated and published by the ICDAS Foundation over the last decade; ICDAS also has a menu of options, including 3 levels of caries lesion classification, radiographic scoring and an integrated, risk-based caries management system ICCMS. (Pitts NB, Ekstrand KR. International Caries Detection and Assessment System [ICDAS] and its International Caries Classification and Management System [ICCMS]: Methods for staging of the caries process and enabling dentists to manage caries. *Community Dent Oral Epidemiol* 2013;41[1]:e41-e52. Pitts NB, Ismail AI, Martignon S, Ekstrand K, Douglas GAV, Longbottom C. ICCMS Guide for Practitioners and Educators. Available at: https://www.icdas.org/uploads/ICCMS-Guide_Full_Guide_US.pdf. Accessed April 13, 2015.)
‡ "Cervical and root" includes any smooth surface lesion above or below the anatomical crown that is accessible through direct visual/tactile examination.
§ Simulated radiographic images.
¶ E0-E2, D1-D3 notation system.[33]
# R0, RA1-RA3, RB4, and RC5-RC6 ICCMS radiographic scoring system (RC6 = into pulp). (Pitts NB, Ismail AI, Martignon S, Ekstrand K, Douglas GAV, Longbottom C. ICCMS Guide for Practitioners and Educators. Available at: https://www.icdas.org/uploads/ICCMS-Guide_Full_Guide_US.pdf. Accessed April 13, 2015.)

Figure 6: Classification of dental caries (Young *et al*. (38))

Score	**Clinical stage**	**Radiograph**
0	Sound	No radiolucency
1	First visual change in enamel	Radiolucency in outer ½ of the enamel
2	Distinct visual change in enamel	Radiolucency in inner ½ of the enamel
3	Localize enamel breakdown	Radiolucency limited to the outer ⅓ of dentin
4	Underlying dentin shadow	Radiolucency reaching the middle ⅓ of dentin
5	Distinct cavity with visible dentin	Radiolucency reaching the inner ⅓ of dentin, clinically cavitated
6	Extensive cavity with visible dentin	Radiolucency into the pulp, clinically cavitated

Table 2: Classification of ICDA ((Gomez *et al*. (39))

Compared with another similar system called “International Caries Detection and Assessment System” (ICDAS), the classification is also based on clinical stage and severity, but it is more precise, containing 6 categories according to the histological extent of the lesion in the tooth (Table 2) (38) (39)

Also, according to the state of the caries, ADA has divided lesions into two types: active lesion and inactive lesion. In active lesion, we can find plaque accumulation with yellowish color and rough surface; meanwhile in inactive lesion, the color is much darker, but the surface is smooth, and won’t cause any gingival inflammation when the lesion is located near to the gingiva (38).

The dmf index is another method used to measure the experience of caries. It is determined by the total number of teeth through clinical examination: d for tooth decay, m for missing (due to decay), and f for filled in individuals (40)

3.2 Early childhood caries

Early childhood caries (ECC) refers to the presence of any caries, missing (due to caries) or filled tooth surface in the primary teeth of 6 year old children or younger (41). The definition of severe early childhood caries (S-ECC) indicates that any signs of smooth surfaces caries can be found in children under 3 years of age. Or in children with a decay, missing and filled index surface scores (dmfs) of the primary maxillary anterior teeth ≥ 4 at age of 3, ≥ 5 at age of 4, or ≥ 6 at age of 5 (29).

Epidemiologic data showed that the prevalence of ECC is high worldwide, whether in the developed or developing countries (29)(42). High prevalence of ECC has an important impact on either their health, or their cost of the treatment. Once the children suffer from ECC, several treatments are required, including restorative treatment, extraction if the tooth cannot be restored, and space maintainers (42). The most common clinical symptoms include pain, color changes, and loss of tooth structure (Figure 7). In radiograph, we might find out the abscess under. These may lead to the following complications, including difficulty eating, which can result in weight loss and even bone mass loss (41).

Figure 7: Structural loss in ECC (Evans *et al*. (55))

The appearance of ECC is similar to other types of dental caries, starting from white spots on the surface, where near the gingiva, accumulating most of the dental biofilm of *S. mutans*. But the main difference is that due to the thin immature layer of enamel, ECC spreads widely and rampantly. ECC usually affects the teeth by following the eruption sequence of the primary maxillary incisor, maxillary first molar, canine teeth, and last second molar (41)

3.2.1 Hypoplasia-associated severe early childhood caries

Hypoplasia-associated severe early childhood caries (HAS-ECC) is considered as a new classification of Severe early childhood caries (S-ECC). Enamel Hypoplasia (EHP) is one of the developmental defects of enamel (DDE), which is defined as a defect of the formation of mineralized tissue during tooth development. This defect usually affects the primary incisors, canine and first molar, corresponding to the developmental stage and degree (43). The main etiology of DDE is heredity, but premature birth, medications and infections may also be related. DDE can affect both hypoplasia and hypo-mineralization, which can be found in the clinical manifestations of insufficient enamel in the pits, grooves or large areas of the tooth, leading to the rough surfaces that tend to accumulate biofilm. The latter will lead to a decrease in mineral content, resulting in a decrease in resistance to acidic environments, and easily lead to dental caries (41).

EHP is difficult to detect because dental caries and damaged enamel surfaces often appear at the same time, but the important difference is that the primary teeth with EHP have been damaged before eruption, which leads to lesions caused by EHP earlier than caries (43). Teeth with EHP are vulnerable, and due to its structure, it's easily colonized by dental biofilm and promotes the enamel defects or early caries, which leads to what we call Hypoplasia-associated ECC (HAS-ESS). But not all EHP teeth are going to result in HAS-ESS, the cariogenic diet plays an essential factor in this process (Figure 8).

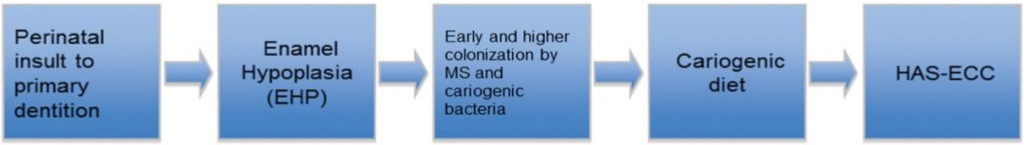


Figure 8: Process of HAS-ECC (Caufield *et al.* 2012 (43))

The cause of ECC is always related to carbohydrate fermentation in dental biofilm (mainly *S. mutans*). Therefore, though the accumulation of tooth biofilm is necessary, without cariogenic diet, EHP will not develop into HAS-ESS (43).

3.2.2 Risk factors and preventions

Sugar has always been the main risk factor for ECC, excessive sugar intake or frequent snacking are highly related to early childhood caries (41). The use of pacifiers containing sweetened fluids at night can also increase the risk because saliva flow is reduced during sleep. Similarly, although breastfeeding can help babies get more immunoglobulin (especially IgA), studies showed that breastfeeding for longer than 12 months may increase the risk of ECC (41)(42).

The developmental defects are also a factor of ECC (41)(42). Children suffering from EHP can increase the number of dental biofilms accumulating on the teeth surfaces. When combined with a high number of sugar consumption and without a proper hygiene technique, it can be highly associated with ECC.

The acquisition of *Streptococcus mutans* in young children is usually transmitted vertically through parents (especially mothers), but it may also be transmitted from caregivers, siblings, and other children (41). The “window of infectivity” is defined as the earliest time of initial colonization with *S. mutans* in children's oral cavities. In 1993, the data had shown that the period of window of infectivity was between the age of 19 months to 31 months during the tooth eruption in 1993. The latest research showed that the early acquisition can be advanced to the age of 16 months, and the earlier tooth eruption is the key factor (44). Other study showed that children with history or their caregivers or siblings who have severe caries may also increase the risk of ECC (33).

ECC prevention techniques and education are needed for parents and children before the initiation of the disease. Knowledge of risk factors and prevention must provide, including sugar restrictions for children under 2 year of age, avoiding night use of sugary pacifiers or bottles and avoid breastfeeding longer than 12 months (41)(42). Using at least 1000 ppm fluoridated toothpaste with an appropriate "smear size" toothpaste for children under 3 years old, and "pea-size" for children 3-6 years old to reinforce oral hygiene (Figure 9)(42).

Children with a higher risk of dental caries, fluoride varnishes and dental sealants can also be performed in dental clinics. Once the lesions appear, restorative treatments are needed to avoid further infection. Resin cement composites, glass (modified) ionomer cement can be carried out as the conservative treatment, in which the release of fluoride can inhibit secondary caries. A full-cover metal crown can be used if children with high caries risk (42).

“Smear size” for children under age 3

“Pea size” for children 3-6.

Figure 9: The size of dentifrices according to age. (Tinanoff *et al*. (42))

Objective

The aim of this thesis is to describe and review the relationship between the microbiota and dental caries, and to evaluate the potential biomarkers for early childhood caries detection.

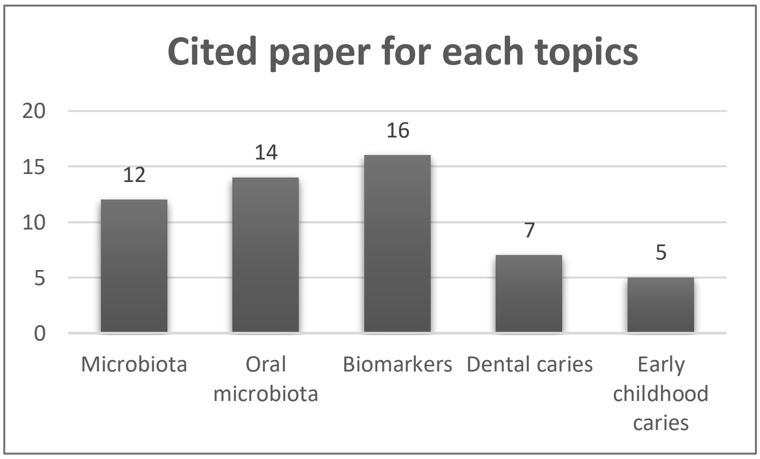
For this purpose, the following objectives were proposed:

- Primary is to understand the knowledge of microbiota, biomarkers and dental caries, especially early childhood caries.
- To study the characteristics and functions of saliva in oral cavity, including the salivary flow rate, buffer capacity, microorganisms present in the saliva and the salivary proteins.
- Secondary objective is to evaluate the relationship between the saliva and dental caries as the potential biomarkers of early childhood caries assessment.

Materials and Methods

The majority of sources came from electronic databases such as PubMed, library database of selective publication at Universidad Europea de Madrid and Google Scholar. Keywords used include: “microbiota”, “oral microbiota”, “biomarkers”, “saliva”, “caries” and “early childhood caries”. These keywords were combined with further “and” and “or” for more relevant articles. Advanced search has been restricted from 2005 to 2020 presented in language English, except for an article in 1983 to demonstrate the figure of the result.

A total of 68 articles were initially found and reviewed. However, 14 articles were excluded due to either duplication or low relevance to the topic. The reasons for excluding these articles include a mismatch between the research participants and the topic that the participants were over 6 years of age, which is unfavorable in this thesis, or the information is directly unrelated to the topic.



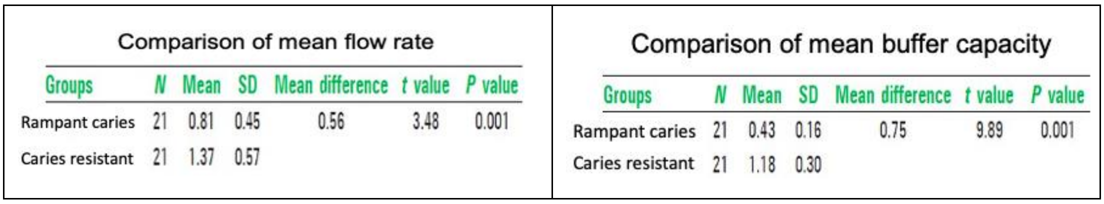
Keywords: microbiota, oral microbiota, biomarkers, saliva, caries, early childhood caries.

RESULTS AND DISCUSSION

Saliva is widely used as a biofluid for the identification of biomarkers of dental caries because is a not invasive methods well accepted by patients and due to the easy collection of the sample. According to its properties, in saliva we can find physical biomarkers (saliva flow rate, buffer capacity) and biological biomarkers, including microorganisms in saliva and salivary proteins (45).

1. <u>Early childhood Caries and salivary flow rate and buffer capacity</u>

To study the relation between salivary flow rate, buffer capacity and early childhood caries, several studies have been done. Kuriakose *et al*. in 2013 conducted a study with 42 children of age range of 3-5 years, comparing the relation between in patients with ECC saliva flow rate (FR) and buffer capacity (BC). Figure 10 shows that both salivary buffer capacity and flow rate are significantly lower in ECC-affected children than children with caries resistance (P value=0.001) (46). Therefore, in this study, they found the relation between salivary flow rate and buffer capacity are highly related to the dental caries.



Comparison of mean flow rate

Groups	*N*	Mean	SD	Mean difference	*t* value	*P* value
Rampant caries	21	0.81	0.45	0.56	3.48	0.001
Caries resistant	21	1.37	0.57			

Comparison of mean buffer capacity

Groups	*N*	Mean	SD	Mean difference	*t* value	*P* value
Rampant caries	21	0.43	0.16	0.75	9.89	0.001
Caries resistant	21	1.18	0.30			

Figure 10: Comparison of ECC with FR and BC (Kuriakose *et al*. (46))

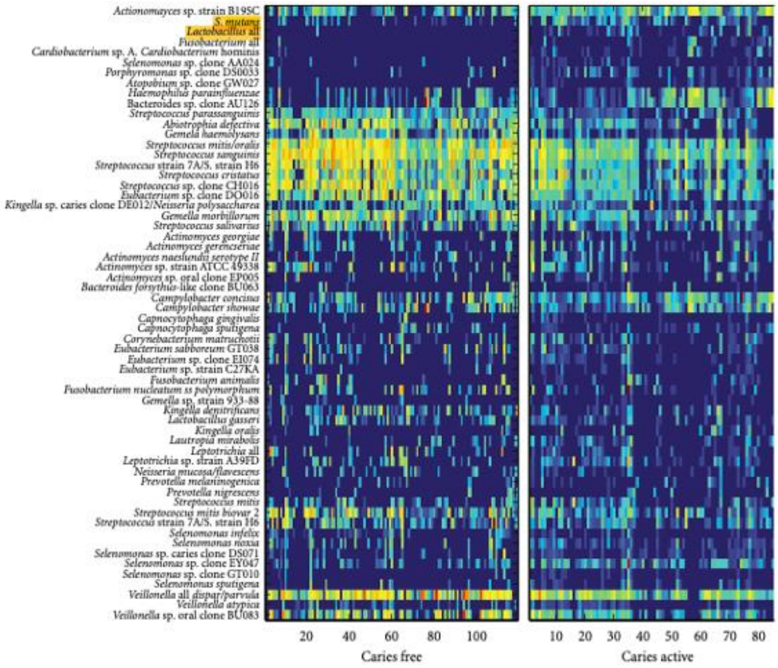
However, in another study, Abbas *et al*. conducted a case-sectional and case-control study with 77 children (age from 3-6 years) and divided into three groups: caries-free, children with early childhood caries and children with severe early childhood caries. Table 3 shows that the correlation of salivary pH, buffer capacity, salivary flow rate and ECC are not significantly related because the P value is > 0.05 among three groups, demonstrating that even though saliva is an important factor in maintaining oral health, no conclusive results were observed in this study (47).

Group	**pH**	**p-value**	**Buffer capacity**	**p-value**	**Salivary Flow Rate**	**p-value**
Healthy	6.98 ± 0.67	0.719	5.07 ± 1.10	0.904	1.28 ± 0.75	0.323
ECC	7.03 ± 0.75		5.27 ± 1.34		1.45 ± 1.03	
ECC-S	6.90 ± 0.86		5.11 ± 1.08		1.20 ± 0.90	

Table 3: Comparison of Salivary pH, buffer capacity and Salivary flow rate in children without ECC, with ECC and with S-ECC. (Abbas *et al*. (47))

2. Early childhood caries and dental microorganisms

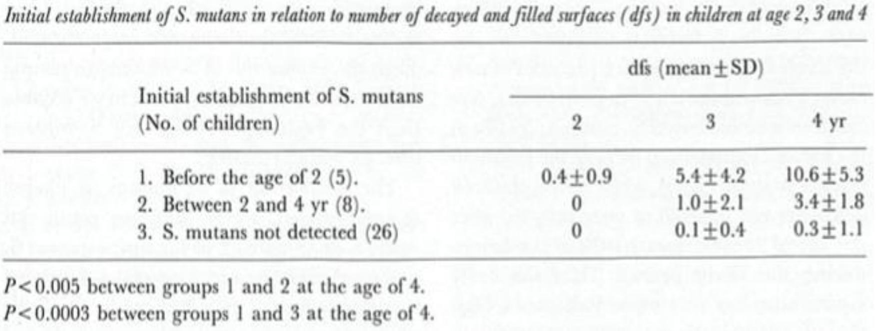
To study the distribution of microorganisms in the oral cavity, and to learn which type of microorganisms have a strong impact in the development of early childhood caries. Hart *et al*. conducted analysis study in which they compared the types of microorganisms in children with or without dental caries. Figure 11 shows that caries-free group patients had more species in their oral cavity compared with the caries affected group. The results also found indicated that in the caries-affected group, *S. mutans* and *Lactobacillus* were more abundant than caries-free group (48).



Brighter colors indicate higher abundance level of the bacteria measured

Figure 11: Bacterial species in caries-free children and caries-affected children. (Hart *et al*, 2011. (48))

Another study had found out that the presence of *Streptococcus. mutans* in preschool children without dental caries will increase the incidence of dental caries (29). In addition, in a longitudinal study with 39 children, showing that the first colonization of *S. mutans* was found before the age of 2, and by the age of 4, the score of decayed and filling surface (dfs) (the modification of the dmf score that only counts the decay and filling) was higher than the group of the first colonization of *S. mutans* occurred between 2 and 4 years of age. (P value <0.05) (Figure 12) (49). Therefore, the initial colonization of *Streptococcus* *mutans* is an important factor, leading to *S. mutans* can be considered as a biomarker.



Initial establishment of S. mutans in relation to number of decayed and filled surfaces (dfs) in children at age 2, 3 and 4

Initial establishment of S. mutans (No. of children)	dfs (mean±SD) 2	3	4 yr
1. Before the age of 2 (5).	0.4±0.9	5.4±4.2	10.6±5.3
2. Between 2 and 4 yr (8).	0	1.0±2.1	3.4±1.8
3. S. mutans not detected (26)	0	0.1±0.4	0.3±1.1

P<0.005 between groups 1 and 2 at the age of 4.
P<0.0003 between groups 1 and 3 at the age of 4.

Figure 12: Colonization of *S. mutans* in children between 2-4 years old. (Alaluusua *et al*. (49))

Zhu *et al.* conducted a study in 2018 to investigate the most recurrent microorganisms in early childhood caries. To demonstrate their hypothesis, 28 children were recruited, 15 of which were diagnosed with ECC and 13 were not diagnosed with ECC. After 12 months of follow-up, all patients were divided into 3 groups: ECC recurrence group (ER), non-ECC recurrence group (NER) and no ECC group (EF). Their research showed that in the ER and NER groups, 4 bacteria showed the most significant differences, including *Capnocytophaga, Fusobacterium, Leptotrichia increased in ECC* and *Prevotella decreased in ECC* , indicating that these four species can potentially be used as a biomarker for recurrence ECC (Figure. 13) (50).

However, in figure 13 the ER group shows abundances of *Corynebacterium, Alloprevotella, Kingella, Porphyromonas, Actinomyces* and *Neisseria*. Among them, the number of *Kingella* and *Neisseria* increased the most, meaning that both are highly associated with the recurrence caries.

Contrastingly, compared with the ER group, the NER group had the lowest level of *Veillonella, Lautropia, Rothia, Haemophilus,* and *Streptococcus*, where *Rothia* and *Haemophilus* have the largest difference in abundance. As a result, species of *Kingella*, *Neisseria*, *Rothia* and *Haemophilus* can be considered as biomarkers of recurrent ECC.

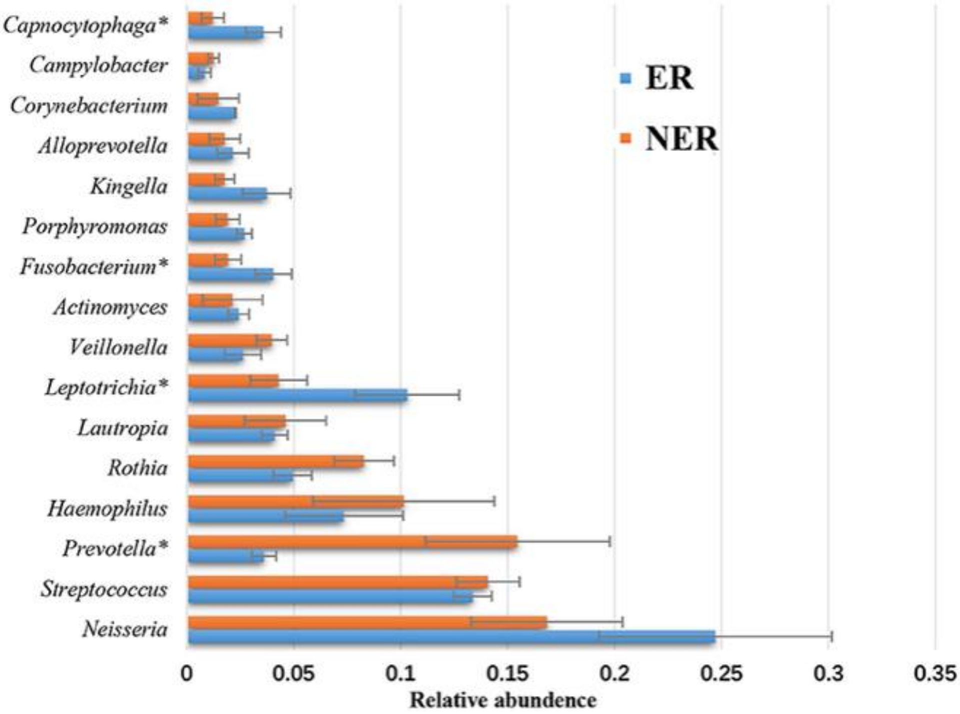


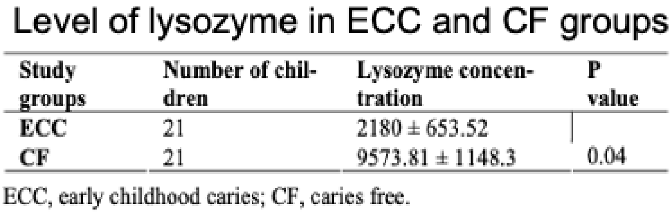
Figure 13: Comparison of bacteria in groups ER and NER. (Zhu *et al*. (50))

3. Salivary proteins as biomarkers in Early Childhood Caries

Two studies conducted by Abdullah *et al*. and Sruthi *et al.*, revealed that salivary proteins can be used as biomarkers in ECC. The authors found that

Cathelicidin LL-37, Histatin 1, Statherin, Mucin 7, Proline-rich proteins and immunoglobulins can help reduce the colonization of the *S. mutans* and protect the teeth from dental caries, thereby can be used as a biomarker for dental caries. However, they also pointed out that although other proteins (such as Lactoferrin and Lysozyme, etc.) had antibacterial activity by reducing or killing bacteria, studies showed that the relationship with dental caries were not strong, therefore they can’t be used as biomarkers and more studies are still needed, and (28,34).

Moslemi *et al*. studied the relationship between lactoferrin and lysozyme and ECC in 42 children (between 36 and 71 months of age). Among them, 21 children had no dental caries and 21 had ECC. In these 21 children affected by the ECC, 15 received restorative treatment. Figure 14 shows that children affected by ECC had higher level of lysozyme than children without ECC, but there is no significant difference in the level of lactoferrin that were found in the two groups (Figure 15) (51), showing that lysozyme could be used as the potential biomarker for ECC when comparing between caries-free and caries-affected group.

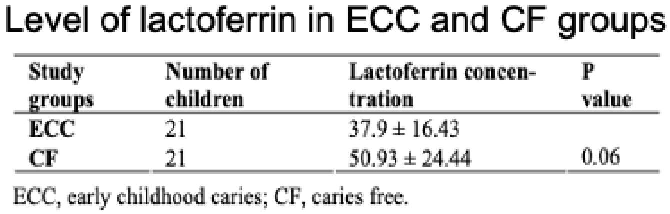


Level of lysozyme in ECC and CF groups

Study groups	Number of children	Lysozyme concentration	P value
ECC	21	2180 ± 653.52	0.04
CF	21	9573.81 ± 1148.3	

ECC, early childhood caries; CF, caries free.

Figure 14: Level of lysozyme in ECC and children without ECC (Moslemi *et al*. (51))



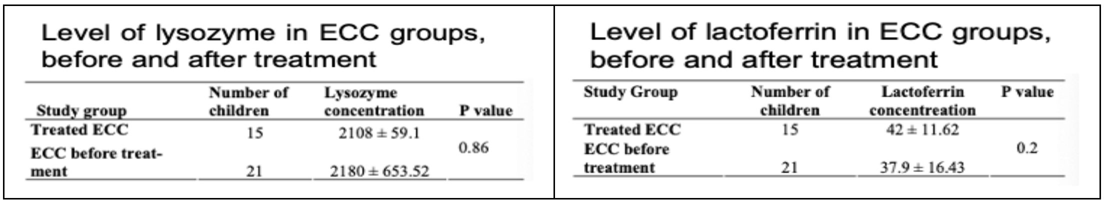
Level of lactoferrin in ECC and CF groups

Study groups	Number of children	Lactoferrin concentration	P value
ECC	21	37.9 ± 16.43	0.06
CF	21	50.93 ± 24.44	

ECC, early childhood caries; CF, caries free.

Figure 15. Level of lactoferrin in ECC and children without ECC (Moslemi *et al*. (51))

However, as showed in figures 16 and 17, the levels of lactoferrin and lysozyme do not decrease after treatment, with the P value > 0.05, indicating that the relationship of lactoferrin and lysozyme were not associated before and after treatment.



Level of lysozyme in ECC groups, before and after treatment

Study group	Number of children	Lysozyme concentration	P value
Treated ECC	15	2108 ± 59.1	0.86
ECC before treatment	21	2180 ± 653.52	

Level of lactoferrin in ECC groups, before and after treatment

Study Group	Number of children	Lactoferrin concentreation	P value
Treated ECC	15	42 ± 11.62	0.2
ECC before treatment	21	37.9 ± 16.43	

Figure 16 and 17: Levels of lysozyme and lactoferrin before treatment and after. (Moslemi *et al*. (51))

Sun *et al.* conducted a study with a total of 30 stimulated saliva samples on 10 S-ECC children aged 3-5 years old, and divided them into 3 groups (before treatment, 1 week after treatment and 4 weeks after restorative treatment). They revealed that the level of Histatin-1 is higher in children after the treatment

(P value <0.005), indicating the strong correlation between Histatin-1 and the absence of dental caries (52) (Figure. 18).

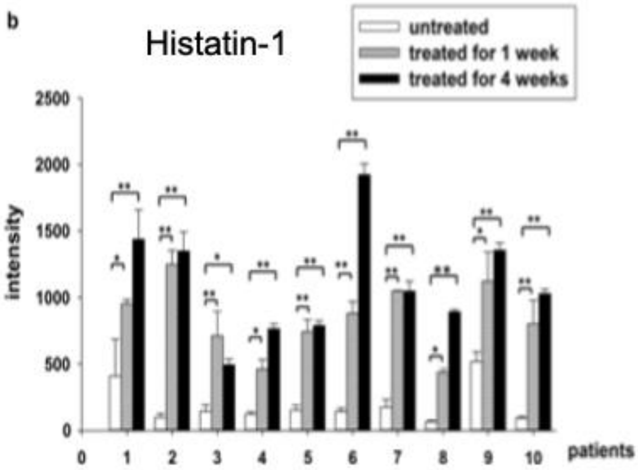


Figure 18: Histogram of Histatin-1 from 3 groups, before treatment, 1 week and 4 weeks after the treatment. (Sun *et al*. (52))

Study conducted by Sharma *et al.* in 2017 of 25 children with ECC to detect the level of inflammatory cytokines (IL6, IL8 and TNF-α) in three groups: before treatment, after treatment, and control. Figure 19 shows that all cytokine levels are higher in children with ECC, and levels decreased in post-treatment, indicating a positive correlation with ECC. (P value<0.005) (53).

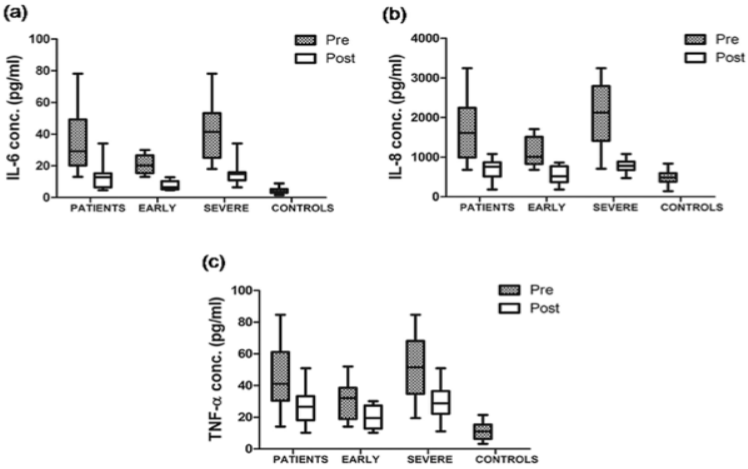
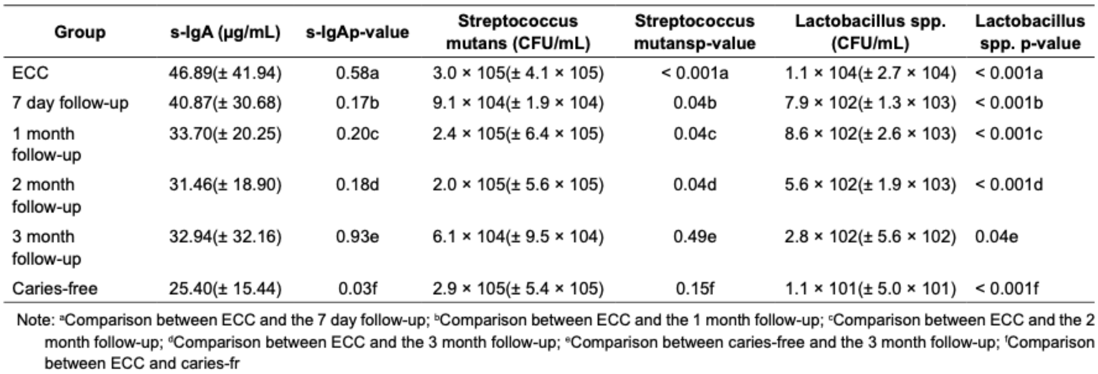


Figure 19: Level of IL6, IL8 and TNF-α in different group samples. (Sharma *et al*. (53))

Letieri *et al.* conducted another study to assess the level of Immunoglobulin A (IgA) in children affected by ECC before and after restorative treatment, with a total of 46 children of age between 24 to 71 months and divided them into two groups: caries-free and ECC-affected. Figure 20 shows that compared with the caries-free group, the levels of IgA, *Streptococcus mutans* and *Lactobacillus* are higher in the ECC-affected group (P value <0.05). (54)

However, the levels of IgA and *S. mutans* do not show a significant reduction in the ECC-affected group in the 3-month follow-up period, indicating that there is no correlation between IgA and *S. mutans* before and after the treatment. (54)



Group	s-IgA (μg/mL)	s-IgAp-value	Streptococcus mutans (CFU/mL)	Streptococcus mutansp-value	Lactobacillus spp. (CFU/mL)	Lactobacillus spp. p-value
ECC	46.89(± 41.94)	0.58a	3.0 × 105(± 4.1 × 105)	< 0.001a	1.1 × 104(± 2.7 × 104)	< 0.001a
7 day follow-up	40.87(± 30.68)	0.17b	9.1 × 104(± 1.9 × 104)	0.04b	7.9 × 102(± 1.3 × 103)	< 0.001b
1 month follow-up	33.70(± 20.25)	0.20c	2.4 × 105(± 6.4 × 105)	0.04c	8.6 × 102(± 2.6 × 103)	< 0.001c
2 month follow-up	31.46(± 18.90)	0.18d	2.0 × 105(± 5.6 × 105)	0.04d	5.6 × 102(± 1.9 × 103)	< 0.001d
3 month follow-up	32.94(± 32.16)	0.93e	6.1 × 104(± 9.5 × 104)	0.49e	2.8 × 102(± 5.6 × 102)	0.04e
Caries-free	25.40(± 15.44)	0.03f	2.9 × 105(± 5.4 × 105)	0.15f	1.1 × 101(± 5.0 × 101)	< 0.001f

Note: [a]Comparison between ECC and the 7 day follow-up; [b]Comparison between ECC and the 1 month follow-up; [c]Comparison between ECC and the 2 month follow-up; [d]Comparison between ECC and the 3 month follow-up; [e]Comparison between caries-free and the 3 month follow-up; [f]Comparison between ECC and caries-fr

Figure 20: Levels of IgA, *Streptococcus mutans* and *Lactobacillus* of caries-free and ECC-affected children before and after the treatment. (Letieri *et al.* (54))

Conclusions

- Saliva is a biofluid that can be used as a biomarker to detect caries due to the factors of easy collection, non-invasive and well accepted by patients.

