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FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

MONOGRAFIA DE INVESTIGAÇÃO – ARTIGO DE REVISÃO BIBLIOGRÁFICA

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SALIVARY PROTEINS AS SUSCEPTIBILITY FACTORS FOR DENTAL CARIES

Helena Margarida Gonçalves Melo

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Resumo

Introdução: A cárie dentária é uma doença infeciosa, caracterizada por uma destruição gradual dos tecidos dentários por produtos bacterianos. Dada a sua etiologia multifatorial assume uma elevada complexidade nos mecanismos envolvidos na sua patogénese, e a saliva um papel relevante na manutenção das condições fisiológicas da cavidade oral. Atendendo à diversidade molecular da saliva, acredita-se ser possível usar este fluido no diagnóstico e prevenção, especialmente as proteínas salivares que começam a ser vistas como uma possível fonte de biomarcadores para doenças como a cárie dentária. Assim, seria possível classificar indivíduos como suscetíveis à cárie ao identificar certas proteínas como fatores de suscetibilidade, sublinhando a importância da prevenção da doença em fases pré-assintomáticas.

Objetivo: Documentar quais as principais proteínas salivares que parecem ser consideradas fatores de suscetibilidade da cárie dentária, qual o seu papel no mecanismo da cárie e qual a possibilidade de as usar como biomarcadores de suscetibilidade para esta doença.

Material e métodos: Foi realizada uma pesquisa com recurso à PubMed, usando palavras-chave e termos MesH em várias combinações. Foram escolhidas quatro proteínas salivares de entre as mais reportadas na literatura – lactoferrina, proteínas ricas em prolina, cistatina S e imunoglobulina A.

Desenvolvimento: Foram encontrados estudos cujos resultados reportavam uma correlação entre a presença / níveis da proteína e a experiência de cárie. Contudo, convém referir que o contrário também se verificou, ou seja, alguns trabalhos de investigação não encontraram qualquer relação entre as variáveis. As diferenças ao nível dos protocolos utilizados na análise da saliva, seleção e divisão dos pacientes em diferentes grupos, entre outros, poderão estar na origem de algumas contradições observadas.

Conclusão: Embora haja resultados contraditórios, no futuro, o objetivo passa por analisar maiores amostras e seguir um protocolo estandardizado. Só assim será possível perceber a verdadeira relevância da utilização de proteínas salivares como marcadores da suscetibilidade para a cárie.

Palavras-chave

Cárie dentária; suscetibilidade; proteínas salivares; lactoferrina; cistatina S; proteínas ricas em prolina; imunoglobulina A.

Abstract

Introduction: Dental caries is an infectious disease, characterized by a gradual destruction of the dental tissues by bacterial products. Considering its multifactorial etiology, caries assumes a high complexity in the mechanisms involved in its pathogenesis, and saliva plays a relevant role in maintaining the physiological conditions of the oral cavity. Due to the molecular diversity of saliva, it is believed that it is possible to use this fluid in diagnosis and prevention, specially the salivary proteins which are beginning to be seen as a possible source of biomarkers for diseases as dental caries. Thus, it would be possible to classify individuals as "caries susceptible" by identifying the presence of certain proteins in saliva as susceptibility factors, underlining the importance of disease prevention in pre-asymptomatic stages.

Aim: To document which are the main salivary proteins that seem to be considered susceptibility factors of dental caries, what is their role in the mechanism of caries and which are able to be used as biomarkers of susceptibility for this disease.

Material and methods: It was performed a search in PubMed Medline using keywords and MesH terms in several permutations. Four salivary proteins were chosen among the most reported in the literature – lactoferrin, proline-rich proteins, cystatin S, immunoglobulin A.

Development: It was found many studies whose results reported a correlation between the presence/ levels of the protein and caries experience. The opposite also applies, that means, there is reference of investigations that didn't found relation between the selected variables. The differences in the used protocols to analyze the saliva, and to select and divide the subjects in the different groups, among others, may account for some of the observed contradictions.

Conclusions: Although there are contradictory results, in future the aim is to analyze larger samples and follow standardized protocols. Only by this way it will be possible to understand the true relevance of the utilization of salivary proteins as susceptibility markers for dental caries.

Keywords

Dental caries; susceptibility; salivary proteins; lactoferrin; cystatin S; proline-rich proteins; immunoglobulin A.

