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ZNAČAJ KATJONSKIH ANTIMIKROBNIH PEPTIDA PLJUVAČKE U PROCENI RIZIKA ZA POJAVU KARIJESA KOD DECE: PREGLED LITERATURE

THE SIGNIFICANCE OF SALIVARY CATIONIC ANTIMICROBIAL PEPTIDES IN CARIES RISK ASSESSMENT IN CHILDREN: A LITERATURE REVIEW

Branislava B. Stojković[1,2], Marija Lj. Igić[1,2], Olivera R. Tričković Janjić[1,2], Ana N. Igić[1], Zorana M. Veličković[1], Simona M. Stojanović[2,3], Milica S. Petrović[4], Miloš S. Kostić[5], Nikola D. Živković[6], Milan S. Spasić[2,3]

[1]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, PREVENTIVNA I DEČJA STOMATOLOGIJA, NIŠ, SRBIJA
[2]KLINIKA ZA DENTALNU MEDICINU, NIŠ, SRBIJA
[3]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, ORALNA HIRURGIJA, NIŠ, SRBIJA
[4]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, ORALNA MEDICINA I PARODONTOLOGIJA, NIŠ, SRBIJA
[5]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, INSTITUT ZA IMUNOLOGIJU, NIŠ, SRBIJA
[6]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, CENTAR ZA PATOLOGIJU I PATOLOŠKU ANATOMIJU, KLINIČKI CENTAR, NIŠ, SRBIJA

[1]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, PREVENTIVE AND PEDIATRIC DENTISTRY, NIŠ, SERBIA
[2]CLINIC OF DENTAL MEDICINE, NIŠ, SERBIA
[3]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, ORAL SURGERY, NIŠ, SERBIA
[4]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, ORAL MEDICINE AND PERIODONTOLOGY, NIŠ, SERBIA
[5]FACULTY OF MEDICINE, UNIVERSITY OF NIŠ, INSTITUTE OF IMMUNOLOGY, NIŠ, SERBIA
[6]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, CENTER OF PATHOLOGY AND PATHOLOGICAL ANATOMY, CLINICAL CENTER NIŠ, SERBIA

Sažetak

Uvod: *Procena rizika od pojave karijesa predstavlja esencijalni korak u savremenom kliničkom pristupu u zbrinjavanju karijesa. Osnovni je preduslov za razvoj preventivno-profilaktičkih mera i strategija primarne prevencije karijesa. Njena pouzdanost je uslovljena poznavanjem relevantnih faktora rizika za pojavu karijesa – indikatora rizika od pojave karijesa i prediktora pojave karijesa.*
Cilj rada: *Cilj ovog rada bio je da se pregledom dostupnih originalnih radova analizira značaj salivarnog nivoa katjonskih antimikrobnih peptida kao indikatora rizika od pojave karijesa i prediktora pojave karijesa u dečjem uzrastu, odnosno njihova moguća uloga u proceni rizika od pojave karijesa kod dece.*
Materijal i metode: *Analizom su obuhvaćeni originalni radovi na engleskom jeziku u kojima je isptivana povezanost salivarnog nivoa katjonskih antimikrobnih peptida i karijesa u dečjem uzrastu. Izabrane su tri baze podataka (PubMed, Scopus i Web of Science), a pretraga je vršena korišćenjem sledećih ključnih reči: pljuvačka I katjonski peptidi /antimimikrobni peptidi /AMP/ defenzini/ katelicidin/histatin/staterin/ adrenomeduli/ azurocidin i dentalni karijes i deca / dečji uzrast.*
Zaključak: *Katjonski antimikrobni peptidi mogli bi imati značaj indikatora rizika od pojave karijesa u dečjem uzrastu. Nažalost, za sada nema pouzdanih dokaza o njihovom karijes prediktivnom značaju. Potrebne su dodatne dobro dizajnirane studije preseka i dugoročne longitudinalne studije koje bi precizirale značaj salivarnih katjonskih antimikrobnih peptida, ali i drugih neimunoglobulinskih antimikrobnih proteina pljuvačke, kao pouzdanih indikatora rizika od pojave karijesa i prediktora pojave karijesa u dečjem uzrastu. Time bi se definisao njihov značaj kao karijes rizik biomarkera rizika i njihova potencijalna primena u proceni rizika od pojave karijesa kod dece.*

Ključne reči: *pljuvačka, karijes, katjonski antimikrobni peptidi, karijes rizik indikatori, prediktori karijesa*