- Early childhood caries (ECC) refers to the sign of caries, missing due to decay and filled surface in children at the age of 6 years old or younger. High sugar intake is the main cause of the ECC, other causes include acquisitions of the oral microbiota, developmental defects and breastfeeding longer than 12 months.

- There are more than 700 oral microbiotas in the oral cavity, maintaining commensal relationship with the host. When a state of imbalance has presented, various infections might occur.

- Nowadays, biomarkers are used as a preventive method to study and evaluate the possible causes of the infection, and to early detect and prevent of the development of the diseases.

- *Streptococcus mutans* is the most common microbiota in the oral cavity*,* and also is the most important bacteria that affects dental caries, followed by *Lactobacillus* and *Actinomycetes*. *S. mutans* can be used as a biomarker of

ECC, but it is not very effective for children who have undergone restorative treatment.

- Salivary proteins such as Cathelicidin LL-37, Histatin 1, Statherin, Mucin 7, Proline-rich proteins, immunoglobulins and inflammatory cytokines have been shown to have an impact on the detection of the ECC; However, there is no positive correlation between lysozyme, lactoferrin and ECC, therefore they cannot be considered as biomarkers.
- There is still controversy between articles discussing the relationship between the salivary flow rate, buffer capacity and ECC, therefore, they cannot become potential biomarkers.

Responsibility

The present work reviewed the Microbiota as possible biomarkers to detect early caries disease. The use of microbiota as biomarker is a new powerful tool to detect and prevent the onset of caries especially during childhood. This work also highlights the responsibility of the practitioners, to inform and educate parents and children in order to prevent the onset of caries that can degenerate and produce more severe pathologies. Dental programs are essential in social events and schools in order to provide information and educate about a proper oral hygiene techniques and oral examinations.

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Annexes

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Research
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The Human Microbiota in Health and Disease

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ABSTRACT

Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases. We also review important advances in techniques associated with microbial research, such as DNA sequencing, metabonomics, and proteomics combined with computation-based bioinformatics. Current research on the human microbiota has become much more sophisticated and more comprehensive. Therefore, we propose that research should focus on the host-microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

1. Introduction

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6]. Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota [7,8] (Fig.1).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7,9–23] via several mechanisms. First, the microbiota has the potential to increase energy extraction from food [24], increase nutrient harvest [9,10], and alter appetite signaling [25,26]. The microbiota contains far more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes and biochemical pathways [9]. In addition, a large proportion of the metabolic microbiotic processes that are beneficial to the host are involved in either nutrient acquisition or xenobiotic processing, including the metabolism of undigested carbohydrates and the biosynthesis of vitamins [10]. Second, the human microbiota also provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances [11–13]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [14,16]. For example, germ-free (GF) animals have abnormal numbers of several immune cell types, deficits in local and systemic lymphoid structures, poorly formed spleens and lymph

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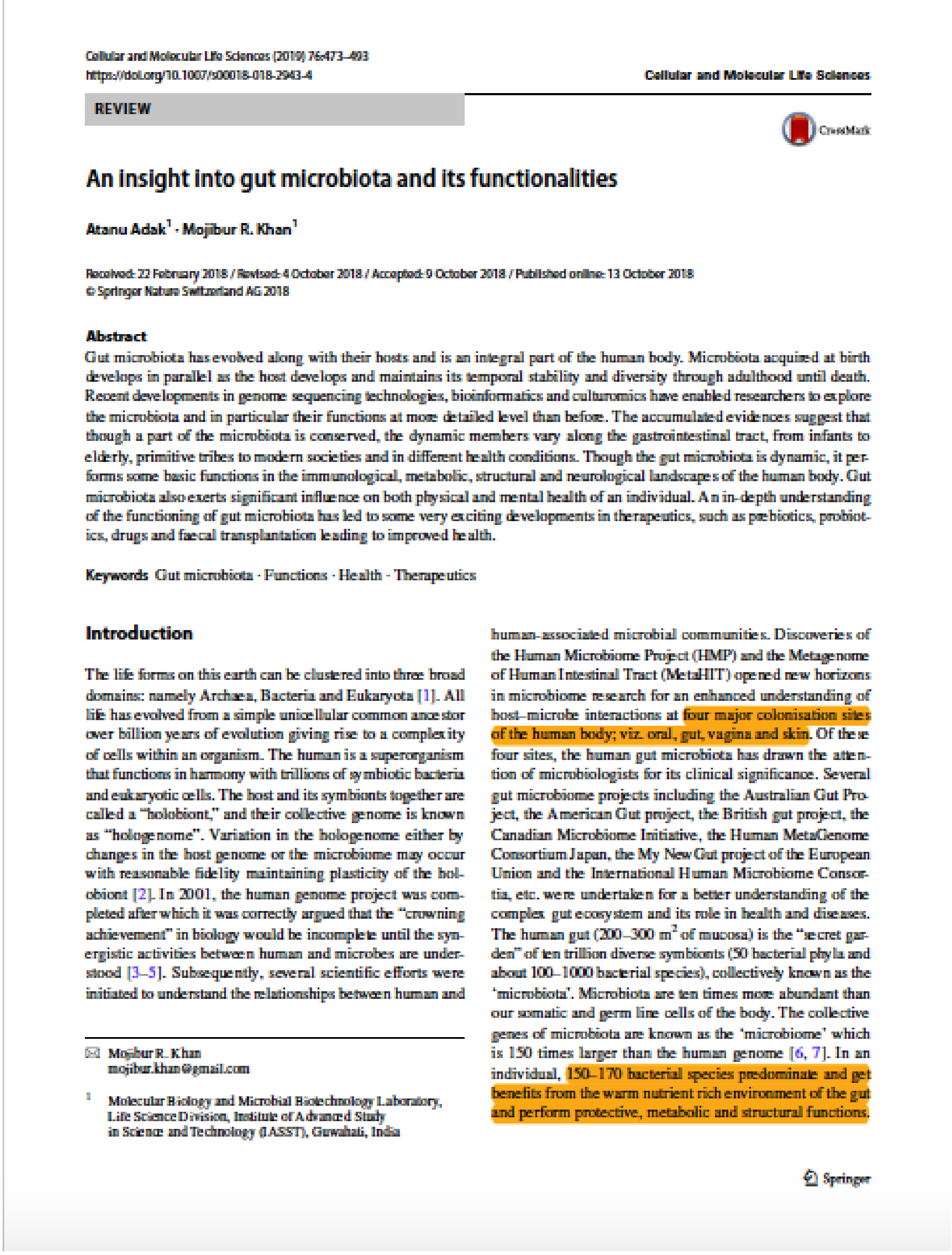

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Cellular and Molecular Life Sciences

REVIEW

An insight into gut microbiota and its functionalities

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Abstract

Gut microbiota has evolved along with their hosts and is an integral part of the human body. Microbiota acquired at birth develops in parallel as the host develops and maintains its temporal stability and diversity through adulthood until death. Recent developments in genome sequencing technologies, bioinformatics and culturomics have enabled researchers to explore the microbiota and in particular their functions at more detailed level than before. The accumulated evidences suggest that though a part of the microbiota is conserved, the dynamic members vary along the gastrointestinal tract, from infants to elderly, primitive tribes to modern societies and in different health conditions. Though the gut microbiota is dynamic, it performs some basic functions in the immunological, metabolic, structural and neurological landscapes of the human body. Gut microbiota also exerts significant influence on both physical and mental health of an individual. An in-depth understanding of the functioning of gut microbiota has led to some very exciting developments in therapeutics, such as prebiotics, probiotics, drugs and faecal transplantation leading to improved health.

Keywords Gut microbiota · Functions · Health · Therapeutics

Introduction

The life forms on this earth can be clustered into three broad domains: namely Archaea, Bacteria and Eukaryota [1]. All life has evolved from a simple unicellular common ancestor over billion years of evolution giving rise to a complexity of cells within an organism. The human is a superorganism that functions in harmony with trillions of symbiotic bacteria and eukaryotic cells. The host and its symbionts together are called a "holobiont," and their collective genome is known as "hologenome". Variation in the hologenome either by changes in the host genome or the microbiome may occur with reasonable fidelity maintaining plasticity of the holobiont [2]. In 2001, the human genome project was completed after which it was correctly argued that the "crowning achievement" in biology would be incomplete until the synergistic activities between human and microbes are understood [3–5]. Subsequently, several scientific efforts were initiated to understand the relationships between human and human-associated microbial communities. Discoveries of the Human Microbiome Project (HMP) and the Metagenome of Human Intestinal Tract (MetaHIT) opened new horizons in microbiome research for an enhanced understanding of host–microbe interactions at four major colonisation sites of the human body; viz. oral, gut, vagina and skin. Of these four sites, the human gut microbiota has drawn the attention of microbiologists for its clinical significance. Several gut microbiome projects including the Australian Gut Project, the American Gut project, the British gut project, the Canadian Microbiome Initiative, the Human MetaGenome Consortium Japan, the My New Gut project of the European Union and the International Human Microbiome Consortia, etc. were undertaken for a better understanding of the complex gut ecosystem and its role in health and diseases. The human gut (200–300 m^2 of mucosa) is the "secret garden" of ten trillion diverse symbionts (50 bacterial phyla and about 100–1000 bacterial species), collectively known as the 'microbiota'. Microbiota are ten times more abundant than our somatic and germ line cells of the body. The collective genes of microbiota are known as the 'microbiome' which is 150 times larger than the human genome [6, 7]. In an individual, 150–170 bacterial species predominate and get benefits from the warm nutrient rich environment of the gut and perform protective, metabolic and structural functions.

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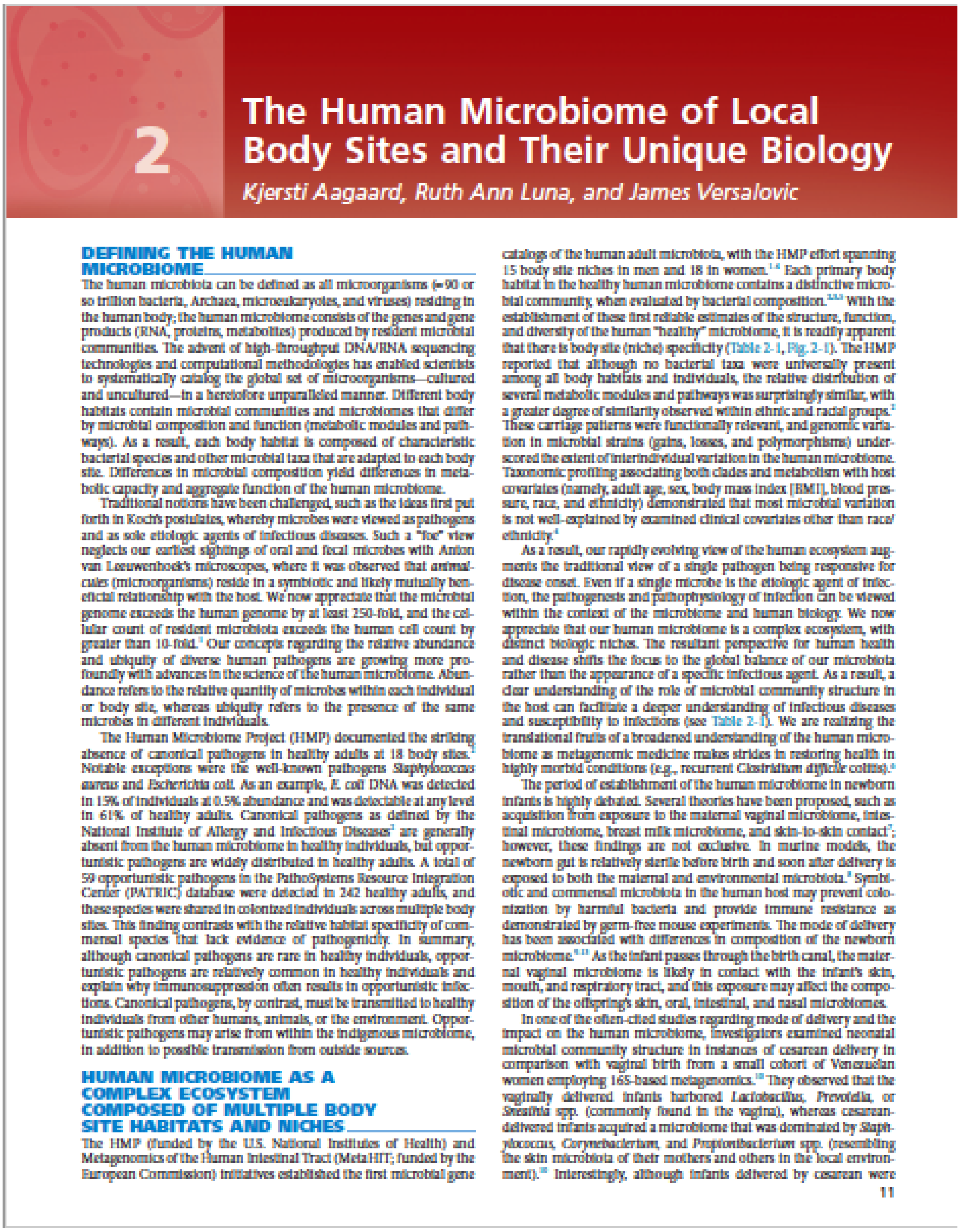

2

The Human Microbiome of Local Body Sites and Their Unique Biology

Kjersti Aagaard, Ruth Ann Luna, and James Versalovic

DEFINING THE HUMAN MICROBIOME

The human microbiota can be defined as all microorganisms (≈90 or so trillion bacteria, Archaea, microeukaryotes, and viruses) residing in the human body; the human microbiome consists of the genes and gene products (RNA, proteins, metabolites) produced by resident microbial communities. The advent of high-throughput DNA/RNA sequencing technologies and computational methodologies has enabled scientists to systematically catalog the global set of microorganisms—cultured and uncultured—in a heretofore unparalleled manner. Different body habitats contain microbial communities and microbiomes that differ by microbial composition and function (metabolic modules and pathways). As a result, each body habitat is composed of characteristic bacterial species and other microbial taxa that are adapted to each body site. Differences in microbial composition yield differences in metabolic capacity and aggregate function of the human microbiome.

Traditional notions have been challenged, such as the ideas first put forth in Koch's postulates, whereby microbes were viewed as pathogens and as sole etiologic agents of infectious diseases. Such a "foe" view neglects our earliest sightings of oral and fecal microbes with Anton van Leeuwenhoek's microscopes, where it was observed that animalcules (microorganisms) reside in a symbiotic and likely mutually beneficial relationship with the host. We now appreciate that the microbial genome exceeds the human genome by at least 250-fold, and the cellular count of resident microbiota exceeds the human cell count by greater than 10-fold.[1] Our concepts regarding the relative abundance and ubiquity of diverse human pathogens are growing more profoundly with advances in the science of the human microbiome. Abundance refers to the relative quantity of microbes within each individual or body site, whereas ubiquity refers to the presence of the same microbes in different individuals.

The Human Microbiome Project (HMP) documented the striking absence of canonical pathogens in healthy adults at 18 body sites.[2] Notable exceptions were the well-known pathogens *Staphylococcus aureus* and *Escherichia coli*. As an example, *E. coli* DNA was detected in 15% of individuals at 0.5% abundance and was detectable at any level in 61% of healthy adults. Canonical pathogens as defined by the National Institute of Allergy and Infectious Diseases[3] are generally absent from the human microbiome in healthy individuals, but opportunistic pathogens are widely distributed in healthy adults. A total of 59 opportunistic pathogens in the PathoSystems Resource Integration Center (PATRIC) database were detected in 242 healthy adults, and these species were shared in colonized individuals across multiple body sites. This finding contrasts with the relative habitat specificity of commensal species that lack evidence of pathogenicity. In summary, although canonical pathogens are rare in healthy individuals, opportunistic pathogens are relatively common in healthy individuals and explain why immunosuppression often results in opportunistic infections. Canonical pathogens, by contrast, must be transmitted to healthy individuals from other humans, animals, or the environment. Opportunistic pathogens may arise from within the indigenous microbiome, in addition to possible transmission from outside sources.

HUMAN MICROBIOME AS A COMPLEX ECOSYSTEM COMPOSED OF MULTIPLE BODY SITE HABITATS AND NICHES

The HMP (funded by the U.S. National Institutes of Health) and Metagenomics of the Human Intestinal Tract (MetaHIT; funded by the European Commission) initiatives established the first microbial gene catalogs of the human adult microbiota, with the HMP effort spanning 15 body site niches in men and 18 in women.[1-4] Each primary body habitat in the healthy human microbiome contains a distinctive microbial community, when evaluated by bacterial composition.[2,3,5] With the establishment of these first reliable estimates of the structure, function, and diversity of the human "healthy" microbiome, it is readily apparent that there is body site (niche) specificity (Table 2-1, Fig. 2-1). The HMP reported that although no bacterial taxa were universally present among all body habitats and individuals, the relative distribution of several metabolic modules and pathways was surprisingly similar, with a greater degree of similarity observed within ethnic and racial groups.[2] These carriage patterns were functionally relevant, and genomic variation in microbial strains (gains, losses, and polymorphisms) underscored the extent of interindividual variation in the human microbiome. Taxonomic profiling associating both clades and metabolism with host covariates (namely, adult age, sex, body mass index [BMI], blood pressure, race, and ethnicity) demonstrated that most microbial variation is not well-explained by examined clinical covariates other than race/ethnicity.[4]

As a result, our rapidly evolving view of the human ecosystem augments the traditional view of a single pathogen being responsive for disease onset. Even if a single microbe is the etiologic agent of infection, the pathogenesis and pathophysiology of infection can be viewed within the context of the microbiome and human biology. We now appreciate that our human microbiome is a complex ecosystem, with distinct biologic niches. The resultant perspective for human health and disease shifts the focus to the global balance of our microbiota rather than the appearance of a specific infectious agent. As a result, a clear understanding of the role of microbial community structure in the host can facilitate a deeper understanding of infectious diseases and susceptibility to infections (see Table 2-1). We are realizing the translational fruits of a broadened understanding of the human microbiome as metagenomic medicine makes strides in restoring health in highly morbid conditions (e.g., recurrent *Clostridium difficile* colitis).[6]

The period of establishment of the human microbiome in newborn infants is highly debated. Several theories have been proposed, such as acquisition from exposure to the maternal vaginal microbiome, intestinal microbiome, breast milk microbiome, and skin-to-skin contact[7]; however, these findings are not exclusive. In murine models, the newborn gut is relatively sterile before birth and soon after delivery is exposed to both the maternal and environmental microbiota.[8] Symbiotic and commensal microbiota in the human host may prevent colonization by harmful bacteria and provide immune resistance as demonstrated by germ-free mouse experiments. The mode of delivery has been associated with differences in composition of the newborn microbiome.[9-11] As the infant passes through the birth canal, the maternal vaginal microbiome is likely in contact with the infant's skin, mouth, and respiratory tract, and this exposure may affect the composition of the offspring's skin, oral, intestinal, and nasal microbiomes.

In one of the often-cited studies regarding mode of delivery and the impact on the human microbiome, investigators examined neonatal microbial community structure in instances of cesarean delivery in comparison with vaginal birth from a small cohort of Venezuelan women employing 16S-based metagenomics.[10] They observed that the vaginally delivered infants harbored *Lactobacillus*, *Prevotella*, or *Sneathia* spp. (commonly found in the vagina), whereas cesarean-delivered infants acquired a microbiome that was dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. (resembling the skin microbiota of their mothers and others in the local environment).[10] Interestingly, although infants delivered by cesarean were

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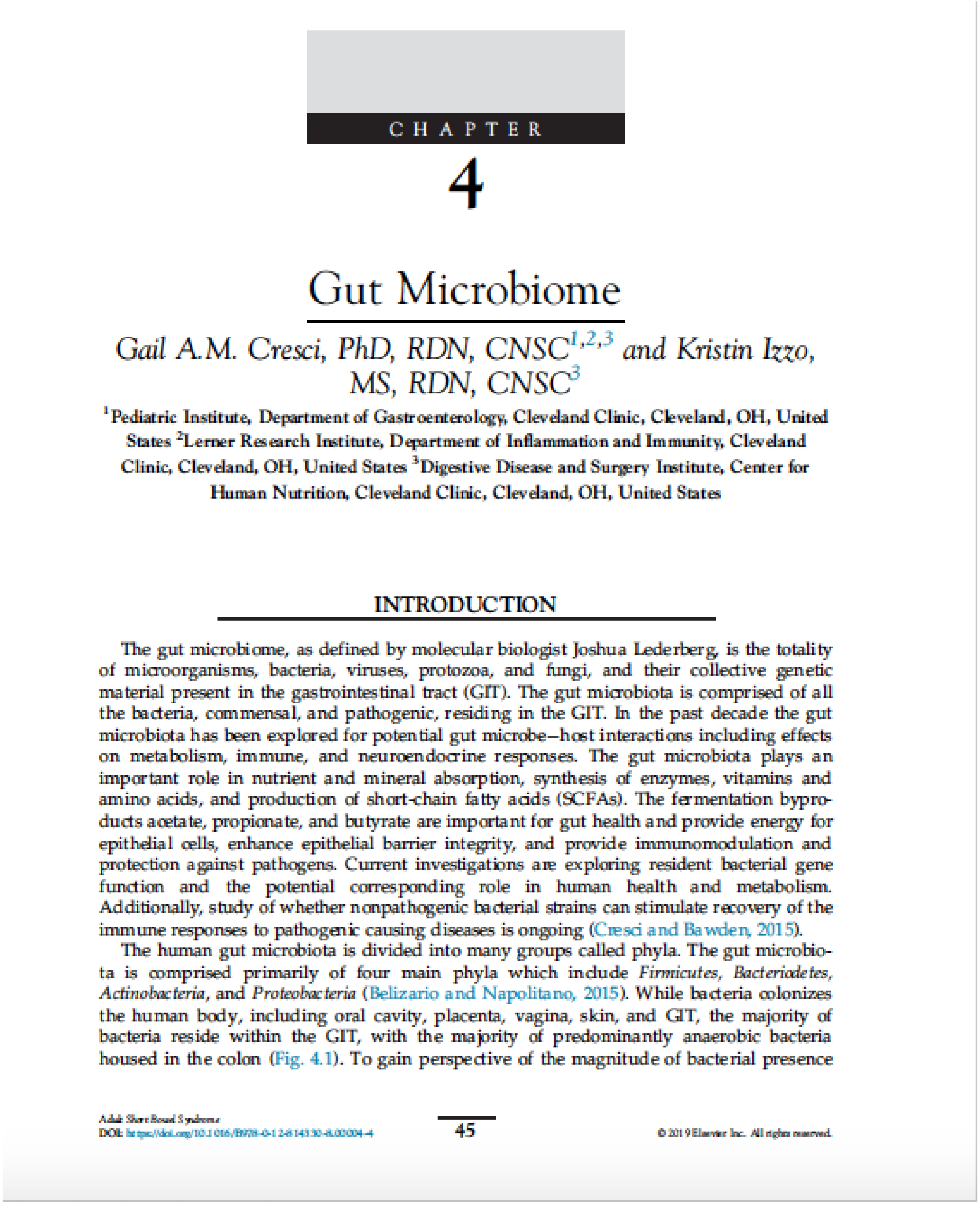

CHAPTER

4

Gut Microbiome

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INTRODUCTION

The gut microbiome, as defined by molecular biologist Joshua Lederberg, is the totality of microorganisms, bacteria, viruses, protozoa, and fungi, and their collective genetic material present in the gastrointestinal tract (GIT). The gut microbiota is comprised of all the bacteria, commensal, and pathogenic, residing in the GIT. In the past decade the gut microbiota has been explored for potential gut microbe–host interactions including effects on metabolism, immune, and neuroendocrine responses. The gut microbiota plays an important role in nutrient and mineral absorption, synthesis of enzymes, vitamins and amino acids, and production of short-chain fatty acids (SCFAs). The fermentation byproducts acetate, propionate, and butyrate are important for gut health and provide energy for epithelial cells, enhance epithelial barrier integrity, and provide immunomodulation and protection against pathogens. Current investigations are exploring resident bacterial gene function and the potential corresponding role in human health and metabolism. Additionally, study of whether nonpathogenic bacterial strains can stimulate recovery of the immune responses to pathogenic causing diseases is ongoing (Cresci and Bawden, 2015).

The human gut microbiota is divided into many groups called phyla. The gut microbiota is comprised primarily of four main phyla which include *Firmicutes*, *Bacteriodetes*, *Actinobacteria*, and *Proteobacteria* (Belizario and Napolitano, 2015). While bacteria colonizes the human body, including oral cavity, placenta, vagina, skin, and GIT, the majority of bacteria reside within the GIT, with the majority of predominantly anaerobic bacteria housed in the colon (Fig. 4.1). To gain perspective of the magnitude of bacterial presence

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The Early Settlers: Intestinal Microbiology in Early Life

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Keywords

infants, microbiota, human physiology, nutrition, immune system, metabolism

Abstract

The human intestinal microbiota forms an integral part of normal human physiology, and disturbances of the normal gut microbiology have been implicated in many health and disease issues. Because newborns are essentially sterile, their microbiota must establish and develop from the very first days of life. The first colonizers play an important role in the development of the ecosystem and may impact the long-term composition and activity of the microbiota. These first settlers obviously develop and proliferate dependent on host characteristics and diet, but other factors can also significantly contribute to this vital biological process. Considering the importance of the microbiota for the human immune, metabolic, and neurological systems, it is important to understand the dynamics and driving determinants of this development. This review gives a global overview of our current understanding of the different factors impacting the intestinal microbiology in early life.

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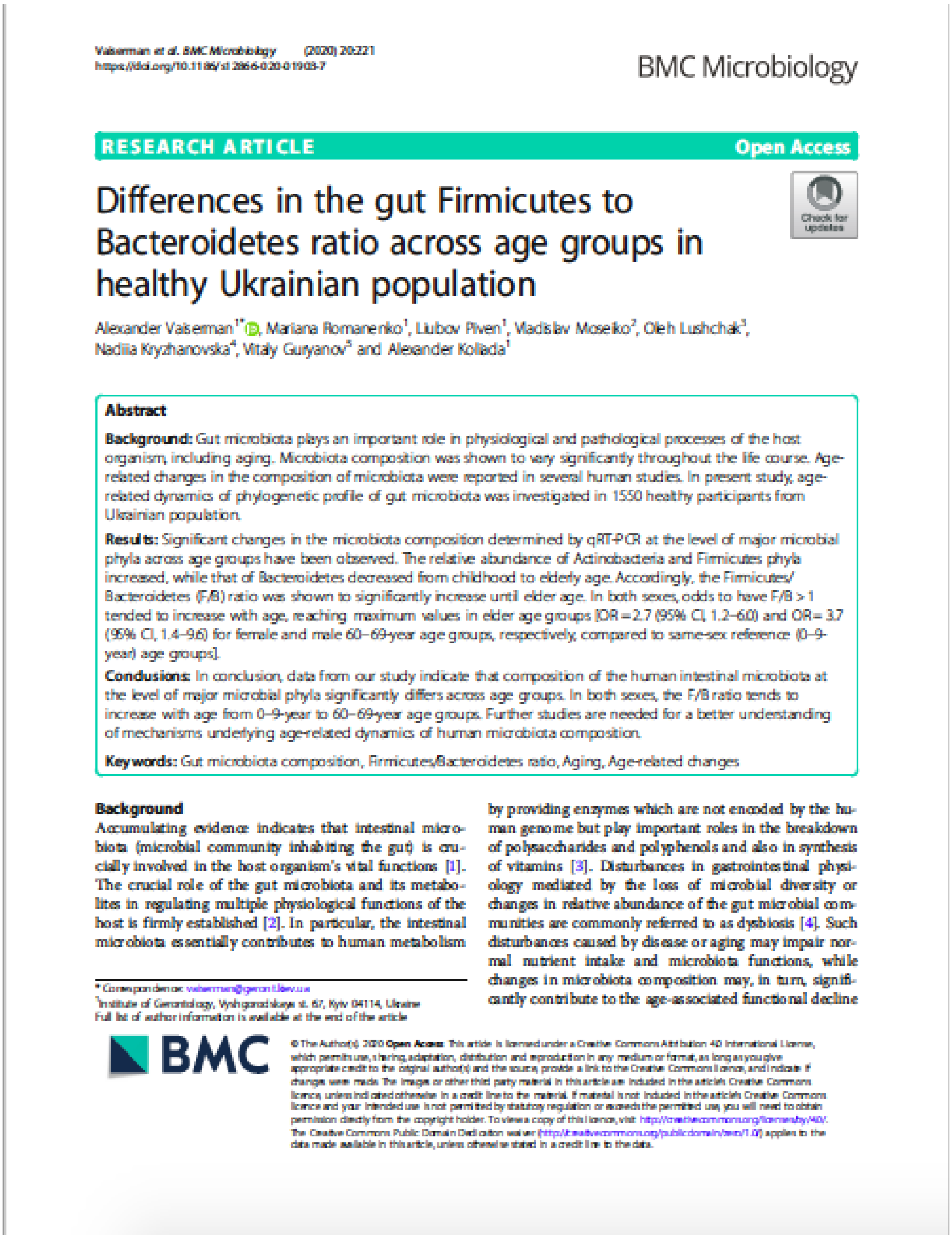

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BMC Microbiology

RESEARCH ARTICLE Open Access

Differences in the gut Firmicutes to Bacteroidetes ratio across age groups in healthy Ukrainian population

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Abstract

Background: Gut microbiota plays an important role in physiological and pathological processes of the host organism, including aging. Microbiota composition was shown to vary significantly throughout the life course. Age-related changes in the composition of microbiota were reported in several human studies. In present study, age-related dynamics of phylogenetic profile of gut microbiota was investigated in 1550 healthy participants from Ukrainian population.

Results: Significant changes in the microbiota composition determined by qRT-PCR at the level of major microbial phyla across age groups have been observed. The relative abundance of Actinobacteria and Firmicutes phyla increased, while that of Bacteroidetes decreased from childhood to elderly age. Accordingly, the Firmicutes/Bacteroidetes (F/B) ratio was shown to significantly increase until elder age. In both sexes, odds to have F/B > 1 tended to increase with age, reaching maximum values in elder age groups [OR = 2.7 (95% CI, 1.2–6.0) and OR = 3.7 (95% CI, 1.4–9.6) for female and male 60–69-year age groups, respectively, compared to same-sex reference (0–9-year) age groups].

Conclusions: In conclusion, data from our study indicate that composition of the human intestinal microbiota at the level of major microbial phyla significantly differs across age groups. In both sexes, the F/B ratio tends to increase with age from 0–9-year to 60–69-year age groups. Further studies are needed for a better understanding of mechanisms underlying age-related dynamics of human microbiota composition.

Keywords: Gut microbiota composition, Firmicutes/Bacteroidetes ratio, Aging, Age-related changes

Background

Accumulating evidence indicates that intestinal microbiota (microbial community inhabiting the gut) is crucially involved in the host organism's vital functions [1]. The crucial role of the gut microbiota and its metabolites in regulating multiple physiological functions of the host is firmly established [2]. In particular, the intestinal microbiota essentially contributes to human metabolism by providing enzymes which are not encoded by the human genome but play important roles in the breakdown of polysaccharides and polyphenols and also in synthesis of vitamins [3]. Disturbances in gastrointestinal physiology mediated by the loss of microbial diversity or changes in relative abundance of the gut microbial communities are commonly referred to as dysbiosis [4]. Such disturbances caused by disease or aging may impair normal nutrient intake and microbiota functions, while changes in microbiota composition may, in turn, significantly contribute to the age-associated functional decline

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microorganisms

MDPI

Review

The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel disease

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Abstract: The two most important bacterial phyla in the gastrointestinal tract, Firmicutes and Bacteroidetes, have gained much attention in recent years. The Firmicutes/Bacteroidetes (F/B) ratio is widely accepted to have an important influence in maintaining normal intestinal homeostasis. Increased or decreased F/B ratio is regarded as dysbiosis, whereby the former is usually observed with obesity, and the latter with inflammatory bowel disease (IBD). Probiotics as live microorganisms can confer health benefits to the host when administered in adequate amounts. There is considerable evidence of their nutritional and immunosuppressive properties including reports that elucidate the association of probiotics with the F/B ratio, obesity, and IBD. Orally administered probiotics can contribute to the restoration of dysbiotic microbiota and to the prevention of obesity or IBD. However, as the effects of different probiotics on the F/B ratio differ, selecting the appropriate species or mixture is crucial. The most commonly tested probiotics for modifying the F/B ratio and treating obesity and IBD are from the genus *Lactobacillus*. In this paper, we review the effects of probiotics on the F/B ratio that lead to weight loss or immunosuppression.

