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ODNOS RELATIVNIH POVRŠINA GORNJEG CENTRALNOG SEKUTIĆA I LICA

THE RELATIONSHIP BETWEEN THE RELATIVE SURFACE OF THE MAXILLARY CENTRAL INCISORS AND THE FACE

Marko Igić

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Sažetak

Uvod: *Centralni sekutići, najkrupniji, najistureniji i najvidljivi zubi, posebno utiču na estetiku zubne nadoknade.*
Cilj *ovog rada je upoređivanje relativne površine odabranog dela lica i relativne površine gornjeg centralnog sekutića.*
Materijal i metode: *Ispitivanje je vršeno kompjuterskom analizom fotografija lica 200 ispitanika, 100 ženskog i 100 muškog pola, kao i fotografija njihovih gipsanih modela gornjih zuba.*
Rezultati: *Upoređivanje relativne površine lica i relativne površine vestibularne površine centralnih sekutića ukazalo je na postojanje određenog odnosa među njima. Taj odnos je kod muškaraca 1,21, dok je kod žena 1,19.*
Zaključak: *Primena što većeg broja parametara može nam olakšati pravilan izbor veličine zuba prilikom izrade protetskih nadoknada. Dobijeni rezultati mogu se iskoristiti pri rekonstrukciji lica kao parametar ukoliko osoba poseduje zube, ili obrnuto za rekonstrukciju zuba kod bezubih osoba.*

Ključne reči: *Centralni sekutići, lice, površina, odnos*

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Abstract

Introduction: *Central incisors, the largest, most protruding and visible, affect denture aesthetics the most.*
The aim *of this paperwork was to compare the relative surface of a particular part of the face and the relative surface of the maxillary central incisors.*
Material and methods: *Measurements were done by computer analysis of face photographs of 200 subjects, 100 women and 100 men, as well as photos of their upper teeth plaster models.*
Results: *The comparison of the relative surface of the face and the vestibular surface of central incisors indicated the existence of a certain relationship between them. That relationship was 1.21 in men, and 1.19 in women.*
Conclusion: *The application of as many parameters may make the right choice of the size of teeth for dentures during their preparation easier. These results may be used during face reconstruction if an individual possesses all teeth, or vice versa, for the teeth reconstruction in edentulous individuals.*

Key words: *Central incisors, face, surface, relationship*

Uvod

Niz je elemenata koji predstavljaju činioce lepote, ali je u svakom slučaju neophodan sklad elemenata ljudskog tela. Postoji bezbroj elemenata koji mogu biti usklađeni, pa tako i veliki broj merenja i teorija koje se ovim bave.

Kako je pored funkcije žvakanja za protetičke nadoknade bitna i njihova estetika, postoji i niz teorija o usklađenosti pojedinih elemenata pre svega ljudskog lica i zuba, a naročito gornjih centralnih sekutića, koji su najkrupniji, najistureniji i najviše vidljivi, pa zato i utiču najviše na estetiku zubne nadoknade.

U dostupnoj literaturi nema podataka o merenjima površina lica i zuba i njihovom upoređivanju, te ni odgovarajućih rezultata.

Cilj ovog rada je upoređivanje relativne površine odabranog dela lica i relativne površine gornjeg centralnog sekutića.

Materijal i metode

Merenja su izvršena kod 200 ispitanika, 100 ženskog i 100 muškog pola. Birane su osobe čije lice deluje skladno, a koje nemaju vidljive deformitete lica, ožiljke ili izrazitu asimetričnost. Takođe nisu uzimane u obzir ekstremne osobe, bilo premale ili prevelike težine, jer se to odražava i na izgled lica.

Ispitanici su bili starosti između 18 i 25 godina, bez vidljivih znakova abrazije zuba.

U radu se pošlo od pretpostavke da ukoliko postoji sklad drugih delova tela verovatno postoji i sklad zuba i lica. U mnogim teorijama (kao što je npr. Williamsova teorija) upoređuju se oblici lica i zuba i pri tom nalazi manji ili veći sklad.

I pored toga što vestibularne površine zuba i samo lice nije moguće izmeriti, jer su granice nepoznate, jasno se može videti njihova kontura, što odgovara dvodimenzionalnom objektu. U radu je izvršeno merenje površina ovih dvodimenzionalnih – u ravni projektovanih objekata.

Da bi moglo da se izvrši upoređivanje površina lica i centralnih sekutića bilo je neophodno da se oni na neki način usklade. To je izvršeno tako što je definisan pravougaonik koji služi kao osnova pri snimanju lica i zuba.

Snimani objekat je smeštan u opisani pravougaonik tako da se poklapa sa njegovom širinom. Zatim je ispitanik rotirao glavu gore-dole tako da se dobije njena najveća vertikalna dimenzija, uz nepromenjenu širinu.

Introduction

There is an array of elements representing beauty factors, however, it is necessary that these elements of the human body are in harmony. There are countless elements that could be harmonized, and thus a large number of measurements and theories that deal with this matter.

In addition to mastication, aesthetics is also essential for dentures. Consequently, there is a wide range of theories on the harmony of certain elements, primarily of the human face and teeth, especially the maxillary central incisors, which are the largest, most protruding and visible, and therefore affect denture aesthetics the most.

The existing literature provides us neither with data on face and teeth surface measurements and their comparison, nor with adequate results.

The aim of this paperwork was to compare the relative surface of a particular part of the face and the relative surface of the maxillary central incisors..

Material and methods

Measurements were done in 200 subjects, 100 women and 100 men. Subjects with a harmonious face, with no visible deformities, scars or pronounced asymmetry were selected. Furthermore, extreme subjects, either too thin or overweight, were not taken into consideration, since that is also reflected on their faces.

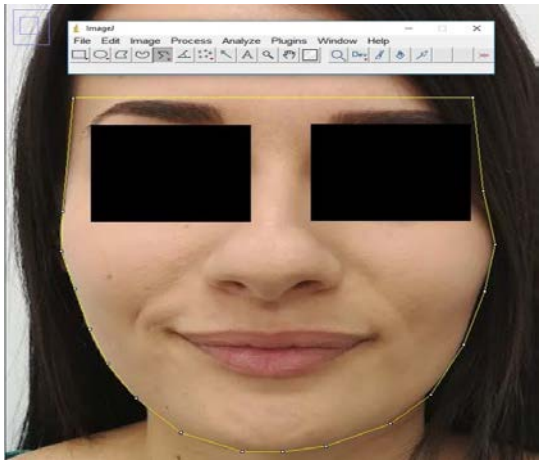
Subjects were between 18 and 25 years of age, with no visible signs of dental abrasion. The paperwork started form the assumption that if other body parts are in harmony, most probably the teeth and the face are in harmony as well. Many theories (e.g. the Williams theory) compare the shapes of the face and teeth and find that there is a certain degree of harmony between them.

Even though the vestibular surface of the teeth and the face itself are not possible to measure because their boundaries are unknown, their contours can clearly be seen, which corresponds to a two-dimensional object. In this paperwork, we measured the surface of these two-dimensional objects – in the projection plane.

In order to compare the surface of the face and central incisors, first it was necessary to harmonize them in a way. It was done by defining a rectangle which served as the basis for face and teeth imaging.

To je učinjeno i sa modelom prednjih zuba dobijenim na osnovu otiska uzetog tom ispitaniku. Da bi moglo da se vrši pravilno pozicioniranje gipsanog modela, po horizontali i vertikali, on je postavljen na postolje koje omogućava pokrete u svim pravcima. Zatim je, pri adekvatnom povećanju, dakle kada bi se širina zuba poklopila sa širinom orijentacionog pravougaonika, uz najveću dužinu, izvršeno snimanje.