Corresponding author:
Assitent Prof. Branislava B. Stojković, DMD, PhD
University of Niš, Faculty of Medicine
Clinic of dental medicine, Preventive and pediatric dentistry
81 Dr Zorana Djindjića Blvd.
E-mail: branislava.stojkovic@medfak.ni.ac.rs
Phone:+381637012340

Abstract

Introduction: *Caries risk assessment is the essential step in the modern clinical approach to caries management. It is a basic prerequisite for the development of preventive measures and strategies for the primary prevention of caries. Its reliability is conditioned by the knowledge of relevant caries risk factors - indicators and predictors of caries.*
Aim: *After reviewing the available literature, the paperwork aimed to analyse the importance of salivary levels of cationic antimicrobial peptides as indicators and predictors of caries in children, i.e., their possible role in caries risk assessment in children.*
Material and methods: *The analysis included original articles in English which studied the association of salivary levels of cationic antimicrobial peptides with caries in children. Three databases were selected: PubMed, Scopus, and Web of Science, and the search was performed using the following keywords: saliva cationic peptides /antimicrobial peptides /AMP/ defensins/ cathelicidin/histatin/statherin/adrenomedullin/azurocidin AND dental caries AND children/children age.*
Conclusion: *Cationic antimicrobial peptides could be important caries risk indicators in children. Unfortunately, there is currently no reliable evidence of their caries predictive value. Well-designed cross-sectional and long-term longitudinal studies are still required to clarify the significance of salivary cationic antimicrobial peptides as reliable caries risk indicators and caries predictors in children. At the same time, this would define their significance as caries risk biomarkers and their potential application in caries risk assessment in children.*

Key words: *saliva, caries, cationic antimicrobial peptides, caries risk indicators, caries predictors*

Uvod

Karijes je najučestalije oralno oboljenje. Rezultat je interakcije bakterija i ugljenih hidrata koja se tokom vremena odvija na površini zuba. Međutim, etiologija karijesa znatno je složenija i, osim interakcije zub–mikroorganizam–ugljeni hidrat, uključuje veliki broj demografskih, socijalnih, bihevioralnih, ekonomskih faktora i faktora sredine (od individualnog nivoa, preko nivoa porodice i uže zajednice, pa sve do makro ili državnog nivoa)[1-4].

Širom sveta, karijes i dalje predstavlja ozbiljan socio-ekonomski i javnozdravstveni problem, te je njegova primarna prevencija osnovni zadatak pedontološke struke[5]. U oblasti prevencije karijesa posebno je naglašen esencijalni značaj procene rizika od pojave karijesa kao prvog koraka u savremenom kliničkom pristupu u zbrinjavanju karijesa[6,7]. S obzirom na to da je njen primarni cilj rana identifikacija pojedinca u riziku od pojave karijesa, smatra se bazičnim preduslovom za razvoj preventivno-profilaktičkih strategija i mera za primarnu prevenciju ovog oboljenja. Pouzdanost procene rizika od nastanka karijesa uslovljena je poznavanjem relevantnih faktora rizika od pojave karijesa, tj. markera čija je povezanost sa prisustvom i/ili pojavom karijesa dokazana statističkim metodama. Ukoliko je povezanost utvrđena studijama preseka, ove varijable predstavljaju indikatore rizika od pojave karijesa; ako je utvrđena longitudinalnim studijama, varijable predstavljaju prediktore pojave karijesa[8]. Identifikovano je preko 106 kliničkih (prethodno iskustvo sa karijesom, stanje oralne higijene), bioloških (sastav i osobine pljuvačke, sastav i osobina dentalnog biofilma), socio-demografskih, bihevioralnih i sredinskih indikatora rizika od pojave karijesa[9,10]. Prema dosadašnjim istraživanjima, najveći značaj u predikciji nastanka karijesa pokazali su prethodno iskustvo sa karijesom i salivarni nivo *S. mutansa*[11-13].

Istraživanja u oblasti procene rizika od pojave karijesa aktuelna su pošto nijedna varijabla kao solo faktor nije pokazala istovremeno visok značaj pouzdanog indikatora i prediktora rizika od pojave karijesa. Posebno je aktuelno definisanje karijes rizik biomarkera – biološkog parametra koji se može kvantifikovati i samostalno, kao solo faktor, koristiti u procesu procene rizika od pojave karijesa. Tu ulogu mogle bi imati mnoge komponente pljuvačke. Zbog infektivne etiologije karijesa istraživanja su naročito usmerena na analizu neimuno-globulinskih antimikrobnih proteina pljuvačke.