1. Introduction

Dental caries is one of the most prevalent infectious diseases worldwide, with a multifactorial etiology.(1-4) The bacteria are considered the main etiologic factor, but there are additional factors that have been shown to influence the disease's progression, as for example, an insufficient level of oral hygiene by the subject or the amount of carbohydrate ingested.(5, 6) The combination of these factors can lead to an imbalance resulting in the demineralization of tooth structure and, therefore, the onset of caries lesions. Thus, it's necessary the interaction of several factors, under certain conditions, in a given period of time, for its clinical expression.(7)

Included in host factors is saliva, which plays an important role in maintaining the physiological conditions of the oral cavity and it is composed by an inorganic component, which includes certain ions such as calcium, phosphate, fluoride, and an organic one, which comprises proteins and other minority molecules.(8, 9) In addition to its protective function, salivary proteins can interact with bacteria in the oral cavity, affording receptors for bacterial adhesion. These proteins, which form complexes that differ in structure and function, play an important role in bacterial agglutination and clearance, in addition to their antibacterial properties.(10, 11)

In order to develop a caries lesion, it's necessary, firstly, the formation of the biofilm and, therefore, the microorganism must adhere to the tooth surface (either enamel or restorative material).(12) For this, it is necessary the formation of the so-called acquired salivary pellicle. The acquired salivary pellicle is a thin film of 0.1 to 1.0 micrometers of thickness that works as a basis for the development of bacterial plaque, made up of proteins derived from the saliva, as for example statherin, proline-rich proteins and mucins that bind to the hydroxyapatite.(9, 13) In addition to these proteins, there are other minority protein constituents such as amylase, carbonic anhydrate, cystatins, secretory immunoglobulin A, lysozyme and bacteria-derived glucosyltransferase (GTF).(13)

Due to the diversity of molecules present in saliva, it is believed that it is possible to take important information about caries disease and not use this biological fluid just as a standard laboratory test. Most of these studies have been realized at the expense of conventional biochemical techniques, but actually there has been recourse to proteomic techniques which involve the study of proteins on a larger scale instead of specific protein groups.(14) Saliva and, specially, the salivary proteins begin to be seen as a possible source of biomarkers for oral diseases, in which is included dental caries.(15)

Thus, it would be possible to classify an individual as a 'caries susceptible individual' by identifying the presence of certain salivary proteins in saliva (susceptibility factors for caries).(5)

This study aims to document which are the main salivary proteins that seem to be considered susceptibility factors of dental caries, what is their role in the mechanism of caries and what are able to be used, in the future, as biomarkers for this oral disease.

2. Material and methods

It was performed a search on the National Library of Medicine of the United States of America (PUBMED)'s electronic database, which resulted in 28 of 268 articles. Keywords and MesH terms were used in several combinations for the identification of relevant literature: "dental caries", "tooth demineralization", "dental enamel solubility" "salivary proteins", "saliva", "dental caries susceptibility", "proteome", "proteins risk indicators", "biomarkers". Sixteen studies were also selected, being considered relevant and related with the objective of this work, from monographs and books. All the literature considered relevant fills the following inclusion criterions: published between 1990 and 2013; written in English, Spanish or Portuguese and conducted in humans. Opinion articles, articles whose objective does not frame in the base questions of this review or those that could not be accessed because of the lack of license were excluded.

3. Development

3.1 Dental caries

Dental caries is one of the most prevalent infectious diseases worldwide, with a multifactorial etiology.(1-4) In a simplistic way it is characterized by a gradual destruction of the dental hard tissues, by bacterial products resulting, in the final stages, in the formation of a cavity. Etiologically, dental caries assumes a multifactorial nature, which means that it involves the presence of several factors and their interactions, in a given period of time, for its clinical expression. There are primary factors, in which are included bacteria – the main etiologic factor of dental caries – that are essential for the onset of the disease, and the secondary factors that have influence at the level of the disease's progression, such as the quality of oral hygiene, the subject's health status, the presence of fluoride in the oral cavity, among others.(7)

The primary factors can be grouped in factors relating to the agent (oral microflora), to the host (saliva, dental morphology) and the environmental (diet).(7, 16, 17) Isolated, these parameters don't originate the caries lesions; however its interaction, in a given period of time, may promote it.(7) The development of dental caries requires the presence of bacteria organized under the tooth structure, the so-called bacterial plaque. (3, 16) Among the several species of bacteria present in the oral cavity, those that seem to be more cariogenically active are *Streptococcus mutans* and *Lactobacillus spp* that use carbohydrates, especially sucrose from diet, for fermentation, producing organic acids namely lactic, formic, propionic and acetic acids as among others (7, 18) These acids create an acidic environment that will promote the dissolution of the inorganic material of the teeth. Furthermore, this kind of bacteria presents several virulence factors(19), allowing them to subsist in those acidic environments, and the ability to synthesize compounds such as glucan polymers , for example, important to adherence and carcinogenicity (13).