Kako bi se izračunala površina lica ispitanika korišćen je softver ImageJ. Slika lica ispitanika je učitavana u program, a zatim je primenom odgovarajućeg alata vršeno označavanje dela lica čija će se površina izračunavati (Slika 1.). Prethodno je vršena kalibracija alata, kako ne bi došlo do odstupanja u dimenzijama. Merenje lica vršeno je od gornje ivice obrva do donje ivice brade. Nakon toga je vršeno merenje površine odabranog centralnog sekutića, na isti način kao i merenje površine lica.



Slika / Fig 1.
Slika lica ispitanika sa označenom površinom za merenje
The image of the face of a subject with marked surface to be calculated

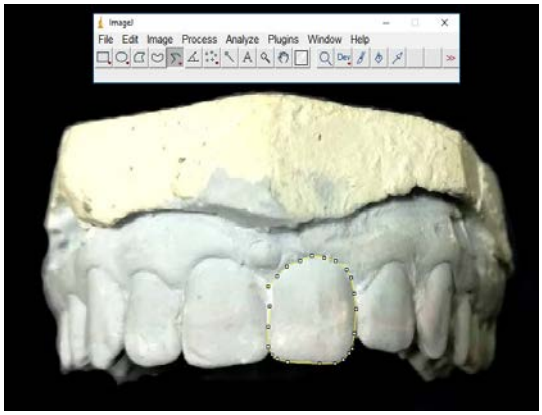
Rezultati

Upoređivanje relativne površina lica i relativne površine vestibularne površine centralnih sekutića ukazalo je na postojanje određenog odnosa među njima. Rezultati merenja predstavljeni su u Tabeli 1.

The recorded object was placed in the described rectangle to match its width. Next, the subject moved their head up and down to get its largest vertical dimension, whereas the width remained unchanged.

The same was done with the model of the front teeth obtained based on the teeth impression mold taken from the subject. In order to position a plaster model well, both horizontally and vertically, it was placed on a stand which allows movements in all directions. Then, with an adequate increase, that is, when the width of the teeth matched the width of the orientation rectangle, its longest side, dental imaging was performed.

ImageJ software was used to measure the surface of the face of the subjects. The image of the face of a subject was loaded into the program, and then the part of the face whose surface needed to be calculated was marked using appropriate tools (Fig 1.). Previously, the calibration of the tools was done to avoid possible deviations in dimensions. The face was measured from the upper eyebrow line to the lower chin line. Next, the surface of the selected central incisor was measured, in the same way as the surface of the face.



Slika / Fig 2.
Slika gipsanog modela gornjih zuba sa označenim sekutićem za merenje
The image of a plaster model of upper teeth with marked incisor to be calculated

Results

The comparison of the surface of the face and the vestibular surface of central incisors indicated the existence of a certain relationship between them. The measurement results are presented in Table 1.

	MUŠKARCI MALES	ŽENE FEMALES
BROJ / NUMBER	100	100
PROSEČNA POVRŠINA ZUBA (U PIKSELIMA) AVERAGE TEETH SURFACE (IN PIXELS)	88320	85240
STANDARDNA DEVIJACIJA STANDARD DEVIATION	7389	7945
PROSEČNA POVRŠINA LICA (U PIKSELIMA) AVERAGE FACE SURFACE (IN PIXELS)	72991	71630
STANDARDNA DEVIJACIJA STANDARD DEVIATION	5024	6831
ODNOS POVRŠINA SURFACE RATIO	1.21	1.19

Tabela / Table 1.
Jedinice mere i razmere
Measurement results and obtained ratio

Diskusija

U prirodi postoji određeni sklad elemenata čijim proučavanjem su se ljudi, a naročito umetnici bavili od davnina. Svakako da takav sklad poseduje i ljudsko telo, a za stomatologe je posebno važan sklad zuba i ostalih delova tela, pre svega lica. Ljudsko telo je manje ili više zaobljeno, praktično skoro da nema nekih jasno izraženih geometrijskih oblika. I čovekova glava i njegovi zubi su manje ili više zaobljeni, te zato lice i vidljiva - vestibularna strana zuba nemaju jasne granice u odnosu na ostale elemente glave ili zuba. Iako su ove površine manje ili više ispupčene, pri susretu sa nekom osobom iz daljine praktično vidimo dvodimenzionalnu sliku, koja sa približavanjem postaje sve više jasno trodimenzionalna. Ako se posmatrač udalji da bi jasno video lice neće dobro videti zube i obrnuto, ako se približi da bi video zube neće moći da pogledom obuhvati celo lice.

Kako u dostupnoj literaturi nema podataka o istraživanjima ove vrste, nije bilo moguće upoređjivanje sa rezultatima drugih autora.

U literature se uglavnom nalaze podaci o jednodimenzionalnim merenjima pa su tako upoređivani dužina i širina pojedinih ili grupe zuba sa određenim elementima lica. Često je vršeno upoređivanje širine centralnih sekutića sa širinom filtruma[1], interpupilarnim razmakom[2,3], bizigomatičnom širinom[4,5]. Sterrett je čak vršio upoređivanje dimenzija centralnog sekutića i visine ispitanika[6].

Discussion

There is certain harmony of elements in the nature which has been studied for ages by people, especially artists. It is certain that the human body also possesses such harmony, and the harmony between the teeth and other body parts, especially the face, is particularly important for dentists. The human body is more or less rounded, practically there are no clearly pronounced geometrical shapes. Moreover, the human head and their teeth are also more or less rounded, therefore the face and the visible – vestibular side of the teeth do not have clear boundaries regarding other elements of the head or teeth. Even though these surfaces are more or less protruding, when we see a person from a distance we practically see a two-dimensional image, which evidently becomes three-dimensional as we approach. If the observer moves away to see the face clearly, they won't see the teeth well, and vice versa, if they approach to see the teeth, they will not be able to see the entire face.

Given that the existing literature does not contain data on this type of research, it was not possible to compare our results with the results of other authors. The literature mostly contains data on one-dimensional measurements, thus the length and width of an individual tooth or groups of teeth were compared to certain facial elements. The width of central incisors was often compared with the philtrum width[1], interpupillary distance[2,3], bizygomatic width[4,5]. Sterrett even compared dimensions of central incisors with

Lombardi je još početkom 1970-ih predložio kriterijume kojih bi se trebalo pridržavati prilikom izrade protetskih nadoknada[7,8].

Apsolutna površina zuba ili površina lica nisu istraživani jer su te vrednosti jako varijabilne kod različitih osoba, pa je zato ispitivan samo njihov sklad. Iz istog razloga u obzir nisu uzimane osobe sa izrazitim odstupanjem od proseka – jako mršave i jako pune osobe.

Zaključak

Primena što većeg broja parametara može nam olakšati pravilan izbor veličine zuba prilikom izrade protetskih nadoknada.

Dobijeni rezultati odnosa merenih površina zuba i lica (1,19 za žene i 1,21 za muškarce, što u proseku čini 1,2) pokazuju da među ovim površinama postoji određeni sklad. Ovi rezultati mogu se iskoristiti pri rekonstrukciji lica ukoliko osoba poseduje zube, ili obrnuto za rekonstrukciju zuba kod bezubih osoba.

Zahvalnica

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the height of subjects6. Back in the early 1970s, Lombardi suggested criteria that should be met when making dentures[7,8].

The absolute surface of the teeth or the surface of the face was not studied since those values were quite variable in different individuals, and therefore, only their harmony was examined. Individuals with a significant deviation from the average, i.e. very thin and overweight individuals, were not taken into consideration for the same reason.

Conclusion

The application of as many parameters may make the right choice of the size of teeth for dentures during their preparation easier.

The obtained results of the relationship between the measured surface of the teeth and the face (1.19 for women and 1.21 for men, 1.2 on average) reveal that there is a certain degree of harmony between them. These results may be used during face reconstruction if an individual possesses all teeth, or vice versa, for the reconstruction in edentulous individuals.