Introduction

Caries is the most common oral disease. It is the result of the interaction between bacteria and carbohydrates on the tooth surface over time. However, the aetiology of caries is much more complex and includes, in addition to the tooth-microorganism-carbohydrate interaction, a wide range of demographic, behavioural, social, economic, and environmental factors (from the individual level, through the family and immediate community level, and up to the macro or state level)[1-4].

Caries has long been a serious socio-economic and public health problem worldwide, and its primary prevention still is the fundamental task of pedontology[5]. Regarding caries prevention, the essential importance of caries risk assessment - the first step in the modern clinical approach to caries treatment - is especially emphasized[6,7]. Its primary goal is the early detection of caries in an individual at risk, and it is therefore considered a basic prerequisite for the development of preventive-prophylactic strategies and measures for the primary prevention of this disease. The reliability of caries risk assessment depends on the knowledge of relevant caries risk factors, i.e. markers proven by statistical methods to be associated with the presence and/or occurrence of caries. If the association has been determined by cross-sectional studies, these variables represent caries risk indicators, and if it has been determined by longitudinal studies, the variables then represent caries predictors[8]. Over 106 clinical (previous caries experience, oral hygiene status), biological (salivary composition and properties, dental biofilm composition and properties), socio-demographic, behavioural, and environmental caries risk indicators have been identified[9,10]. However, research has revealed that so far previous caries experience and salivary levels of *S. mutans* have shown the greatest caries predictive value so far[11-13].

Research in the field of caries risk assessment is still ongoing given that no single variable has yet proven to be both a reliable indicator and predictor of caries risk. The definition of a solo caries risk biomarker-biological parameter that can be quantified and used as such in the caries risk assessment process is particularly topical. Many salivary components could play this role.

Due to the infectious aetiology of caries, research is particularly focused on the examination of non-globulin antimicrobial salivary proteins.

Ovi proteini, zajedno sa ostalim komponentama nespecifične urođene imunosti usne duplje, predstavljaju prvu liniju odbrane zuba kao domaćina od invazije kariogenim mikroorganizmima. Otkriven je velik broj ovih proteina, koji su prema mehanizmu delovanja grupisani u sedam funkcionalnih klasa[14]. Jednu od njih čine katjonski antimikrobni peptidi. To su mali, pozitivno naelektrisani, antimikrobni peptidi sastavljeni od 12 do 50 amino-kiselina[15]. Važni predstavnici ove funkcionalne grupe su defenzini, katelicidini, histatini, staterini, adrenomedulin i azurocidin[14]. Defenzine karakteriše prisustvo 6–8 cisteinskih rezidua. U usnoj duplji identifikovane su dve supfamilije defenzina: mijelodidni α-defenzini (*human neutrophil peptides*-HNP 1, 2, 3, 4) i β-defenzini (*human β-defensin*-hBD 1, 2, 3, 4). Kod čoveka je prisutna samo jedna biološki aktivna forma katelicidina; to je LL-37, u čiji sastav, za razliku od defenzina, ne ulazi cistein. Histatini su histidinom bogati peptidi; u humanoj oralnoj sredini detektovani su histatin 1, 3 i 5. Staterin ima 43 amino-kiseline i sprečava kristalizaciju kalcijum-fosfata. Azurocidin je prisutan u azurofilnim granulama neutrofila, dok su glavni izvor adrenomedulina oralne epitelne ćelije. Oralni katjonski antimikrobni peptidi pokazuju sva tri vida antimikrobne zaštite. Aktivno učestvuju u biološkoj kontroli mnogih oralnih infektivnih bolesti, uključujući karijes i gingivo-parodontalna oboljenja, kao i u procesu zarastanja postekstrakcionih rana[16-18]. Njihova aktivnost je usmerena i na *S. mutans* i na druge kariogene bakterije[16]. U usnoj duplji su detektovani i u gingivalnoj tečnosti i u pljuvački. Njihova salivarna sekrecija u najvećoj meri izazvana je bakterijskim agensima[15]. Sugerisano je da ovi salivarni peptidi mogu imati ulogu karijes rizik biomarkera i, shodno tome, ulogu u proceni rizika od pojave karijesa u dečjem uzrastu[19,20]. Međutim, podaci iz literature su oprečni.