Keywords: probiotics; Firmicutes; Bacteroidetes; dysbiosis; obesity; inflammation

1. Introduction

In the human body, trillions of microorganisms live in symbiosis with the host and are mainly located in the gastrointestinal tract, skin, saliva, oral mucosa, conjunctiva, and vagina [1]. Microorganisms that inhabit the gastrointestinal tract (i.e., gut microbiota) number approximately 1×10^{14} [2] and play an essential role in intestinal homeostasis, development, and protection against pathogens. Furthermore, their presence in the gut is associated with immunomodulatory and metabolic reactions [3]. Gut microbiota consists of bacteria, yeasts, and viruses. Bacteria in the gut are represented by more than 1000 species that belong to six dominant phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Bacteria from the phyla Firmicutes and Bacteroidetes are the most common, representing 90% of the gut microbiota [4]. The gut microbiota of a healthy individual differs in different parts of the gastrointestinal tract and changes with time due to aging (including infant development) and environmental factors such as dietary habits, lifestyle, and antibiotic consumption. Large differences in microbiota composition exist among individuals, with the differences attributed to age, ethnicity, lifestyle, and diet [4,5]. Different microbiota are classified into three distinct enterotypes [6]. Such variations are considered physiological and consistent with healthy microbiota. Nevertheless, changes in microbiota composition are often related to diseases, also termed dysbioses. However, the causality between altered microbiota and various diseases is often unclear.

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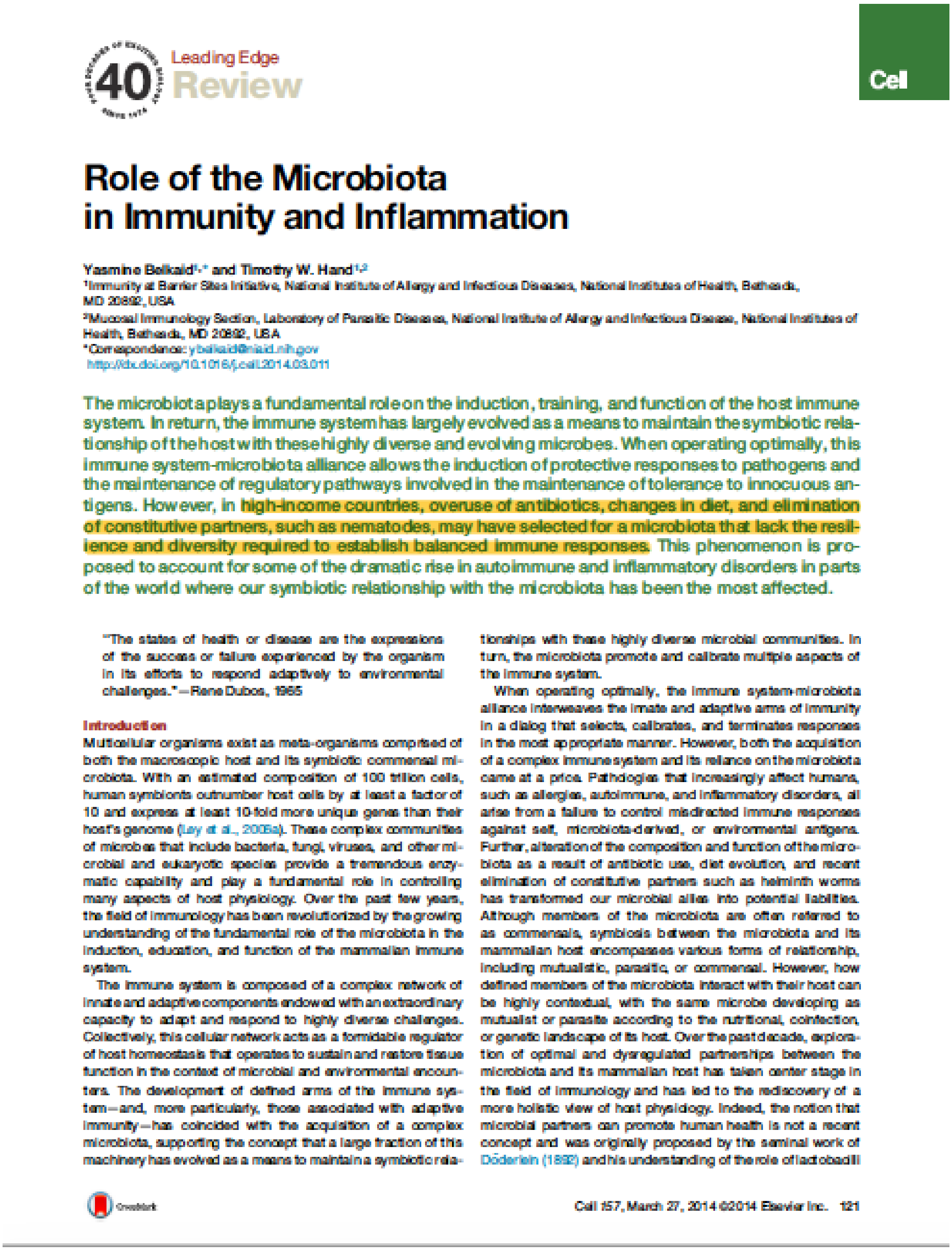

Leading Edge
Review

Cell

Role of the Microbiota in Immunity and Inflammation

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The microbiota plays a fundamental role on the induction, training, and function of the host immune system. In return, the immune system has largely evolved as a means to maintain the symbiotic relationship of the host with these highly diverse and evolving microbes. When operating optimally, this immune system-microbiota alliance allows the induction of protective responses to pathogens and the maintenance of regulatory pathways involved in the maintenance of tolerance to innocuous antigens. However, in high-income countries, overuse of antibiotics, changes in diet, and elimination of constitutive partners, such as nematodes, may have selected for a microbiota that lack the resilience and diversity required to establish balanced immune responses. This phenomenon is proposed to account for some of the dramatic rise in autoimmune and inflammatory disorders in parts of the world where our symbiotic relationship with the microbiota has been the most affected.

> "The states of health or disease are the expressions of the success or failure experienced by the organism in its efforts to respond adaptively to environmental challenges."—Rene Dubos, 1965

Introduction

Multicellular organisms exist as meta-organisms comprised of both the macroscopic host and its symbiotic commensal microbiota. With an estimated composition of 100 trillion cells, human symbionts outnumber host cells by at least a factor of 10 and express at least 10-fold more unique genes than their host's genome (Ley et al., 2006a). These complex communities of microbes that include bacteria, fungi, viruses, and other microbial and eukaryotic species provide a tremendous enzymatic capability and play a fundamental role in controlling many aspects of host physiology. Over the past few years, the field of immunology has been revolutionized by the growing understanding of the fundamental role of the microbiota in the induction, education, and function of the mammalian immune system.

The immune system is composed of a complex network of innate and adaptive components endowed with an extraordinary capacity to adapt and respond to highly diverse challenges. Collectively, this cellular network acts as a formidable regulator of host homeostasis that operates to sustain and restore tissue function in the context of microbial and environmental encounters. The development of defined arms of the immune system—and, more particularly, those associated with adaptive immunity—has coincided with the acquisition of a complex microbiota, supporting the concept that a large fraction of this machinery has evolved as a means to maintain a symbiotic relationships with these highly diverse microbial communities. In turn, the microbiota promote and calibrate multiple aspects of the immune system.

When operating optimally, the immune system-microbiota alliance interweaves the innate and adaptive arms of immunity in a dialog that selects, calibrates, and terminates responses in the most appropriate manner. However, both the acquisition of a complex immune system and its reliance on the microbiota came at a price. Pathologies that increasingly affect humans, such as allergies, autoimmune, and inflammatory disorders, all arise from a failure to control misdirected immune responses against self, microbiota-derived, or environmental antigens. Further, alteration of the composition and function of the microbiota as a result of antibiotic use, diet evolution, and recent elimination of constitutive partners such as helminth worms has transformed our microbial allies into potential liabilities. Although members of the microbiota are often referred to as commensals, symbiosis between the microbiota and its mammalian host encompasses various forms of relationship, including mutualistic, parasitic, or commensal. However, how defined members of the microbiota interact with their host can be highly contextual, with the same microbe developing as mutualist or parasite according to the nutritional, coinfection, or genetic landscape of its host. Over the past decade, exploration of optimal and dysregulated partnerships between the microbiota and its mammalian host has taken center stage in the field of immunology and has led to the rediscovery of a more holistic view of host physiology. Indeed, the notion that microbial partners can promote human health is not a recent concept and was originally proposed by the seminal work of Döderlein (1892) and his understanding of the role of lactobacilli

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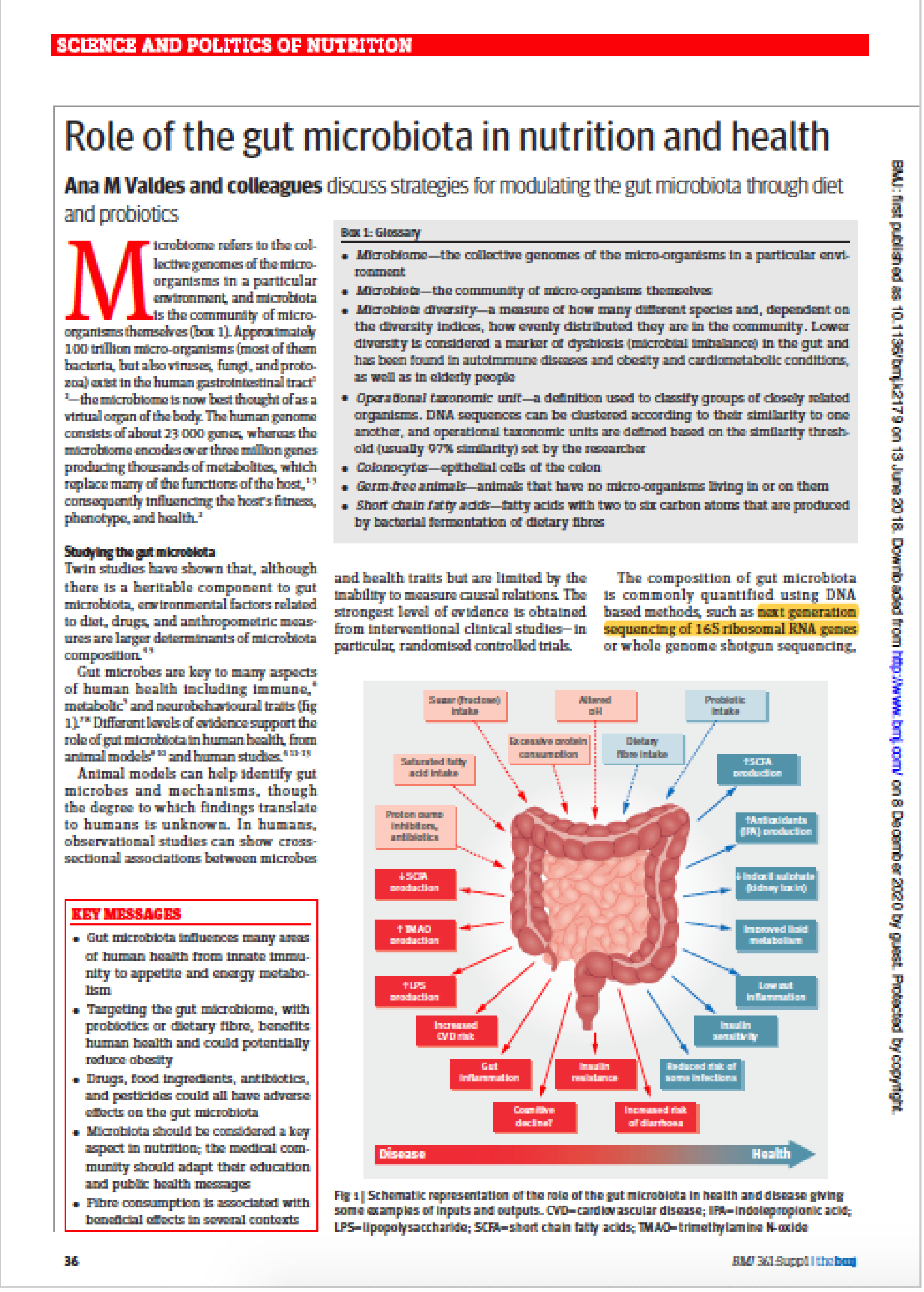

Role of the gut microbiota in nutrition and health

Ana M Valdes and colleagues discuss strategies for modulating the gut microbiota through diet and probiotics

Microbiome refers to the collective genomes of the micro-organisms in a particular environment, and microbiota is the community of micro-organisms themselves (box 1). Approximately 100 trillion micro-organisms (most of them bacteria, but also viruses, fungi, and protozoa) exist in the human gastrointestinal tract[1] [2]—the microbiome is now best thought of as a virtual organ of the body. The human genome consists of about 23 000 genes, whereas the microbiome encodes over three million genes producing thousands of metabolites, which replace many of the functions of the host,[1] [3] consequently influencing the host's fitness, phenotype, and health.[2]

Studying the gut microbiota

Twin studies have shown that, although there is a heritable component to gut microbiota, environmental factors related to diet, drugs, and anthropometric measures are larger determinants of microbiota composition.[4] [5]

Gut microbes are key to many aspects of human health including immune,[6] metabolic[3] and neurobehavioural traits (fig 1).[7] [8] Different levels of evidence support the role of gut microbiota in human health, from animal models[9] [10] and human studies.[4] [11-15]

Animal models can help identify gut microbes and mechanisms, though the degree to which findings translate to humans is unknown. In humans, observational studies can show cross-sectional associations between microbes and health traits but are limited by the inability to measure causal relations. The strongest level of evidence is obtained from interventional clinical studies—in particular, randomised controlled trials.

The composition of gut microbiota is commonly quantified using DNA based methods, such as next generation sequencing of 16S ribosomal RNA genes or whole genome shotgun sequencing.

KEY MESSAGES

- Gut microbiota influences many areas of human health from innate immunity to appetite and energy metabolism
- Targeting the gut microbiome, with probiotics or dietary fibre, benefits human health and could potentially reduce obesity
- Drugs, food ingredients, antibiotics, and pesticides could all have adverse effects on the gut microbiota
- Microbiota should be considered a key aspect in nutrition; the medical community should adapt their education and public health messages
- Fibre consumption is associated with beneficial effects in several contexts

Box 1: Glossary

- *Microbiome*—the collective genomes of the micro-organisms in a particular environment
- *Microbiota*—the community of micro-organisms themselves
- *Microbiota diversity*—a measure of how many different species and, dependent on the diversity indices, how evenly distributed they are in the community. Lower diversity is considered a marker of dysbiosis (microbial imbalance) in the gut and has been found in autoimmune diseases and obesity and cardiometabolic conditions, as well as in elderly people
- *Operational taxonomic unit*—a definition used to classify groups of closely related organisms. DNA sequences can be clustered according to their similarity to one another, and operational taxonomic units are defined based on the similarity threshold (usually 97% similarity) set by the researcher
- *Colonocytes*—epithelial cells of the colon
- *Germ-free animals*—animals that have no micro-organisms living in or on them
- *Short chain fatty acids*—fatty acids with two to six carbon atoms that are produced by bacterial fermentation of dietary fibres

Fig 1 | Schematic representation of the role of the gut microbiota in health and disease giving some examples of inputs and outputs. CVD=cardiovascular disease; IPA=indolepropionic acid; LPS=lipopolysaccharide; SCFA=short chain fatty acids; TMAO=trimethylamine N-oxide

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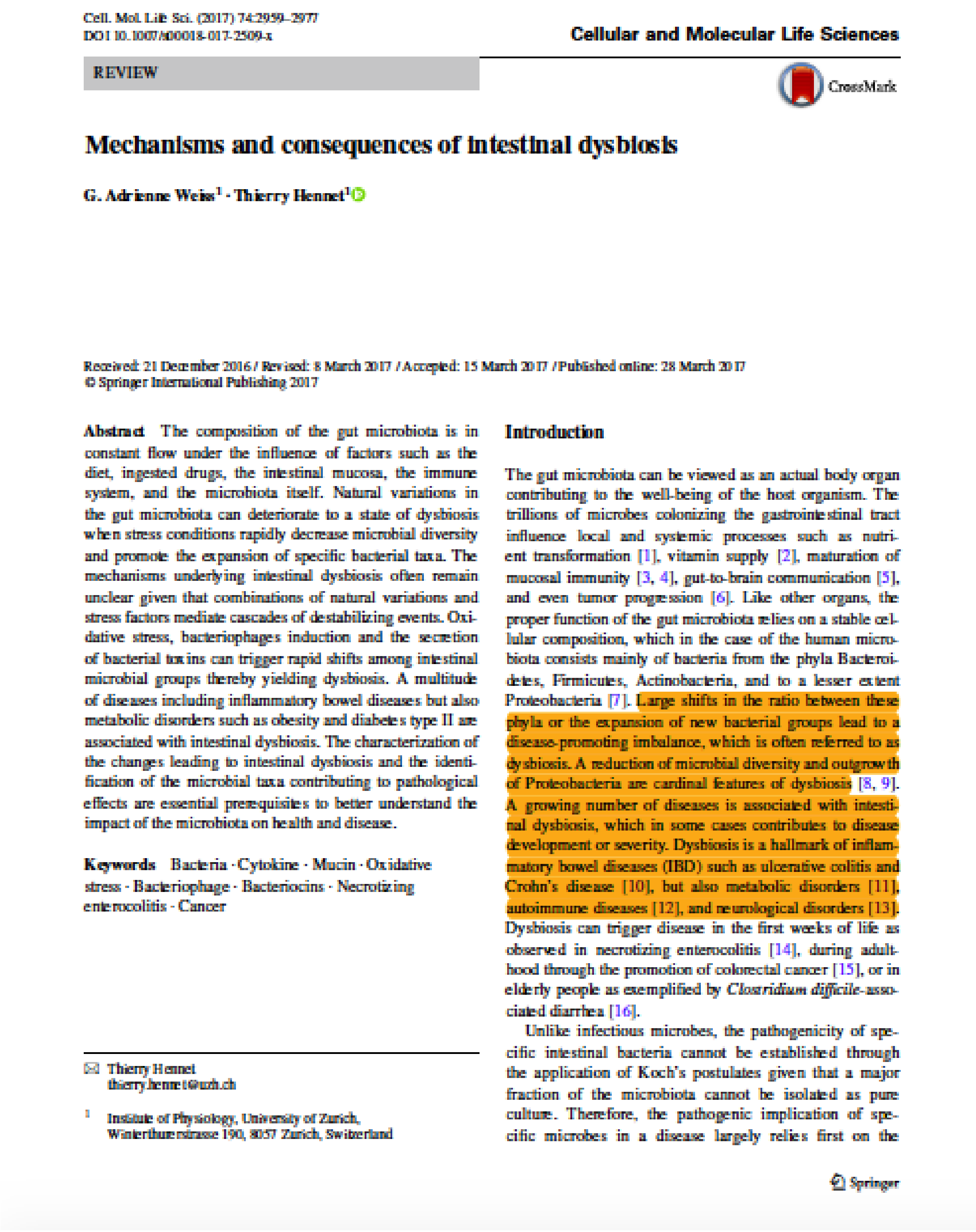

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Cellular and Molecular Life Sciences

REVIEW

CrossMark

Mechanisms and consequences of intestinal dysbiosis

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Abstract The composition of the gut microbiota is in constant flow under the influence of factors such as the diet, ingested drugs, the intestinal mucosa, the immune system, and the microbiota itself. Natural variations in the gut microbiota can deteriorate to a state of dysbiosis when stress conditions rapidly decrease microbial diversity and promote the expansion of specific bacterial taxa. The mechanisms underlying intestinal dysbiosis often remain unclear given that combinations of natural variations and stress factors mediate cascades of destabilizing events. Oxidative stress, bacteriophages induction and the secretion of bacterial toxins can trigger rapid shifts among intestinal microbial groups thereby yielding dysbiosis. A multitude of diseases including inflammatory bowel diseases but also metabolic disorders such as obesity and diabetes type II are associated with intestinal dysbiosis. The characterization of the changes leading to intestinal dysbiosis and the identification of the microbial taxa contributing to pathological effects are essential prerequisites to better understand the impact of the microbiota on health and disease.

Keywords Bacteria · Cytokine · Mucin · Oxidative stress · Bacteriophage · Bacteriocins · Necrotizing enterocolitis · Cancer

Introduction

The gut microbiota can be viewed as an actual body organ contributing to the well-being of the host organism. The trillions of microbes colonizing the gastrointestinal tract influence local and systemic processes such as nutrient transformation [1], vitamin supply [2], maturation of mucosal immunity [3, 4], gut-to-brain communication [5], and even tumor progression [6]. Like other organs, the proper function of the gut microbiota relies on a stable cellular composition, which in the case of the human microbiota consists mainly of bacteria from the phyla Bacteroidetes, Firmicutes, Actinobacteria, and to a lesser extent Proteobacteria [7]. Large shifts in the ratio between these phyla or the expansion of new bacterial groups lead to a disease-promoting imbalance, which is often referred to as dysbiosis. A reduction of microbial diversity and outgrowth of Proteobacteria are cardinal features of dysbiosis [8, 9]. A growing number of diseases is associated with intestinal dysbiosis, which in some cases contributes to disease development or severity. Dysbiosis is a hallmark of inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease [10], but also metabolic disorders [11], autoimmune diseases [12], and neurological disorders [13]. Dysbiosis can trigger disease in the first weeks of life as observed in necrotizing enterocolitis [14], during adulthood through the promotion of colorectal cancer [15], or in elderly people as exemplified by *Clostridium difficile*-associated diarrhea [16].

Unlike infectious microbes, the pathogenicity of specific intestinal bacteria cannot be established through the application of Koch's postulates given that a major fraction of the microbiota cannot be isolated as pure culture. Therefore, the pathogenic implication of specific microbes in a disease largely relies first on the

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Current Understanding of Dysbiosis in Disease in Human and Animal Models

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and Emiko Mizoguchi, MD, PhD[*,†,‡]

Abstract: Inflammatory bowel disease (IBD) is an intestinal inflammatory condition that affects more than 2 million people in the United States. Although the etiology and pathogenesis of IBD are still largely unknown, dysregulated host/enteric microbial interactions are requisite for the development of IBD. So far, many researchers have tried to identify a precise relationship between IBD and an imbalance of the intestinal microbiota, termed "dysbiosis." Despite extensive efforts, it is still largely unknown about the interplay among microbes, their hosts, and their environments, and whether dysbiosis is a causal factor or an effect of IBD. Recently, deep-sequencing analyses of the microbiota in patients with IBD patients have been instrumental in characterizing the strong association between dysbiosis and IBD development, although it is still unable to identify specific-associated species level changes in most cases. Based on many recent reports, dysbiosis of the commensal microbiota is implicated in the pathogenesis of several diseases, including IBD, obesity, and allergic disorders, in both human and animal models. In this review article, the authors have focused on explaining the multiple types of dysbiosis, as well as dysbiosis-related diseases and potential treatments to apply this knowledge to understand a possible cause and potentially find therapeutic strategies for IBD as well as the other dysbiosis-related diseases.

(Inflamm Bowel Dis 2016;22:1137–1150)

Key Words: dysbiosis, inflammatory bowel disease, colorectal cancer, fecal microbiota, IBD-susceptibility genes

The adult human gut contains about 10^{14} bacterial cells with more than 1000 different bacterial species. There are several major divisions of bacteria found in the normal intestinal microflora, with the most dominant groups being Bacteroidetes and Firmicutes. The gut houses both bacteria that are protective and some bacteria that could be potentially harmful to the host (Fig. 1). In normal conditions of healthy individuals, there is cross talk and cross-regulation between the host and the microbiota that reside in the gut, which creates a homeostatic balance of bacteria so that the gastrointestinal tract remains healthy and free from overgrowth of potentially pathogenic bacteria. The microbiota has a commensal relationship with the host; the bacteria thrive in the rich environment of the gut while the host benefits from multiple functions provided by the bacteria.

The homeostatic balance of the intestinal microflora is extremely beneficial to the host, however, if there is a change in the microbial composition that causes a drastic imbalance between the beneficial and potentially pathogenic bacteria, the gut becomes vulnerable to pathogenic insult with gut microbial alterations. This imbalance in the microbial equilibrium is termed "dysbiosis," which has been further defined as a disturbance to gut microbiota homeostasis due to an imbalance in the flora itself, changes in their functional composition and metabolic activities, or changes in their local distribution.[1,2] In general, dysbiosis can be categorized into 3 different types: (1) loss of beneficial organisms, (2) excessive growth of potentially harmful organisms, and (3) loss of overall microbial diversity. It has been found that these 3 types are not mutually exclusive and can occur at the same time, which is most often the case. Dysbiosis has been implicated in a wide range of diseases including inflammatory bowel disease (IBD), obesity, allergic disorders, type 1 diabetes mellitus, autism, obesity, and colorectal cancer (CRC) in both human and animal models. This review will mainly focus on the implication found between dysbiosis and IBD such as Crohn's disease (CD) and ulcerative colitis in addition to the selected dysbiosis-associated diseases.

DYSBIOSIS-ASSOCIATED DISEASES

Inflammatory Bowel Disease

For many years, researchers have been trying to discover a mono-associated cause of IBD. As a result, there are 3 major pathogens that have been found to be associated the most with

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Nutrition, oxidative stress and intestinal dysbiosis: Influence of diet on gut microbiota in inflammatory bowel diseases

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Background. Microbiota refers to the population of microorganisms (bacteria, viruses and fungi) that inhabit the entire gastrointestinal tract, more particularly the colon whose role is to maintain the integrity of the intestinal mucosa and control the proliferation of pathogenic bacteria. Alteration in the composition of the gut microbiota is called dysbiosis. Dysbiosis redisposes to inflammatory bowel diseases such as ulcerative colitis, Crohn disease and indeterminate colitis.
Methods. The purpose of this literature review is to elucidate the influence of diet on the composition of the gastrointestinal microbiota in the healthy gut and the role of diet in the development of dysbiosis.
Conclusion. The "Western diet", in particular a low - fiber high fat/high carbohydrate diet is one factor that can lead to severe dysbiosis. In contrast, "mediterranean" and vegetarian diets that includes abundant fruits, vegetables, olive oil and oily fish are known for their anti-inflammatory effects and could prevent dysbiosis and subsequent inflammatory bowel disease.

Key words: inflammatory bowel diseases, colorectal cancer, intestinal dysbiosis, gut microbiota, healthy diet

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INTRODUCTION

Many areas of current research focus on the the influence and effects of dietary habits on human health, especially in Western countries, where he largest proportions of so- called "junk food" consumers reside. The "western diet", mainly refined carbohydrates, simple sugars and saturated fats, is linked to various metabolic disorders.

The purpose of this review is to illustrate how dietary habits can influence the composition of the intestinal microbiota leading to dysbiosis and inflammatory bowel disease (IBD). After a brief overview of the gut anatomy and the role of the microbiota in healthy gut, this review depicts how the microbiota composition varies based on diet and how this in turn may lead to the onset of systemic metabolic disorders and/or IBD.

GUT MORPHOLOGY

The habitat of the gut microbiota is represented by the intestine. The latter consists of two major portions: the small intestine, divided into duodenum, jejunum and ileum responsible for digestion and nutrient absorption and the large intestine, divided into cecum, colon and rectum is responsible for water absorption and formation of feces. The intestinal wall is composed of four superimposed layers: from the outermost layer, we find the serosa, the muscularis propria, the submucosa and finally the mucosa. The small intestine has villi created mainly in enterocytes which show on their surface, protrusions called microvilli. These protrusions increase the absorption surface and thus facilitate the digestive process and the absorption of nutrients. The large intestine epithelium comprises colonocytes similar to the enterocytes. However, their main function is to absorb water. Colonocytes are arranged to form along goblet cells, simple tubular glands which secrete neutral mucus to lubricate the intestinal contents. The highest concentration of bacteria is found in the large intestine. For a more detailed description, please refer to specialized books[1,2].

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GENERAL

The oral microbiota – a mechanistic role for systemic diseases

G. Jia,[1] A. Zhi,[2] P. F. H. Lai,[1] G. Wang,[1] Y. Xia,[1] Z. Xiong,[1] H. Zhang,[1] N. Che[3] and L. Ai*[1]

Key points

Provides an overview on basic composition and distribution of oral microbiota.	Elucidates the underlying mechanisms of endogenous and exogenous factors on oral microbiota and oral health.	Reviews oral microbiota and its implications for systemic diseases.	Summarises the improvement of clinical diagnosis and treatment based on microbial community information.

Human oral microbiota is the ecological community of commensal, symbiotic, and pathogenic microorganisms found in the oral cavity. Oral microbiota generally exists in the form of a biofilm and plays a crucial role in maintaining oral homeostasis, protecting the oral cavity and preventing disease development. Human oral microbiota has recently become a new focus research for promoting the progress of disease diagnosis, assisting disease treatment, and developing personalised medicines. In this review, the scientific evidence supporting the association that endogenous and exogenous factors (diet, smoking, drinking, socioeconomic status, antibiotics use and pregnancy) modulate oral microbiota. It provides insights into the mechanistic role in which oral microbiota may influence systemic diseases, and summarises the challenges of clinical diagnosis and treatment based on the microbial community information. It provides information for noninvasive diagnosis and helps develop a new paradigm of personalised medicine. All these benefit human health in the post-metagenomics era.

Introduction

The oral cavity is a connection channel between outside environments and the respiratory tract and digestive tract. It provides an appropriate temperature, humidity, and nutrition for microorganism colonisation. The human oral microbiome has been extensively studied as part of the Human Microbiome Project. The oral microbiome has an essential role in maintaining a normal oral ecological balance and in the development of oral diseases. There is abundant evidence supporting the theory that endogenous and exogenous factors are closely related to oral microbiota and systemic diseases.[1,2] Studies on dietary behaviours demonstrate a fundamental aspect of the oral disease paradigm.[3] Lifestyles and diets including smoking, alcohol drinking and consuming spicy food, and antibiotic treatments can persistently alter commensal microbial communities.[4] The resultant microbial disturbances may increase pathogen susceptibility.[5]

The disturbance of the oral microbiota-ecology balance in the host usually causes a series of oral infectious diseases including dental caries, apical periodontitis, periodontal diseases, pericoronitis, and craniofacial bone osteomyelitis. Oral microbiota is also associated with several systemic diseases, namely cardiovascular disease, pneumonia, heart disease, rheumatoid arthritis, pancreatic cancer, colorectal cancer, oesophageal cancer, stroke, and adverse pregnancy outcomes. Accordingly, oral microbiota has been considered as a potential biomarker for human diseases. Relationships between oral microbiota and systemic diseases are essential and need to be elucidated, in order to provide a reasonable diagnosis basis for disease prevention and treatments.

This article mainly discusses the mechanisms for how endogenous and exogenous factors modulate oral microbiota, provides insights into their roles in the influence of oral microbiota on systemic diseases, and summarises the challenges for clinical diagnosis and treatment.

Basic composition and distribution of oral microbiota

The oral microbiome can be classified into core microbiome and variable microbiome. The core microbiome is similar for all individuals and comprised of the predominant species at different sites of the healthy body. The variable microbiome is different between individuals in response to unique lifestyles and phenotypic and genotypic determinants.

For newborns, within five minutes of birth, bacterial communities in the oral cavity and other body habitats are very similar to each other.[6] Types of microorganisms are closely decided by the delivery mode.[7] In addition, the mother's oral microbiota is the most important source of infants' and young children's oral microbiota by successful vertical transmission.[7,8] As ageing continues, babies and children form a wide variety of oral microorganisms in response to different diets, lifestyles, environments and so on.[9]

The oral cavity contains over 700 microbial species as well as commensal

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Oral microbiota: A new view of body health

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ABSTRACT

Oral microbiota is an important part of the human microbiota. Oral microbes can be colonized into the intestine in various ways. Oral microbiota is associated with a variety of oral diseases. Recently, increasing evidence has shown that the oral microbiota is closely related to the physical state of humans, such as diabetes, obesity, and cancer. In the future, oral microbiota will become a new target for improving the physical state of humans.

1. Introduction

About 700 kinds of microorganisms exist in the human mouth, and these microbes constitute the human oral microbiota. It is one of the most complex microbial communities in the human body [1].

In recent years, with the completion of the Human Microbiota Program [2,3], people have become increasingly aware of oral microbes [4] but have not further analyzed oral microbiota in oral diseases such as caries [5], periodontal disease [6], and oral cancer [7]. There is evidence that oral microbiota is also closely related to systemic diseases [8], including rheumatoid arthritis (RA) [9], adverse pregnancy outcomes [10], and cardiovascular disease [11]. Notably, a large number of oral microorganisms enter the downstream digestive tract from the oral cavity through saliva, and they present a particularly close relationship with digestive diseases [12].

Oral microbiota can be used as targets to treat oral and systemic diseases. This article will discuss the relationship between oral microbiota and gut microbiota.