The action that the pH decrease (elicited by of the acidogenic bacterial activity in enamel-biofilm interface) can have over enamel – mainly made up of mineral matter (hydroxyapatite) – depends not only on the amount of carbohydrates available but also on a series of other factors such as the type and frequency of ingestion of carbohydrates, the number of active caries lesions and the thickness of the bacterial plaque.(7)

These events can lead to the dissolution of dental tissues with the release of calcium and phosphate ions to the external surroundings.(7) This phenomenon is called demineralization which is counterbalanced by the opposite process of remineralization that occurs, partly, due to the buffer capacity and mineral composition of saliva. Fluoride is an example of an ion that promotes the remineralization of some areas previously liable to demineralization, allowing the formation of fluorohydroxyapatite and fluroapatite crystals. In addition to intervening in the process of remineralization, fluoride inhibits demineralization and plays a bacteriostatic role, inducing metabolic and growth alterations, inhibiting bacterial replication.(20)

Thus, the imbalance of this dynamic equilibrium between demineralization and remineralization may overhang for successive episodes of demineralization that leads to the onset of caries lesions.(7)

3.2 Saliva

Saliva is the fluid secreted from salivary glands. These glands are divided in major glands that include parotid, submandibular and sublingual glands, and minor glands located at almost every spots of oral cavity. Saliva is produced as a sterile fluid mostly composed by water (99% of its composition) and other organic and inorganic components whose composition displays inter and intra-variations according to its origin and conditions of production.(15, 21, 22) The inorganic component includes certain ions such as calcium, phosphate, fluoride, sodium, potassium, chloride, bicarbonate, among others, while in the organic component are present the salivary proteins and glycoproteins which will be discussed ahead.(8, 9)

Different from the concept of saliva, there is the so-called whole saliva that represents the biological fluid that surrounds oral mucosa and dental surfaces, which comprises not only exocrine secretions from salivary glands but also other non-glandular contributions such as gingival exudation, nourishing remains, epithelial cells or microorganisms of oral cavity.(23)

This complex solution assumes a multifunctional role concerning to the homeostasis of oral environment. If on one hand it exerts an action of self-cleaning the oral tissues, saliva also contributes for the maintenance of dental structure’s integrity regulating the mineral availability, so important for the remineralization process. The buffer capacity of saliva is a detachable parameter on neutralization of organic acids, contributing to dental enamel protection at the expense of bicarbonate/ carbonic acid and phosphate systems, mainly, maintaining pH values of oral cavity near to neutrality.(13) Also over microorganisms, saliva exerts an antibacterial, antifungal and antiviral action, considering immunological or non-immunological components as, for example, some proteins such as histatins, lysozyme or certain immunoglobulins.(21)

Clinically, salivary flow is a relevant characteristic that seems to be related with the salivary composition.(24) A decrease on the volume of saliva secreted by salivary glands seems to contribute for increased caries susceptibility and it can be caused by many causes, namely an alteration in salivary glands, autoimmune diseases such as Sjögren's syndrome, environmental factors as tobacco or alcohol, certain pharmacological treatments or, simply, physiological situations like circadian rhythms or aging.(21, 23)

Considering all these factors, saliva appears as an important biologic fluid due to its intricate composition, in addition to the fact that, the sampling is a simple and noninvasive process that presents a unique potential in the field of clinical diagnosis of oral and systemic diseases. (15, 21, 25)

3.3 Salivary proteins

If the maintenance of the integrity of the enamel is achieved, mainly, at the expense of inorganic components of saliva, the salivary organic components exert, as above-mentioned, a multiplicity of functions associated to the oral microflora, nourishment and also to the preservation of dental tissues. The majority of the organic substances are proteins, whose secretion differs among the different salivary glands.(9, 15, 26)