Cilj rada bio je da se pregledom dostupnih originalnih radova analizira značaj salivarnog nivoa katjonskih antimikrobnih peptida kao indikatora rizika od pojave karijesa i prediktora pojave karijesa u dečjem uzrastu i, posledično, njihova moguća uloga u proceni rizika od pojave karijesa kod dece.

Uloga salivarnih katjonskih antimikrobnih peptida u biološkoj kontroli karijesa

Kao što je istaknuto, katjonski antimikorbni peptidi efikasno deluju i na kariogene mikroorganizme.

Together with other components of the non-specific innate immunity of the oral cavity, they represent the first line of defence of the tooth against invasion by cariogenic microorganisms. A large number of these proteins, which are according to the mechanism of action grouped into 6 functional classes, have been discovered[14]. One of the classes consists of cationic antimicrobial peptides, small, positively charged, antimicrobial peptides composed of 12 to 50 amino acids[15]. Important representatives of this functional group are defensins, cathelicidins, histatins, statherins, adrenomedullin, and azurocidin. Defensins are characterised by the presence of 6−8 cysteine residues. Two defensin subfamilies have been identified in the oral cavity: myeloid α defensins (*human neutrophil peptides*-HNP 1, 2, 3 and 4) and β defensins (*human β defensin*-hBD 1, 2, 3, 4). Only one biologically active form of cathelicidin, LL-37, is present in humans, which, unlike defensin, does not contain cysteine. Histatins are histidine-rich peptides and histatin 1, 3, 5 are detected in the human oral environment. Statherin contains 43 amino acids and prevents the crystallization of calcium phosphate. Azurocidin is present in azurophilic granules of neutrophils, whereas oral epithelial cells are the main source of adrenomedullin. Oral cationic antimicrobial peptides exhibit all three types of antimicrobial protection. They are actively involved in the control of numerous oral infectious diseases including dental caries, gingivo-periodontal diseases as well as in the healing process of post-extraction wounds[16−18]. Their activity is also directed against *S. mutans* and other cariogenic bacteria. In the oral cavity, they are detected both in the gingival fluid and saliva[15]. Their salivary secretion is mostly caused by bacterial pathogens. It has been suggested that these salivary peptides may act as caries risk biomarkers and therefore play a role in caries risk assessment in children[19,20]. However, data from the available literature are conflicting in this regard.

The aim. The article aimed to analyse the importance of salivary levels of cationic antimicrobial peptides as indicators and predictors of caries in children, and consequently their possible role in caries risk assessment in children.

The role of salivary cationic antimicrobial peptides in the biological control of caries

As pointed out, cationic antimicrobial peptides also effectively act on cariogenic microorganisms.

Od svih kariogenih mikroorganizama, na dejstvo ovih peptida najosetljiviji su *S. mutans* i *S. sobrinus*[21]. Istraživanja pak pokazuju da od svih pomenutih peptida α-defenzini, hBD2 i LL-37 imaju najveći antimikrobni potencijal usmeren na *S. mutans*[16]. Pokazuju jako baktericidno dejstvo, mada je njihov mehanizam antibakterijskog dejstva prilično složen. Pre svega, oni direktno deluju na kariogene bakterije tako što ekstracelularnim mehanizmina menjaju propustljivost membrane bakterije, a intracelularnim mehanizmima inhibiraju intracelularne procese bakterija (sinteza DNK, RNK, proteina)[22]. Osim toga, ovi peptidi mogu stimulisati i modulirati stečeni imunski odgovor (utiču na produkciju citokona, Ig A, Ig G itd.) i na taj način indirektno ostvariti antibakterijsko dejstvo[22,23].

Katjonski antimikrobni peptidi učestvuju u sprečavanju formiranja dentalnog biofilma tako što utiču na kolonizaciju biofilma određenim mikroorganizmima (uključujući i *S. mutans*)[24]. Smatra se da se ovi peptidi lokalno stvaraju kako bi aktivno učestvovali u odbrani od supragingivalnog dentalnog biofilma odgovornog za nastanak karijesa[25].

Iako je mehanizam dejstva skoro identičan za sve katjonske antimikrobne peptide pljuvačke, neki od njih imaju i dodatna svojstva kojima doprinose biološkoj kontroli karijesa; tako staterini ometaju kristalizaciju kalcijum-fosfata i time doprinose redukciji formiranja dentalnog biofilma[24]. Kod histatina se prvenstveno izdvajaju antimikotična svojstva, mada je utvrđeno da mogu snažno inhibirati i kariogene bakterije[24].