In the future, oral microbiota may become a new target for the treatment of certain diseases.

2. Oral microbiota

The oral cavity is a complex environment that encompasses distinct, small microbial habitats, such as teeth, buccal mucosa, soft and hard palate, and tongue, which form a species-rich heterogeneous ecological system [13] (Fig. 1A). Numerous microorganisms exist in the mouth, among which are bacteria, fungi, and viruses. Bacteria are the main inhabitants of the mouth [14]; they primarily comprise bacteria of the Firmicutes, Bacillus, Proteobacteria, and Actinomycetes [15]. Unlike gut microbiota, these types of bacteria do not change significantly. Diet and the environment have a great impact on gut microbiota [16] but exert minimal effect on the composition of oral bacteria. Healthy people from different countries have similar compositions of oral microbiota. In the human mouth, 85 species of fungi can be found. Among these fungi, the most important one is *Candida* [17]. *Candida* is neutral when the oral microbiota is normal; however, when the oral microbiota balance is broken, *Candida* will seek the opportunity to attack oral tissue. *Candida* forms a biofilm with *Streptococcus* to play a pathogenic role [18]. Viruses, mainly phages, are also part of the oral microbiota [19]. The type of phage in the mouth is constant during all stages of life [20]. Other non-original viruses may also appear in the mouth when certain diseases exist in the human body. The most common is the mumps virus [21] and HIV [22]. Oral bacteria are the main components of the oral microbiota. Common oral bacteria include *Streptococcus mutans*, *Porphyromonas gingivalis*, *Staphylococcus*, and *Lactobacillus* [23]. *S. mutans* is the main component of the oral microbiota, and it is one of the main components of dental plaque [24]. It is also the main pathogen of caries, which is a bacterial infectious disease that occurs in hard tissues of the teeth and has the highest incidence among oral diseases [25]. *P. gingivalis* is a non-glycolytic Gram-negative anaerobic bacterium that is a periodontal pathogen. Untreated *P. gingivalis*

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Review

Antibiotic resistance of bacterial biofilms

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ABSTRACT

A biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA. Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the body's defence system. The persistence of, for example, staphylococcal infections related to foreign bodies is due to biofilm formation. Likewise, chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients is caused by biofilm-growing mucoid strains. Characteristically, gradients of nutrients and oxygen exist from the top to the bottom of biofilms and these gradients are associated with decreased bacterial metabolic activity and increased doubling times of the bacterial cells: it is these more or less dormant cells that are responsible for some of the tolerance to antibiotics. Biofilm growth is associated with an increased level of mutations as well as with quorum-sensing-regulated mechanisms. Conventional resistance mechanisms such as chromosomal β-lactamase, upregulated efflux pumps and mutations in antibiotic target molecules in bacteria also contribute to the survival of biofilms. Biofilms can be prevented by early aggressive antibiotic prophylaxis or therapy and they can be treated by chronic suppressive therapy. A promising strategy may be the use of enzymes that can dissolve the biofilm matrix (e.g. DNase and alginate lyase) as well as quorum-sensing inhibitors that increase biofilm susceptibility to antibiotics.

1. Introduction

Biofilm-growing bacteria cause chronic infections [1] characterised by persistent inflammation and tissue damage [2]. Chronic infections, including foreign-body infections, are infections that (i) persist despite antibiotic therapy and the innate and adaptive immune and inflammatory responses of the host and (ii) in contrast to colonisation, are characterised by an immune response and persisting pathology (Table 1).

2. Occurrence and architecture of bacterial biofilms

Foreign-body infections are characterised by biofilm growth of bacteria on the outer and/or inner surface of the foreign body (Table 2). Biofilm growth also occurs on natural surfaces such as teeth [3], heart valves (endocarditis) [4], in the lungs of cystic fibrosis (CF) patients causing chronic bronchopneumonia [2], in the middle ear in patients with persistent otitis media [5], in chronic rhinosinusitis [6], in chronic osteomyelitis and prosthetic joint infections [7–9], in intravenous (i.v.) catheters and stents [10] and in chronic wounds [11,12] (Fig. 1). The microbes in biofilms are kept together by a self-produced biopolymer matrix. The matrix contains polysaccharides, proteins and DNA originating from the microbes, and the bacterial consortium can consist of one or more species living in a sociomicrobiological way [1,2,14,15]. The matrix is important since it provides structural stability and protection to the biofilm. Development of bacterial biofilms over time has been intensively studied in vitro by confocal scanning laser microscopy employing green fluorescent protein (GFP)-tagged bacteria. This technique has been combined with advanced in silico image analysis to produce three-dimensional images of the biofilm [16–18]. As an example, *Pseudomonas aeruginosa* produces a mature in vitro biofilm in 5–7 days (Fig. 2).

Development of an in vitro biofilm is initiated by planktonic (freely moving) bacteria that reversibly attach to a surface, which may be covered by a layer of, for example, proteins (a pellicle) [3,20]. At this stage, the bacteria are still susceptible to antibiotics and this is in accordance with the success of perioperative antibiotic prophylaxis, e.g. for alloplastic surgery. The next step is irreversible binding to the surface within the next few hours and multiplication of the bacteria, which form microcolonies on the surface and begin to produce a polymer matrix around the microcolonies [20]. The biofilm grows in thickness (up to 50 μm) and under in vitro conditions mushroom-like or tower-like structures are often observed

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Oral Biofilm Architecture on Natural Teeth

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Abstract

Periodontitis and caries are infectious diseases of the oral cavity in which oral biofilms play a causative role. Moreover, oral biofilms are widely studied as model systems for bacterial adhesion, biofilm development, and biofilm resistance to antibiotics, due to their widespread presence and accessibility. Despite descriptions of initial plaque formation on the tooth surface, studies on mature plaque and plaque structure below the gum are limited to landmark studies from the 1970s, without appreciating the breadth of microbial diversity in the plaque. We used fluorescent in situ hybridization to localize in vivo the most abundant species from different phyla and species associated with periodontitis on seven embedded teeth obtained from four different subjects. The data showed convincingly the dominance of *Actinomyces* sp., *Tannerella forsythia*, *Fusobacterium nucleatum*, *Spirochaetes*, and *Synergistetes* in subgingival plaque. The latter proved to be new with a possibly important role in host-pathogen interaction due to its localization in close proximity to immune cells. The present study identified for the first time in vivo that *Lactobacillus* sp. are the central cells of bacterial aggregates in subgingival plaque, and that *Streptococcus* sp. and the yeast *Candida albicans* form corncob structures in supragingival plaque. Finally, periodontal pathogens colonize already formed biofilms and form microcolonies therein. These in vivo observations on oral biofilms provide a clear vision on biofilm architecture and the spatial distribution of predominant species.

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Introduction

Oral microbial biofilms are three-dimensional structured bacterial communities [1] attached to a solid surface like the enamel of the teeth, the surface of the root or dental implants [2] and are embedded in an exo-polysaccharide matrix [3]. Oral biofilms are exemplary and served as a model system for bacterial adhesion [4,5] and antibiotic resistance [6].

The appreciation of the complex nature of oral biofilms was highlighted decades ago by the work of Listgarten and co-workers who described the architecture of biofilms by light and electron microscopy on epoxy resin crowns and extracted teeth [7,8]. Supragingivally, on the enamel, they observed the formation of columnar micro-colonies with their long axis perpendicular to the crown surface. Gram-positive cocci dominated these columns and occasionally, some isolated branching filaments were found after one day of growth. After one week filaments appeared on top of the columns. After three weeks, the biofilm was predominantly filamentous without any sign of cocci left. Filaments seemed to have colonized and subsequently replaced the predominantly coccoid population. A loose layer of so-called corncobs covered the three-week-old biofilm. Corncobs were thought to be bacterial aggregates with a central filamentous cell surrounded by cocci attached to it. After two months, the general features of the biofilm resembled those found at the three weeks time point. Most noticeably was the gingival area, where a fuzzy layer of spirochetes covered the biofilm. This fuzzy layer contained bacterial aggregates resembling test-tube brushes. There were rough and fine types of these brushes. In a study examining biofilm structure at varying degrees of periodontal health, the gingivitis and periodontitis associated biofilms resembled largely the two months old plaque on epoxy resin crowns. Filamentous bacteria were predominant in the biofilm. Between the adhered biofilm and the soft tissue of the pocket, a layer without a well-defined extracellular matrix was observed. This layer consisted of spirochetes, flagellated bacteria and test-tube brushes [8]. The major hindrance of these electron microscopy studies was the inability to identify the species in the biofilm, corncobs or test-tube brushes.

Using fluorescent *in situ* hybridization (FISH), it was shown for the first time *in vivo* that initial biofilm formation was the result of co aggregation and adhesion between *Streptococcus* spp. and *Actinomyces* spp. [9]. In a later study with the same technique, it was shown *in situ*, that after seven days the proportion of streptococci decreased and the proportion of *Fusobacterium nucleatum* increased [10]. Subgingival biofilms formed on expanded polytetrafluoroethylene carriers that had been inserted into the depth of periodontal pockets have been studied with FISH with only two probes, one with specificity for a large group of oral treponemes and the other recognizing all oral bacteria [11]. The bacterial diversity in the oral cavity is estimated to be more than 700 different species and phylotypes, belonging to nine phyla; *Deferribacteres*, *Spirochaetes*, *Fusobacteria*, *Actinobacteria*, *Firmicutes*, *Bac-*

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REVIEW ARTICLE

Dental biofilm infections – an update

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Larsen T, Fiehn N-E. Dental biofilm infections – an update. APMIS 2017; 125: 376–384.

Teeth are colonized by oral bacteria from saliva containing more than 700 different bacterial species. If removed regularly, the dental biofilm mainly comprises oral streptococci and is regarded as resident microflora. But if left undisturbed, a complex biofilm containing up to 100 bacterial species at a site will build up and may eventually cause development of disease. Depending on local ecological factors, the composition of the dental biofilm may vary considerably. With access to excess carbohydrates, the dental biofilm will be dominated by mainly gram-positive carbohydrate-fermenting bacteria causing demineralization of teeth, dental caries, which may further lead to inflammation and necrosis in the pulp and periapical region, i.e., pulpitis and periapical periodontitis. In supra- and subgingival biofilms, predominantly gram-negative, anaerobic proteolytic bacteria will colonize and cause gingival inflammation and breakdown of supporting periodontal fibers and bone and ultimately tooth loss, i.e., gingivitis, chronic or aggressive periodontitis, and around dental implants, peri-implantitis. Furthermore, bacteria from the dental biofilm may spread to other parts of the body by bacteremia and cause systemic disease. Basically, prevention and treatment of dental biofilm infections are achieved by regular personal and professional removal of the dental biofilm.

Key words: Dental biofilm; oral biofilm; dental caries; gingivitis; periodontal disease; oral disease.

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More than 700 different bacterial species have been detected in the oral cavity of humans (1). Saliva contains 10^8 to 10^9 bacteria per milliliter, and some of these adhere to the teeth and initiate formation of a dental biofilm, previously called dental plaque. Generally, the dental biofilm is similar to biofilms elsewhere in the body, where bacteria colonize tissue surfaces or artificial implants and are embedded in a self-produced extracellular matrix of exopolymers (polysaccharides and proteins) and DNA

(2-4). There are, however, also differences from biofilms at other sites of the body (5).

Dental plaque and its relation to oral health and diseases have been studied for decades. A search in PubMed with the Mesh words "dental plaque" and "dental biofilm" yields 22.968 and 3.097 hits, respectively, and the earliest papers date back to 1946 and 1981. With the search words "lung biofilm", "urogenital biofilm", "hemodialysis biofilms", and "catheter biofilms", the number of hits are 639, 271, 203, and 1.386, respectively (searched on April 21, 2016), and early articles date from 1984 to 1989. So, dental biofilm research has been a pioneer in the field of human biofilm research.

The dental biofilm causes diseases in the teeth and their supporting tissues, i.e., dental caries and periodontal diseases. Dental caries is characterized by a demineralization of the teeth without concurrent inflammation in surrounding tissues, while its sequels if left untreated, pulpitis and apical periodontitis are infections. Similarly, the periodontal diseases, such as gingivitis, periodontitis, and peri-implantitis, induce an inflammatory response. Each of these biofilm-induced dental diseases will be described including the principles of biofilm control/elimination. Biofilm-induced infections on the oral mucosa are not included in this article, whereas bacteria in dental biofilms causing infections at other locations of the body are mentioned.

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The oral microbiome and the immunobiology of periodontal disease and caries

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ABSTRACT

The composition of the oral microbiome differs from one intraoral site to another, reflecting in part the host response and immune capacity at each site. By focusing on two major oral infections, periodontal disease and caries, new principles of disease emerge. Periodontal disease affects the soft tissues and bone that support the teeth. Caries is a unique infection of the dental hard tissues. The initiation of both diseases is marked by an increase in the complexity of the microbiome. In periodontitis, pathobionts and keystone pathogens such as *Porphyromonas gingivalis* appear in greater proportion than in health. As a keystone pathogen, *P. gingivalis* impairs host immune responses and appears necessary but not sufficient to cause periodontitis. Historically, dental caries had been causally linked to *Streptococcus mutans*. Contemporary microbiome studies now indicate that singular pathogens are not obvious in either caries or periodontitis. Both diseases appear to result from a perturbation among relatively minor constituents in local microbial communities resulting in dysbiosis. Emergent consortia of the minor members of the respective microbiomes act synergistically to stress the ability of the host to respond and protect. In periodontal disease, host protection first occurs at the level of innate gingival epithelial immunity. Secretory IgA antibody and other salivary antimicrobial systems also act against periodontopathic and cariogenic consortia. When the gingival immune response is impaired, periodontal tissue pathology results when matrix metalloproteinases are released from neutrophils and T cells mediate alveolar bone loss. In caries, several species are acidogenic and aciduric and appear to work synergistically to promote demineralization of the enamel and dentin. Whereas technically possible, particularly for caries, vaccines are unlikely to be commercialized in the near future because of the low morbidity of caries and periodontitis.

1. Introduction

The digestive system begins with the oral cavity where food and microorganisms are introduced, mixed with salivary proteins and digestive enzymes, swallowed, and enter the lower gastrointestinal (GI) tract to be further digested. In the oral environment, several unique ecological niches can be mapped where microorganisms establish in consortial communities. Failing to establish in oral communities, some environmental microorganisms simply transit to the lower GI tract.

Despite continuous shedding of superficial epithelial layers, the oral mucosae are persistently colonized by microorganisms growing in unique ecological niches. One distinctive feature of the oral cavity is the surface of the tooth or tooth enamel (Fig. 1). This non-shedding surface supports the growth and maturation of a complex microbial biofilm. The nutrient foundations of the microbiota surviving on mucosae or within the tooth biofilm are the proteins and glycoproteins of saliva, and the carbohydrates, proteins and lipids of dietary food. Since the teeth are anchored to the jaws, but grow out of the gums or gingivae, serum proteins that exude at the gingival sulcus (the junction of the tooth and the gingiva; Fig. 2) are an additional source of nutrients in specific ecological niches. In this review, we will discuss the composition of the microbiota that has shed from oral surface niches into saliva, biofilm communities on the tooth enamel, and within gingival sulcus. The current literature reveals that contrary to what occurs in the GI tract, initiation of oral infectious diseases and disease status are associated with increased diversity and richness of the microbiota. Oral health is associated with low diversity and richness within the microbial community. This review also highlights the host response to the oral microbiomes in specific niches by

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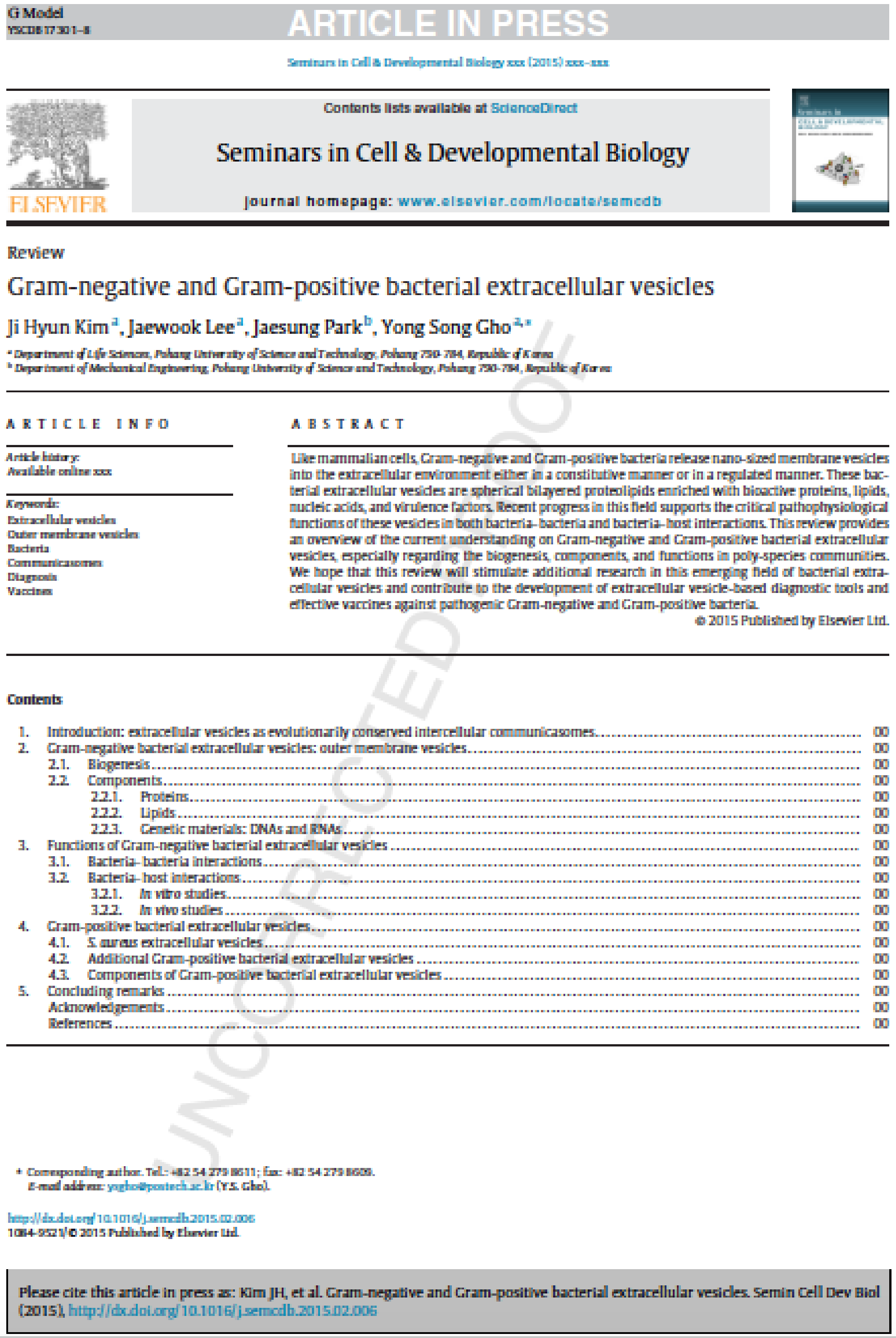

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Review

Gram-negative and Gram-positive bacterial extracellular vesicles

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ABSTRACT

Like mammalian cells, Gram-negative and Gram-positive bacteria release nano-sized membrane vesicles into the extracellular environment either in a constitutive manner or in a regulated manner. These bacterial extracellular vesicles are spherical bilayered proteolipids enriched with bioactive proteins, lipids, nucleic acids, and virulence factors. Recent progress in this field supports the critical pathophysiological functions of these vesicles in both bacteria–bacteria and bacteria–host interactions. This review provides an overview of the current understanding on Gram-negative and Gram-positive bacterial extracellular vesicles, especially regarding the biogenesis, components, and functions in poly-species communities. We hope that this review will stimulate additional research in this emerging field of bacterial extracellular vesicles and contribute to the development of extracellular vesicle-based diagnostic tools and effective vaccines against pathogenic Gram-negative and Gram-positive bacteria.

Contents

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Review

Human oral microbiota and its modulation for oral health

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ABSTRACT

The oral microbiome is an important part of the human microbiome. The oral cavity contains several significantly different niches with distinct microbial communities. A wide range of microorganisms inhabit the human oral cavity, including bacteria, fungi, viruses, archaea and protozoa. These microorganisms form a complex ecological community that influences oral and systemic health. The most prevalent oral diseases, dental caries and periodontal diseases, are microbiota-associated diseases. Moreover, increasing evidences have supported that many systemic diseases are associated with disturbances in the oral ecosystem, such as diabetes, cardiovascular diseases and tumors. The current control of dental plaque-related diseases is nonspecific and is centered on the removal of plaque by mechanical means. Due to this realization about the oral microbiome, several new methods based on the modulation of the microbiome that aim at maintaining and reestablishing a healthy oral ecosystem have been developed.

1. Introduction

Human are superorganisms composed of both their own cells and microbial cells. The number of microorganisms residing on or in the human body is tenfold over that of the body's own cells [1]. These commensal microorganisms contribute to host health by resisting pathogens, maintaining homeostasis and modulating the immune system [2]. The National Institute of Health (NIH) of the United States (US) initiated the Human Microbiome Project (HMP) to characterize the human microbiome more completely and determine the association between changes of microbiome and health/disease [3]. The oral microbiome is one of the important parts of the human microbiome, and it refers specifically to the microorganisms residing in the human oral cavity [4].

The oral cavity has been considered to possess the second most complex microbiota in human body, only behind the colon [5]. The oral microbiome is highly diverse, including bacteria, fungi, viruses, archaea and protozoa. Approximately 700 species are present in the oral cavity, and most of them are indigenous [6]. Among them, approximately 54% have been cultivated and named, 14% are cultivated but unnamed, and 32% are known only as uncultivated phylotypes (from the Human Oral Microbiome Database). An increasing number of studies have demonstrated that the oral microbiota plays a vital role in the pathogenesis and development of many oral and systemic diseases.

In this review, we describe the microbial diversity of the oral cavity, expound microbial communities of different oral niches and present evidences that have confirmed the relationship between oral bacterial community shifts and oral or systemic diseases. Moreover, several prevention and treatment methods based on oral microbiota modulation are discussed.

2. Oral microbiome composition

2.1. Bacteria

Bacteria account for the main portion of oral microorganisms, and the major knowledge of the composition of oral bacteria comes from past culture-dependent methods. Culture-dependent techniques led to the identification of specific microorganisms thought to have a causative role in caries and periodontitis [5]. However, these data substantially underestimated the composition of the oral microbiome. The development of culture-independent methods, particularly targeting 16S ribosomal RNA, has expanded our awareness of the great richness and diversity of the oral microbiome. A list of oral bacteria with a description of their characteristics and genomic information are available from the Human Oral Microbiome Database website at www.homd.org. The oral bacterial community is dominated by the six major phyla, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes

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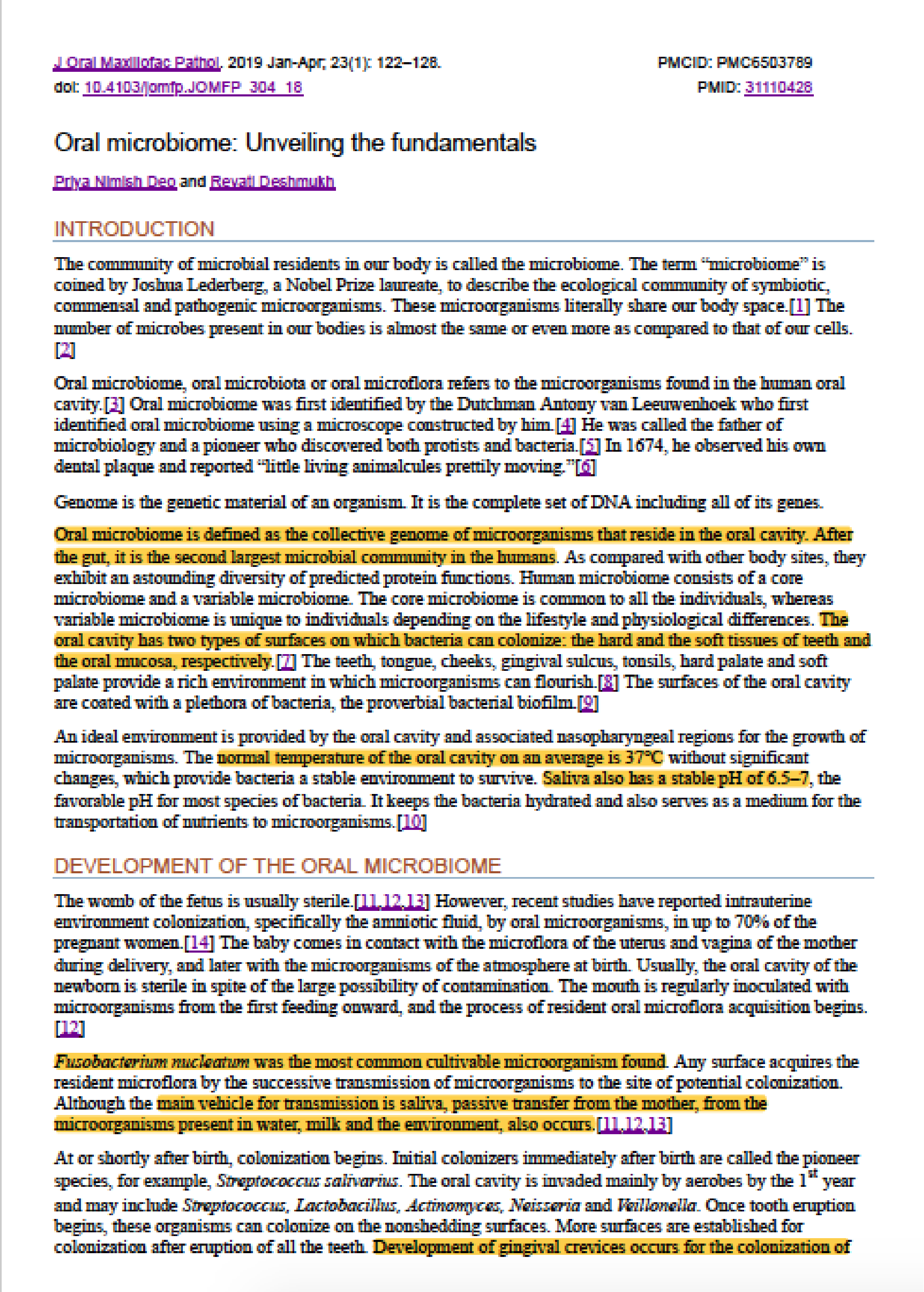

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Oral microbiome: Unveiling the fundamentals

Priya Nimish Deo and Revati Deshmukh

INTRODUCTION

The community of microbial residents in our body is called the microbiome. The term "microbiome" is coined by Joshua Lederberg, a Nobel Prize laureate, to describe the ecological community of symbiotic, commensal and pathogenic microorganisms. These microorganisms literally share our body space.[1] The number of microbes present in our bodies is almost the same or even more as compared to that of our cells.[2]

Oral microbiome, oral microbiota or oral microflora refers to the microorganisms found in the human oral cavity.[3] Oral microbiome was first identified by the Dutchman Antony van Leeuwenhoek who first identified oral microbiome using a microscope constructed by him.[4] He was called the father of microbiology and a pioneer who discovered both protists and bacteria.[5] In 1674, he observed his own dental plaque and reported "little living animalcules prettily moving."[6]

Genome is the genetic material of an organism. It is the complete set of DNA including all of its genes.

Oral microbiome is defined as the collective genome of microorganisms that reside in the oral cavity. After the gut, it is the second largest microbial community in the humans. As compared with other body sites, they exhibit an astounding diversity of predicted protein functions. Human microbiome consists of a core microbiome and a variable microbiome. The core microbiome is common to all the individuals, whereas variable microbiome is unique to individuals depending on the lifestyle and physiological differences. The oral cavity has two types of surfaces on which bacteria can colonize: the hard and the soft tissues of teeth and the oral mucosa, respectively.[7] The teeth, tongue, cheeks, gingival sulcus, tonsils, hard palate and soft palate provide a rich environment in which microorganisms can flourish.[8] The surfaces of the oral cavity are coated with a plethora of bacteria, the proverbial bacterial biofilm.[9]

An ideal environment is provided by the oral cavity and associated nasopharyngeal regions for the growth of microorganisms. The normal temperature of the oral cavity on an average is 37°C without significant changes, which provide bacteria a stable environment to survive. Saliva also has a stable pH of 6.5–7, the favorable pH for most species of bacteria. It keeps the bacteria hydrated and also serves as a medium for the transportation of nutrients to microorganisms.[10]

DEVELOPMENT OF THE ORAL MICROBIOME

The womb of the fetus is usually sterile.[11,12,13] However, recent studies have reported intrauterine environment colonization, specifically the amniotic fluid, by oral microorganisms, in up to 70% of the pregnant women.[14] The baby comes in contact with the microflora of the uterus and vagina of the mother during delivery, and later with the microorganisms of the atmosphere at birth. Usually, the oral cavity of the newborn is sterile in spite of the large possibility of contamination. The mouth is regularly inoculated with microorganisms from the first feeding onward, and the process of resident oral microflora acquisition begins.[12]

Fusobacterium nucleatum was the most common cultivable microorganism found. Any surface acquires the resident microflora by the successive transmission of microorganisms to the site of potential colonization. Although the main vehicle for transmission is saliva, passive transfer from the mother, from the microorganisms present in water, milk and the environment, also occurs.[11,12,13]

At or shortly after birth, colonization begins. Initial colonizers immediately after birth are called the pioneer species, for example, *Streptococcus salivarius*. The oral cavity is invaded mainly by aerobes by the 1st year and may include *Streptococcus, Lactobacillus, Actinomyces, Neisseria* and *Veillonella*. Once tooth eruption begins, these organisms can colonize on the nonshedding surfaces. More surfaces are established for colonization after eruption of all the teeth. Development of gingival crevices occurs for the colonization of

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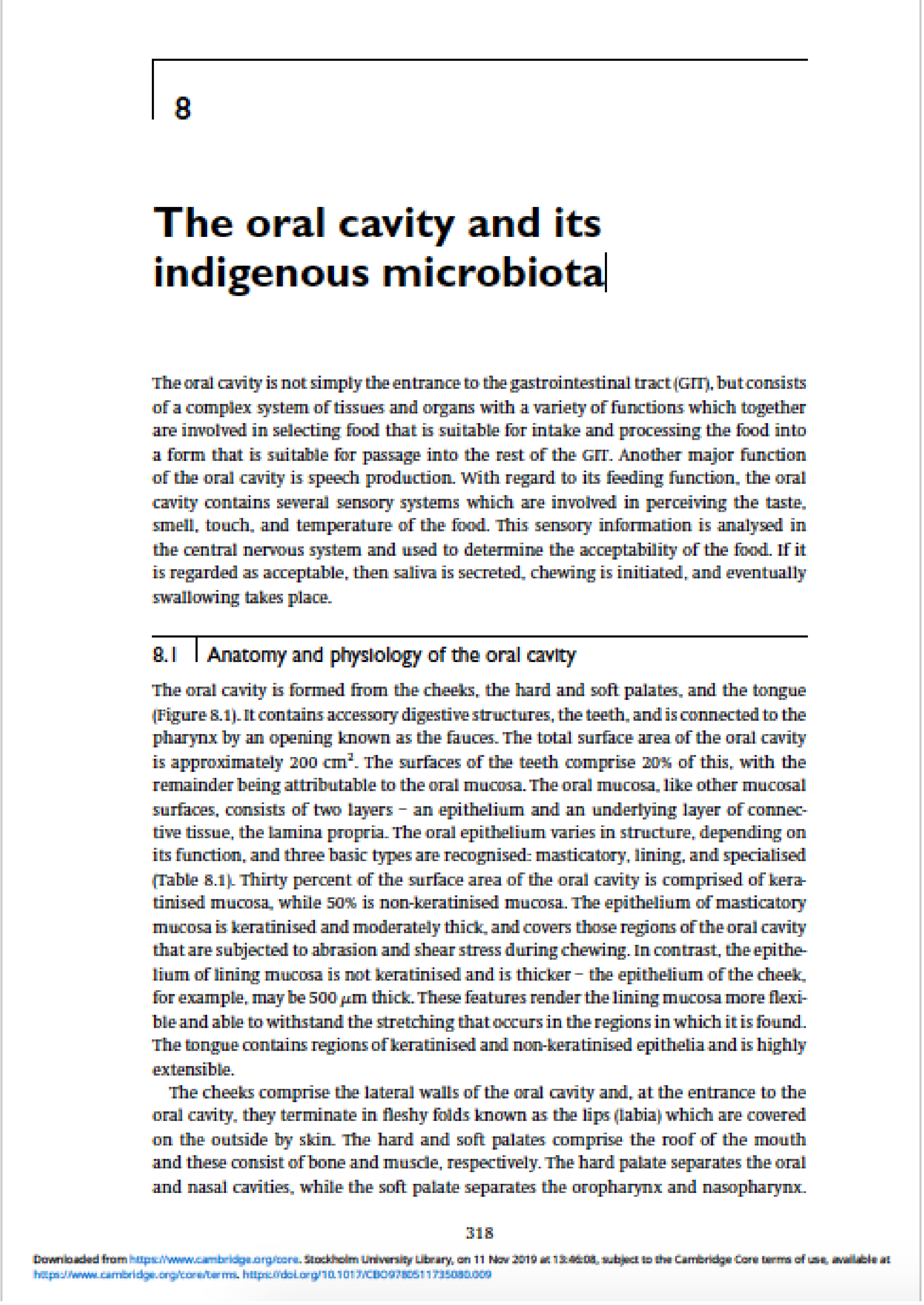

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The oral cavity and its indigenous microbiota

The oral cavity is not simply the entrance to the gastrointestinal tract (GIT), but consists of a complex system of tissues and organs with a variety of functions which together are involved in selecting food that is suitable for intake and processing the food into a form that is suitable for passage into the rest of the GIT. Another major function of the oral cavity is speech production. With regard to its feeding function, the oral cavity contains several sensory systems which are involved in perceiving the taste, smell, touch, and temperature of the food. This sensory information is analysed in the central nervous system and used to determine the acceptability of the food. If it is regarded as acceptable, then saliva is secreted, chewing is initiated, and eventually swallowing takes place.