While the biologic profile of most of salivary proteins is not fully characterized, it is known that a series of proteins, such as mucins, proline-rich proteins, statherins, histatins, lactoferrin, agglutinins, among others, have an antibacterial, antifungal and antiviral action (direct and/ or indirect).(9, 27-29)

Immunoglobulins account for 5-15% of total salivary proteins, being immunoglobulin A (IgA) the most prevalent subclass. Its function resides, mainly, in the inhibition of the bacterial adherence and subsequent colonization, blocking the surfaces involved in the binding process. This action relies in its affinity to antigens of microorganisms, with which it forms macromolecular aggregates, promoting its clearance.(9) Associated to this immunoglobulin is, often, another protein – agglutinin - responsible for the bacterial agglutination phenomenon at the level of the oral cavity.(9, 29)

Mucins are glycoproteins synthetized by the acinar cells of salivary glands, that represent 20-30% of the total proteins of unstimulated saliva.(9) There are two kinds of mucins - MUC5B and MUC7 – that can be distinguished not only by their structure but also by their location and function.(9) MUC5B integrates the composition of acquired salivary pellicle, contributing to the proton-barrier function(30) and lubrication of dental surface(9), whereas MUC7 can bind to a wide range of bacterial species, as *Streptococcus mutans*(31), contributing to the bacterial aggregation. In addition, both mucins seem to intervene in the protection against viral agents.(32, 33)

Another important class of proteins are the so-called proline-rich proteins, being about 15-20% of secreted proteins from parotid gland. They are divided into acidic, basic and glycosylated families and present a proline content between 25-42%.(9, 34) The acidic proline-rich proteins inhibit the precipitation of calcium phosphate salts and the growth of hydroxyapatite crystals, similarly to statherin, another protein present in the salivary fluid. Statherin is, in fact, one of the proteins with stronger calcium-binding activity and a potent inhibitor of precipitation.(35) The acidic proline-rich proteins take part of the acquired enamel pellicle and, in addition to its intervention in the calcium and hydroxyapatite homeostasis, participate in selective bacterial adhesion to dental enamel.(36-39) Also, cystatins are involved in the regulation of the salivary and enamel calcium equilibrium. These same proteins also act as protease inhibitors, playing an antiviral role and an indirect antibacterial action.(9, 29)

Enzymes are, as well, in the limelight with significant proprieties. As examples of proteins with enzymatic activity and antimicrobial functions there are lactoperoxidase and lysozyme.(9)The first participates in the oxidation reaction (that occurs in the presence of hydrogen peroxide) of the salivary thiocyanate ion and which results in the release of hipothiocianite, an ion capable of turning bacterial cells inactive. (9, 36-39) Lysozyme hydrolyzes the cell walls of some bacteria, interfering also at the level of the bacterial membrane cell permeability.(9, 29)

Lactoferrin and alpha-amylases are other examples of enzymes present in saliva. Lactoferrin is one of the non-specific host factors and its bacteriostatic action is based on its high affinity for the iron present in saliva, depriving some bacteria of this essential element to their survival. Moreover, this protein has effects at the level of the bacterial membranes, as well as a modular of inflammatory and immune processes.(40) The salivary alpha-amylases - in addition to its primary activity that is based on starch digestion - seem to contribute to the formation of dental biofilm, since these enzymes have binding sites for some oral *Streptococcus* species, as for example the *Streptococcus gordonni*.(40)

Histatins are a group of histidine-rich peptides, synthesized in the parotid and submandibular glands, with a broad spectrum of bactericide and fungicide functions. These effects occur by the change of permeability and structure of biological membranes of some strains of microorganisms present in the oral cavity.(29) It is suggested the involvement of these peptides in the formation of the acquired pellicle.(8)

In spite of all these proteins that play a role in the defense of the oral cavity (which include immune and antimicrobial responses), there are studies that suggest a correlation between some salivary proteins and caries experience.(41) Although in the majority of the cases the results may not be completely conclusive, it is believed potential role that these parameters can assume in the diagnosis and prevention of dental caries. In this context, in the next chapters four relevant salivary proteins will be presented.