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IN VITRO PROCENA KAZEIN FOSFOPEPTIDA – AMORFNOG KALCIJUM FOSFATA (CPP-ACP) U PREVENCIJI BELE MRLJE

IN VITRO ASSESSMENT OF CASEEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE (CPP - ACP) IN PREVENTION OF WHITE SPOT LESIONS

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Sažetak

Uvod: *Ortodonske bravice i drugi ortodontski elementi, otežavaju oralni higijenu olakšavaju akumulaciju dentalnog plaka što povećava rizik od karijesa u toku ortodontskog tretmana.*
Cilj: *ovog istraživanja bio je da se utvrdi koncentracija Ca i Mg u veštačkoj pljuvački posle preventivnog tretmana.*
Metode: *Laboratorijsko ispitivanje (in vitro) obavljeno je na 90 zdravih zuba ekstrahovanih iz ortodontskih razloga. Formirane su tri grupe od po trideset zuba. Zubi iz svake grupe su podeljeni na polovine u buko-lingvalnom pravcu. Na taj način formirani su i ispitivani i kontrolni uzorak, oba od jednog istog zuba. Brayice su zalepljene pomoću GC Fuji OrthoTM LC (GC Amerika Cikago, III),glasjonomer cementom modifikovanim smolom, a zubi su zatim čuvani u veštačkoj pljuvački. Jednom dnevno zubi su premazivani topikalnim gelom - CG Tooth Mousse u trajanju od 5 minuta, a zatim vraćeni u veštačku pljuvačku . Preventivni tretman je sproveden u određenim intervalima od 1, 3 i 6 meseci. Posle svakog perioda ispitivanja, veštačka pljuvačka u kojoj su uzorci čuvani je bila korišćena za određivanje koncentracije Ca i Mg pomoću plamene atomske apsorpcione spektrofotometrije.*
Rezultati: *Rezultati ovog (in vitro) istraživanja pokazali su da su koncentracije Ca i Mg u veštačkoj pljuvački značajno povećane čak i nakon prvog meseca od primene ovog sredstva koje oslobađa Ca i druge minerale (uključujući cement bez fluora koji se koristi za lepljenje bravica) sa maksimalnom vrednošću nakon tri meseca primene. Ove vrednosti su bile značajno niže nakon šest meseci, verovatno kao rezultat njegove apsorpcije u gleđi. Rezultati za Mg su isti kao i za Ca, a vrednost se povećava, kao i njena stabilnost u pljuvački nakon prvog meseca.*
Zaključci: *Rezultati ove in vitro studije jasno ukazuju da preventivni materijali koji se koriste u fiksnom ortodontskom tretmanu inhibiraju demineralizaciju gleđi oko bravica i ortodontskih prstenova. Preporuka za njihovu upotrebu kao dodatnih preventivnih metoda / sredstava je očigledna pored primarne oralne higijene.*

Ključne reči: *zubni karijes, preventivni tretman, koncentracija Ca i Mg*

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Abstract

Introduction: *Brackets and various orthodontic elements that are used during the treatment, make the oral hygiene difficult and the accumulation in dental plaque much easier.Increasing the risk of dental caries during orthodontic treatment.*
The aim: *of this study was to determine the concentration of Ca and Mg in artificial saliva after preventive treatment.*
Methods: *The laboratory examination (in vitro) was performed in 90 healthy teeth extracted for orthodontic reasons. Three groups of thirty teeth were formed. The teeth from each group were separated in half in bucco-lingual direction. Thus, the control and test specimens were obtained from the same teeth. The brackets were bonded with GC Fuji OrthoTM LC (GC America Chicago, III), a resin-modified glass ionomer cement and the teeth then were stored in artificial saliva. Once per day, the teeth were coated with topical gel - CG Tooth Mousse in duration of 5 minutes and then returned to artificial saliva. Preventive treatment was at certain intervals of 1, 3 and 6 months. After each study period, the artificial saliva where the samples were stored was used for evaluation of Ca and Mg concentration by flame atomic absorption spectrophotometry.*
Results: *The results of this study (in vitro) showed that the concentrations of Ca and Mg in artificial saliva were significantly increased even after the first month of application of this means which released Ca and other minerals (including fluoride free cement used to bond the brackets) with maximum value after a three-month application. These values were significantly lower after six months, probably as a result of its absorption into the enamel. The results for Mg are the same as for Ca, and the value is increased as well as its stability in saliva after the first month.*
Conclusions: *The results of this in vitro study clearly indicate that preventive materials used in fixed orthodontic treatment inhibit the demineralization of enamel around brackets and orthodontic rings. Evidently, their use as additional preventive methods/tools, besides primary oral hygiene, is recommended.*

Key words: *dental caries, preventive treatment, Ca and Mg concentration*

Uvod

Veliki broj pacijenata u okviru mlade populacije rešava postojeće funkcionalne i estetske probleme ortodontskim tretmanom. Kontrola demineralizacije u gleđi oko ortodontskih bravica i traka tokom fiksne ortodontske terapije je značajan klinički problem. Bravice i različiti ortodontski elementi (elastični, plastični, naglavci, opruge), koji se koriste tokom tretmana, otežavaju oralnu higijenu i znatno olakšavaju akumulaciju zubnog plaka.

Povećanje b roja kariogenih bakterija takođe povećava rizik od karijesa tokom ortodontskog tretmana . Jedno od mogućih rešenja u sprečavanju dekalcifikacije gleđi oko bravica tokom fiksno-ortodontskog tretmana je kontinuirana upotreba 10% Kazein fosfopeptid amorfnog kalcijum fosfata - gel za zube GC Tooth Mousse (Dental Cream GC Tooth Mousse). Reynolds je izvestio da je CPP-ACP, koji je dobijen iz mlečnog kazeina, apsorbovan kroz površinu gleđi, uticao na procese demineralizacije-remineralizacije[1]. Nedavno istraživanje pokazalo je da se ovakav efekat delimično može postići proteinom kazeina koji se naziva CPP, koji prenosi kalcijumove i fosfatne jone "zarobljene" u njemu, u obliku APP[2]. Ovaj kompleks CPP-ACP otpušta bioraspoložive jone kalcijuma i fosfata. Postoji mogućnost da antikariogena aktivnost CPP-ACP, ojačana inkorporacijom kompleksa u zubni plak i na površinu zuba, služi kao rezervoar kalcijuma i fosfata.CPP-ACP se vezuje za bakterijski zid i površinu zuba[3]. U slučaju intraoralnog povećanja kiselosti, oslobađaju se kalcijumovi i fosfatni joni, postižući prezasićenost jona u pljuvački i zatim taloženjem kalcijum-fosfatnog jedinjenja na eksponiranoj površini zuba[4]. Pored toga, razgradnja CPP može pomoći da se poveća pH (pufer) proizvodnjom amonijaka; pored toga, može sprečiti adheziju bakterija na površinu zuba i odložiti formiranje biofilma[5]. Ne postoji pregledni rad na Cochran bazi o ulozi CPP-ACP u demineralizaciji i remineralizaciji površine zuba. Ipak, nekoliko *in vitro* studija je pokazalo da proizvodi koji sadrže CPP-ACP smanjuju demineralizaciju i pojačavaju remineralizaciju[6-8]. CPP-ACP se može dodati u žvakaće gume, paste i kreme[9]. U početnoj karijesnoj leziji, sa eliminacijom etiološkog faktora (zubnog plaka), sa jedne strane, i sprovođenjem maksimalnih preventivnih mera sa druge, (oralna higijena i preventivni tretman sa GC

Background

A large number of patients within the young population solve their existing functional and aesthetic problems with orthodontic treatment. The control of demineralization in enamel around orthodontic brackets and bands during fixed orthodontic treatment is a significant clinical problem. Brackets and various orthodontic elements (elastic, plastic, sleeves, springs) that are used during the treatment, make the oral hygiene difficult and the accumulation in dental plaque much easier.