8.1 Anatomy and physiology of the oral cavity

The oral cavity is formed from the cheeks, the hard and soft palates, and the tongue (Figure 8.1). It contains accessory digestive structures, the teeth, and is connected to the pharynx by an opening known as the fauces. The total surface area of the oral cavity is approximately 200 cm^2. The surfaces of the teeth comprise 20% of this, with the remainder being attributable to the oral mucosa. The oral mucosa, like other mucosal surfaces, consists of two layers – an epithelium and an underlying layer of connective tissue, the lamina propria. The oral epithelium varies in structure, depending on its function, and three basic types are recognised: masticatory, lining, and specialised (Table 8.1). Thirty percent of the surface area of the oral cavity is comprised of keratinised mucosa, while 50% is non-keratinised mucosa. The epithelium of masticatory mucosa is keratinised and moderately thick, and covers those regions of the oral cavity that are subjected to abrasion and shear stress during chewing. In contrast, the epithelium of lining mucosa is not keratinised and is thicker – the epithelium of the cheek, for example, may be 500 μm thick. These features render the lining mucosa more flexible and able to withstand the stretching that occurs in the regions in which it is found. The tongue contains regions of keratinised and non-keratinised epithelia and is highly extensible.

The cheeks comprise the lateral walls of the oral cavity and, at the entrance to the oral cavity, they terminate in fleshy folds known as the lips (labia) which are covered on the outside by skin. The hard and soft palates comprise the roof of the mouth and these consist of bone and muscle, respectively. The hard palate separates the oral and nasal cavities, while the soft palate separates the oropharynx and nasopharynx.

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ORIGINAL ARTICLE

The oral metagenome in health and disease

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The oral cavity of humans is inhabited by hundreds of bacterial species and some of them have a key role in the development of oral diseases, mainly dental caries and periodontitis. We describe for the first time the metagenome of the human oral cavity under health and diseased conditions, with a focus on supragingival dental plaque and cavities. Direct pyrosequencing of eight samples with different oral-health status produced 1 Gbp of sequence without the biases imposed by PCR or cloning. These data show that cavities are not dominated by *Streptococcus mutans* (the species originally identified as the ethiological agent of dental caries) but are in fact a complex community formed by tens of bacterial species, in agreement with the view that caries is a polymicrobial disease. The analysis of the reads indicated that the oral cavity is functionally a different environment from the gut, with many functional categories enriched in one of the two environments and depleted in the other. Individuals who had never suffered from dental caries showed an over-representation of several functional categories, like genes for antimicrobial peptides and quorum sensing. In addition, they did not have *mutans* streptococci but displayed high recruitment of other species. Several isolates belonging to these dominant bacteria in healthy individuals were cultured and shown to inhibit the growth of cariogenic bacteria, suggesting the use of these commensal bacterial strains as probiotics to promote oral health and prevent dental caries.

The ISME Journal (2012) 6, 46–56; doi:10.1038/ismej.2011.85; published online 30 June 2011
Subject Category: microbe–microbe and microbe–host interactions
Keywords: metagenomics; human microbiome; dental caries; *Streptococcus mutans*; pyrosequencing; probiotics

Introduction

The oral cavity of humans is inhabited by hundreds of bacterial species, most of which are commensal and required to keep equilibrium in the mouth ecosystem. However, some of them have a key role in the development of oral diseases, mainly dental caries and periodontal disease (Marsh, 2010). Oral diseases initiate with the growth of the dental plaque, a biofilm formed by the accumulation of bacteria in a timely manner together with the human salivary glycoproteins and polysaccharides secreted by the microbes (Marsh, 2006). The subgingival plaque, located within the neutral or alkaline subgingival sulcus, is typically inhabited by anaerobic Gram negatives and is responsible for the development of gingivitis and periodontitis. The supragingival dental plaque is formed on the teeth surfaces by acidogenic and acidophilic bacteria, which are responsible for dental caries. This is considered the most extended infectious disease in the world, affecting over 80% of the human population (Petersen, 2004). A poor oral health has also been related to the stomach ulcers, gastric cancer or cardiovascular disease, among others (Watabe *et al.*, 1998; Wu *et al.*, 2000). It is therefore surprising that no efficient strategies to combat oral diseases have been developed, despite their dramatic impact on human health. Some of the main reasons that oral pathogens have not been eradicated are related to the difficulty of studying the microbial communities inhabiting the oral cavity: First, the complexity of the ecosystem (several hundreds of species have been reported with multiple interaction levels) makes the potential pathogenical species difficult to target (Socransky *et al.*, 1998); second, not a single ethiological agent can be identified as in classical, Koch's postulates diseases. This has been clearly shown in periodontal disease, where at least three bacterial species that belong to very different taxonomic groups (the so-called 'red complex' of periodontal pathogens) are known to be involved in the illness (Darveau, 2010); and third, a large proportion of oral bacteria cannot be cultured (Paster *et al.*, 2001), and therefore traditional microbiological approaches give an incomplete picture of the natural communities inhabiting the

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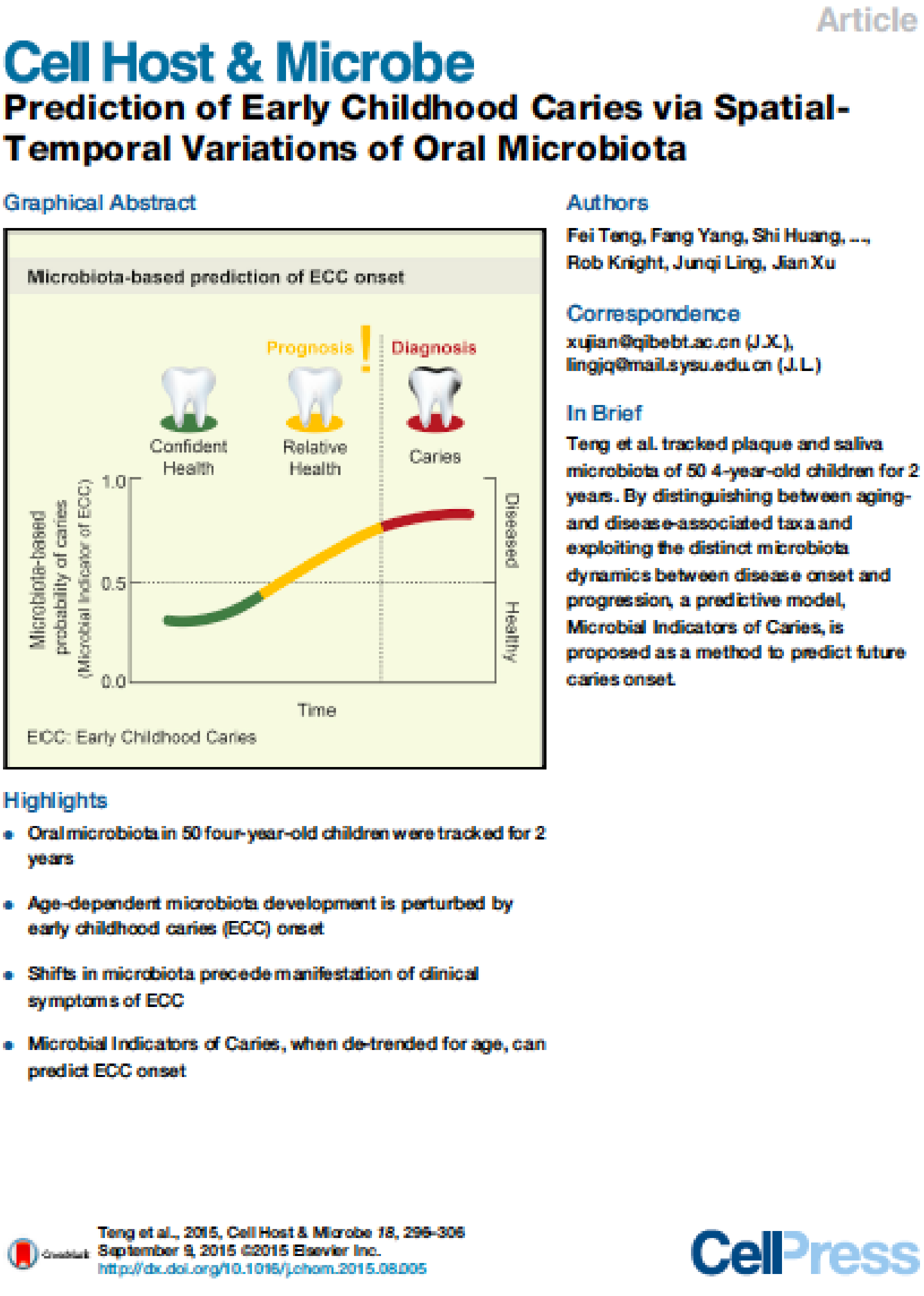

Article

Cell Host & Microbe

Prediction of Early Childhood Caries via Spatial-Temporal Variations of Oral Microbiota

Graphical Abstract

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In Brief

Teng et al. tracked plaque and saliva microbiota of 50 4-year-old children for 2 years. By distinguishing between aging- and disease-associated taxa and exploiting the distinct microbiota dynamics between disease onset and progression, a predictive model, Microbial Indicators of Caries, is proposed as a method to predict future caries onset.

Highlights

- Oral microbiota in 50 four-year-old children were tracked for 2 years
- Age-dependent microbiota development is perturbed by early childhood caries (ECC) onset
- Shifts in microbiota precede manifestation of clinical symptoms of ECC
- Microbial Indicators of Caries, when de-trended for age, can predict ECC onset

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Microbial Risk Indicators of Early Childhood Caries

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The aim of this study was to use molecular identification methods, such as 16S RNA gene sequence and reverse-capture checkerboard hybridization, for identification of the bacteria associated with dental caries and with dental health in a subset of 204 twins aged 1.5 to 7 years old. A total of 448 plaque samples (118 collected from caries-free subjects and 330 from caries-active subjects) were used for analysis. We compared the bacteria found in biofilms of children exhibiting severe dental caries, with different degrees of lesion severity, with those found in biofilms of caries-free children. A panel of 82 bacterial species was selected, and a PCR-based reverse-capture checkerboard method was used for detection. A simple univariate test was used to determine the overabundance and underabundance of bacterial species in the diseased and in the healthy groups. Features identified with this univariate test were used to construct a probabilistic disease prediction model. Furthermore, a method for the analysis of global patterns of gene expression was performed to permit simultaneous analysis of the abundance of significant species by allowing cross-bacterial comparisons of abundance profiles between caries-active and caries-free subjects. Our results suggested that global patterns of microbial abundance in this population are very distinctive. The top bacterial species found to be overabundant in the caries-active group were *Actinomyces* sp. strain B19SC, *Streptococcus mutans*, and *Lactobacillus* spp., which exhibited an inverse relationship to beneficial bacterial species, such as *Streptococcus parasanguinis*, *Abiotrophia defectiva*, *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus sanguinis*.

The mechanisms of dental caries manifestation are complex and are triggered at various levels, i.e., genetic, behavioral, environmental, and microbial. Understanding the role of specific bacterial species and subspecies is important for creating a complete model of caries etiology. Dental plaque is a microbial biofilm community consisting of hundreds of distinct organisms that are ubiquitous in the oral cavity and that colonize the tooth surfaces. Cariogenic microorganisms initially colonize the dental biofilm early in life and can subsequently emerge, under favorable environmental conditions, to cause disease (12). Conversely, studies have shown that if pits and fissures in occlusal surfaces are initially colonized by a noncariogenic bacterial flora, these microorganisms may confer protection to the host by physically occupying the niche and blocking the colonization of cariogenic organisms, such as *Streptococcus mutans*, thereby preventing the onset and development of dental decay (4, 5).

In previous studies, conventional culturing methods have been used to show that well-known species, such as *Streptococcus mutans* and *Lactobacillus* spp., are associated with dental caries (13). These species have been reported as potential contributors to caries onset and development. More recently, advanced molecular methods of bacterial identification, such as PCR techniques and 16S rRNA gene sequencing analysis, have become available and have revealed that the bacterial involvement in the development of dental caries is more complex than previously believed (2).

The aim of this study was to use molecular identification methods, such as 16S rRNA gene sequence and reverse-capture checkerboard hybridization, for identification of the bacteria associated with dental caries and health in infants and children. We compared the bacteria found in biofilms of caries-active children with different degrees of lesion severity with those found in biofilms of caries-free children. In addition, we compared biofilms of healthy surfaces of caries-active subjects with biofilms of healthy surfaces of caries-free subjects. A simple univariate test was used to determine the overabundance and underabundance of bacterial species in the diseased and in the healthy groups. Features identified with this univariate test were used to construct a probabilistic disease prediction model. With proper machine learning-based evaluation, we found that this model was successful in utilizing biofilm bacterial risk indicators to predict disease and health. Our modeling approach splits a data set into two groups of samples, a training and a test set. Because we evaluated the model by performing learning (classifier construction) in the training set and evaluated in the test set, the performance of the prediction model could be statistically validated and, thus, could be generalized.

MATERIALS AND METHODS

Subject population and sample. The study population consisted of a twin cohort from low socioeconomic urban families who resided in the city of Montes Claros, State of Minas Gerais, Brazil. City water supplies have fluoride levels of

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REVIEW

The oral cavity microbiota: between health, oral disease, and cancers of the aerodigestive tract

Pierre Le Bars, Sébastien Matamoros, Emmanuel Montassier, Françoise Le Vacon, Gilles Potel, Assem Soueidan, Fabienne Jordana, and Marie-France de La Cochetière

Abstract: Many studies show that the human microbiome plays a critical role in the chronic pathologies of obesity, inflammatory bowel diseases, and diabetes. More recently, the interaction between cancer and the microbiome has been highlighted. Most studies have focused on the gut microbiota because it represents the most extensive bacterial community, and the body of evidence correlating it with gut syndromes is increasing. However, in the strict sense, the gastrointestinal (GI) tract begins in the oral cavity, and special attention should be paid to the specific flora of this cavity. This study reviewed the current knowledge about the various microbial ecosystems of the upper part of the GI tract and discussed their potential link to carcinogenesis. The overall composition of the microbial communities, as well as the presence or absence of "key species", in relation to carcinogenesis is addressed. Alterations in the oral microbiota can potentially be used to predict the risk of cancer. Molecular advances and the further monitoring of the microbiota will increase our understanding of the role of the microbiota in carcinogenesis and open new perspectives for future therapeutic and prophylactic modalities.

Key words: upper aerodigestive, microbiome, oral cavity, carcinogenesis.

Résumé : Plusieurs études montrent que le microbiome humain joue un rôle essentiel dans les pathologies chroniques que sont l'obésité, les maladies inflammatoires de l'intestin et le diabète. Plus récemment, l'interaction entre le cancer et le microbiome a été soulignée. La plupart des études se sont concentrées sur le microbiote intestinal car il représente la communauté bactérienne la plus étendue, et l'ensemble des preuves le corrélant avec les syndromes de l'intestin est en croissance. Cependant, au sens strict, le tractus gastro-intestinal (GI) commence dans la cavité orale et une attention spéciale devrait être portée à la flore spécifique de cette cavité. Cette étude fait la synthèse des connaissances actuelles relatives à divers écosystèmes microbiens de la partie supérieure du tractus GI et discute de leur lien potentiel à la carcinogenèse. La composition globale des communautés microbiennes, de même que la présence ou l'absence « d'espèces clés » relativement à la carcinogenèse sont soulevées. Des modifications du microbiote oral peuvent potentiellement être utilisées pour prédire les risques de cancer. Les percées moléculaires et la surveillance accrue du microbiote accroîtront notre compréhension du rôle du microbiote dans la carcinogenèse et ouvriront de nouvelles perspectives en vue de modalités thérapeutiques et prophylactiques futures. [Traduit par la Rédaction]

Mots-clés : voie aérodigestives supérieures, microbiome, cavité orale, carcinogenèse.

Introduction

Every year there are 12.7 million new cancer cases worldwide, and an estimated 16.1% are linked to infections. Approximately 15%–20% of human tumors are initiated by inflammation-driven processes (Francescone et al. 2014).

The gastrointestinal (GI) tract can be considered not only a pipeline system from the oral cavity to the anus but also an entry point for nutrients and a point of contact between the immune system and the environment. Various microenvironments are scattered along this tract, each having a specific ecosystem with its own microbiome.

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What are biomarkers?

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Current Opinion in HIV and AIDS 2010, 5:463–466

Purpose of review
This article provides working definitions and a conceptual framework to understand the roles of biomarkers in clinical research.

Recent findings
The definitions of the terms discussed in this article – medical signs, symptoms, biomarkers, surrogate endpoints, clinical endpoints, validation – are still under discussion, as are their relationships to each other, but broad consensus has developed in the past decade and a half about the necessity of distinguishing between, in particular, surrogate and clinical endpoints.

Summary
This article outlines the major definitions of the key terms in this field and considers select cases in which misunderstandings about the terms led to flawed research conclusions.

Keywords
biomarkers, clinical endpoints, surrogate endpoints

Curr Opin HIV AIDS 5:463–466

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Introduction

The use of biomarkers in basic and clinical research as well as in clinical practice has become so commonplace that their presence as primary endpoints in clinical trials is now accepted almost without question. In the case of specific biomarkers that have been well characterized and repeatedly shown to correctly predict relevant clinical outcomes across a variety of treatments and populations, this use is entirely justified and appropriate. In many cases, however, the 'validity' of biomarkers is assumed when, in fact, it should continue to be evaluated and reevaluated. This article will consider the current conceptual status of biomarkers as clinical and diagnostic tools and as surrogate endpoints in clinical research with the goal of providing context for interpreting studies that rely heavily on such biological measures.

What is a biomarker?

The term 'biomarker', a portmanteau of 'biological marker', refers to a broad subcategory of medical signs, that is, objective indications of medical state observed from outside the patient, which can be measured accurately and reproducibly. Medical signs stand in contrast to medical symptoms, which are limited to those indications of health or illness perceived by patients themselves. There are several more precise definitions of biomarkers in the literature, and they fortunately overlap considerably. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [1••]. A joint venture on chemical safety, the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labour Organization, has defined a biomarker as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease' [2]. An even broader definition takes into account not just incidence and outcome of disease, but also the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients. In their report on the validity of biomarkers in environmental risk assessment, the WHO has stated that a true definition of biomarkers includes 'almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction' [3]. Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues. Medical signs have a long history of use in clinical practice – as old as medical practice itself – and biomarkers are merely the most objective, quantifiable medical signs modern laboratory science allows us to measure reproducibly. The use of biomarkers, and in particular laboratory-measured

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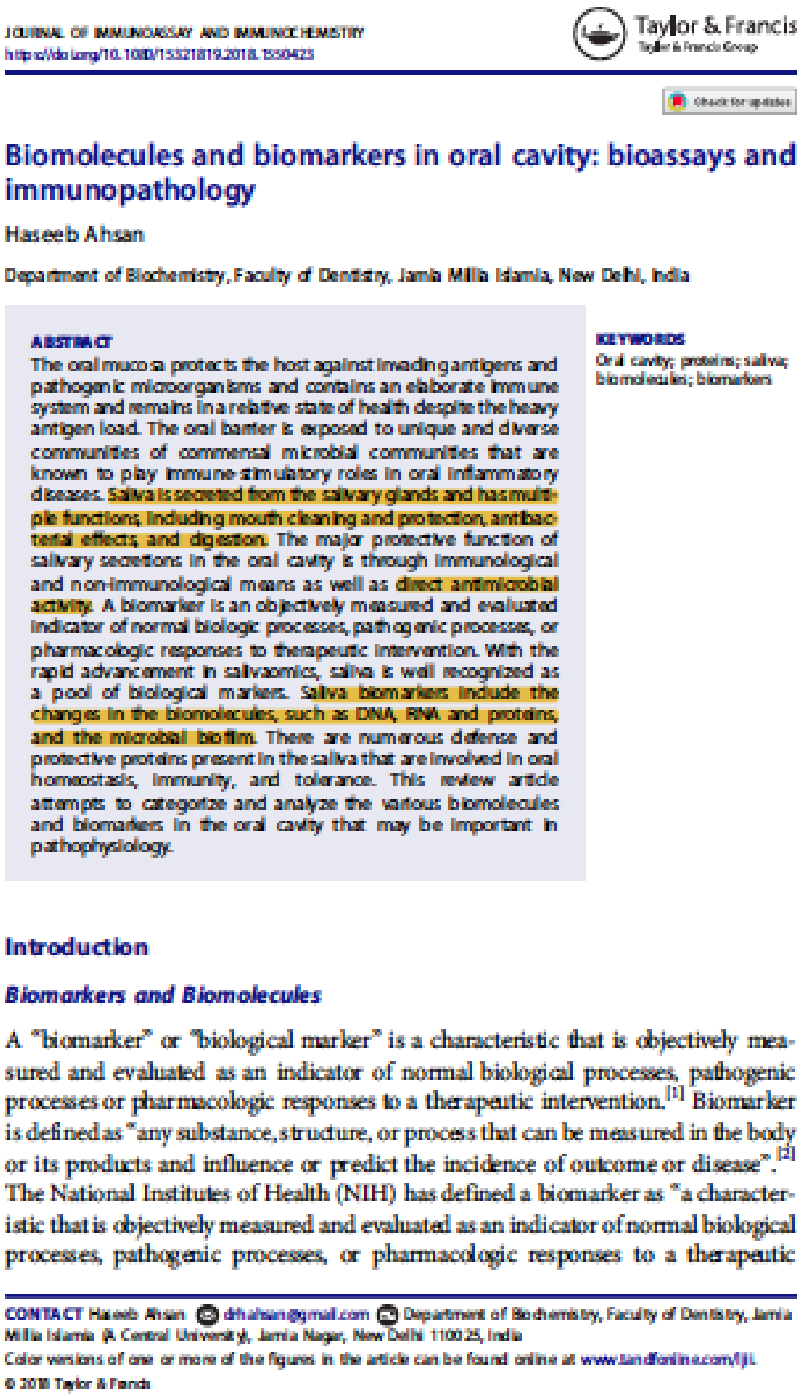

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Biomolecules and biomarkers in oral cavity: bioassays and immunopathology

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ABSTRACT
The oral mucosa protects the host against invading antigens and pathogenic microorganisms and contains an elaborate immune system and remains in a relative state of health despite the heavy antigen load. The oral barrier is exposed to unique and diverse communities of commensal microbial communities that are known to play immune-stimulatory roles in oral inflammatory diseases. Saliva is secreted from the salivary glands and has multiple functions, including mouth cleaning and protection, antibacterial effects, and digestion. The major protective function of salivary secretions in the oral cavity is through immunological and non-immunological means as well as direct antimicrobial activity. A biomarker is an objectively measured and evaluated indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention. With the rapid advancement in salivaomics, saliva is well recognized as a pool of biological markers. Saliva biomarkers include the changes in the biomolecules, such as DNA, RNA and proteins, and the microbial biofilm. There are numerous defense and protective proteins present in the saliva that are involved in oral homeostasis, immunity, and tolerance. This review article attempts to categorize and analyze the various biomolecules and biomarkers in the oral cavity that may be important in pathophysiology.

KEYWORDS
Oral cavity; proteins; saliva; biomolecules; biomarkers

Introduction

Biomarkers and Biomolecules

A "biomarker" or "biological marker" 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.[1] Biomarker is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[2] The National Institutes of Health (NIH) has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljii.

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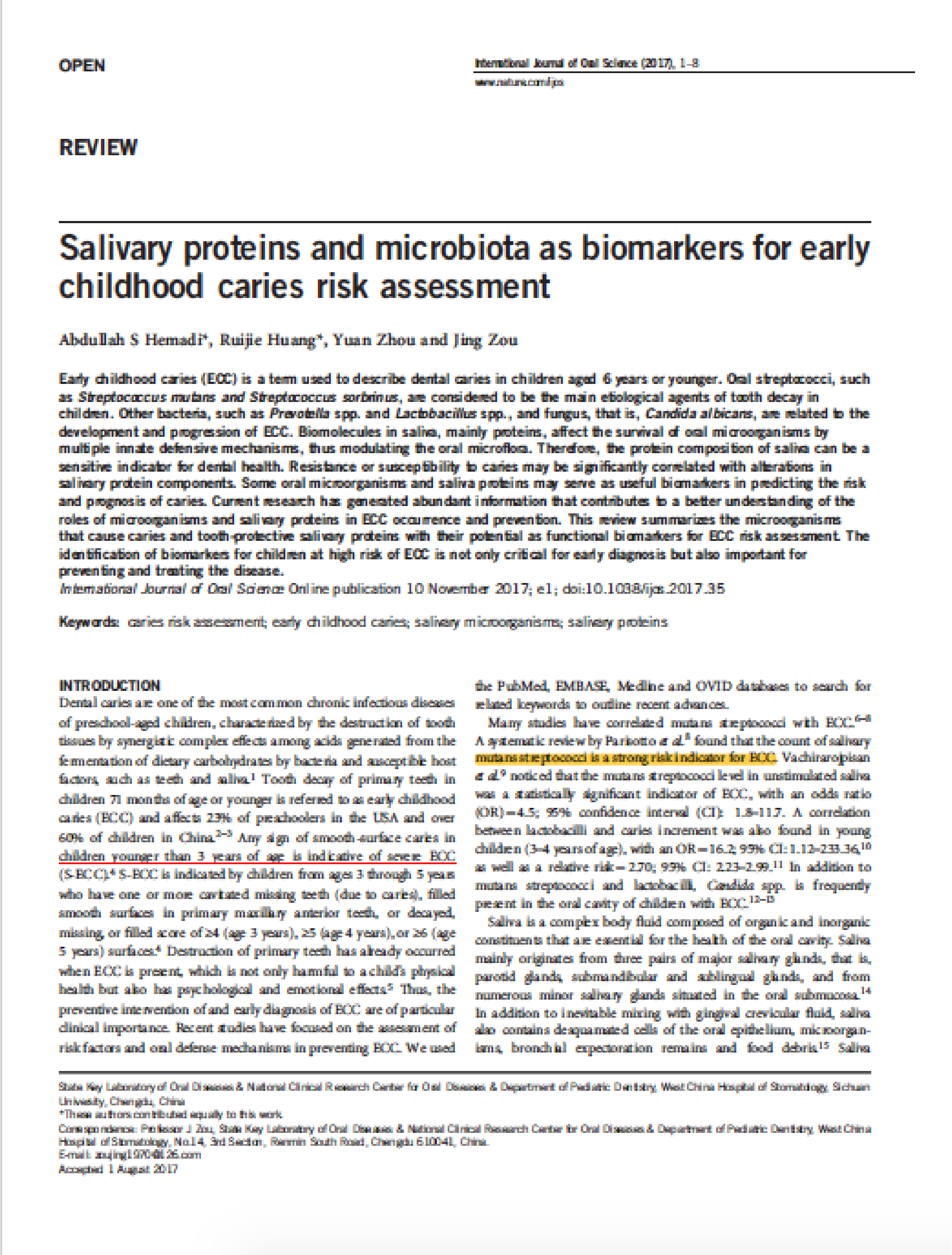

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International Journal of Oral Science (2017), 1–8
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REVIEW

Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment

Abdullah S Hemadi*, Ruijie Huang*, Yuan Zhou and Jing Zou

Early childhood caries (ECC) is a term used to describe dental caries in children aged 6 years or younger. Oral streptococci, such as *Streptococcus mutans and Streptococcus sorbrinus*, are considered to be the main etiological agents of tooth decay in children. Other bacteria, such as *Prevotella* spp. and *Lactobacillus* spp., and fungus, that is, *Candida albicans*, are related to the development and progression of ECC. Biomolecules in saliva, mainly proteins, affect the survival of oral microorganisms by multiple innate defensive mechanisms, thus modulating the oral microflora. Therefore, the protein composition of saliva can be a sensitive indicator for dental health. Resistance or susceptibility to caries may be significantly correlated with alterations in salivary protein components. Some oral microorganisms and saliva proteins may serve as useful biomarkers in predicting the risk and prognosis of caries. Current research has generated abundant information that contributes to a better understanding of the roles of microorganisms and salivary proteins in ECC occurrence and prevention. This review summarizes the microorganisms that cause caries and tooth-protective salivary proteins with their potential as functional biomarkers for ECC risk assessment. The identification of biomarkers for children at high risk of ECC is not only critical for early diagnosis but also important for preventing and treating the disease.

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Keywords: caries risk assessment; early childhood caries; salivary microorganisms; salivary proteins

INTRODUCTION

Dental caries are one of the most common chronic infectious diseases of preschool-aged children, characterized by the destruction of tooth tissues by synergistic complex effects among acids generated from the fermentation of dietary carbohydrates by bacteria and susceptible host factors, such as teeth and saliva.[1] Tooth decay of primary teeth in children 71 months of age or younger is referred to as early childhood caries (ECC) and affects 23% of preschoolers in the USA and over 60% of children in China.[2–3] Any sign of smooth-surface caries in children younger than 3 years of age is indicative of severe ECC (S-ECC).[4] S-ECC is indicated by children from ages 3 through 5 years who have one or more cavitated missing teeth (due to caries), filled smooth surfaces in primary maxillary anterior teeth, or decayed, missing, or filled score of ≥4 (age 3 years), ≥5 (age 4 years), or ≥6 (age 5 years) surfaces.[4] Destruction of primary teeth has already occurred when ECC is present, which is not only harmful to a child's physical health but also has psychological and emotional effects.[5] Thus, the preventive intervention of and early diagnosis of ECC are of particular clinical importance. Recent studies have focused on the assessment of risk factors and oral defense mechanisms in preventing ECC. We used the PubMed, EMBASE, Medline and OVID databases to search for related keywords to outline recent advances.