3.3.1. Lactoferrin

Lactoferrin, also known as lactotransferrin, is a glycoprotein from the transferrin family with a molecular weight of 80 kDa, which has two homologous globular regions that reversibly bind to iron. Due to this fact, lactoferrin is considered as one of the non-specific host defense mechanisms, since it makes, as mentioned above, the iron unavailable for bacterial use. Besides, lactoferrin participates in the modulation of processes like bacterial aggregation and development of dental biofilm, which result in the inhibition of adhesion of microorganisms, mainly *Streptococcus mutans*. (42, 43)

Actually, there are some studies that try to relate the levels of some proteins, as lactoferrin, and caries experience, in order to assess what is their role in caries susceptibility; however contradictory results have been reported. Sikorska *et al* (44) verified, in a study with 15 years-old individuals, that the DMFS index (decayed, missing or filled surfaces of teeth) increased with an increase in the levels of lactoferrin, being this rise simultaneous with a decrease on salivary flow (one of the main factors associated with caries lesions).

Other studies exist, such as the one of Vitorino *et al* (45) that showed a positive correlation between a high DMFT index (decayed, missing or filled teeth) and high levels of lactoferrin and other proteins. Also Mass *et al* (46) found an association amongst the presence of lactoferrin and dental caries. A similar finding was reported by Felizardo *et al*(47). In this case, it was used DMFT index again and the authors performed the analysis of proteins by electrophoresis. The results showed a positive correlation between lactoferrin and DMFT index different from 0 (although lactoferrin was not always present). One interesting point to emphasize relates to the fact that lactoferrin seems to be expressed most significantly in the presence of restored teeth (one of the components of the DMFT index).

In another perspective, Azevedo *et al* (48) performed a study with 110 children in order to evaluate a possible association between a lactotransferrin gene polymorphism and dental caries. The results pointed to the existence of an association with caries susceptibility, however it should be taken into account that dental caries is a multifactorial disease and a protein gene polymorphism does not explain by itself the occurrence of disease, as well as the fact that polymorphisms in genes that encode other proteins with antimicrobial compound can also contribute for caries.

Taken together, these studies present similar results, although they use distinct methods and variables, namely the type of collected saliva (stimulated/ not stimulated), methodologies (ELISA, electrophorese, among others) and caries indices (DMFT/ DMF-S).

Contrarily, there are studies that sought to analyze the relationship between some salivary factors, in which are included lactoferrin, and found no association with dental caries (49-51).

3.3.2. Proline-rich proteins

As above-mentioned, saliva contains proline-rich proteins whose composition in terms of amino acids includes glycine, glutamine and proline, among others, and where proline confers them an extended chain conformation.(52)

These proteins are divided into acidic, basic and glycosylated families, being their action related to the process of remineralization and also to bacterial adhesion. Whereas the acidic proline-rich proteins are found in all the major salivary glands secretions, the basic proline-rich proteins are present, at the level of oral cavity, only in parotid secretions.(8)

Acidic proline-rich proteins possess a 30-amino acid N-terminal domain which adheres to teeth surfaces, eliciting a conformational modification that exposes a binding site for bacteria within another domain.(53-56) Basic proteins do not adhere to the tooth surface but can bind to bacteria.(57-60) It should be noted that these proteins may be expressed in different combinations (polymorphisms) by genes that encode them.(34) The PRH1 gene, for example, is a gene that encodes acidic proline-rich proteins and *Db* is one of the alleles encoded by it. This allelic acidic proline-rich proteins variant has been correlated with high dental caries experience, being pointed as a caries susceptibility factor.(61, 62) Inclusively, Jonasson *et al*(61) related another protein - the salivary scavenger receptor cysteine-rich glycoprotein gp-340 I – with caries-prone subjects, and they verified that this phenotype, in addition to coincide with a high incidence of dental caries, also seemed to be over represented in subjects positives for *Db*. This alellic variant is referenced in other previous studies, with results that show that subjects containing *Db* in saliva exhibit a better adhesion of *Streptococcus mutans* to the acquire pellicle and are more associated with cases of dental caries.(63, 64)

Thus, these proteins have been studied over the past years in order to verify a possible relationship in dental caries susceptibility but, once again, differences in the reported results are found.

Vitorino *et al*(65) assessed the presence and amount of salivary peptides in two distinct groups (caries-free and caries-susceptible groups) and noticed a higher proteolytic activity in caries-susceptible group and, consequently, low quantities of proteins, in which are included proline-rich proteins. This observation suggests an increase of caries susceptibility when these proteins are in lower concentrations.