Istaknuto je da su pojedini autori ukazivali na potencijalnu ulogu salivarnih katjonskih antimikrobnih peptida kao biomarkera rizika od pojave karijesa u dečjem uzrastu i, shodno tome, na značajnu ulogu u proceni rizika od pojave karijesa kod dece[19-20]. Međutim, da bi se okvalifikovali kao pouzdani biomarkeri rizika od pojave karijesa, važno je dokazati njihov značaj kao indikatora rizika od pojave karijesa i prediktora pojave karijesa.

Salivarni katjonski antimikrobni peptidi kao indikatori rizika od pojave karijesa u dečjem uzrastu

Da bi varijabla imala značaj indikatora rizika od nastanka karijesa, potrebno je studijama preseka pokazati njenu povezanost sa karijesom[8].

Veliki broj studija preseka ispitivao je povezanost salivarnog nivoa katjonskih antimikrobnih peptida pljuvačke sa prisustvom karijesa[26-41].

It has been found that of all the cariogenic microorganisms, *S. mutans* and *S. sobrinus* are the most sensitive to cationic antimicrobial peptides[21]. Studies, on the other hand, show that of all these peptides, α defensins, hBD2, and LL-37 have the greatest antimicrobial potential against *S. mutans*[16]. They express a strong bactericidal effect, even though their mechanism of antibacterial action is quite complex. Primarily, they directly affect cariogenic bacteria by altering the permeability of the bacterial membrane by extracellular mechanisms, i.e., by inhibiting the intracellular processes of bacteria by intracellular mechanisms (synthesis of DNA, RNA, proteins)[22]. In addition, these peptides can stimulate and modulate the acquired immune response (affect the production of cytokines, IG A, Ig G, etc.) and thus indirectly achieve an antibacterial effect[22,23].

Cationic antimicrobial peptides are involved in the prevention of dental biofilm formation by interfering with biofilm colonization by certain microorganisms including *S. mutans*[24]. It is thought that these peptides are generated locally to actively participate in the defence against the supragingival dental biofilm responsible for caries[25].

Even though the mechanism of action is almost identical for all salivary cationic antimicrobial peptides, some of them have additional properties that contribute to the biological control of caries. Thus, statherins interfere with the crystallization of calcium phosphate and thereby contribute to the reduction of dental biofilm formation[24]. Histatins are primarily considered to have antimycotic properties, but it has been determined that they can strongly inhibit cariogenic bacteria as well[24].

As mentioned, it has been suggested that salivary cationic antimicrobial peptides could act as caries risk biomarkers in children and, accordingly, play a significant role in caries risk assessment in children[19–20]. However, to qualify them as reliable caries risk biomarkers, it is important to prove their significance as caries risk indicators and caries predictors.

Salivary cationic antimicrobial peptides as caries risk indicators in children

For a variable to be a significant caries risk indicator, its association with the presence of caries must be demonstrated in cross-sectional studies[8]. A great number of cross-sectional studies have examined the association of salivary levels of cationic antimicrobial peptides with the presence of caries[26–41].

Najveći broj njih analizirao je povezanost karijesa sa salivarnim nivoom α-defenzina HNP1-3[26-35] i katelicidina LL-37[26,27,30,33-40]; u nešto manjem broju studija ispitivala se povezanost sa nivoom β-defenzina hBD2[27,29,30,36,37,41] i hBD3[26,27,29,30,33-36]. Takođe, ispitivana je povezanost karijesa sa salivarnim nivoom staterina[29,40] i histatina[29,30,36,41]. Ni u jednoj studiji nije ispitivana povezanost salivarnog nivoa adrenomedulina i azurocidina sa karijesom u dečjem uzrastu. Povezanost navedenih parametara u nekima od ovih studija ispitivana je kod dece u ranom detinjstvu[28-32,36,39,41], a u nekima od njih kod dece školskog uzrasta i adolescenata[26,27,37,40]; bilo je i studija koje su obuhvatile širok raspon godina, posmatrajući rano detinjstvo, školski uzrast i adolescenciju istovremeno[33-35,38].