Many studies have correlated mutans streptococci with ECC.[6–8] A systematic review by Parisotto *et al.*[8] found that the count of salivary mutans streptococci is a strong risk indicator for ECC. Vachirarojpisan *et al.*[9] noticed that the mutans streptococci level in unstimulated saliva was a statistically significant indicator of ECC, with an odds ratio (OR)=4.5; 95% confidence interval (CI): 1.8–11.7. A correlation between lactobacilli and caries increment was also found in young children (3–4 years of age), with an OR=16.2; 95% CI: 1.12–233.36,[10] as well as a relative risk=2.70; 95% CI: 2.23–2.99.[11] In addition to mutans streptococci and lactobacilli, *Candida* spp. is frequently present in the oral cavity of children with ECC.[12–13]

Saliva is a complex body fluid composed of organic and inorganic constituents that are essential for the health of the oral cavity. Saliva mainly originates from three pairs of major salivary glands, that is, parotid glands, submandibular and sublingual glands, and from numerous minor salivary glands situated in the oral submucosa.[14] In addition to inevitable mixing with gingival crevicular fluid, saliva also contains desquamated cells of the oral epithelium, microorganisms, bronchial expectoration remains and food debris.[15] Saliva

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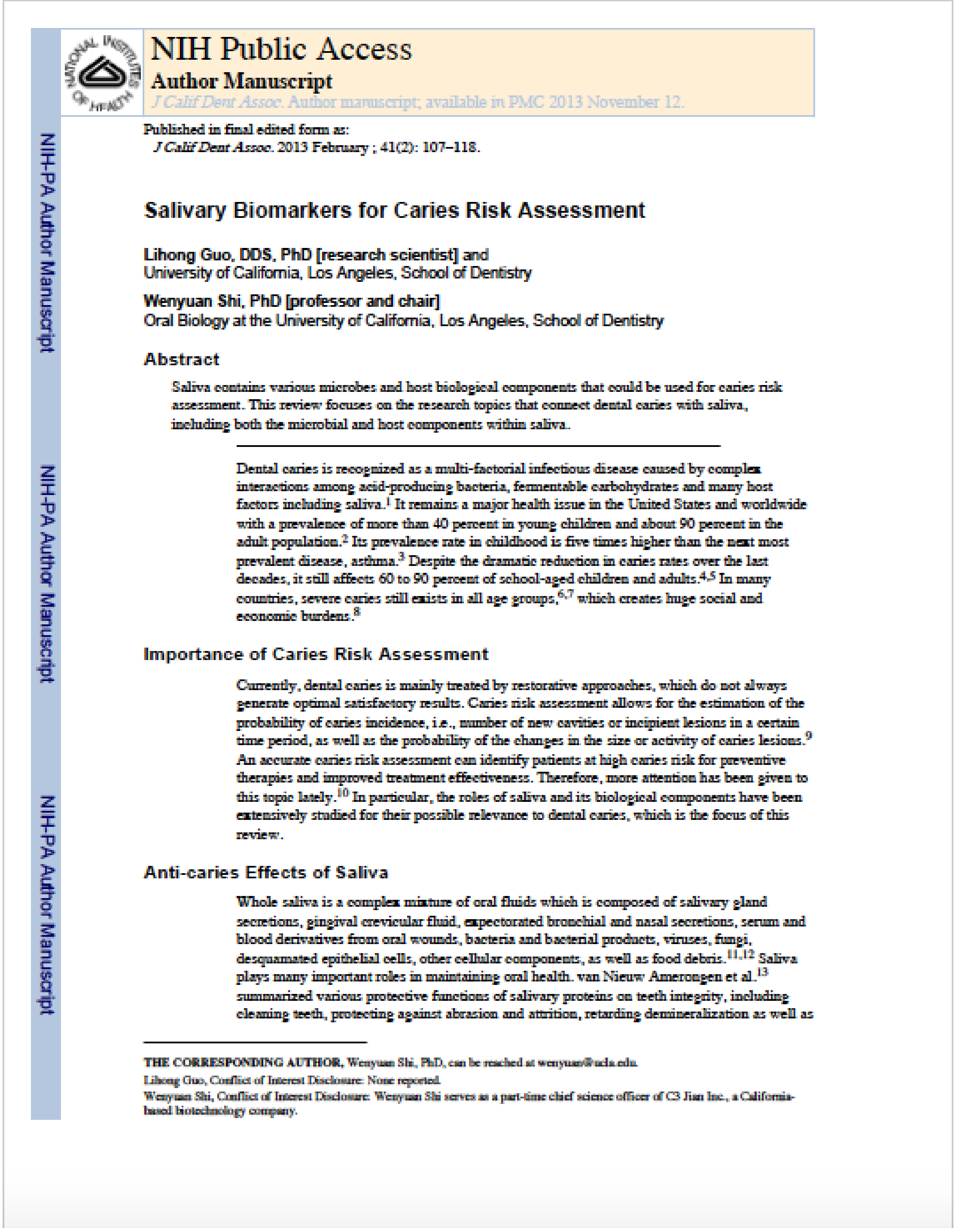

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Salivary Biomarkers for Caries Risk Assessment

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Abstract

Saliva contains various microbes and host biological components that could be used for caries risk assessment. This review focuses on the research topics that connect dental caries with saliva, including both the microbial and host components within saliva.

Dental caries is recognized as a multi-factorial infectious disease caused by complex interactions among acid-producing bacteria, fermentable carbohydrates and many host factors including saliva.[1] It remains a major health issue in the United States and worldwide with a prevalence of more than 40 percent in young children and about 90 percent in the adult population.[2] Its prevalence rate in childhood is five times higher than the next most prevalent disease, asthma.[3] Despite the dramatic reduction in caries rates over the last decades, it still affects 60 to 90 percent of school-aged children and adults.[4,5] In many countries, severe caries still exists in all age groups,[6,7] which creates huge social and economic burdens.[8]

Importance of Caries Risk Assessment

Currently, dental caries is mainly treated by restorative approaches, which do not always generate optimal satisfactory results. Caries risk assessment allows for the estimation of the probability of caries incidence, i.e., number of new cavities or incipient lesions in a certain time period, as well as the probability of the changes in the size or activity of caries lesions.[9] An accurate caries risk assessment can identify patients at high caries risk for preventive therapies and improved treatment effectiveness. Therefore, more attention has been given to this topic lately.[10] In particular, the roles of saliva and its biological components have been extensively studied for their possible relevance to dental caries, which is the focus of this review.

Anti-caries Effects of Saliva

Whole saliva is a complex mixture of oral fluids which is composed of salivary gland secretions, gingival crevicular fluid, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses, fungi, desquamated epithelial cells, other cellular components, as well as food debris.[11,12] Saliva plays many important roles in maintaining oral health. van Nieuw Amerongen et al.[13] summarized various protective functions of salivary proteins on teeth integrity, including cleaning teeth, protecting against abrasion and attrition, retarding demineralization as well as

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Minireview

Saliva diagnostics – Current views and directions

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Impact statement

The purpose of this mini-review is to make an update about the present and future applications of saliva as a diagnostic biofluid in many fields of science such as dentistry, medicine and pharmacotherapy. Using saliva as a fluid for diagnostic purposes would be a huge breakthrough for both patients and healthcare providers since saliva collection is easy, non-invasive and inexpensive. We will go through the current main diagnostic applications of saliva, and provide a highlight on the emerging, newly developing technologies and tools for cancer screening, detection and monitoring.

Abstract

In this review, we provide an update on the current and future applications of saliva for diagnostic purposes. There are many advantages of using saliva as a biofluid. Its collection is fast, easy, inexpensive, and non-invasive. In addition, saliva, as a "mirror of the body," can reflect the physiological and pathological state of the body. Therefore, it serves as a diagnostic and monitoring tool in many fields of science such as medicine, dentistry, and pharmacotherapy. Introduced in 2008, the term "Salivaomics" aimed to highlight the rapid development of knowledge about various "omics" constituents of saliva, including: proteome, transcriptome, micro-RNA, metabolome, and microbiome. In the last few years, researchers have developed new technologies and validated a wide range of salivary biomarkers that will soon make the use of saliva a clinical reality. However, a great need still exists for convenient and accurate point-of-care devices that can serve as a non-invasive diagnostic tool. In addition, there is an urgent need to decipher the scientific rationale and mechanisms that convey systemic diseases to saliva. Another promising technology called liquid biopsy enables detection of circulating tumor cells (CTCs) and fragments of tumor DNA in saliva, thus enabling non-invasive early detection of various cancers. The newly developed technology—electric field-induced release and measurement (EFIRM) provides near perfect detection of actionable mutations in lung cancer patients. These recent advances widened the salivary diagnostic approach from the oral cavity to the whole physiological system, and thus point towards a promising future of salivary diagnostics for personalized individual medicine applications including clinical decisions and post-treatment outcome predictions.

Keywords: Saliva, diagnostics, trancriptomics, point-of-care, liquid biopsy, biomarkers

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Introduction

Saliva (whole saliva [WS], oral fluids [OFs]) is an acidic (pH = 6–7) biological fluid composed of secretions from the three major salivary glands (parotid, submandibular, sublingual) and from minor glands (i.e. labial, buccal, lingual, and palatal tissues), gingival crevicular fluid, cell debris, plaque, bacteria, nasal and bronchial secretions, lining cells, blood and exogenous substances.[1,2] It contains 99% water, 0.3% proteins and both 0.2% inorganic and organic substances.[3] The most prevalent inorganic components include: sodium, potassium, calcium, magnesium, chloride, and carbonates, while the organic components comprise amylases, peroxidase, lipase, mucins, lysozyme, lactoferins, kallikreins, cystatins, hormones, and growth factors.[4] In a healthy individual, the daily salivary secretion is estimated to be between 0.5 and 1.5 L.[5]

Saliva plays an important role in many biological functions such as perception of oral sensations (i.e. taste, temperature and touch), lubrication, chewing, swallowing, and digestion. In addition, it enhances remineralization of tooth enamel and prevents demineralization due to its buffering capacity.[6,7]

Saliva also protects oral mucosa against biological, mechanical, and chemical factors, as well as against bacterial, viral, and fungal infections, thus maintaining the oral cavity ecosystem remain in balance.[8,9]

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Oral biomarkers in the diagnosis and progression of periodontal diseases

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Abstract
Periodontitis is a disease characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium. Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease The long-term aim is that treatment and prevention of periodontal disease will be founded on diagnostic tests based on aetiopathogenic factors rather than just clinical experience. Clinical measurements used in diagnosis of periodontal diseases are often of limited usefulness in that they are indications of previous periodontal disease rather than the present disease activity. Biochemical mediators in oral fluids like saliva and gingival crevicular fluid (GCF) are highly beneficial in the determination of current periodontal status. These substances known as biomarkers help in determination of inflammatory mediator levels, as they are good indicators of inflammatory activity. This review highlights recent advances in the use of salivary and gingival crevicular fluid (GCF) biomarker-based disease diagnostics that focus on the identification of active periodontal disease.

Keywords: Periodontitis; gingival crevicular fluid; biomarkers.

Introduction

Periodontal diseases are heterogeneous and include a variety of infections and inflammatory lesions. Notably, periodontitis is a prevalent disease of man that is characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to apical migration of the junctional epithelium. The microbial nature of many periodontal diseases has been recognized long ago. More recently, it has been realized that the host related factors might be the keys to understanding of the disease processes in periodontitis. Periodontal disease progression is episodic in nature on a tooth site level; however, the risk of periodontal disease is principally patient based rather than site based (Champagne et al 2003).

Bacterial virulence factors either result in degradation of host tissues or cause the release of biologic mediators from host tissue cells that lead to host tissue destruction. Mediators produced as a part of host response that contribute to tissue destruction include proteinases, cytokines and prostaglandins. Also, a variety of enzymes produced by periodontal microorganism cause tissue destruction.

Locally, presence of bacteria adjacent to gingival crevice and the intimate contact of bacterial lipopolysaccharide with host cells trigger monocytes, polymorphonucleoleukocytes, macrophages and other cells to release inflammatory mediators such as IL-1, TNF-α, and prostaglandin E_2. IL-1 and TNF-α have an important role in periodontal tissue destruction and PGE_2 appears to partly responsible for bone loss associated with periodontal diseases (Miyasaki 2004).

Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease (Kinane 2000). A goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location and severity. These findings serve as a basis for treatment planning and provide essential data during periodontal maintenance and disease monitoring phases of treatment.

Traditional clinical measurements (probing pocket depth, bleeding on probing, clinical attachment loss, plaque index, radiographs) used for periodontal diagnosis are often of limited usefulness in that they are indicators of previous periodontal disease rather than present disease activity. There is a need for development of new diagnostic tests that can detect the presence of active disease, predict

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Dental caries

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Dental caries, otherwise known as tooth decay, is one of the most prevalent chronic diseases of people worldwide; individuals are susceptible to this disease throughout their lifetime. Dental caries forms through a complex interaction over time between acid-producing bacteria and fermentable carbohydrate, and many host factors including teeth and saliva. The disease develops in both the crowns and roots of teeth, and it can arise in early childhood as an aggressive tooth decay that affects the primary teeth of infants and toddlers. Risk for caries includes physical, biological, environmental, behavioural, and lifestyle-related factors such as high numbers of cariogenic bacteria, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, inappropriate methods of feeding infants, and poverty. The approach to primary prevention should be based on common risk factors. Secondary prevention and treatment should focus on management of the caries process over time for individual patients, with a minimally invasive, tissue-preserving approach.

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Dental caries is one of the most common preventable childhood diseases; people are susceptible to the disease throughout their lifetime.[1–4] It is the primary cause of oral pain and tooth loss.[3,5] It can be arrested and potentially reversed in its early stages, but is often not self-limiting and without proper care, caries can progress until the tooth is destroyed.[6] Therefore, physicians and other health-care providers should be familiar with dental caries and its causes. The aim of this Seminar is to enhance physicians' knowledge of the dental caries process and its management; to encourage physicians to incorporate relevant aspects of caries prevention and control into their daily practice, and to educate physicians about when to refer patients to a dentist.

Definition

Dental caries is the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates.[4,6] The signs of the carious demineralisation are seen on the hard dental tissues, but the disease process is initiated within the bacterial biofilm (dental plaque) that covers a tooth surface. Moreover, the very early changes in the enamel are not detected with traditional clinical and radiographic methods. Dental caries is a multifactorial disease that starts with microbiological shifts within the complex biofilm and is affected by salivary flow and composition, exposure to fluoride, consumption of dietary sugars, and by preventive behaviours (cleaning teeth). The disease is initially reversible and can be halted at any stage, even when some dentine or enamel is destroyed (cavitation), provided that enough biofilm can be removed. Dental caries is a chronic disease that progresses slowly in most people. The disease can be seen in both the crown (coronal caries) and root (root caries) portions of primary and permanent teeth, and on smooth as well as pitted and fissured surfaces. It can affect enamel, the outer covering of the crown; cementum, the outermost layer of the root; and dentine, the tissue beneath both enamel and cementum. Caries in primary teeth of preschool children is commonly referred to as early childhood caries.

The terms dental caries or caries can be used to identify both the caries process and the carious lesion (cavitated or non-cavitated) that is formed as a result of that process.[7,8] In daily practice, dental practitioners, other health-care providers, and patients often refer to an established caries lesion as a cavity in the tooth. The cavity, or decayed surface, is the sequela of the disease process and is a sign of fairly advanced disease.[8] Dental caries is a continuum of disease states of increasing severity and tooth destruction that ranges from sub-clinical sub-surface changes at the molecular level to lesions with dentinal involvement, either with an intact surface or obvious cavitation[9,10,11,12] (figure 1). Assessment of the presence or absence of dental caries is dependent on the diagnostic cutoff points selected; this decision greatly affects practitioners' treatment decisions. Carious lesions are the outcome of events that progress over time.[7]

Search strategy and inclusion criteria

Sources of information for this Seminar were: (1) systematic reviews of dental caries (cariology), including the Cochrane Library, Centre for Reviews and Dissemination, University of York (restoration longevity), and the NIH Consensus Development Conference on Diagnosis and Management of Dental Caries Throughout Life; (2) formally constructed and peer reviewed consensus development papers and statements published in the Proceedings from the International Consensus Workshop on Caries Clinical Trials; (3) summaries of peer-reviewed reviews, such as proceedings of the 50th Anniversary Congress of the European Organisation for Caries Research, Cariology in the 21st Century and a specialist review on caries diagnostic literature; (4) MEDLINE database through PubMed to identify papers containing the term dental caries and associated definitions, epidemiological considerations, aetiological agents, pathogenic factors, and risk factors; and (5) as additional sources, comprehensive textbooks on dental caries.

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DENTAL CARIES: AN UPDATED MEDICAL MODEL OF RISK ASSESSMENT

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Dental caries is a transmissible, complex biofilm disease that creates prolonged periods of low pH in the mouth, resulting in a net mineral loss from the teeth. Historically, the disease model for dental caries consisted of mutans streptococci and *Lactobacillus* species, and the dental profession focused on restoring the lesions/damage from the disease by using a surgical model. The current recommendation is to implement a risk-assessment-based medical model called CAMBRA (caries management by risk assessment) to diagnose and treat dental caries. Unfortunately, many of the suggestions of CAMBRA have been overly complicated and confusing for clinicians. The risk of caries, however, is usually related to just a few common factors, and these factors result in common patterns of disease. This article examines the biofilm model of dental caries, identifies the common disease patterns, and discusses their targeted therapeutic strategies to make CAMBRA more easily adaptable for the privately practicing professional. (J Prosthet Dent 2014;111:280-285)

Dental caries is a transmissible biofilm dysfunction of the teeth marked by prolonged periods of low pH, which results in a net mineral loss.[1] Historically, the disease model for dental caries consisted of mutans streptococci and *Lactobacillus* species.[2] However, more recent scientific evidence indicates that the disease is more complex than this model suggests and that it has traits in common with other biofilm diseases.

Biofilm research using DNA sequencing identification of bacteria has identified some 40 bacterial species to date as having a role in dental caries, and that list continues to grow. In recent independent studies, *Bifidobacterium* species, *Scardovia wiggsiae*, *Slackia exigua*, and *Propionibacterium acidifaciens* have been implicated.[3-5] Next-generation sequencing technologies promise to add to these species as the biofilm model of dental caries becomes better understood. Dental caries also has potential systemic effects.[6] Studies from randomly collected coronary plaque specimens during surgery indicate that when found in the mouth, the most common oral bacteria found in the coronary plaque is also *Streptococcus mutans*.[6] The authors concluded that *S mutans* was responsible for most bacterial endocarditis and that by comparison, the presence of periodontal pathogens was negligible. *S mutans* is also able to invade endothelial cells directly by means of its *cnm* (collagen-binding protein) gene.[7] Further studies have also implicated caries-causing bacteria in impaired cognitive function, ulcerative colitis, and accelerated plaque growth after angioplasty.[8-10] Dental caries also has apparent hereditary characteristics and genetic associations.[11,12] Early studies found that individuals with the G20A polymorphism for beta-defensin-1, a salivary bacteriolytic enzyme, had 5 times the decayed, missing, and filled teeth (DMFT) scores seen in those with other variations of this gene.[11] Hereditary associations with the *TAS2R38* taste-bud gene increase the risk for dental caries.[12] A recent genome-wide-association study indicated multiple gene site associations with an increased risk for caries, the strongest of which was *LYZL2* (lysozyme-like 2), which encodes another bacteriolytic enzyme.[13] The data from this study also indicated 5 distinct patterns of decay geographically in the mouth, with the *LYZL2* gene being associated with carious lesions only in the mandibular incisors.[13] Additional genetic associations have been attributed to a mutation in matrix metalloproteinase 13 (*MMP13*) and the HLA antigen allele HLA-DQ2.[14,15] Regardless of how complex the biofilm disease model becomes, however, dental caries still means prolonged periods of low pH, resulting in a net mineral loss from the teeth. With the continued development of next-generation sequencing technologies, examining the biofilm and its metabolic outcome differently will be possible. Nyvad et al[16] have explored the novel idea of viewing the biofilm as a single organism, as first proposed by Buchen.[17] Biofilm is a collection of distinct and separate organisms, but it behaves collectively as one superorganism. As such, it is less important to identify which specific bacterial species are present. Instead, the authors proposed a metagenomic study to identify which genes were present in the biofilm in total. The genes that are active produce the proteins resulting in metabolic output from the biofilm. In the case of dental caries, the concern is that acid

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This study was presented to the American Academy of Restorative Dentistry, Chicago, Ill, February 2013.

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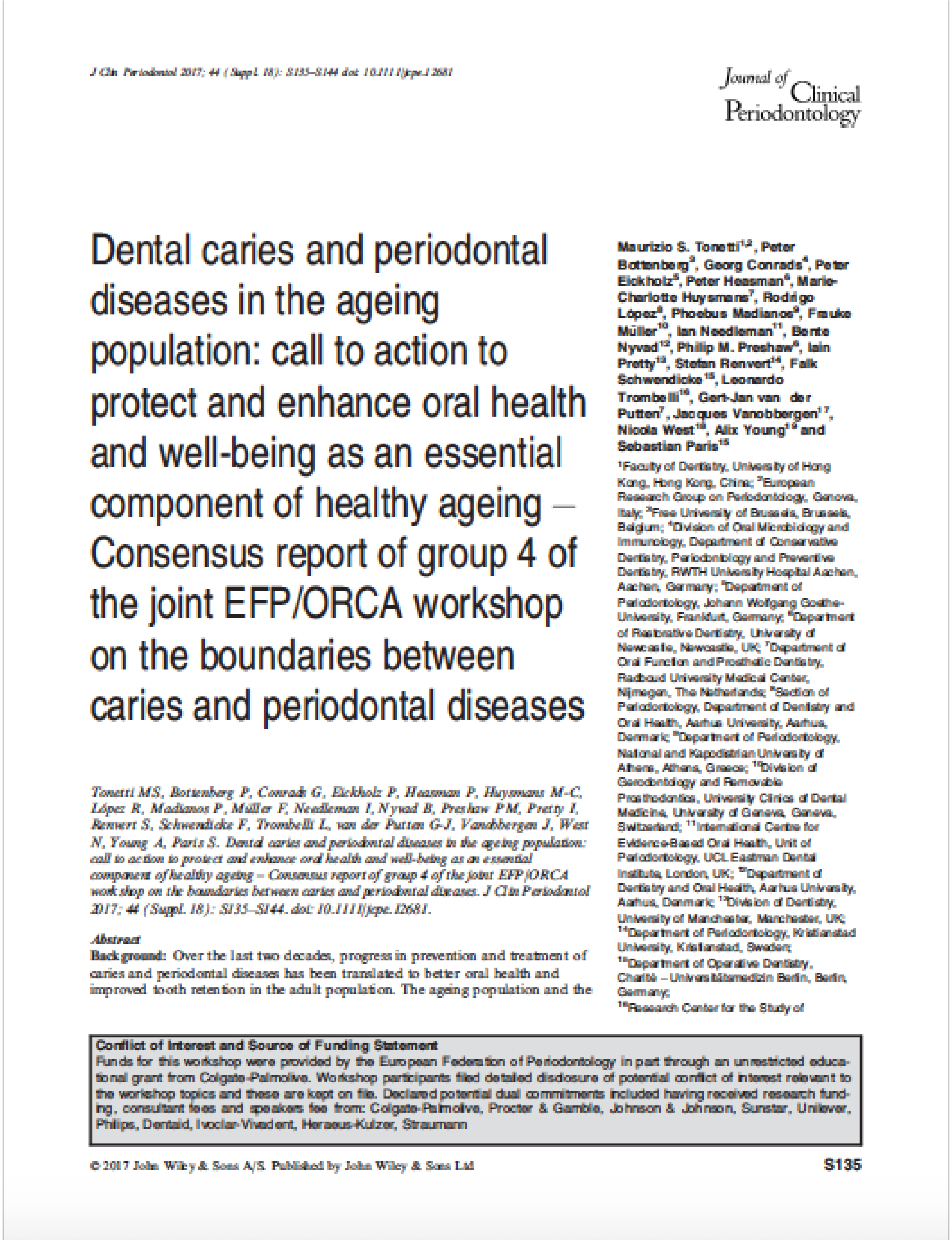

J Clin Periodontol 2017; 44 (Suppl. 18): S135–S144 doi: 10.1111/jcpe.12681

Journal of Clinical Periodontology

Dental caries and periodontal diseases in the ageing population: call to action to protect and enhance oral health and well-being as an essential component of healthy ageing – Consensus report of group 4 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases

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Abstract

Background: Over the last two decades, progress in prevention and treatment of caries and periodontal diseases has been translated to better oral health and improved tooth retention in the adult population. The ageing population and the

Conflict of Interest and Source of Funding Statement

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Review

Oral and dental health care of oral cancer patients: hyposalivation, caries and infections

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S U M M A R Y

Oral cancer and its treatment can cause a variety of problems to patients, also as regards maintaining their daily oral hygiene. Surgery mutilates tissues which may hamper cleaning the teeth and mucosal surfaces. The patient may have complicated reconstructive structures that also need continuous attention. Radiotherapy-induced hyposalivation further complicates the situation and decreases the quality of life. Consequently, dental caries, mucosal diseases such as candidosis and sialadenitis become problematic to treat. Hence every effort should be focused on prevention. In caries prevention intensified fluoride therapy together with dietary counseling is needed. Oral cancer patients also need to be frequently referred to dental hygienists for professional cleaning. Drinking enough daily and moisturizing mucosal surfaces with commercial dry-mouth products, vegetable oils, milk products and respective topical agents need to be individually recommended. In addition, patients with severe dry mouth cases may also benefit from the prescription of pilocarpine tablets. In oral candidosis, the microbiological diagnosis must be confirmed before administration of antifungal drugs in order to avoid the selection pressure to resistant strains.

Introduction

Oral cancer and particularly its treatment can cause problems for the daily maintenance of oral health. Surgical treatment mutilates tissue anatomy and radiotherapy may cause mucositis, tissue constriction and irreversible damage to salivary glands with subsequent dry mouth. Cytostatic drugs affect both the local and systemic defensive factors leading easily to persistent or masked infections. Hyposalivation and xerostomia not only affect dental health but also burden the patient with oral dryness or mucosal pain, reduces taste and smell, increases the risk for dental and mucosal infections, and cause problems of speaking and mastication thus decreasing the quality of life.[1] Reconstructive surgery together with prosthetic and dental rehabilitation causes further problems to the patient but also a frequent need to modify treatment plans by the professional oral health team. Consequently, guidelines and recommendations for treating oral health problems of oral cancer patients have been presented; especially for preventing infections and other dental diseases.[2]

The cornerstone in maintaining satisfactory oral and dental health is daily self-care of oral hygiene. In this, the cancer patients often need hands-on advice regularly controlled by the dentist or dental hygienist (Fig. 1). These patients also need frequent dental appointments based on their individual needs (Fig. 2). In particular the complicated reconstructive structures and devices the patient may have after surgery call for close attention by the oral health team.

It is highly important to emphasize the necessity of dental clinical examination of patients before cancer treatment is started in order to properly target the individual oral health problems and to advise the patient about anticipated problems.[3,4] Of the many problems the patient with oral cancer faces, this review focuses on hyposalivation, dental caries and oral candidosis. We also give some recommendations for their management mainly based on our own hospital practice.

Sialoadenitis and hyposalivation

Treatment of oral cancer often leads to impairment of salivary function. Irradiation and cytostatic drugs lead to sialoadenitis which in turn may lead to irreversible damage of secretion and subsequent hyposalivation. Poor general health renders cancer patients liable to bacterial sialadenitis.[3] This condition calls for knowledge of possible etiology with special diagnostic methods such as computer tomography scan and magnetic resonance imaging. The management of bacterial sialadenitis often means hospitalization to the patient.

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ORIGINAL RESEARCH
Pediatric Dentistry

Incidence of dental caries in primary dentition and risk factors: a longitudinal study

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Abstract: The objectives of this prospective, longitudinal, population-based study were to estimate the incidence of dental caries in the primary dentition, identify risk factors and determine the proportion of children receiving dental treatment, through a two-year follow up. The first dental exam was conducted with 381 children aged one to five years, at health centers during immunization campaigns; 184 of them had dental caries and 197 had no caries experience. The second exam was carried out two years later at a nursery or at home with the same individuals who participated in the first exam. The diagnosis of dental caries was performed using the dmft criteria. Parents were interviewed regarding socioeconomic indicators. Descriptive, bivariate and adjusted Poisson regression analyses were performed. Among the 381 children, 234 were reexamined after two years (non-exposed: 139; exposed: 95). The overall incidence of dental caries was 46.6%. The greatest incidence of dental caries was found in the group of children with previous caries experience (61.1%). Among the children without dental caries in the first exam, 36.7% exhibited caries in the second exam. The majority of children (72.6%) received no treatment for carious lesions in the two-year interval between examinations. Children with previous dental caries (RR: 1.52, 95%CI: 1.12-2.05) had a greater risk of developing new lesions, compared with the children without previous dental caries. The incidence of dental caries was high and most of children's caries were untreated. Previous caries experience is a risk factor for developing new carious lesions in children.

Keywords: Dental Caries; Tooth, Deciduous; Oral Hygiene; Incidence.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Dental caries is one of the most frequent chronic conditions in childhood. Approximately 50% of preschool children in different countries have caries experience.[1] This estimate is confirmed in studies conducted in Brazil, where prevalence rates range from 20.3%[2] to 53.6%.[3] Dental caries exerts a negative impact on the quality of life of both the child and the family,[2] and is considered a public health problem.

The etiology of dental caries in childhood is associated with eating habits,[4,5] irregular tooth brushing[6,7] and socioeconomic indicators.[8] Children from economically vulnerable families have a higher

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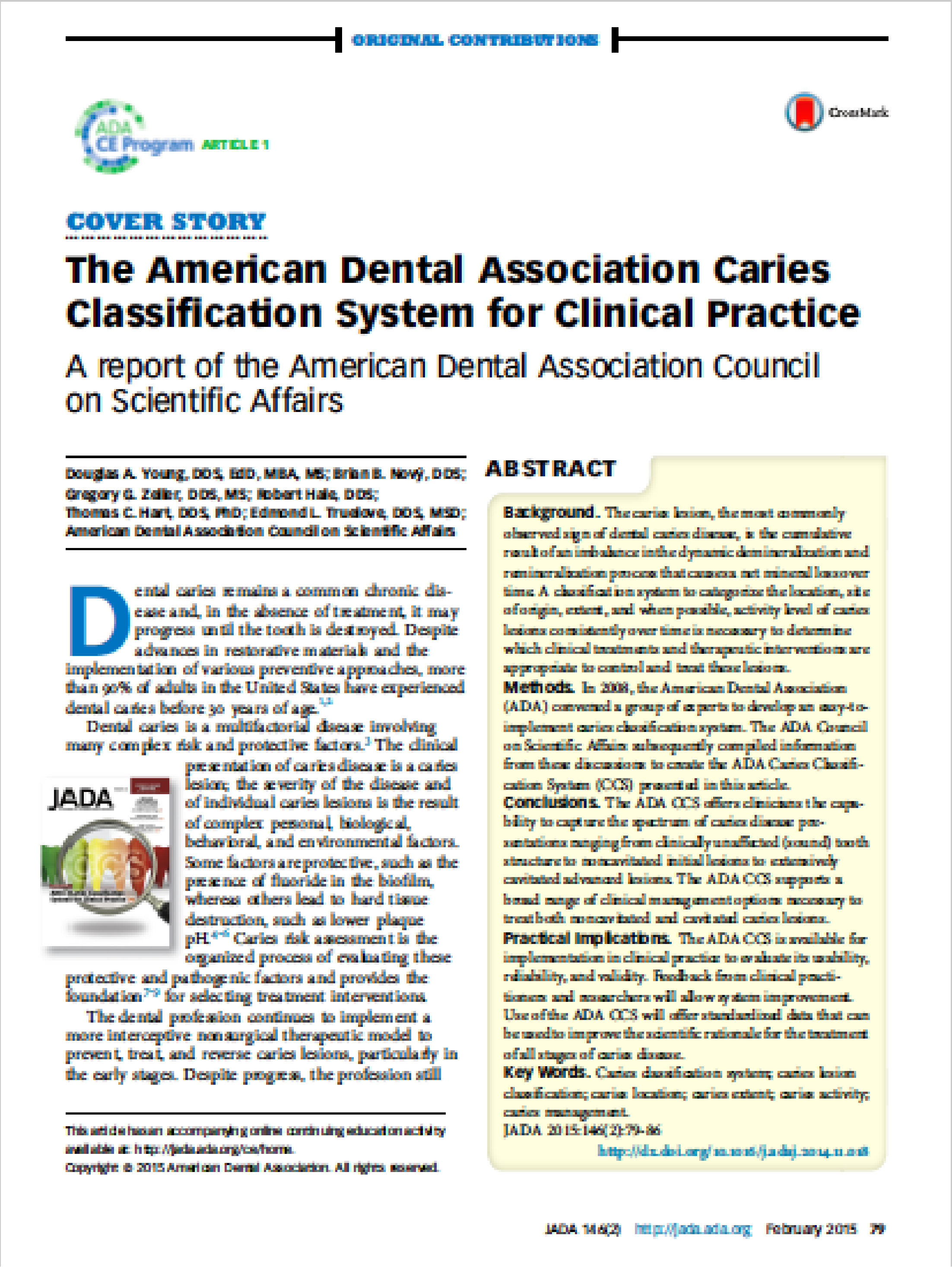

ORIGINAL CONTRIBUTIONS

ADA CE Program ARTICLE 1

CrossMark

COVER STORY

The American Dental Association Caries Classification System for Clinical Practice

A report of the American Dental Association Council on Scientific Affairs

Douglas A. Young, DDS, EdD, MBA, MS; Brian B. Nový, DDS; Gregory G. Zeller, DDS, MS; Robert Hale, DDS; Thomas C. Hart, DDS, PhD; Edmond L. Truelove, DDS, MSD; American Dental Association Council on Scientific Affairs

Dental caries remains a common chronic disease and, in the absence of treatment, it may progress until the tooth is destroyed. Despite advances in restorative materials and the implementation of various preventive approaches, more than 90% of adults in the United States have experienced dental caries before 30 years of age.[1,2]

Dental caries is a multifactorial disease involving many complex risk and protective factors.[3] The clinical presentation of caries disease is a caries lesion; the severity of the disease and of individual caries lesions is the result of complex personal, biological, behavioral, and environmental factors. Some factors are protective, such as the presence of fluoride in the biofilm, whereas others lead to hard tissue destruction, such as lower plaque pH.[4-6] Caries risk assessment is the organized process of evaluating these protective and pathogenic factors and provides the foundation[7-9] for selecting treatment interventions.

The dental profession continues to implement a more interceptive nonsurgical therapeutic model to prevent, treat, and reverse caries lesions, particularly in the early stages. Despite progress, the profession still

JADA

ABSTRACT

Background. The caries lesion, the most commonly observed sign of dental caries disease, is the cumulative result of an imbalance in the dynamic demineralization and remineralization process that causes net mineral loss over time. A classification system to categorize the location, site of origin, extent, and when possible, activity level of caries lesions consistently over time is necessary to determine which clinical treatments and therapeutic interventions are appropriate to control and treat these lesions.

Methods. In 2008, the American Dental Association (ADA) convened a group of experts to develop an easy-to-implement caries classification system. The ADA Council on Scientific Affairs subsequently compiled information from these discussions to create the ADA Caries Classification System (CCS) presented in this article.