In the same perspective, a study conducted with Mexican students correlated high DMFT indices with a significant reduction or absence of acidic proline-rich protein-1, differing from those with lower DMFT indices.(66) This fact, according to the authors, can be explained based on the assumption that the proline-rich proteins and some other proteins contribute to the formation of the salivary acquired pellicle and, therefore, a lower output of these same proteins can modulate the composition of bacterial plaque and affect the permeability barrier. Furthermore, this difference found between subjects with and without caries experience was similar to that reported by Bhalla and co-workers(67). In this latter study, samples from caries-free children revealed, by gel electrophoresis, a higher number of proline-rich protein bands when compared with samples of subjects with early childhood caries.

Also, Tenovuo(68) observed that acidic proline-rich proteins were significantly correlated with low DMFT index values which, according to the authors, emphasize the protective role of these proteins, also suggested by the results from Banderas-Tarabay *et al*(66). All these results go against the opinion of Vukosavljevic and co-workers(25) who affirmed that the analysis of protein composition of a subject can give information about which proteins are up or down regulated in order to accurately diagnose a patient.(69)

Vitorino *et al*(45), in 2005, obtained correlation coefficients that show a consistent correlation between some proteins (among whom are included acidic proline-rich proteins) and the lowest values of DMFT of their sample. Considering their known role as enamel pellicle precursors, these results reinforce, one more time, the protective role of proline-rich proteins.

Ayad *et al*(70) examined the phenotypes of proline-rich proteins expressed by caries-susceptible subjects and by individuals of caries-free group. The results were consistent with the idea that the proteolytic processing of the proteins secreted by parotid is different between two groups. These differences, according to them, can be due to a distinct expression of protease, protease inhibitors or polymorphisms. All these hypotheses remain to be elucidated.

In fact, later, Levine *et al*(34) concluded that early childhood caries may be associated with the absence of certain basic proline-rich proteins alleles. In the analysis of the results, there was a greater breakdown of basic proline-rich proteins in adults with severe caries and one of the possible explanations can be based on differences in the activity of certain proteases such as cathepsin H. This protease intervenes in cleavage processes, to which the basic proline-rich proteins are very susceptible. Since these proteases are inhibited by cystatins S and C, the lower the activity of cystatins, the greater will be the activity of the cathepsin and, in turn, the proline-rich proteins will be smaller. This causes a less efficient acid neutralization because the smaller the availability of basic proline-rich proteins, the lower will be the number of basic residues adherent to the acid-producing streptococci.(34)

As a consequence, Levine and co-workers believe to be pertinent the sequencing of the exons of the proline-rich proteins genes, as well as those of cathepsin H and cystatins, since it can be used to identify children more susceptible to severe caries. Next it will be addressed the specific case of cystatin S.

3.3.3. Cystatin S

Salivary cystatins are multifunctional proteins, with molecular masses between 13-14 kDa, that can be divided into five major groups (S, S1, S2, SA, SN) and two minor (C and D).(71) They are, mainly, secreted by submandibular and sublingual glands, although parotid also has a little contribution in its secretion, namely, in the production of cystatin D, probably a specific salivary protein.(72)

Generally, cystatins are strong cysteine protease inhibitors; however this inhibitory activity seems to be weaker concerning to cystatin S.(73) While cystatins like SN or SA seem to play an important role in the regulation of proteolytic events (they inhibit the lysosomal cathepsins (B, C, H, L) *in vitro*), cystatin S participates in the regulation of the salivary and enamel calcium equilibrium, along with statherin.(71) Thus, according to Lupi *et al*(71), the identification and quantification of different cystatins in saliva, as well as possible derived and truncated forms, can be useful as an oral health indicator.

Therefore, Vitorino *et al*(45) tried to analyze whether the salivary composition of a subject could be relevant in relation to the development of dental caries, among other infectious diseases of the oral cavity. Their results showed a significant correlation between the quantity of cystatin S and samples from subjects belonging to the caries-free group. This finding seems to strengthen, according to the same authors, the hypotheses that the cystatins may be important from the standpoint of prevention.

An opposite comment applies to the data of Rudney *et al*(35), which used a proteomic approach to conclude that the levels of a truncated form of cystatin S (AA1-8), as well as the levels of statherin can be assumed as potential risk indicators for dental caries.