Veći broj pomenutih studija ukazao je na povezanost salivarnog nivoa katjonskih peptida sa prisustvom karijesa u dečjem uzrastu; to ih čini potencijalno značajnim indikatorima rizika od nastanka karijesa. Uglavnom je povezanost sa prisustvom karijesa kod dece utvrđena za HNP1-3[26,29,31,32,35], hBD2[37,41], hBD3[28,35,36] i LL-37[35,36,38,39]. Angarita Diaz MP i sar.[40] i Ribeiro TR26 ukazali su na moguću povezanost karijesa i staterina pljuvačke, Sun i sar.[30] na moguću vezu karijesa i histatina, dok su Jurczak i sar.[41] ukazali na moguću povezanost histatina 5 i karijesa kod dece. Međutim, u jednom broju studija ispitivana veza nije utvrđena ni za jedan analizirani katjonski antimikrobni peptid[27,28,33,34].

Interesantno je da su neke od ovih studija utvrdile da je kod dece sa prisutnim karijesom salivarni nivo peptida veći nego kod dece bez karijesa (pozitivna korelacija). Nasuprot tome, u određenim studijama ustanovljeno je da je kod dece sa karijesom salivarni nivo ispitivanih peptida niži nego kod dece bez karijesa (obrnuta korelacija). Iako otežavaju utvrđivanje značaja salivarnih katjonskih peptida kao indikatora rizika od pojave karijesa, ovakvi oprečni rezultati donekle su bili očekivani. Najpre, salivarni katjonski antimikrobni peptidi deo su nespecifične imunosti usne duplje i njihova salivarna ekspresija može biti izazvana bakterijama, ali i inflamatornim agensima i povredama[15]; stoga, oni pokazuju velike varijacije u salivarnom nivou, na šta su pojedini autori i uputili[41]. Potom, njihov salivarni nivo menja se sa uzrastom dece, odnosno sa sazrevanjem imunskog odgovora; na to su pojedini autori ukazali u svojim radovima[38,42].

Zatim, usna duplja je dinamična sredina sa čestim promenama pH i sredina u kojoj pozitivno nalektrisani katjonski peptidi,

The majority of these have analysed the association of caries with salivary levels of α-defensin HNP1-3[26–35] and cathelicidin LL-37[26,27,30,33–40], whereas a slightly smaller number of studies have focused on β-defensins hBD2[27,29,30,36,37,41] and hBD3[26,27,29,30,33–36]. In addition, the association of caries with the salivary level of statherin[26,27,29,30,33–36], histatins[29,30,36,41] has been examined. No studies have examined the link between the salivary levels of adrenomedullin and azurocidin and caries in children. Some of these studies have examined the association of these parameters in early childhood[28–32,36,39,41], some in school children and adolescents[26,27,37,40], while others have covered a wide range of years, including early childhood, school age, and adolescence simultaneously[33–35,38].

A larger number of these studies have shown a correlation between the salivary level of cationic peptides and the presence of caries in childhood, which classifies them as potentially significant caries risk indicators. In particular, HNP1-3[26,29,31,32,35], hBD2[37,41], hBD3[28,35,36], and LL-37[35,36,38,39] have been found to be associated with the presence of caries in children. Angarita Diaz MP et al.[40] and Ribeiro TR[26] suggested a possible association between caries and salivary statherin, Sun et al.[30] specified a possible connection between caries and histatin 1, whereas Jurczak et al.[41], indicated a possible connection between histatin 5 and caries in children. However, some studies failed to prove the relationship with any of the analysed cationic antimicrobial peptides[27,28,33,34].

Interestingly, some of these studies found that salivary levels of the peptides were higher in children with caries than in those without (positive correlation). On the contrary, some concluded that salivary levels of the peptides tested were lower in children with than in those without caries (inverse correlation). Although this makes it difficult to determine the significance of salivary cationic peptides as caries risk indicators, the conflicting results are somewhat to be expected. Firstly, salivary cationic antimicrobial peptides are part of the nonspecific immunity of the oral cavity and their salivary expression can be caused not only by bacteria but also by inflammatory agents and injuries[15]. That is why they show great variations in the salivary level, as some authors have pointed out[41].

Furthermore, their salivary level changes with the age of children, i.e., with the maturation of the immune response, as some authors have pointed out[38,42].

pri fiziološkim pH pljuvačke, mogu stupiti u interakciju sa različitim komponentama pljuvačke (NaCl, mucini, jednovalentni i dvovalentni joni...), što se može odraziti na koncentraciju njihovih aktivnih formi u pljuvački[22,43,44]. Naposletku, oprečni rezultati u pogledu ispitivane povezanosti mogu se pripisati i različitim kriterijumima određivanja statusa karijesa kod pojedinca (kep/KEP, kips/KIps, ICDAS, aktivitet karijesa).