Conclusions. The ADA CCS offers clinicians the capability to capture the spectrum of caries disease presentations ranging from clinically unaffected (sound) tooth structure to noncavitated initial lesions to extensively cavitated advanced lesions. The ADA CCS supports a broad range of clinical management options necessary to treat both noncavitated and cavitated caries lesions.

Practical Implications. The ADA CCS is available for implementation in clinical practice to evaluate its usability, reliability, and validity. Feedback from clinical practitioners and researchers will allow system improvement. Use of the ADA CCS will offer standardized data that can be used to improve the scientific rationale for the treatment of all stages of caries disease.

Key Words. Caries classification system; caries lesion classification; caries location; caries extent; caries activity; caries management.

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This article has an accompanying online continuing education activity available at: http://jada.ada.org/ce/home.

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BMC Oral Health

PROCEEDINGS Open Access

Detection and diagnosis of the early caries lesion

J Gomez

From Prevention in practice - making it happen
Cape Town, South Africa. 29 June 2014

Abstract

The purpose of this manuscript is to discuss the current available methods to detect early lesions amenable to prevention. The current evidenced-based caries understanding, based on biological concepts, involves new approaches in caries detection, assessment, and management that should include non-cavitated lesions.
Even though the importance of management of non-cavitated (NC) lesions has been recognized since the early 1900s, dental caries has been traditionally detected at the cavitation stage, and its management has focused strongly on operative treatment. Methods of detection of early carious lesions have received significant research attention over the last 20 years. The most common method of caries detection is visual-tactile. Other non-invasive techniques for detection of early caries have been developed and investigated such as Quantitative Light-induced Fluorescence (QLF), DIAGNOdent (DD), Fibre-optic Transillumination (FOTI) and Electrical Conductance (EC). Based on previous systematic reviews, the diagnosis of NCCLs might be more accurately achieved in combination of the visual method and the use of other methods such as electrical methods and QLF for monitoring purposes.

Introduction

Dental caries is the most prevalent chronic disease worldwide. When initial lesions are taken into account into the clinical assessment, only few individuals are truly unaffected. In most industrialized countries 60-90% of school-aged children are affected and nearly 100% of the adult population is affected [1]. However, over the recent years, the patterns of disease presentation have changed. The progression of non-cavitated lesions seems to be slower [2], allowing preventive strategies to be implemented when the lesions have the greatest opportunity to arrest. Traditional methods combined with more sensitive methods may improve the caries diagnosis and also help the clinician in monitoring non-operative treatments. Also, clinical trials involving thousands of subjects and for long periods of time are today unrealistic and the use of cavitated endpoints questionable [3].

Clinical caries measures involving "pre-cavitation" lesions have been in fact reported in caries clinical trials since 1965 [4] and have been described and used in clinical research and practice already for more than 50 years [5]. However, some approaches still used in dental practice and in clinical trials have focused on detecting lesions at a cavitation stage informing only restorative decisions [6].

Several conferences have also been held during the past years focused on caries detection and management. In the last Consensus on Diagnosis and Management of Dental Caries, the inability to accurately identify early caries lesions and the need for a change in the system with respect to the non-surgical management of non-cavitated lesions was highlighted [7]. The Consensus Panel concluded the evidence-base for current methods of detection and activity assessment of non-cavitated lesions was not sufficiently strong to recommend their formal adoption [8].

An International Consensus Workshop on Caries Clinical Trials (ICW-CCT) [9] concluded among others:

- Lesion detection implies an objective method of determining whether or not the disease is present, lesion assessment which aims to characterize once it has been detected and caries diagnosis which implies a human professional summation of all available data.
- Visual diagnosis is the standard of caries diagnosis; the use of additional methods should be explored further.

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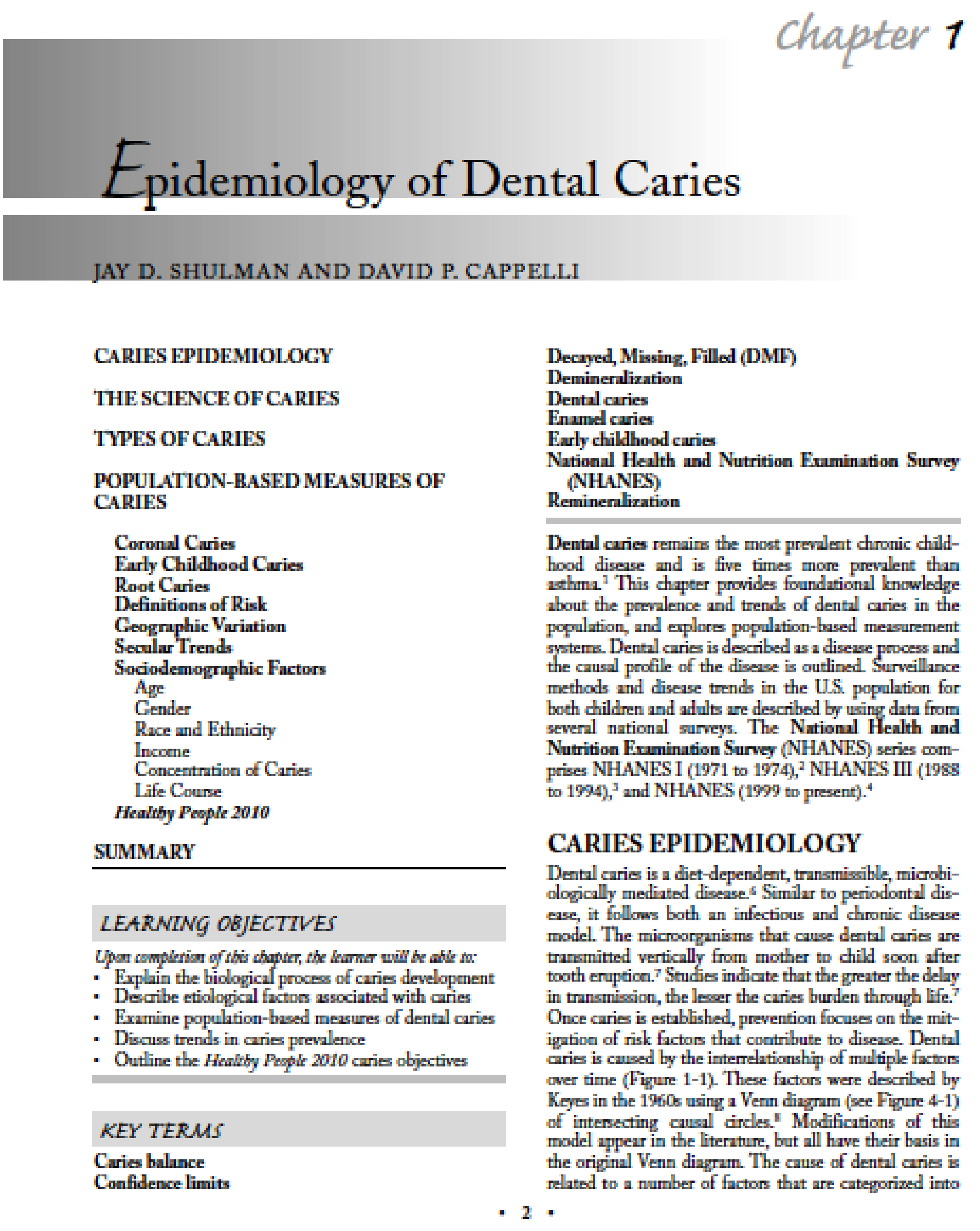

Chapter 1

Epidemiology of Dental Caries

JAY D. SHULMAN AND DAVID P. CAPPELLI

LEARNING OBJECTIVES

Upon completion of this chapter, the learner will be able to:

- Explain the biological process of caries development
- Describe etiological factors associated with caries
- Examine population-based measures of dental caries
- Discuss trends in caries prevalence
- Outline the *Healthy People 2010* caries objectives

KEY TERMS

Caries balance
Confidence limits
Decayed, Missing, Filled (DMF)
Demineralization
Dental caries
Enamel caries
Early childhood caries
National Health and Nutrition Examination Survey (NHANES)
Remineralization

Dental caries remains the most prevalent chronic childhood disease and is five times more prevalent than asthma.[1] This chapter provides foundational knowledge about the prevalence and trends of dental caries in the population, and explores population-based measurement systems. Dental caries is described as a disease process and the causal profile of the disease is outlined. Surveillance methods and disease trends in the U.S. population for both children and adults are described by using data from several national surveys. The **National Health and Nutrition Examination Survey** (NHANES) series comprises NHANES I (1971 to 1974),[2] NHANES III (1988 to 1994),[3] and NHANES (1999 to present).[4]

CARIES EPIDEMIOLOGY

Dental caries is a diet-dependent, transmissible, microbiologically mediated disease.[6] Similar to periodontal disease, it follows both an infectious and chronic disease model. The microorganisms that cause dental caries are transmitted vertically from mother to child soon after tooth eruption.[7] Studies indicate that the greater the delay in transmission, the lesser the caries burden through life.[7] Once caries is established, prevention focuses on the mitigation of risk factors that contribute to disease. Dental caries is caused by the interrelationship of multiple factors over time (Figure 1-1). These factors were described by Keyes in the 1960s using a Venn diagram (see Figure 4-1) of intersecting causal circles.[8] Modifications of this model appear in the literature, but all have their basis in the original Venn diagram. The cause of dental caries is related to a number of factors that are categorized into

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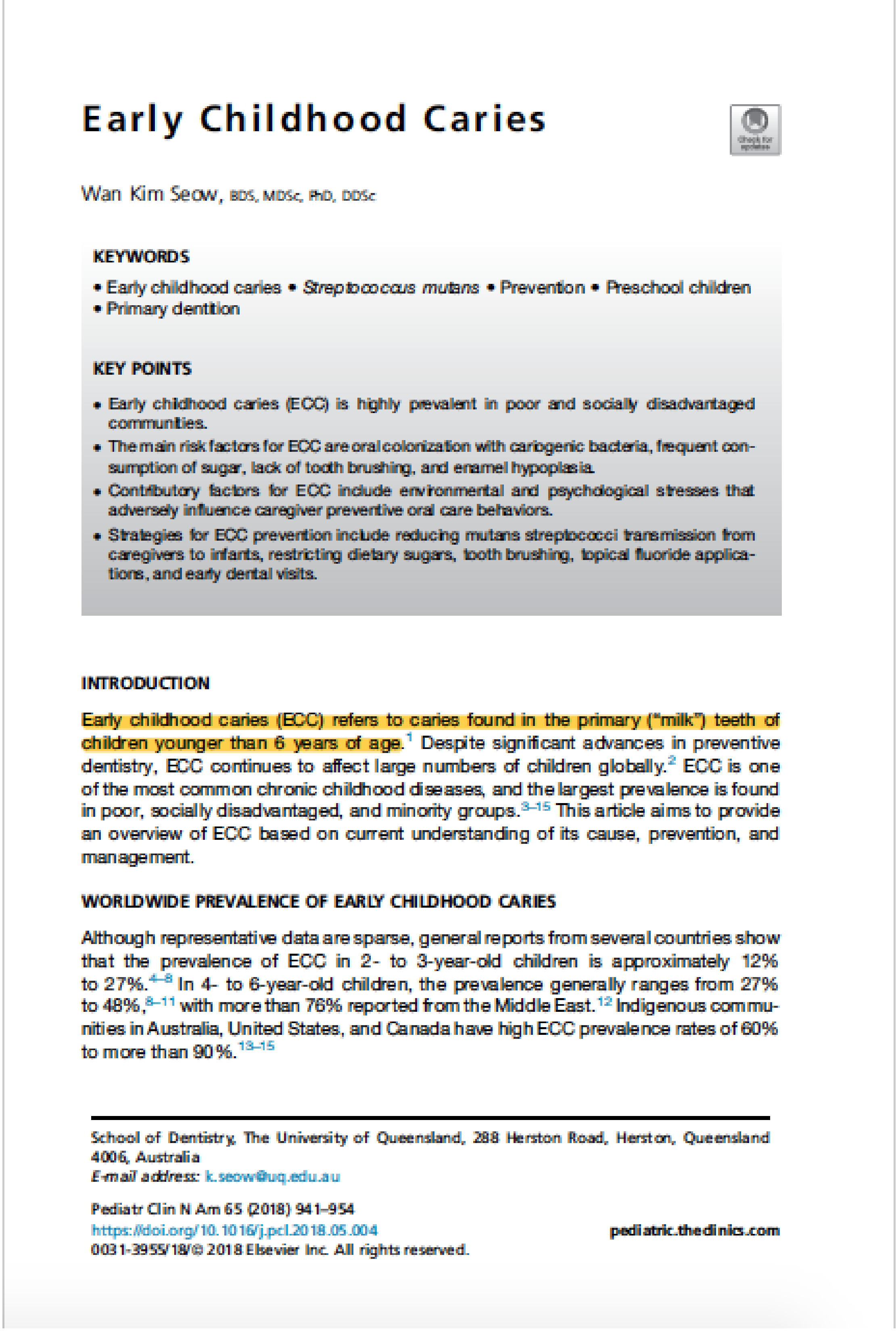

Early Childhood Caries

Wan Kim Seow, BDS, MDSc, PhD, DDSc

KEYWORDS

- Early childhood caries • *Streptococcus mutans* • Prevention • Preschool children
- Primary dentition

KEY POINTS

- Early childhood caries (ECC) is highly prevalent in poor and socially disadvantaged communities.
- The main risk factors for ECC are oral colonization with cariogenic bacteria, frequent consumption of sugar, lack of tooth brushing, and enamel hypoplasia.
- Contributory factors for ECC include environmental and psychological stresses that adversely influence caregiver preventive oral care behaviors.
- Strategies for ECC prevention include reducing mutans streptococci transmission from caregivers to infants, restricting dietary sugars, tooth brushing, topical fluoride applications, and early dental visits.

INTRODUCTION

Early childhood caries (ECC) refers to caries found in the primary ("milk") teeth of children younger than 6 years of age.[1] Despite significant advances in preventive dentistry, ECC continues to affect large numbers of children globally.[2] ECC is one of the most common chronic childhood diseases, and the largest prevalence is found in poor, socially disadvantaged, and minority groups.[3–15] This article aims to provide an overview of ECC based on current understanding of its cause, prevention, and management.

WORLDWIDE PREVALENCE OF EARLY CHILDHOOD CARIES

Although representative data are sparse, general reports from several countries show that the prevalence of ECC in 2- to 3-year-old children is approximately 12% to 27%.[4–8] In 4- to 6-year-old children, the prevalence generally ranges from 27% to 48%,[8–11] with more than 70% reported from the Middle East.[12] Indigenous communities in Australia, United States, and Canada have high ECC prevalence rates of 60% to more than 90%.[13–15]

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REVIEW ARTICLE

WILEY INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective

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Background: This paper is a summary of the proceedings of the International Association of Paediatric Dentistry Bangkok Conference on early childhood caries (ECC) held in 3-4 November 2018.
Aim: The paper aims to convey a global perspective of ECC definitions, aetiology, risk factors, societal costs, management, educational curriculum, and policy.
Design: This global perspective on ECC is the compilation of the state of science, current concepts, and literature regarding ECC from worldwide experts on ECC.
Results: Early childhood caries is related to frequent sugar consumption in an environment of enamel adherent, acid-producing bacteria in a complex biofilm, as well as developmental defects of enamel. The seriousness, societal costs, and impact on quality of life of dental caries in pre-school children are enormous. Worldwide data show that ECC continues to be highly prevalent, yet infrequently treated. Approaches to reduce the prevalence include interventions that start in the first year of a child's life, evidence-based and risk-based management, and reimbursement systems that foster preventive care.
Conclusions: This global perspective on ECC epidemiology, aetiology, risk assessment, global impact, and management is aimed to foster improved worldwide understanding and management of ECC.

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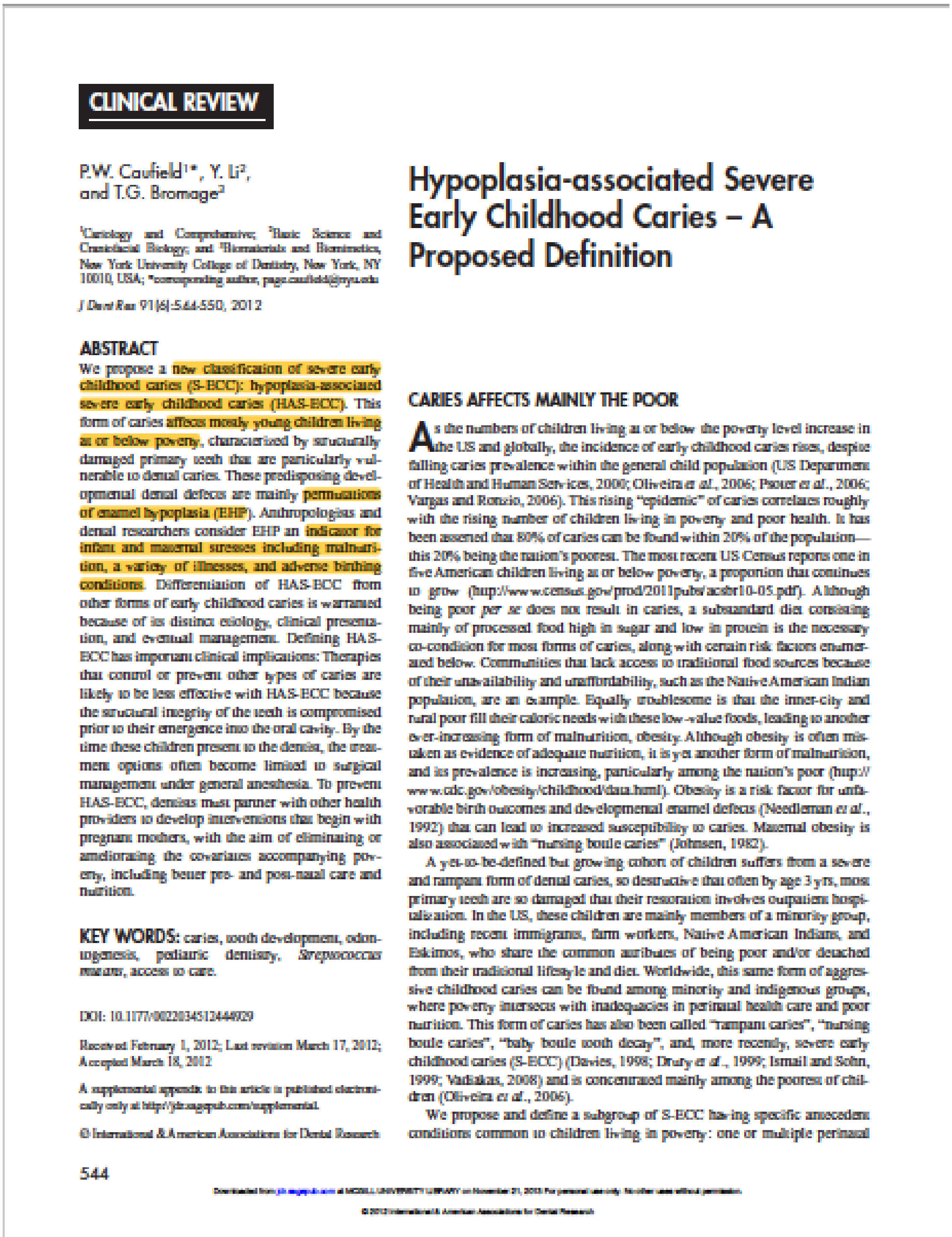

CLINICAL REVIEW

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and T.G. Bromage[3]

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J Dent Res 91(6):544-550, 2012

Hypoplasia-associated Severe Early Childhood Caries – A Proposed Definition

ABSTRACT

We propose a new classification of severe early childhood caries (S-ECC): hypoplasia-associated severe early childhood caries (HAS-ECC). This form of caries affects mostly young children living at or below poverty, characterized by structurally damaged primary teeth that are particularly vulnerable to dental caries. These predisposing developmental dental defects are mainly permutations of enamel hypoplasia (EHP). Anthropologists and dental researchers consider EHP an indicator for infant and maternal stresses including malnutrition, a variety of illnesses, and adverse birthing conditions. Differentiation of HAS-ECC from other forms of early childhood caries is warranted because of its distinct etiology, clinical presentation, and eventual management. Defining HAS-ECC has important clinical implications: Therapies that control or prevent other types of caries are likely to be less effective with HAS-ECC because the structural integrity of the teeth is compromised prior to their emergence into the oral cavity. By the time these children present to the dentist, the treatment options often become limited to surgical management under general anesthesia. To prevent HAS-ECC, dentists must partner with other health providers to develop interventions that begin with pregnant mothers, with the aim of eliminating or ameliorating the covariates accompanying poverty, including better pre- and post-natal care and nutrition.

KEY WORDS: caries, tooth development, odontogenesis, pediatric dentistry, *Streptococcus mutans*, access to care.

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

CARIES AFFECTS MAINLY THE POOR

As the numbers of children living at or below the poverty level increase in the US and globally, the incidence of early childhood caries rises, despite falling caries prevalence within the general child population (US Department of Health and Human Services, 2000; Oliveira *et al.*, 2006; Psoter *et al.*, 2006; Vargas and Ronzio, 2006). This rising "epidemic" of caries correlates roughly with the rising number of children living in poverty and poor health. It has been asserted that 80% of caries can be found within 20% of the population—this 20% being the nation's poorest. The most recent US Census reports one in five American children living at or below poverty, a proportion that continues to grow (http://www.census.gov/prod/2011pubs/acsbr10-05.pdf). Although being poor *per se* does not result in caries, a substandard diet consisting mainly of processed food high in sugar and low in protein is the necessary co-condition for most forms of caries, along with certain risk factors enumerated below. Communities that lack access to traditional food sources because of their unavailability and unaffordability, such as the Native American Indian population, are an example. Equally troublesome is that the inner-city and rural poor fill their caloric needs with these low-value foods, leading to another ever-increasing form of malnutrition, obesity. Although obesity is often mistaken as evidence of adequate nutrition, it is yet another form of malnutrition, and its prevalence is increasing, particularly among the nation's poor (http://www.cdc.gov/obesity/childhood/data.html). Obesity is a risk factor for unfavorable birth outcomes and developmental enamel defects (Needleman *et al.*, 1992) that can lead to increased susceptibility to caries. Maternal obesity is also associated with "nursing bottle caries" (Johnsen, 1982).

A yet-to-be-defined but growing cohort of children suffers from a severe and rampant form of dental caries, so destructive that often by age 3 yrs, most primary teeth are so damaged that their restoration involves outpatient hospitalization. In the US, these children are mainly members of a minority group, including recent immigrants, farm workers, Native American Indians, and Eskimos, who share the common attributes of being poor and/or detached from their traditional lifestyle and diet. Worldwide, this same form of aggressive childhood caries can be found among minority and indigenous groups, where poverty intersects with inadequacies in perinatal health care and poor nutrition. This form of caries has also been called "rampant caries", "nursing bottle caries", "baby bottle tooth decay", and, more recently, severe early childhood caries (S-ECC) (Davies, 1998; Drury *et al.*, 1999; Ismail and Sohn, 1999; Vadiakas, 2008) and is concentrated mainly among the poorest of children (Oliveira *et al.*, 2006).

We propose and define a subgroup of S-ECC having specific antecedent conditions common to children living in poverty: one or multiple perinatal

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Journal of Oral Microbiology

ORIGINAL ARTICLE

Genotypic characterization of initial acquisition of *Streptococcus mutans* in American Indian children

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Background: Severe-early childhood caries (S-ECC) is one of the most common infectious diseases in children and is prevalent in lower socio-economic populations. American Indian children suffer from the highest levels of S-ECC in the United States. Members of the mutans streptococci, *Streptococcus mutans*, in particular, are key etiologic agents in the development of caries. Children typically acquire *S. mutans* from their mothers and early acquisition is often associated with higher levels of tooth decay.

Methods: We have conducted a 5-year birth cohort study with a Northern Plains Tribe to determine the temporality and fidelity of *S. mutans* transmission from mother to child in addition to the genotypic diversity of *S. mutans* in this community. Plaque samples were collected from 239 mother/child dyads at regular intervals from birth to 36 months and *S. mutans* were isolated and genotyped by arbitrarily primed-polymerase chain reaction (AP-PCR).

Results: Here we present preliminary findings from a subset of the cohort. The focus for this paper is on initial acquisition events in the children. We identified 17 unique genotypes in 711 *S. mutans* isolates in our subset of 40 children, 40 mothers and 14 primary caregivers. Twelve of these genotypes were identified in more than one individual. *S. mutans* colonization occurred by 16 months in 57.5% of the children and early colonization was associated with higher decayed, missing and filled surface (DMFS) scores ($p = 0.0007$). Children colonized by *S. mutans* shared a common genotype with their mothers 47.8% of the time. While multiple genotypes were common in adults, only 10% of children harbored multiple genotypes.

Conclusion: These children acquire *S. mutans* at an earlier age than the originally described 'window of infectivity' and often, but not exclusively, from their mothers. Early acquisition is associated with both the caries status of the children and the mothers.

Keywords: *caries; etiologic agents; American Indian; children;* Streptococcus mutans; *genotypic diversity; genotypes; oral microbiology; severe early childhood caries*

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There is growing evidence of a substantial oral health disparities problem in young children in our society (1–5). While dental caries continues to burden youth and adults at all ages, there is disparaging evidence of a devastating disease called severe-early childhood caries (S-ECC) in lower socio-economic and minority children in the United States and worldwide (6). The highest prevalence of this disease occurs in American Indian (AI) children. The most recent surveys have revealed an extremely high level of S-ECC in AI children (2, 7–9). By 5 years of age, 75% of AI children had significant caries experience with three times the severity of the national average (8). Many studies have now shown that American Indian children are at the highest risk of caries compared to any other racial/ethnic group (7, 10–12).

Many studies have shown that the mutans streptococci exhibit a number of virulence factors that can lead to the development of caries (13–17). These organisms produce copious amounts of lactic acid in the presence of dietary sucrose, which leads to demineralization of the tooth

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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,

~ 341 ~

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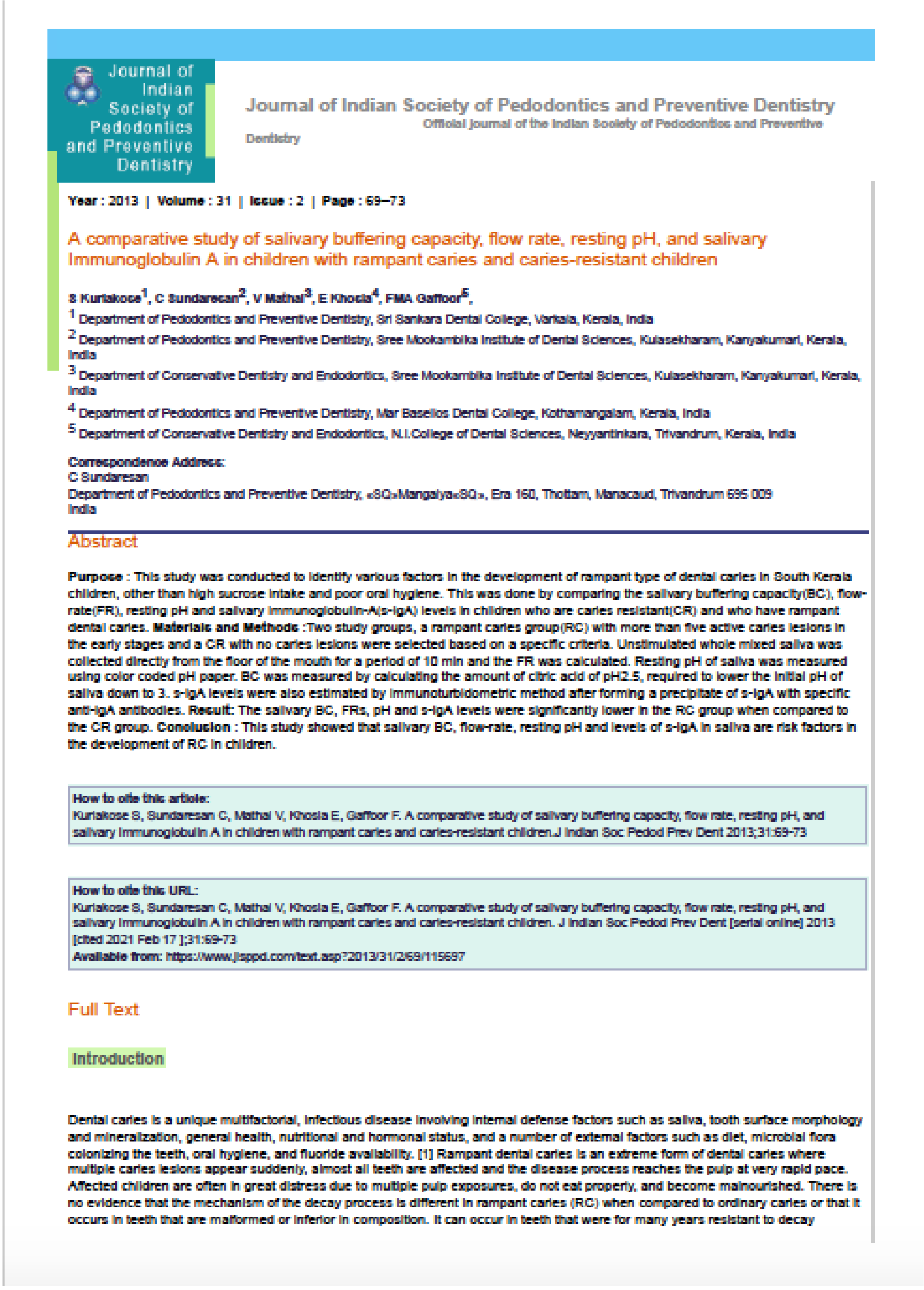

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A comparative study of salivary buffering capacity, flow rate, resting pH, and salivary Immunoglobulin A in children with rampant caries and caries-resistant children

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India

Abstract

Purpose : This study was conducted to identify various factors in the development of rampant type of dental caries in South Kerala children, other than high sucrose intake and poor oral hygiene. This was done by comparing the salivary buffering capacity(BC), flow-rate(FR), resting pH and salivary Immunoglobulin-A(s-IgA) levels in children who are caries resistant(CR) and who have rampant dental caries. **Materials and Methods** :Two study groups, a rampant caries group(RC) with more than five active caries lesions in the early stages and a CR with no caries lesions were selected based on a specific criteria. Unstimulated whole mixed saliva was collected directly from the floor of the mouth for a period of 10 min and the FR was calculated. Resting pH of saliva was measured using color coded pH paper. BC was measured by calculating the amount of citric acid of pH2.5, required to lower the initial pH of saliva down to 3. s-IgA levels were also estimated by immunoturbidometric method after forming a precipitate of s-IgA with specific anti-IgA antibodies. **Result:** The salivary BC, FRs, pH and s-IgA levels were significantly lower in the RC group when compared to the CR group. **Conclusion** : This study showed that salivary BC, flow-rate, resting pH and levels of s-IgA in saliva are risk factors in the development of RC in children.

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Full Text

Introduction

Dental caries is a unique multifactorial, infectious disease involving internal defense factors such as saliva, tooth surface morphology and mineralization, general health, nutritional and hormonal status, and a number of external factors such as diet, microbial flora colonizing the teeth, oral hygiene, and fluoride availability. [1] Rampant dental caries is an extreme form of dental caries where multiple caries lesions appear suddenly, almost all teeth are affected and the disease process reaches the pulp at very rapid pace. Affected children are often in great distress due to multiple pulp exposures, do not eat properly, and become malnourished. There is no evidence that the mechanism of the decay process is different in rampant caries (RC) when compared to ordinary caries or that it occurs in teeth that are malformed or inferior in composition. It can occur in teeth that were for many years resistant to decay

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ORIGINAL RESEARCH

Comparison of Some Salivary Characteristics in Iraqi Children with Early Childhood Caries (ECC) and Children without Early Childhood Caries

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Background: Early Childhood Caries (ECC) is a unique form of caries that develops in temporary dentition. It has a multifactorial infectious disease. Saliva is one of the most important factors, which has an important protective effect against tooth decay when its multiple characteristics and functions are normal. The study aimed to compare some salivary characteristics in children with ECC and children without ECC.

Materials and Methods: Case-control, cross-sectional observational study. The 77 pre-schoolers aged 37 to 72 months (12 with ECC, 26 with ECC-S, and 39 without ECC) examined and we collected the stimulated saliva. The pH microelectrode was used to determine pH and buffer capacity; a formula that involves volume, collection time, and specific saliva weight was used to test the salivary flow rate. The potentiometric and phosphate methods were used to determine fluoride through spectrophotometric, colorimetric absorption techniques.

Results: The results got to show that there are no statistically significant differences in pH, buffer capacity, salivary flow rate, and levels of fluoride and phosphate, in children with ECC, ECC-S, and without ECC. The risk factors, such as mother's education, bottle use, brushing frequency, and previous dental care of the child are more important at the time of developing ECC than some salivary variables.