Similarly to other proteins, there is therefore a need of additional studies to confirm the utility of using cystatins or truncated variants as markers for dental caries susceptibility.

3.3.4. Immunoglobulin A

The immunoglobulins are one of the salivary components with an antibacterial action and include different subclasses – IgA, IgG and IgM. About 60% of the total immunoglobulins present in saliva correspond to salivary IgA which is a secretory immunoglobulin that, as above-mentioned, plays a role in the neutralization of toxins and bacterial enzymes and in the inhibition of bacterial adhesion to dental surfaces. (9, 74)

In fact, among salivary immunoglobulins that one most study from the standpoint of dental caries risk is secretory immunoglobulin A (sIgA), noting that relatively to IgG and IgM there is not sufficient evidence to establish a relationship between these immunoglobulins and caries risk.(23) The presence of IgM seems to be more related to mucosa pathologies than to the protection of hard tissues.(75)

The truth is that previous investigations about the role of IgA in the caries process have revealed contradictory results. While some authors did not find any kind of relation between dental caries and IgA levels(74, 76, 77), other studies showed that a high concentration of sIgA is related with a low incidence of caries(78), and the opposite also applies(45, 74, 79-83).

Parkash *et al*(83) reported that increased levels of specific sIgA were related to dental caries, unlike to the levels of total sIgA that do not seem to be good risk indicators. Also Vitorino *et al*(45), similarly to what happened in the case of lactoferrin, found a consistent positive correlation between IgA and the group of caries-susceptible subjects.

On the other hand, there are studies such as the one developed by Shifa and co-workers(74), in which it was not found any correlation between the levels of sIgA and the caries status. Similar results were obtained by Koga-Ito *et al* and Camling *et al*.(76, 77)

In order to determine the protective role of sIgA in the saliva of caries-free children, Chawda *et al*(78) developed a study that found a significant inverse correlation between caries activity and sIgA. Contrarily, Thaweboon *et al*(84) and de Farias *et al*(79) verified that the occurrence of dental caries was associated with an increase of total sIgA. Other studies revealed similar results, namely that dental caries were significantly associated to large amounts of microorganisms and high titers of total salivary immunoglobulins.(80-82)

Once again, the different conclusions obtained in these studies may be attributable to differences in methods, patient selection and tests used, as well as, in the fact that the concentration of this immunoglobulin can change depending on several parameters such as salivary flow, hormonal factors or physical activity.(85)

Although there are different results published, the literature suggests that the study of the role of immunoglobulins may be advantageous for the prevention of oral diseases such as dental caries.(79)

Table I summarizes the description of some selected studies relatively to these proteins.

Table I – Summary of selected studies.

Authors, year, Country	**Caries index**	**Sample size (*n*)**	**Type of saliva**	**Salivary parameters tested**	**Data analysis (tests)**	**Results**
Ayad *et al.*, 2000, USA	DMF-S	DMFS=0:9 DMFS>0:9	Stimulated (parotid)	Basic proline-rich peptide	Mann-Whitney; Fisher's exact test	Differences were found in the phenotypes of proline-rich proteins expressed by subjects with and without caries experience.
Banderas-Tarabay *et al*, 2002, Mexico	DMF-T	DMFS≤4:24 DMFS >10:40	Unstimulated	Electrophoretic pattern and protein composition of saliva	Chi-square test ($\chi 2$);	Subjects with higher DMFT indices presented significant reduction or absence of high-molecular-weight mucin glycoprotein-1 (MG1), low-molecular-weight mucin glycoprotein-2 (MG2), and acidic proline-rich protein-1 (PRP-1), differing from subjects with lower DMFT indices.
Sikorska *et al.*, 2002, Poland	DMF-S	83	Unstimulated	sIgA, lactoferrin and α_1 proteinase inhibitor	For comparison of the results: analysis of variance; Kruskal–Wallis; median test For evaluation of the relationships between individual parameters: Spearman test	Significant relationship was found between the decayed surface index and the levels of lactoferrin, sIgA and α1-proteinase inhibitor
de Farias *et al., 2003, Brazil*	DMF-S	Children with ECC:20 Caries-free children:20	Unstimulated	Total salivary IgA, IgG and IgM; total proteins; amylase activity	U Mann-Whitney test	The presence of ECC was associated with an increase in total salivary IgA.
Vitorino *et al.*, 2005, Portugal	DMF-T/ DMF-S	20	Unstimulated	Peptides saliva composition	Multivariate analysis using Andad software (7.1 version)	Strong correlation between large amounts phosphopeptides (PRP1/3, Histatin 1 and statherin), and the absence of caries. In the caries-susceptible group a high number of peptide fragments was observed.