Increasing the number of cariogenic bacteria also increases the risk of dental caries during orthodontic treatment. One of the possible solutions to prevent the decalcification of enamel around the brackets during the fixed-orthodontic treatment is the continuous use of 10% Casein Phosphopeptide Amorphous Calcium Phosphate (Dental Cream GC Tooth Mousse). Reynolds reported that CPP-ACP, which is derived from milk casein, was absorbed through the enamel surface and affected the demineralization-remineralization processes[1]. Recent research has shown that this is accomplished by a part of the casein protein referred to as CPP, which carries calcium and phosphate ions "stuck" to it, in the form of APP[2]. This complex of CPP-ACP delivers the bioavailable calcium and phosphate ions. It has been suggested that the anticariogenic activity of CPP-ACP relies on the incorporation of nanocomplexes into the dental plaque and on the tooth surface, thereby serving as a calcium and phosphate reservoir. CPP-ACP binds to the bacterial wall and tooth surfaces[3]. In case of an intraoral acid attack, the calcium and phosphate ions are released, reaching a supersaturated state of ions in the saliva and then precipitating a calcium-phosphate compound on the exposed tooth surface[4]. In addition, the breakdown of the CPP can help increase the pH (buffer) by producing ammonia; in addition, it might prevent bacterial adhesion to tooth surfaces and delay formation of biofilms[5]. There is no Cochrane review available on the role of CPP-ACP in demineralization and remineralization. Nonetheless, several in vitro studies have shown that CPP-ACP-containing products decrease demineralization and support remineralization[6-8].

Tooth Mousse), stvaraju se uslovi za dominaciju procesa remineralizacije nad demineralizacijom, čime se zamenjuje deficit minerala[10]. U kiselom okruženju, CPP-ACP jedinjenje oslobađa jone kalcijuma i fosfata, što pomaže supersaturaciji usne duplje. To je potvrđeno rezultatima dobijenim u *in vitro* studiji od strane Zabokova-Bilbilova E[11]. Naime, dobijene veće vrednosti masenog udela Ca u gleđima, posle jednomesečne primene preventivne dentalne kreme GC Tooth Mousse, potvrdile su ovu tvrdnju. Nakon tri meseca od primene preventivnog stomatološkog gela, gleđ pokazuje značajno veće vrednosti ne samo kalcijuma , već i drugih ispitivanih elemenata (Na, K i Mg).

Cilj ove studije bio je da se nakon preventivnog tretmana sa CCP ACP odredi koncentracija Ca i Mg u veštačkoj pljuvačci.

Metode

Laboratorijsko ispitivanje (*u in vitro* uslovima) je sprovedeno na uzorku od 90 zdravih zuba, izvađenih iz ortodontskih razloga. Formirane su tri grupe od po 30 zuba. Zubi iz svake grupe su presečeni na pola u bukolingvalnom pravcu. Prema tome, kontrolni i studijski uzorci su dobijeni od istih zuba. Bravice su za uzorke vezane upotrebom smolom modifikovanog glas jonomer cementa,GC Fuji Ortho^{TM} LC (GC America Chicago, III), a uzorci zuba su zatim na sobnoj temperaturi čuvani u veštačkoj pljuvački[12] sastavljenoj od 20 mmol/l $NaHCO_3$, 3 mmol/l NaH_2PO_4, and 1 mmol/l $CaCl_2$ neutralne pH vrednosti. Jednom dnevno, zubi su premazivani topikalnim GC Tooth Mousse gelom u trajanju od 5 minuta, a zatim vraćani u veštačku pljuvačku. Preventivni tretman je sprovođen u određenim intervalima od 1, 3 i 6 meseci. Nakon svakog perioda ispitivanja, veštačka pljuvačka u kojoj su uzorci čuvani korišćena je za određivanje koncentracije Ca i Mg, primenom plamene atomske apsorpcione spektrofotometrije[13,14], na sledeći način: kreirali smo kalibracioni diagram, utvrđivali nivo Ca i Mg, izračunali koncentraciju prema formuli.

Konstrukcija kalibracionog diagrama

Kalibracioni dijagram je konstruisan korišćenjem metode standardnih rastvora za analizirani element.

CPP-ACP might be incorporated into chewing gums, lozenges, or creams[9]. In the initial carious lesion, with the elimination of the etiological factor (dental plaque), on one hand, and taking of maximum preventive measures, on the other (oral hygiene and preventive treatment with GC Tooth Mousse), conditions are created for the pre-emption of remineralization processes over demineralization, by which the mineral deficit is replaced[10]. In an acidic environment, the CPP-ACP compound releases ions of calcium and phosphate, which helps supersaturation of the oral cavity. This has been confirmed with the results obtained in the in vitro study of Zabokova-Bilbilova E.[11]. Namely, the obtained higher values of the mass fraction of Ca in the enamel after one-month application of the preventive dental cream GC Tooth Mousse have confirmed this statement. The enamel after three months of application of the preventive dental cream shows significantly higher values not only of the calcium, but also of the other examined elements (Na, K and Mg).

The objective in this study was to determine the concentration of Ca and Mg in artificial saliva after preventive treatment.

Methods

The laboratory examination (*in vitro*) was performed in 90 healthy teeth extracted for orthodontic reasons. Three groups of thirty teeth were formed. The teeth from each group were separated in half in bucco-lingual direction. Thus, the control and test specimens were obtained from the same teeth. The brackets were bonded with GC Fuji Ortho^{TM} LC (GC America Chicago, III), a resin-modified glass ionomer cement and the teeth then were stored in artificial saliva[12] consisting of 20 mmol/l $NaHCO_3$, 3 mmol/l NaH_2PO_4, and 1 mmol/l $CaCl_2$ at room temperature, neutral pH. Once per day the teeth were coated with topical gel - CG Tooth Mousse in duration of 5 minutes and then returned to artificial saliva. Preventive treatment was at certain intervals of 1, 3 and 6 months. After each study period, the artificial saliva where the samples were stored was used for determination of Ca and Mg concentration by flame atomic absorption spectrophotometry[13,14], as follows: we created a calibration diagram, determined the level of Ca and Mg and calculated the concentration by the formula.

Regresiona analiza je pokazala funkcionalnu vezu između koncentracije i absorbanse Ca i Mg. Za konstrukciju kalibracionog dijagrama korišćen je standardni rastvor Ca i Mg koncentracije 1mg/L. Srednja apsorbansa za svaki standardni rastvor kalcijuma data je u Tabeli 1 i Figuri 1.

Analitička zavisnost od apsorpcije koncentracije Ca data je sledećom jednačinom:
$A = 0{,}009 \bullet \gamma(Ca)/\ \mu g/mL$
Koeficijent korelacije je bio: 0,9963.

Analitička zavisnost od apsorpcije koncentracije Mg data je sledećom jednačinom: $A = 0{,}1598 \bullet \gamma(Mg)/\mu g/mL + 0{,}0105$

Koefijent korelacije je bio: 0,994. Kalibracioni dijagram za određivanje koncentracije Mg u pljuvački sa FAAP prikazan je na Figuri 2.

Tabela 1.Apsorpcija za odgovarajuće koncentracije Ca u veštačkoj pljuvački

Table 1. Absorbance for appropriate concentrations of Ca in artificial saliva

γ(Ca)/μg/ml	*A*
0	0
1	0.010
3	0.031
5	0.045
10	0.089

Tabela 2.Vrednosti koncentracije Ca u veštačkoj pljuvački ispitivane grupe(mmol/L)

Table 2. Values of the concentration of Ca in the artificial saliva in the examined group (mmol/L)

time	$\overline{X}$	**SD**	**N**
1 month	3.19	0.86	30
3 months	3.86	1.30	30
6 months	1.89	1.21	30

Statistička analiza

Za statističku procenu korišćena je analiza varijanse (ANOVA) kako bi se utvrdilo da li postoji statistički značajna razlika između ispitivanih grupa.