Kada se uzme u obzir sve što je prethodno navedeno, može se smatrati da je precizna procena značaja salivarnih katjonskih peptida kao karijes rizik indikatora u dečjem uzrastu prilično složena. Stoga, potrebno je i nadalje sprovoditi dobro dizajnirane studije preseka sa jasno definisanim kriterijumima za uključenje/isključenje ispitanika. U ovim studijama trebalo bi obuhvatiti veliki broj dece različitih uzrasta i analizirati značaj svih katjonskih antimikrobnih peptida. Takođe, potrebno je istovremeno analizirati ulogu različitih kombinacija salivarnih nivoa ovih peptida kao potencijalnih biomarkera za pojavu karijesa kod dece, koja proizilazi iz kompleksnosti njihovog baktericidnog dejstva. Dalja istraživanja ne bi trebalo da se zaustave samo na analizi salivarnih katjonskih antimikrobnih peptida kao indikatora rizika za pojavu karijesa kod dece, već u njima treba analizirati i značaj ostalih neimunoglobulinskih antimikrobnih peptida kao indikatora rizika za pojavu karijesa kod dece (npr. salivarne peroksidaze, kalprotektin, fibronektin, laktoferin, mucini, cistatini itd.). Naime, do sada je u pljuvački identifikovan veliki broj neimono-globulinskih antimikrobnih peptida, koji su prema mehanizmu delovanja podeljeni u šest funkcionalnih klasa – njima, osim katjonskih peptida, pripadaju bakterijski aglutinini i adahezini (fibronektin, mucini, prolinom bogati proteini...), helatori metalnih jona (laktoferin, kalgranulin), peroksidaze (laktoperoksidaza, salivarna peroksidaza), inhibitori proteaza (cistatini) i proteini koji pokazuju aktivnost protiv zida bakterijskih ćelija (lizozim). Iako svaki od pomenutih proteina ima specifičan mehanizam delovanja (karakterističan za funkcionalnu grupu kojoj pripada), svi oni pokazuju i sinergističko dejstvo u borbi protiv oralnih bakterija, uključujući one kariogene. To znači da svaki od njih učestvuje u biološkoj kontroli karijesa i ima potencijalni značaj kao indikator rizika od pojave karijesa.

Next, it should be taken into account that the oral cavity is a dynamic environment in which the pH value often changes and positively charged cationic antimicrobial peptides, at the physiological pH value of saliva, can interact with various components of saliva (NaCl, mucins, monovalent and divalent ions, etc.) all of which can be reflected in the concentration of their active forms in saliva[22,43,44]. Finally, the obtained conflicting results regarding the studied relationship can be partially attributed to different criteria used to determine the caries status of an individual (number of dmf/DMF teeth, dmft/DMFT index, ICDAS system, caries activity).

Considering all the above, it can be concluded that the precise assessment of the significance of salivary cationic peptides as caries risk indicators in children is quite complex. This still requires well-designed cross-sectional studies with clearly defined subject inclusion/exclusion criteria. These studies should include a great number of children of different ages and analyse the significance of all cationic antimicrobial peptides. In addition, it is necessary to simultaneously analyse the role of different combinations of salivary levels of these peptides as potential caries risk biomarkers in children, due to the complexity of their bactericidal action.

Further research should not stop only at the analysis of salivary cationic antimicrobial peptides as caries risk indicators in children. It is also necessary to analyse the importance of other non-globulin antimicrobial peptides as caries risk indicators in children, such as salivary peroxidases, calprotectin, fibronectin, lactoferrin, mucins, cystatins, etc. In fact, so far, a large number of non-immunoglobulin antimicrobial peptides have been identified in saliva. According to the mechanism of action, they are divided into 6 functional classes, which, in addition to cationic peptides, also include bacterial agglutinins and adhesins (fibronectin, mucins, proline-rich proteins...), metal ion chelators (lactoferrin, calgranulin), peroxidases (lactoperoxidase, salivary peroxidase), protease inhibitors (cystatins), proteins that show activity against the bacterial cell wall (lysozyme). Although each of these proteins has a specific mechanism of action (characteristic of the functional group to which it belongs), they all show a synergistic effect in the fight against oral bacteria, including cariogenic ones. Therefore, each of them participates in the biological control of caries and is potentially important as a caries risk indicator.