Vitorino *et al.*, 2006, Portugal	DMF-T	DMFT=0:16 DMFT > 0:16	Unstimulated	Total protein, protein *per* minute, proline-rich proteins, amylase, lipocalin, IgA, lactoferrin, cystatins S and SN	Spearman test	Significant statistical correlation for cystatins, acidic PRPs and lipocalin-1, and the observed absence of dental caries. Amylase, IgA, and lactoferrin levels were found to correlate with the caries susceptible group.
Jonasson *et al.*, 2007, Sweden	DMF-S	DMFS=0:19 DMFS >5:19	Stimulated	*Db*; Glycoprotein gp-340 I, II, III	Student's unpaired *t* test or ANOVA followed by Tukey's test; Chi-square test; Mann- Whitney U test	The gp-340 I phenotype correlated positively with caries experience and other two phenotypes tended to behave in the opposite way. The gp-340 I protein appeared over represented in subjects positive for Db.
Shifa *et al.*, 2008, India	DMF-T	DMFT=0:10 DMFT≥5:10	Unstimulated	Salivary IgA	Student's *t* -test.	Any correlation between sIgA levels and caries status was found.
Azevedo *et al.*, 2009, Brazil	DMF-T	DMFT=0:48 DMFT ≥1:62	—	—	Chi-square test ($\chi 2$)	Lactotransferrin A/G (exon 2, Lys/Arg) polymorphism was associated with susceptibility to dental caries in 12-year-old students.
Felizardo *et al.*, 2010, Brazil	DMF-T	DMFT=0:27 DMFT > 0:53	Stimulated	Lactoferrin; Lysozyme	Pearson; Spearman; Kruskal–Wallis; Mann-Whitney	Slight association between lysozyme concentrations and DMFT. Positive correlation between lactoferrin and DMFT and restored teeth.
Chawda *et al.*, 2010, India	DMF-T	DMFT=0:10 DMFT=1-5:10 DMFT=6-10:10	Unstimulated	Salivary IgA	ANOVA test	The total salivary concentration of sIgA was statistically significantly higher in the group of caries-free children than that of both the groups with caries experience.
Bhalla *et al.*, 2010, India	DMF-T	DMFT=0:50 DMFT > 6:50	Unstimulated	Salivary flow rate; pH, mean protein concentration; electrophoretic profile of salivary proteins	Chi-square, Fisher's exact and Pearson's chi-square	Free subjects showed a higher number of proline-rich protein bands. A significantly higher number of glycoprotein bands were observed in the whole saliva of subjects with early childhood caries.

Adapted from Martins *et al* (5)

4. Conclusion

Since dental caries appears as one of the most prevalent infectious diseases worldwide, many researchers have been focused on the study of the etiology of this oral disease. Saliva, as a biological fluid that surrounds the tissues of the oral cavity, has been seen as a possible source of biomarkers for oral diseases, in which is included dental caries. In recent years saliva in general, and salivary proteins in particular, have demonstrated potential as risk indicators and biomarkers.

However, the scientific investigations performed until now have shown contradictory and inconsistent results. Firstly, dental caries has, as above-mentioned, a multifactorial etiology, being difficult to link specific salivary parameters as disease risk factors. Besides, there are additional factors which difficult this analysis and act as elements capable of interfere in caries process, as, for example, the inclusion of individuals in oral health programs, exposure to fluoride, among others. Another difficulty which may explain, partly, the present contradictions is related with the redundancy at the level of the function that some salivary proteins present. In addition to this fact, some proteins modulate the functions of others or have the ability to form heterotypic complexes that behave in a distinct way.

Despite the inherent difficulties, it is believed that it is possible to take important information through the analysis of proteins present in saliva in order to be able to classify an individual as a 'caries susceptible individual' and, thus, allow an intervention in pre-asymptomatic stages.

Finally, most of the studies have been realized at the expense of conventional laboratorial techniques. However, nowadays the advanced proteomics technologies may offer a new and more accurate perspective about this issue. In future, the aim is to analyze larger samples of subjects and, as suggested by some authors, following a standardized protocol.

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