Construction of calibration diagram

Calibration diagram was constructed by using a method of standard solutions for the analyzed element. Regression analysis showed functional relationship between concentration and absorbance of Ca and Mg.

For construction of calibration diagram, standard solutions of Ca and Mg with concentration 1 mg/L were used. Means of absorbance for each calcium standard solution are given in Table 1. and Figure 1.

Analytical dependence on absorbance of Ca concentration was given by the following equation:

$A = 0.009\ \gamma(Ca)/\ \mu g/mL$

Correlation coefficient was 0.9963.

Analytical dependence on absorbance of Mg concentration was given by the following equation:

$A = 0.1598\ \gamma(Mg)/\mu g/mL + 0.0105$

Correlation coefficient was 0.994. Calibration diagram for determination of Mg in saliva with FAAP is given in Figure 2.

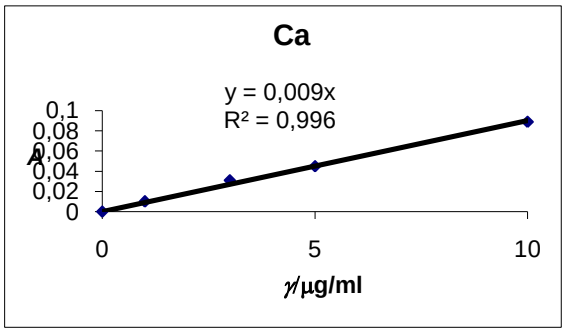

Figura 1.Kalibracioni dijagram za određivanje Ca u pljuvački sa FAAP

Figure 1. Calibration diagram for determination of Ca in saliva with FAAP

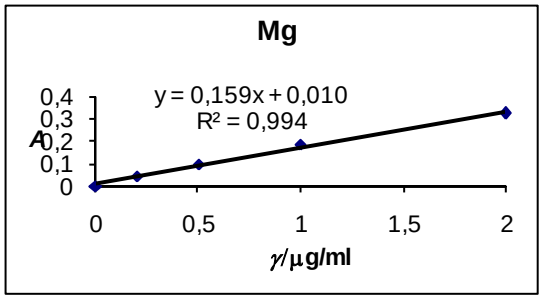

Figura 2.Kalibracioni dijagram za određivanje Mg u pljuvački sa FAAP

Figure 2. Calibration diagram for determination of Mg in saliva with FAAP

Rezultati

U studijskoj grupi utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Ca u veštačkoj pljuvački nakon tretmana od 1,3 i 6 meseci (Analiza varijanse:F=23,013; p=0,00001); (Tabela2., Figura 3.). Tukey (HSD) test zanačjnosti je pokazao da nema statistički značajne razlike između tretmana od 1 i 3 meseca, dok je između tretmana od 1 i 6, i tretmana od 3 i 6 meseci, utvrđena statistički značajna razlika (Tabela 3).

Nakon tretmana od 1,3 i 6 meseci, utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Ca u veštačkoj pljuvački između ispitivane i studijske grupe (Tabela 4, Figura 4).

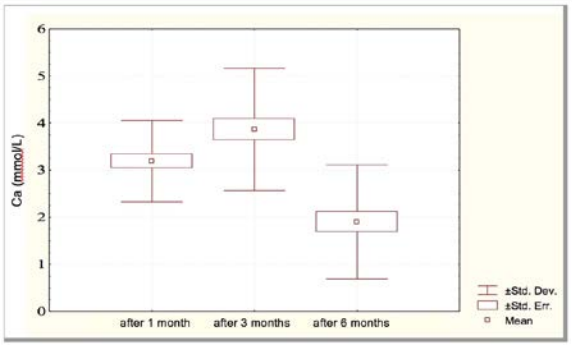

Figura 3.Vrednosti koncentracije Ca u veštačkoj pljuvački ispitivane grupe

Figure 3. Values of the concentration of Ca in the artificial saliva in the examined group

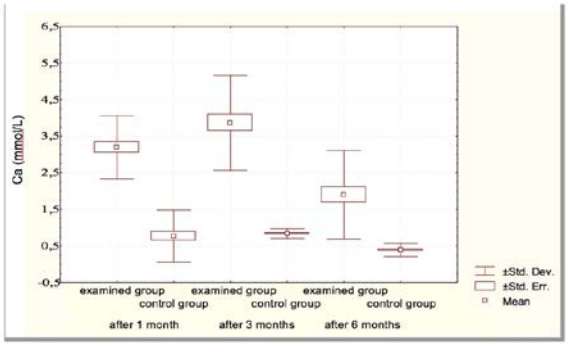

Figura 4. Komparativni prikaz vrednosti koncentracija Ca u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

Figure 4. Comparative overview of the values of the concentration of Ca in the artificial saliva in the examined and control group (mmol/L)

Statistical analysis

For statistical evaluation, a one-way analysis of variance (ANOVA) was initially used to see if there was a significant difference between groups.

Results

In the study group, there were statistically significant differences in relation to the mean values of Ca concentration in the artificial saliva after treatment of 1, 3 and 6 months (Variance analysis: F=23.013; p=0.00001) (Table 2., Figure 3.). The Tukey honest significant difference (HSD) test showed that the differences were not significant for treatment of 1 and 3 months, and for treatment of 1 and 6 months, and treatment of 3 and 6 months they were statistically significant (Table 3.).

After treatment of 1, 3 and 6 months, there were statistically significant differences in relation to the mean values of Ca concentration in the artificial saliva between the examined and the control group (Table 4. and Figure 4.).

Tabela 3. Razlike između vrednosti koncentracije Ca u veštačkoj pljuvački u ispitivanoj grupi

Table 3. Differences between the values of Ca concentration in the artificial saliva in the examined group

time	p
1 - 3 months	0.0634
1 - 6 months	0.00019*
3 - 6 months	0.00010*

*Tukey (HSD) test

Tabela 4. Komparativni prikaz vrednosti koncentracija Ca u veštačkoj pljuvački ispitivane i kontrolne grupe(mmol/L)

Table 4. Comparative overview of the values of the concentration of Ca in the artificial saliva in the examined and control group (mmol/L)

time	examined group		control group		t	p
	$\overline{X}$	SD	$\overline{X}$	SD		
1 month	3.19	0.86	0.76	0.71	11.874	0.00000001*
3 months	3.86	1.30	0.83	0.13	12.696	0.00000001*
6 months	1.89	1.21	0.38	0.17	6.764	0.000001*

*statistically significant differences

U studijskoj grupi nije utvrđena statistički značajna razlika u pogledu srednje vrednosti koncentracije Mg u veštačkoj pljuvački nakon tretmana od 1,3 i 6 meseci (analiza varijanse: F=1,779; p=0,1747) (Tabela 5, Figura 5.). Tukey (HSD) test je pokazao razliku između srednje vrednosti koncentracije Mg u veštačkoj pljuvački tokom tretmana za 1,3 i 6 meseci ponaosob (Tabela 6).

Nakon tretmana od 1,3 i 6 meseci, utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Mg u veštačkoj pljuvački između studijske i kontrolne grupe(Tabela 7. i Figura 6.).

In the study group, there were no statistically significant differences in relation to the mean values of Mg concentration in the artificial saliva after treatment of 1,3 and 6 months (variance analysis: F=1.779; p=0.1747) (Table 5., Figure 5.). Tukey honest significant difference (HSD) test showed differences between mean values of Mg concentration in the artificial saliva during treatment for 1, 3 and 6 months alone (Table 6.).

After treatment of 1, 3 and 6 months, there were statistically significant differences in relation to the mean values of Mg concentration in the artificial saliva between the examined and control group (Table 7. and Figure 6.).