Salivarni katjonski antimikorbni peptidi kao prediktori karijesa u dečjem uzrastu

Značaj katjonskih peptide u predikciji nastanka karijesa ispitivale su samo dve studije. Jedna je analizirala značaj HNP1-3 i LL-37 u predikciji nastanka karijesa u ranom detinjstvu i obuhvatila decu uzrasta od tri godine na početku studije[45]. Druga studija bila je studija grupe naših autora – u njoj je analiziran značaj HNP1-3, hBD-2 i LL-37 u predikciji nastanka karijesa u periodu rane adolescencije, kod dece uzrasta od 11 do 13 godina na početku studije[46]. Nažalost, nijedna studija nije utvrdila značaj ispitivanih salivarnih peptida u predikciji nastanka karijesa.

Međutim, na osnovu rezultata samo dveju dosad sprovedenih studija ne može se negirati potencijalni značaj katjonskih antimikorbnih peptida u predikciji nastanka karijesa. Osim toga, obe studije su analizirale mali broj katjonskih peptida, obuhvatile relativno mali broj ispitanika, a značaj peptida u predikciji nastanka karijesa analizirale jednogodišnjim studijama praćenja. Sve navedeno može predstavljati značajna ograničenja studija, što su i sami autori predočili. Ova činjenica istovremeno ukazuje na neophodnost sprovođenja daljih dobro dizajniranih studija praćenja čiji bi cilj bilo preciziranje značaja katjonskih antimikrobnih peptida pljuvačke kao prediktora karijesa u dečjem uzrastu. Takođe, potrebno je analizirati i značaj koji ostali neimonoglobulinski antimikrobni proteini imaju u predikciji nastanka karijesa.

Zaključak

Sudeći po dosad sprovedenim studijama, salivarni nivo katjonskih antimikrobnih peptida može imati značaj indikatora rizika od pojave karijesa u dečjem uzrastu. Nažalost, za sada nema pouzdanih dokaza o njihovom značaju u predikciji nastanka karijesa u dečjem uzrastu. Na osnovu sprovedenih studija može se zaključiti da su potrebne dodatne dobro dizajnirane studije preseka i dugoročne longitudinalne studije koje bi precizirale značaj salivarnih katjonskih antimikrobnih peptida kao pouzdanih indikatora i prediktora rizika od pojave karijesa u dečjem uzrastu. Istovremeno, time bi se definisali njihov značaj kao biomarkeri rizika od pojave karijesa i njihova potencijalna primena u proceni rizika od pojave karijesa kod dece. U narednim studijima treba analizirati i značaj ostalih neimunoglobulinskih antimikrobnih proteina kao indikatora i prediktora rizika od pojave karijesa kod dece.

Salivary cationic antimicrobial peptides as caries predictors in children

Only two studies dealt with the caries predictive value of cationic peptides. One analysed the caries predictive value of HNP1-3 and LL-37 in early childhood and included children aged 3 at the beginning of the study[45]. The second study is a study by a group of our authors who analysed the caries predictive value of HNP1-3, hBD-2, and LL-37 in early adolescence, in children aged 11−13 at the beginning of the study[46]. Unfortunately, both studies were unable to determine the caries predictive value of the studied salivary peptides.

However, based on the results of only two studies conducted to date, the potential caries predictive value of cationic antimicrobial peptides cannot be denied. In addition, both studies analysed a small number of cationic peptides, included a relatively small number of subjects, and analysed the caries predictive value of the peptides with one-year follow-up studies. All of these may represent significant limitations of the study, as pointed out by the authors themselves. This also suggests the need for further well-designed follow-up studies to clarify the significance of salivary cationic antimicrobial peptides as predictors of caries in children. Also, it is necessary to analyse the caries predictive significance of other non-immunoglobulin antimicrobial proteins.

Conclusion

Based on studies carried out to date, the salivary level of cationic antimicrobial peptides can be an important caries risk indicator in children. Unfortunately, the studies have not confirmed the caries predictive value of these peptides in children. In conclusion, well-designed cross-sectional studies and long-term longitudinal studies are still required to specify the significance of salivary cationic antimicrobial peptides as reliable caries risk indicators and caries predictors in children. At the same time, this would define their value as caries risk biomarkers and their potential application in caries risk assessment in children. Further studies should also analyse the importance of other non-globulin antimicrobial proteins as caries risk indicators and caries predictors in children.

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Conflict of interest statement

The authors declare no conflict of interest.

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