Conclusion: The risk factors, such as mother's education, bottle use, brushing frequency, and previous dental care of the child are more important at the time of developing ECC than some salivary variables such as pH, buffer capacity, salivary flow rate, and levels of fluoride and phosphate.

Keywords: early childhood caries, fluoride, saliva, phosphate, buffer capacity, tooth brushing

Introduction

Dental caries is the most common chronic diseases in many countries because they are a serious Public Health problem because of their high prevalence, impact on individuals and society, American Association of Dental Paediatrics adopted the term "Early Childhood Caries" (ECC) for specific caries modality of temporary dentition, which affects infants and children in preschool age and develops immediately after the first teeth erupted.[1] Dental caries is a chronic pathology with an infectious component occurs both in enamel sub-surfaces and in deeper dental tissues such as dentin and dental pulp, induced by pH variations that lead to the imbalance between demineralization and remineralization in enamel.[2] Although

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Research Article

Identification of Microbial and Proteomic Biomarkers in Early Childhood Caries

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The purpose of this study was to provide a univariate and multivariate analysis of genomic microbial data and salivary mass-spectrometry proteomic profiles for dental caries outcomes. In order to determine potential useful biomarkers for dental caries, a multivariate classification analysis was employed to build predictive models capable of classifying microbial and salivary sample profiles with generalization performance. We used high-throughput methodologies including multiplexed microbial arrays and SELDI-TOF-MS profiling to characterize the oral flora and salivary proteome in 204 children aged 1–8 years ($n = 118$ caries-free, $n = 86$ caries-active). The population received little dental care and was deemed at high risk for childhood caries. Findings of the study indicate that models incorporating both microbial and proteomic data are superior to models of only microbial or salivary data alone. Comparison of results for the combined and independent data suggests that the combination of proteomic and microbial sources is beneficial for the classification accuracy and that combined data lead to improved predictive models for caries-active and caries-free patients. The best predictive model had a 6% test error, >92% sensitivity, and >95% specificity. These findings suggest that further characterization of the oral microflora and the salivary proteome associated with health and caries may provide clinically useful biomarkers to better predict future caries experience.

1. Introduction

Dental caries, the most common disease of childhood, is a complex infectious disease with a multifactorial etiology. The caries process is characterized by interactions between a receptive host and microorganisms with the potential for colonization and pathogenesis. Microbial, genetic, immunological, behavioral, environmental, and socioeconomic factors contribute to risk and determine the occurrence and severity of clinical disease [1, 2]. Of the identified risk factors, the cariogenic oral microbial flora and saliva have received particular research attention.

Microbiological studies conducted in the past four decades have shown that *Streptococcus mutans* is the chief pathogen associated with childhood dental caries onset and that lactobacilli are associated with dental caries progression [3, 4]. Much of this knowledge has been made possible with the use of traditional culturing methods employing selective media for these pathogens. Recent advances employing microbial molecular techniques have allowed for better understanding of the complexity of the flora associated with oral infections, particularly dental caries. More than 750 oral microbial taxa inhabit the oral cavity [5]. Of those, approximately 50% have yet to be cultivated, and many

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Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old

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Alaluusua S, Renkonen O-V: Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res 1983; 91: 453–7.

Abstract – 39 children were studied longitudinally at the age of 2, 3, and 4 yr for the colonization of S. mutans in plaque and saliva and for caries experience. S. mutans was found in 38% of the children, and the predominant serotype group was c/e/f. A total of 16 children got caries before the age of 4. Children who harbored S. mutans in their plaque at the age of 2, appeared to be the most caries-active individuals. Their caries index values (number of decayed, missed and filled surfaces, dmfs = 10.6 ± 5.3) at the age of 4 differed significantly from the values of children who harbored S. mutans later (dmfs = 3.4 ± 1.8, $P < 0.005$) or remained free from S. mutans infection (dmfs = 0.3 ± 1.1, $P < 0.0003$). It was thus concluded that the early establishment of S. mutans in the plaque of primary incisors indicated early and extensive caries attack in young primary dentition.

Key words: dental caries; Streptococcus mutans.

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Studies of Streptococcus mutans strongly suggest its active involvement in the initiation and progression of dental caries; the organism does not become established in the mouths of infants during the first year (1–8). There is a gradual increase in the isolation frequency of S. mutans with age and as the number of teeth and retentive sites on the tooth surfaces increases (4).

Subjects highly infected with S. mutans develop more caries than those with a low level of this organism (7, 9, 10). On the basis of the level of S. mutans infection caries-susceptible patients have been identified and selected for prophylactic and antimicrobial treatment (11–13). Since the oral microflora of infants and young children differs considerably from that of the older children or adults (2, 14), the infection levels of S. mutans indicating high caries risk in older children or adults may not be applicable to younger children.

A longitudinal study was therefore undertaken to evaluate the initial establishment, the isolation frequency and the changes in the proportion of S. mutans in plaque of

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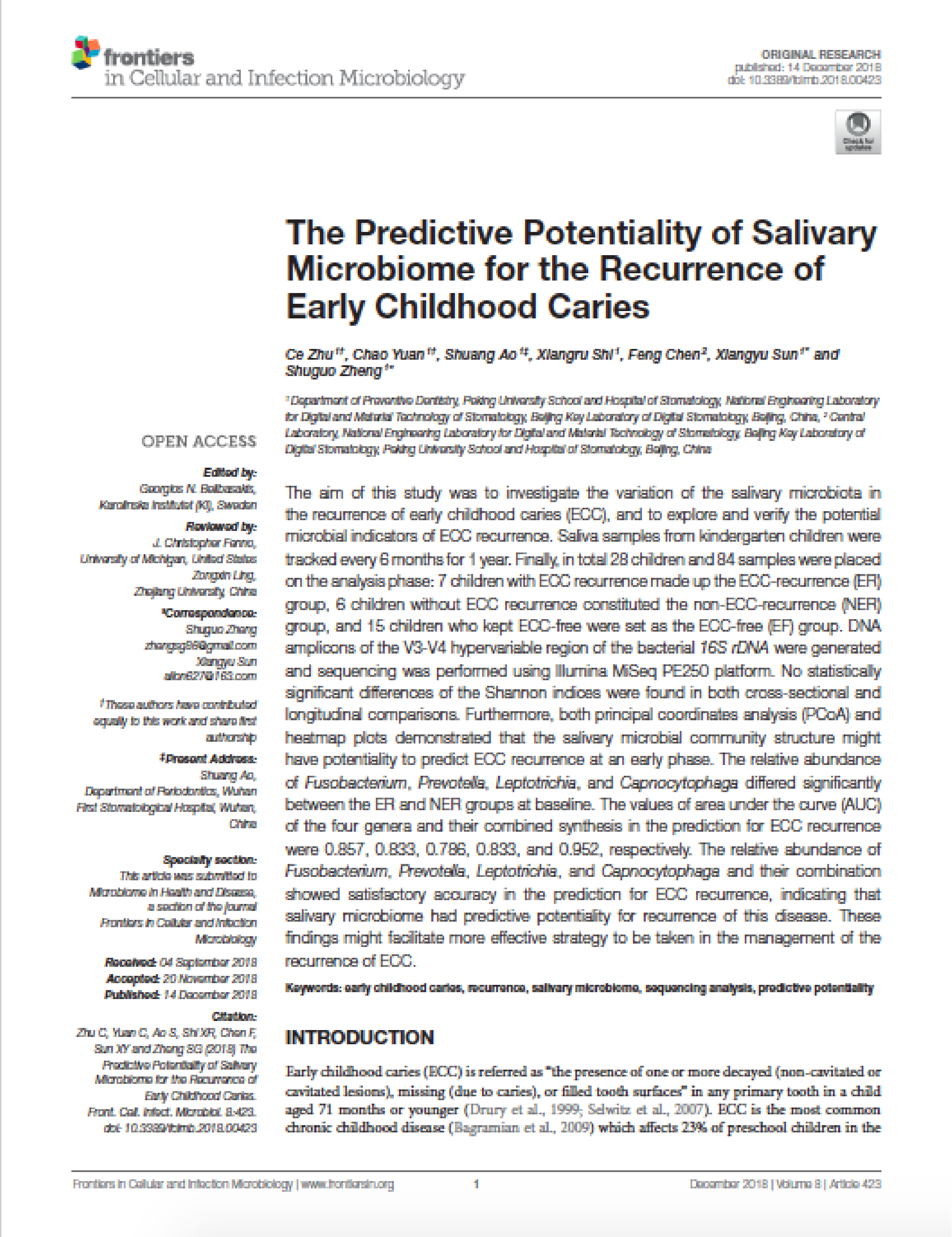

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ORIGINAL RESEARCH
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The Predictive Potentiality of Salivary Microbiome for the Recurrence of Early Childhood Caries

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The aim of this study was to investigate the variation of the salivary microbiota in the recurrence of early childhood caries (ECC), and to explore and verify the potential microbial indicators of ECC recurrence. Saliva samples from kindergarten children were tracked every 6 months for 1 year. Finally, in total 28 children and 84 samples were placed on the analysis phase: 7 children with ECC recurrence made up the ECC-recurrence (ER) group, 6 children without ECC recurrence constituted the non-ECC-recurrence (NER) group, and 15 children who kept ECC-free were set as the ECC-free (EF) group. DNA amplicons of the V3-V4 hypervariable region of the bacterial *16S rDNA* were generated and sequencing was performed using Illumina MiSeq PE250 platform. No statistically significant differences of the Shannon indices were found in both cross-sectional and longitudinal comparisons. Furthermore, both principal coordinates analysis (PCoA) and heatmap plots demonstrated that the salivary microbial community structure might have potentiality to predict ECC recurrence at an early phase. The relative abundance of *Fusobacterium*, *Prevotella*, *Leptotrichia*, and *Capnocytophaga* differed significantly between the ER and NER groups at baseline. The values of area under the curve (AUC) of the four genera and their combined synthesis in the prediction for ECC recurrence were 0.857, 0.833, 0.786, 0.833, and 0.952, respectively. The relative abundance of *Fusobacterium*, *Prevotella*, *Leptotrichia*, and *Capnocytophaga* and their combination showed satisfactory accuracy in the prediction for ECC recurrence, indicating that salivary microbiome had predictive potentiality for recurrence of this disease. These findings might facilitate more effective strategy to be taken in the management of the recurrence of ECC.

Keywords: early childhood caries, recurrence, salivary microbiome, sequencing analysis, predictive potentiality

INTRODUCTION

Early childhood caries (ECC) is referred 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 aged 71 months or younger (Drury et al., 1999; Selwitz et al., 2007). ECC is the most common chronic childhood disease (Bagramian et al., 2009) which affects 23% of preschool children in the

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Original Article

Relationship of Salivary Lactoferrin and Lysozyme Concentrations with Early Childhood Caries

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Abstract

Background and aims. Lysozyme and lactoferrin are salivary proteins which play an important role in innate defense mechanisms against bacteria. This study investigated the association of salivary lysozyme and lactoferrin concentrations with early childhood caries (ECC).

Materials and methods. This study was carried out on 42 healthy children (age range, 36 to 71 months), of whom 21 were caries free (CF) and 21 had ECC. Disposable needle-less syringes were used to collect unstimulated saliva from buccal and labial vestibules. Fifteen children who had ECC were treated completely and their saliva was collected in the same way for the second time, three months after treatment. Lysozyme and lactoferrin concentrations were measured and recorded by the ELISA method. The intergroup comparisons were carried out using chi-square, Student's *t*-test and Wilcoxon signed ranked test. A P-value less than 0.05 was considered as statistically significant.

Results. The mean concentration of lysozyme was significantly higher in CF group compared with that of ECC group (P = 0.04). Although the mean concentration of lactoferrin in ECC group was higher in comparison with ECC group, the difference was not statistically significant (P = 0.06). After dental treatment, the mean concentrations of lysozyme and lactoferrin did not change in comparison with their concentrations before treatment.

Conclusion. ECC may have a relationship with lower concentrations of unstimulated salivary lactoferrin and lysozyme and reduced amounts of these two salivary proteins may be a risk factor for dental caries in children.

Key words: **Dental caries, lactoferrin, lysozyme, saliva.**

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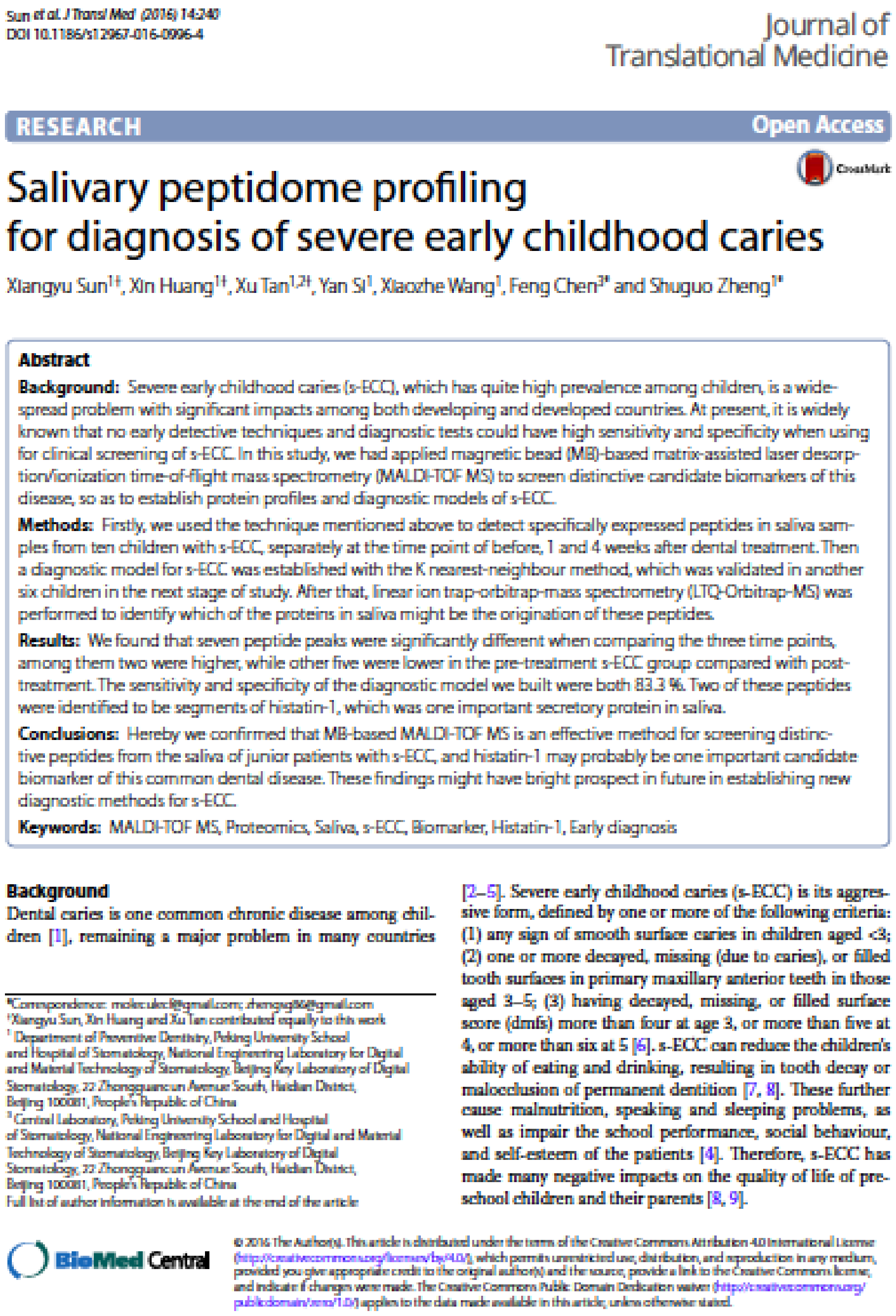

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RESEARCH Open Access

Salivary peptidome profiling for diagnosis of severe early childhood caries

Xiangyu Sun[1†], Xin Huang[1†], Xu Tan[1,2†], Yan Si[1], Xiaozhe Wang[1], Feng Chen[3*] and Shuguo Zheng[1*]

Abstract

Background: Severe early childhood caries (s-ECC), which has quite high prevalence among children, is a widespread problem with significant impacts among both developing and developed countries. At present, it is widely known that no early detective techniques and diagnostic tests could have high sensitivity and specificity when using for clinical screening of s-ECC. In this study, we had applied magnetic bead (MB)-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to screen distinctive candidate biomarkers of this disease, so as to establish protein profiles and diagnostic models of s-ECC.

Methods: Firstly, we used the technique mentioned above to detect specifically expressed peptides in saliva samples from ten children with s-ECC, separately at the time point of before, 1 and 4 weeks after dental treatment. Then a diagnostic model for s-ECC was established with the K nearest-neighbour method, which was validated in another six children in the next stage of study. After that, linear ion trap-orbitrap-mass spectrometry (LTQ-Orbitrap-MS) was performed to identify which of the proteins in saliva might be the origination of these peptides.

Results: We found that seven peptide peaks were significantly different when comparing the three time points, among them two were higher, while other five were lower in the pre-treatment s-ECC group compared with post-treatment. The sensitivity and specificity of the diagnostic model we built were both 83.3 %. Two of these peptides were identified to be segments of histatin-1, which was one important secretory protein in saliva.

Conclusions: Hereby we confirmed that MB-based MALDI-TOF MS is an effective method for screening distinctive peptides from the saliva of junior patients with s-ECC, and histatin-1 may probably be one important candidate biomarker of this common dental disease. These findings might have bright prospect in future in establishing new diagnostic methods for s-ECC.

Keywords: MALDI-TOF MS, Proteomics, Saliva, s-ECC, Biomarker, Histatin-1, Early diagnosis

Background

Dental caries is one common chronic disease among children [1], remaining a major problem in many countries [2–5]. Severe early childhood caries (s-ECC) is its aggressive form, defined by one or more of the following criteria: (1) any sign of smooth surface caries in children aged <3; (2) one or more decayed, missing (due to caries), or filled tooth surfaces in primary maxillary anterior teeth in those aged 3–5; (3) having decayed, missing, or filled surface score (dmfs) more than four at age 3, or more than five at 4, or more than six at 5 [6]. s-ECC can reduce the children's ability of eating and drinking, resulting in tooth decay or malocclusion of permanent dentition [7, 8]. These further cause malnutrition, speaking and sleeping problems, as well as impair the school performance, social behaviour, and self-esteem of the patients [4]. Therefore, s-ECC has made many negative impacts on the quality of life of preschool children and their parents [8, 9].

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Diagnostic potential of inflammatory biomarkers in early childhood caries - A case control study

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A R T I C L E I N F O

Keywords:
Early childhood caries
Il-6
Il-8
TNF-α
Diagnostic marker
Prognostic marker

A B S T R A C T

Background: Early Childhood Caries (ECC) is most common chronic infectious disease of childhood. Diagnosis of dental caries has been limited to clinical, visual and radiographic methods but its inflammatory component remained unexplored. Hence, this study aims to evaluate salivary levels of inflammatory cytokines in children with ECC to assess their potential as non-invasive biomarkers.
Methods: 50 subjects were recruited (25 ECC patients and 25 healthy children). Saliva samples were taken from all subjects and collected again from patients after rehabilitative intervention. Levels of IL-6, IL-8 and TNF-α were determined using ELISA. Cytokines level were statistically correlated with each other and with DMF score along with ROC curve analysis.
Results: Salivary levels of IL-6, IL-8 & TNF-α were significantly higher in patients which got significantly reduced after rehabilitative intervention. Levels of these cytokines were significantly associated with severity of dental caries. These cytokines were correlating with each other along with DMF score upon Spearman correlation. ROC curve reveals optimum sensitivity and specificity of these cytokines for diagnosis in ECC with absolute levels observed for IL-6.
Conclusions: Significant elevation of IL-6, IL-8 and TNF-α with optimum sensitivity and specificity might imply their involvement as potential non-invasive diagnostic/prognostic markers in ECC.

1. Introduction

Early childhood caries (ECC) is a serious socio behavioral and dental problem that affects infants and toddlers [1]. ECC is defined as the presence of 1 or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger.

Severe ECC is expressed in lieu of rampant caries in the presence of at least one of the following criteria:

- Any sign of caries on a smooth surface in children younger than 3 years.
- From ages three through five, one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of greater than or equal to four (age 3), greater than or equal to five (age 4), or greater than or equal to six (age 5) surfaces [2].

The etiology of ECC is multifactorial, caused by 3 primary factors: microorganisms, cariogenic substrate diet (exposure to fermentable carbohydrate), and susceptible host (or tooth), which forms a triad. When these 3 essential factors interact for a considerable period of time, an imbalance in the demineralization and remineralization between tooth surface and the adjacent plaque (biofilm) occurs, resulting in the development of dental caries or ECC.

Studies have suggested that the prevalence of ECC among preschool children is very evident with a percentage of 67.3%. Therefore, there is a need for preventive and curative oral health programs for the same [3,4].

Diagnosis of dental caries has always been limited to clinical, visual and/or radiographic methods but its inflammatory component remained an unexplored frontier, which is an authenticated source for diagnosis at molecular level. Dental caries or trauma can result in an inflammatory response in the dental pulp, characterized by the accumulation of inflammatory cells leading to the release of host

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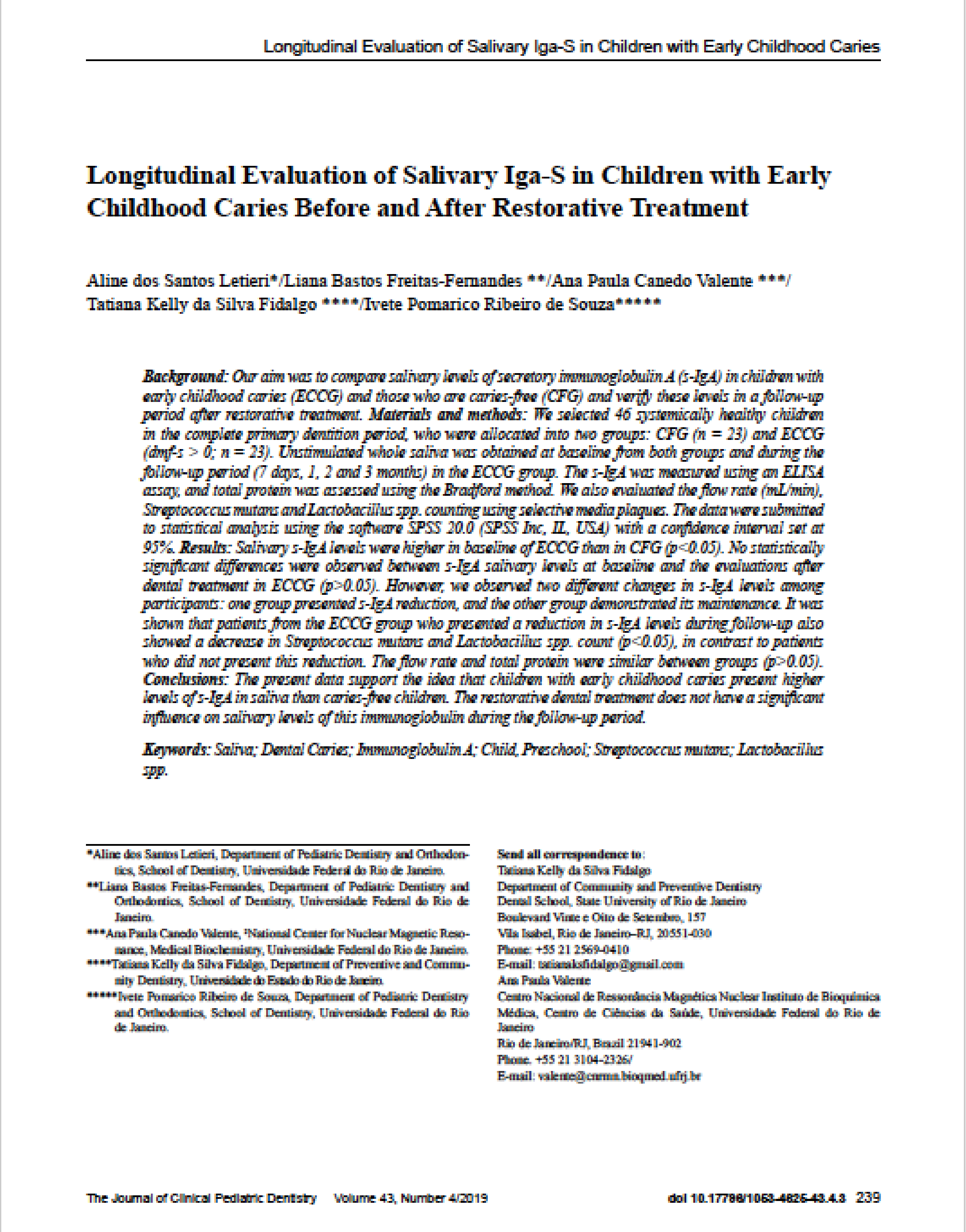

Longitudinal Evaluation of Salivary Iga-S in Children with Early Childhood Caries

Longitudinal Evaluation of Salivary Iga-S in Children with Early Childhood Caries Before and After Restorative Treatment

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Background: *Our aim was to compare salivary levels of secretory immunoglobulin A (s-IgA) in children with early childhood caries (ECCG) and those who are caries-free (CFG) and verify these levels in a follow-up period after restorative treatment.* ***Materials and methods:*** *We selected 46 systemically healthy children in the complete primary dentition period, who were allocated into two groups: CFG (n = 23) and ECCG (dmf-s > 0; n = 23). Unstimulated whole saliva was obtained at baseline from both groups and during the follow-up period (7 days, 1, 2 and 3 months) in the ECCG group. The s-IgA was measured using an ELISA assay, and total protein was assessed using the Bradford method. We also evaluated the flow rate (mL/min), Streptococcus mutans and Lactobacillus spp. counting using selective media plaques. The data were submitted to statistical analysis using the software SPSS 20.0 (SPSS Inc, IL, USA) with a confidence interval set at 95%.* ***Results:*** *Salivary s-IgA levels were higher in baseline of ECCG than in CFG ($p<0.05$). No statistically significant differences were observed between s-IgA salivary levels at baseline and the evaluations after dental treatment in ECCG ($p>0.05$). However, we observed two different changes in s-IgA levels among participants: one group presented s-IgA reduction, and the other group demonstrated its maintenance. It was shown that patients from the ECCG group who presented a reduction in s-IgA levels during follow-up also showed a decrease in Streptococcus mutans and Lactobacillus spp. count ($p<0.05$), in contrast to patients who did not present this reduction. The flow rate and total protein were similar between groups ($p>0.05$).* ***Conclusions:*** *The present data support the idea that children with early childhood caries present higher levels of s-IgA in saliva than caries-free children. The restorative dental treatment does not have a significant influence on salivary levels of this immunoglobulin during the follow-up period.*

Keywords: *Saliva; Dental Caries; Immunoglobulin A; Child, Preschool; Streptococcus mutans; Lactobacillus spp.*

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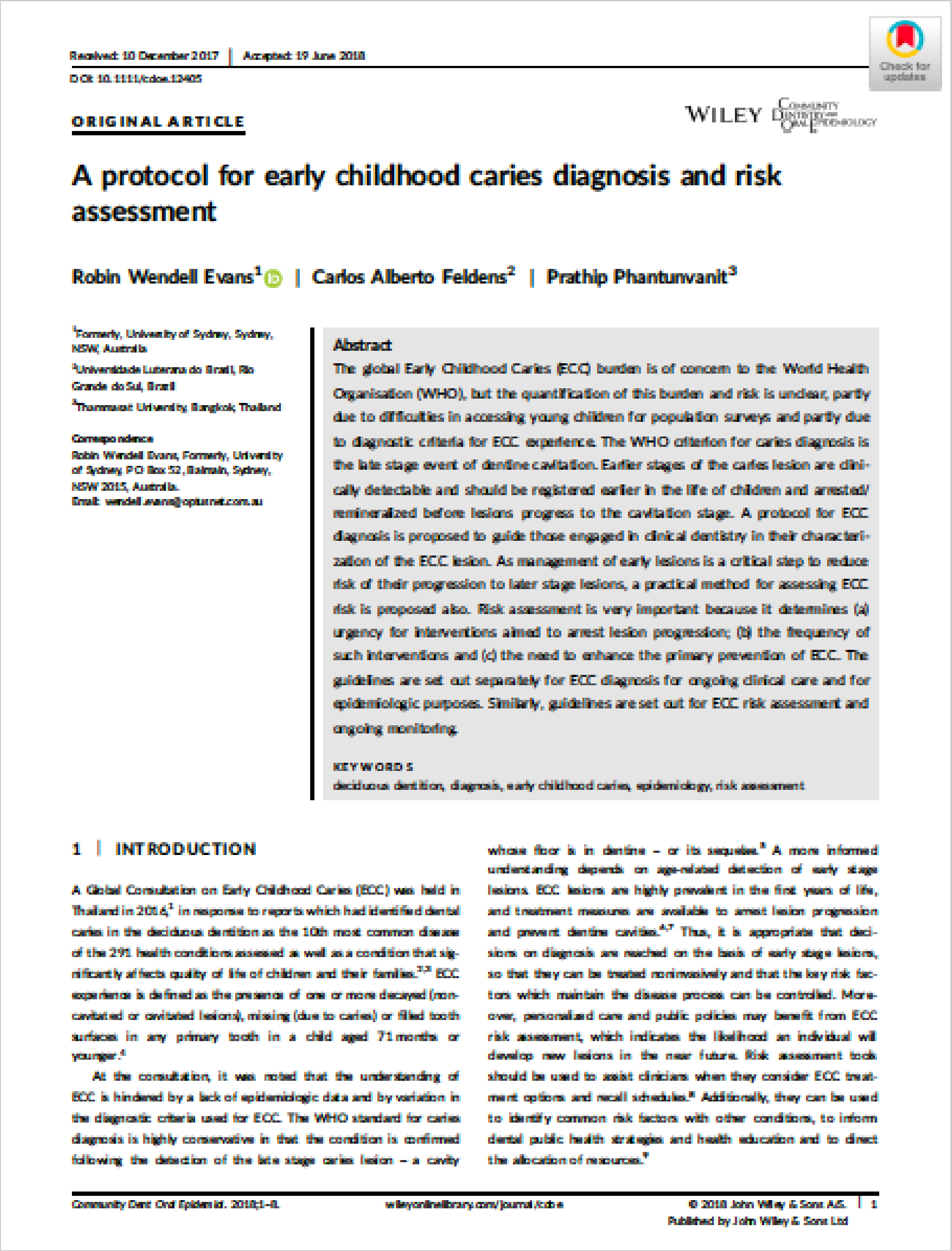

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ORIGINAL ARTICLE

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A protocol for early childhood caries diagnosis and risk assessment

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Abstract

The global Early Childhood Caries (ECC) burden is of concern to the World Health Organisation (WHO), but the quantification of this burden and risk is unclear, partly due to difficulties in accessing young children for population surveys and partly due to diagnostic criteria for ECC experience. The WHO criterion for caries diagnosis is the late stage event of dentine cavitation. Earlier stages of the caries lesion are clinically detectable and should be registered earlier in the life of children and arrested/remineralized before lesions progress to the cavitation stage. A protocol for ECC diagnosis is proposed to guide those engaged in clinical dentistry in their characterization of the ECC lesion. As management of early lesions is a critical step to reduce risk of their progression to later stage lesions, a practical method for assessing ECC risk is proposed also. Risk assessment is very important because it determines (a) urgency for interventions aimed to arrest lesion progression; (b) the frequency of such interventions and (c) the need to enhance the primary prevention of ECC. The guidelines are set out separately for ECC diagnosis for ongoing clinical care and for epidemiologic purposes. Similarly, guidelines are set out for ECC risk assessment and ongoing monitoring.

KEYWORDS
deciduous dentition, diagnosis, early childhood caries, epidemiology, risk assessment

1 | INTRODUCTION

A Global Consultation on Early Childhood Caries (ECC) was held in Thailand in 2016,[1] in response to reports which had identified dental caries in the deciduous dentition as the 10th most common disease of the 291 health conditions assessed as well as a condition that significantly affects quality of life of children and their families.[2,3] ECC experience 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 aged 71 months or younger.[4]

At the consultation, it was noted that the understanding of ECC is hindered by a lack of epidemiologic data and by variation in the diagnostic criteria used for ECC. The WHO standard for caries diagnosis is highly conservative in that the condition is confirmed following the detection of the late stage caries lesion – a cavity whose floor is in dentine – or its sequelae.[5] A more informed understanding depends on age-related detection of early stage lesions. ECC lesions are highly prevalent in the first years of life, and treatment measures are available to arrest lesion progression and prevent dentine cavities.[6,7] Thus, it is appropriate that decisions on diagnosis are reached on the basis of early stage lesions, so that they can be treated noninvasively and that the key risk factors which maintain the disease process can be controlled. Moreover, personalised care and public policies may benefit from ECC risk assessment, which indicates the likelihood an individual will develop new lesions in the near future. Risk assessment tools should be used to assist clinicians when they consider ECC treatment options and recall schedules.[8] Additionally, they can be used to identify common risk factors with other conditions, to inform dental public health strategies and health education and to direct the allocation of resources.[9]

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