Tabela 5. Vrednosti koncentracije Mg u arteficijalnoj pljuvački ispitivane grupe

Table 5. Values of the concentration of Mg in the artificial saliva in the examined group (mmol/L)

time	$\overline{X}$	SD	N
1 month	0.438	0.164	30
3 months	0.427	0.162	30
6 months	0.368	0.129	30

Tabela 6.Razlike u vrednostima koncentracije Mg u veštačkoj pljuvački u ispitivanoj grupi

Table 6. Differences between the values of the concentration of Mg in the artificial saliva in the examined group

time	p
1 - 3 months	0.9633
1 - 6 months	0.1937
3 - 6 months	0.3018

*****Tukey (HSD) test

Tabela 7. Komparativni prikaz vrednosti koncentracija Mg u veštačkoj pljuvački ispitivane i kontrolne grupe(mmol/L)

Table 7. Comparative overview of the values of the concentration of Mg in the artificial saliva in the examined and control group (mmol/L)

time	examined group		control group		t	p
	$\overline{X}$	SD	$\overline{X}$	SD		
1 month	0.438	0.164	0.351	0.154	2.112	0.03894*
3 months	0.427	0.162	0.282	0.096	4.174	0.00010*
6 months	0.368	0.129	0.185	0.092	6.316	0.00001*

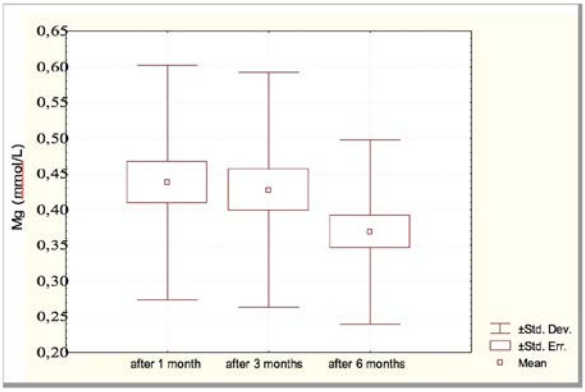

Figura 5.Vrednosti koncentracije Mg u arteficijalnoj pljuvački ispitivane grupe(mmol/L)

Figure 5. Values of the concentration of Mg in the artificial saliva in the examined group (mmol/L)

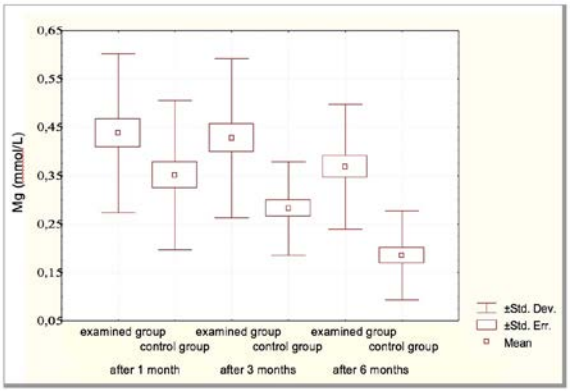

Figura 6. Komparativni prikaz vrednosti koncentracija Mg u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

Figure 6. Comparative overview of the values of the concentration of Mg in the artificial saliva in the examined and control group (mmol/L)

Diskusija

Dental krema GC Tooth Mousse ostvaruje svoj antikariogeni efekat uz pomoć CPP-ACP. Mehanizam delovanja CPP-ACP uključuje sjedinjavanje nanokompleksa u zubnom plaku i na površini gingive, delujući kao rezervoar kalcijuma i fosfata. Rejnoldsove studije[1] ukazuju da CPP-ACP inkorporiran u zubni plak može značajno povećati nivo kalcijuma i fosfatnih jona u plaku. Razlog je dejstvo preventivnog preparata GC Tooth Mousse, koji pomaže u sprečavanju demineralizacije. Ivice gleđi remineralizovane zbog izloženosti CPP-ACP otporne su na lateralnu indukciju kiseline u poređenju sa normalnom remineralizovanom gleđi, jer CPP-ACP ima sposobnost da olakša remineralizaciju lezija makule albe na gleđi zbog prisustva hidroksilapatia. Relativno niska nukleotidna regija lezija tretiranih sa CPP-ACP jedinjenjima, takođe može pokazati bolju kristalizaciju i nižu mikrostrukturu od intaktne gleđne mase. To je efekat koji se očekuje u ortodontskom tretmanu fiksnim aparatom koji je potvrđen istraživanjima više autora.

In vitro podaci pokazuju da redovna upotreba preventivne stomatološke kreme GC Tooth Mousse smanjuje mogućnost demineralizacije gleđi oko bravica i različitih ortodontskih elemenata koji se koriste tokom ortodontskog tretmana. U isto vreme, ona stimuliše proces remineralizacije, što podrazumeva vraćanje minerala izgubljenih iz hidroksilapatita gleđi.

Discussion

Dental Cream GC Tooth Mousse accomplishes its effect by the use of CPP-ACP. The mechanism of action of CPP-ACP involves the unification of the nanocomplex in the dental plaque and the surface of the gum, acting as a calcium and phosphate reservoir. Studies of Reynolds[1] suggest that CPP-ACP incorporated into the dental plaque can significantly increase the levels of calcium and phosphate ions in the plaque. This is due to the action of preventive dental cream GC Tooth Mousse, which helps in prevention of demineralization of the enamel. The enamel blades remineralized by the surface exposure of CPP-ACP are resistant to lateral acid induction compared to normal remineralized enamel, since CPP-ACP has the ability to help remineralization of white spot lesions on the enamel with hydroxylapatite. The relatively low nucleotide region of the lesions treated with CPP-ACP compounds can also show a better crystallization and lower microstructure than an intact enamel. This is the effect expected in the orthodontic treatment with a fixed apparatus which has been confirmed by the research of several authors.

In vitro data show that the regular use of preventive dental cream GC Tooth Mousse reduces the possibility of demineralization of the enamel around the brackets and the various orthodontic elements used during orthodontic treatment. At the same time, it stimulates the process of remineralization, which involves the reverting of minerals lost from the hydroxylapatite of the enamel.

Mnoge laboratorijske studije[15-17], uključujući i ovu, ukazuju na efekat oslobađanja Ca iz veštačke pljuvačke. Rezultati koncentracije kalcijuma u veštačkoj pljuvački pokazuju više vrednosti posle jednomesečne aplikacije dentalne kreme Tooth mousse GC (3,19 mmol/l) u poređenju sa kontrolnom grupom gde je vrednost bila (0,86 mmol/l). Procena rezultata dobijenih posle tromesečne upotrebe GC Tooth Mousse pokazuje statističku značajnost u poređenju sa kontrolnom grupom, pri čemu su vrednosti Ca u veštačkoj pljuvački bili 3,86 mmol/L, dok je koncentracija Ca u kontrolnoj grupi bila 1,30mmol/L. Posle šestomesečnog perioda vrednost kalcijuma u veštačkoj pljuvački u kojoj su čuvani zubi bila je (1,89 mmol/L). Rezultati izričito ukazuju na smanjenje vrednosti kalcijuma u veštačkoj pljuvački u ovom šestomesečnom periodu, što je rezultat veće gleđne apsorpcije .

Posle preventivne preparacije, sa protokom vremena, uz upotrebu paste GC Tooth Mouss, najveće povećanje je zabeleženo u prvom tromesčju, za sve ispitivane mikrolelmente (Ca i Mg). Veštačka pljuvačka u maloj meri sadrži Ca i Mg, važne mikroelemente za maturaciju, što je takođe slučaj i u prirodnoj sredini i ima pozitivan efekat na maturaciju gleđi.

Poređenje vrednosti dobijenih za ispitivane elemente (Ca i Mg), u veštačkoj pljuvački u različitim vremenskim intervalima (posle 1,3 i 6 meseci), sa nalazima drugih autora nije urađeno, zbog oskudnosti takvih podataka u raspoloživim naučnim studijama, što govori u prilog originalnim nalazima predstavljenim u ovoj studiji. U fazi inicijalne karijesne lezije sa eliminacijom etiološkog faktora, (zubni plak) na jednoj strani, i uz primenu maksimalnih preventivnih mera (oralna higijena i preventivni tretman sa GC Tooth Mousse) s druge strane, stvaraju se uslovi za remineralizaciju posle procesa demineralizacije, koji kompenzuju deficit minerala i mogu dostići biološku reparaciju [18,19]. U kiseloj sredini, CPP-ACP jedinjenje oslobađa jone kalcijuma i fosfata, koji pomažu supersaturaciji usne duplje.

Many laboratory studies[15-17], including this study, show the effect of the release of Ca in the artificial saliva. The results of the concentration of Ca in the artificial saliva showed higher Ca values after one-month application of dental cream Tooth Mousse GC (3.19 mmol/L), and they were higher than the concentration in Ca control group (0.86 mmol/L). Evaluation of the data obtained after a three-month application of GC Tooth Mousse showed a statistically significant difference in relation to the control group; the concentration of Ca in the artificial saliva at this time interval was 3.86 mmol/L, compared to the concentration of Ca in the control group (1.30 mmol/L). After a six-month application of the dental cream there was a significantly lower level of calcium in the artificial saliva where the tooth (1.89 mmol/L) was kept. The results pointed out to a significant decrease in Ca in the artificial saliva in this period (six months), as a result of its greater absorption in the enamel.

With the increase in the time interval since the application of the preventative preparation, in this case the dental cream GC Tooth Mousse, the highest increase in three months was observed for all examined microelements (Ca and Mg). The fading of the contents of the artificial saliva with Ca and Mg, important elements for the maturation, suggests that this also occurs in vivo (in the oral environment), with a positive reflection on the maturation of the enamel.

Comparison of the values obtained for the examined elements (Ca and Mg) in the this domain. This, however, speaks in favor of the original findings presented in this study. In the phase of initial carious lesion with the elimination of the etiological factor (dental plaque) on one side, and taking the maximum preventive measures (oral hygiene and preventive treatment with GC Tooth Mousse), on the other hand, conditions are created for the remineralization processes over demineralization, which compensates for the mineral deficit and can reach biological reparations[18,19]. In an acidic environment, the CPP-ACP compound releases ions of calcium and phosphate, which helps supersaturation of the oral emptiness.

Zaključak

Neophodno je očuvati zdravlje zuba i integriteta gleđi u periodu fiksne ortodontske terapije primenom odgovarajućih preventi - vnih mera. Očekivani efekat ovih mera je inhibicija procesa demineralizacije gleđi. Neophodno je da pacijenti obavljaju oralnu higijenu, ali treba naglasiti da je kod ovih pacijenata povremeno potrebno profesio-nalno uklanjanje plaka.

Rezultati ove studije (*in vitro*) pokazali su da su koncentracije Ca i Mg u veštačkoj pljuvački značajno povećane i nakon prvog meseca primene ovog sredstva, koje oslo-bađa Ca i druge minerale (uključujući cement bez fluora, koji se koristi za lepljenje bravica), sa maksimalnom vrednošću posle tromesečne aplikacije. Ove vrednosti su bile značajno niže posle šest meseci, verovatno kao rezultat njegove apsorpcije u gleđi. Rezultati za Mg su isti kao i za Ca, pri čemu se vrenost povećava, kao i njena stabilnost u pljuvački nakon prvog meseca upotrebe.

Rezultati ove *in vitro* studije jasno pokazuju da preventivni materijali koji se koriste u fiksnom ortodontskom tretmanu inhibiraju demineralizaciju gleđi oko bravica i ortodontskih žica. Pored primarne oralne higijene,preporučuje sei njihova upotreba kao dodatne preventivne metode/sredstva.

Conclusion

It is an imperative to preserve the dental health and the integrity of enamel in the period during fixed orthodontic treatment by application of appropriate preventive measures. The expected effect of these measures is the inhibition of the demineralization processes in the enamel. It is essential to practice oral hygiene by the patients, but it has to be emphasized that occasional professional removal of the plaque in these patients is required.

The results of this study (*in vitro*) showed that the concentrations of Ca and Mg in artificial saliva were significantly increased even after the first month of application of this means, which releases Ca and other minerals (including fluoride free cement used to bond the brackets) with maximum value after a three-month application. These values were significantly lower after six months, probably as a result of its absorption into the enamel. The results for Mg are the same as for Ca, and the value is increased as well as its stability in saliva after the first month of use.

The results of this *in vitro* study clearly indicated that preventive materials used in fixed orthodontic treatment inhibited the demineralization of enamel around brackets and orthodontic rings. Evidently, their use as additional preventive methods/tools, besides primary oral hygiene, is recommended.

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EVALUACIJA PRIMENLJIVOSTI PONTOVOG INDEKSA U SRPSKOJ POPULACIJI

EVALUATION OF APPLICABILITY OF PONT'S INDEX IN SERBIAN POPULATION

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Sažetak

Uvod: *Primena Pontovog indeksa u ortodontskoj dijagnostici koristi se za predviđanje idealne širine zubnog luka, neophodne za smeštaj zuba bez teskobe, pravilnu okluziju i postizanje stabilnih rezultata terapije.*
Cilj : *ove studije bio je utvrditi primenljivost Pontovog indeksa u srpskoj populaciji i uporediti dobijene rezultate sa rezultatima ispitivanju u drugim populacijama.*
Materijal i metode: *Analizirano je 200 studijskih modela osoba srpskog porekla (100 osoba muškog pola i 100 osoba ženskog pola). Svi ispitanici su bili sa klasom I po Angleu, bez prethodnog ortodontskog tretmana. Merenje je izvršeno pomoću digitalnog nonijusa (preciznost od 0,01 mm). Izmerene su meziodistalna širina maksilarnih stalnih sekutića, kao i interpremolarna i intermolarna širina maksilarnog luka. T-testom je uporedjena vrednost standardnog Pontovog indeksa i Pontovog indeksa dobijenog u istraživanju, kao i razlike dobijenih vrednosti izmedju polova. Određen je Pearsonov koeficijent korelacije između izmerenih i vrednosti širine zubnih lukova dobijenih korišćenjem Pontove formule.*
Rezultati: *Rezultati ispitivanja pokazali su da su vrednosti za interpremolarnu i za intermolarnu širinu luka, izmerene na studijskim modelima pacijenata kod oba pola, manje nego vrednosti koje su dobijene primenom Pontove formule ($p<0,001$). Pearsonov koeficijent korelacije izmedju izmerenih vrednosti širine luka i vrednosti širine luka prema Pontovoj formuli pored visoke korelacije pokazuje statitički značajnu razliku ($p<0,001$).*
Zaključak: *Dobijeni rezultati ukazuju da Pontov indeks nije primenljiv u srpskoj populaciji, te da su potrebni prilagodjeni populaciono specifični standardi za ovu dijagnostičku metodu.*

Ključne reči: *ortodoncija, Pontov indeks, srpska populacija*

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Abstract

Introduction: *The applicability of Pont's Index n orthodontic patients may guide clinicians in predicting an ideal arch width, necessary to accommodate the dentition, unloose crowding and to produce more stable final results.*
The aim: *of this study was to establish the applicability of Pont's Index to Serbian population and compare the results with those acquired from studies conducted on different ethnic subjects.*
Materials and methods: *Dental casts of 200 Serbian subjects (100 males and 100 females) were used in this study. All subjects had normal Class I occlusion, with no history of orthodontic treatment. Measurements were taken by a digital caliper (precision of 0.01 mm). The mesiodistal widths of the maxillary permanent incisors, as well as interpremolar and intermolar maxillary arch widths, were measured. Using t-test Pont's Index ratios between this study and Pont's study and between genders were compared. Pearson correlation coefficients between measured and arch width values according to Pont's formulae were determined.*
Results: *Although correlations determined between the calculated arch width and corresponding values calculated using Pont's Index were high in all cases for both genders, a statistically significant difference was observed ($p<0.001$). Calculated Pearson coefficient between real and measured arch width are found to be high and statistically significant.*
Conclusion: *Our results suggest that the Pont's Index was not applicable to Serbian population and specific standards for this population might be needed.*

Key words: *orthodontic, Pont's index, Serbian population*

Uvod

Karakteristike ljudskih vilica, zajedno sa zubima, mogu pružiti jake dokaze o postojanju polnog i etničkog dimorfizma[1,2]. Genetski, epigenetski i filogenetski aspekti imaju značajan uticaj na razvoj ovih karakteristika koje mogu imati odlučujuću ulogu kako u forenzičkoj identifikaciji, tako i u ortodontskoj dijagnozi i terapiji[3]. Brojna ispitivanja pokazala su da veličina zuba zavisi od pola[1,4-7] kao i da postoji korelacija između veličine zuba i etničkog porekla[8,9].

U planiranju terapije ortodonti se često suočavaju sa izazovom da predvide idealnu širinu zubnog luka potrebnu za pravilan smeštaj zuba. Podaci dobijeni analizom studijskog modela imaju ključnu ulogu u postavljanju dijagnoze, planiranju terapije i prognozi terapije[10]. Malokluzije praćene teskobom mogu se rešavati širenjem zubnih nizova ili redukcijom zubnog materijala (ekstrakcionom terapijom). Slučajevi rešavani neekstrakcionom terapijom zahtevaju širenje zubnog luka, pa se u ortodontskoj praksi primenjuje nekoliko indeksa i metoda kojima se može predvideti idealna širina zubnog luka, koja omogućava zadovoljavajuće i stabilne rezultate ortodontske terapije. Iako se zasniva na pretpostavci da se odstupanja od normalne okluzije mogu precizno izmeriti i da se ortodontska dijagnoza može zasnivati na matematičkim kalkulacijama, mogućnost predvidjanja širine zubnog luka je korisno dijagnostičko sredstvo. Brojne analize koje se koriste u svakodnevnoj kliničkoj praksi imaju za cilj da omoguće stabilne rezultate predviđanjem idealne širine zubnih lukova[11,12].

Prema Pontu[13] idealna širina zubnog luka neophodna za pravilnu okluziju i odsustvo teskobe predstavlja konstantni odnos izmedju zbira meziodistalnih promera stalnih maksilarnih inciziva (SI) i interpremolarne i intermolarne širine zubnog luka.

IPŠ = SI/0,80

IMŠ = SI/0,64

IPŠ – interpremolarna širina luka

IMŠ – intermolarna širina luka

SI - suma incizivi, zbir meziodistalnih promera maksilarnih stalnih sekutića.

Vrednosti za Pontov indeks (PI) dobijene su na osnovu ispitivanja veličine zuba i vilica na francuskoj populaciji. Uzimajući u obzir da postoje razlike u veličini zuba između etničkih grupa postavlja se pitanje primene ovog indeksa na drugim populacijama[14]. Nalazi nekih istraživača podržavaju upotrebu PI u predviđanju idealne širne zubnog luka[15,16],

Introduction

Human dental bone characteristics, alongside with teeth, can provide strong evidence about sexual and ethnic dimorphism[1,2]. Genetic, epigenetic and phylogenetic aspects have a significant influence on the development of these characteristics, which could play the decisive role in the forensic identification, orthodontic diagnosis and treatment[3]. As previously established by numerous investigations, tooth size is gender-dependant[1,4-7], and the correlation between tooth size and ethnic origin was confirmed[8,9].

In the treatment planning, the orthodontists are frequently challenged to predict the ideal arch width necessary for normal occlusion. Information collected from the dental casts provide a crucial part in the diagnosis, treatment planning and clinical prognosis[10]. Malocclusion with crowding can be resolved by the expansion of dental arch or by the extraction therapy. In some cases where it is uncertain which therapy would be the best, many formulas, indexes and tables can be used to calculate the ideal width of the dental arch. Although based on the assumption that the deviation of normal occlusion can be mathematically determined, the assessment of the ideal width of the dental arch is a useful diagnostic tool. In order to produce more stable results and to predict the ideal arch width, different analysis were conducted in everyday clinical practice[11,12].

Pont[13] described an ideal arch width, required to accommodate the dentition and facilitate crowding, that can be calculated using the following formula:

Interpremolar arch width (IPW) = SI/0.80

Intermolar arch width (IMW) = SI/0.64

SI- suma incisivi, the sum of mesiodistal diameter of maxillary permanent incisors.

However, the applicability of Pont's Index is controversial[14], since Pont's study was conducted on the French population, and considering that the differences between ethnic groups may exist, it is reasonable to test the Pont's Index on other populations as well[14]. The findings of some investigations support the use of Pont's Index in assessment of ideal arch widths[15,16], while others believe that the Pont's Index is not universally valid and its use should be adjusted to the different ethnics groups[16,17-20].

dok drugi smatraju da on nije validan i da se ne može upotrebljavati u kliničkoj praksi[16,17-20].

Ovo je prvo ispitivanje sprovedeno sa ciljem da uporedi dobijene vrednosti sa vrednostima iz literature i da proceni primenu Pontovog indeksa kod ortodontskih pacijenata u srpskoj populaciji.

Materijal i metode

Ispitano je 200 studijskih modela (100 muškaraca i 100 žena), pacijenata srpskog porekla, sa okluzijom I klase, bez prethodnog ortodontskog tretmana starosti od 13 do 16 godina (14,35±1,14). Kriterijumi za izbor pacijenata bili su: prisustvo svih stalnih zuba u oba luka (isključujući druge i treće molare) dovoljno izniklih da omoguće premeravanja meziodistalnog promera, dobar kvalitet studijskih modela, odsustvo abrazije zuba, karijesnih lezija, plombi II klase, odsustvo protetskih ili kompozitnih nadoknada, odsustvo anomalija u obliku, strukturi i razvoju zuba.

Premeravanja su vršena digitalnim nonijusom japanske proizvodnje (Model No. CD6 GS, Mitoyoto, Tokyo) sa preciznošću od 0,01 mm. Sva merenja su izvršena od strane jednog ispitivača sa velikim isksutvom u merenju. Meren je meziodistalni promer četiri maksilarna inciziva i maksilarno interpremolarno (najdublja tačka centralne fisure prvih premolara) i intermolarno rastojanje (najdublja tačka centralne fisure prvih molara). Greška premeravanja određena je poređenjem vrednosti ponovnog merenja deset modela izabranih slučajno, deset dana nakon prvog premeravanja Wilcoxon statističkim testom. Rezultati nisu pokazali statistički signifikantnu razliku između dva premeravanja.

Statistička analiza

Statitički softver IBM SPSS 20 korišćen je za analizu podataka. Prosečne vrednosti, standardna devijacija i koeficijent varijacije su izračunati za muški i ženski pol odvojeno. Širina luka izračunata je za svakog pacijenta prema Pontovoj formuli i odredjen je koeficijent korelacije između izmerenih i izračunatih vrednosti.

To the best of our knowledge, up to now, no study was undertaken to investigate the application of Pont's Index in Serbian population. Thus, this study was initiated in order to estimate the applicability of Pont's Index to the Serbian population.

Materials and methods

For the present study, 200 dental casts (100 males and 100 females) were selected. All the subjects were homogeneous Serbs, 13 to 16 years old (14.35±1.14), with normal class I occlusion and without history of previous orthodontic treatment. The inclusion and exclusion criteria implied: the presence of permanent dentition (excluding second and third molars) with adequately erupted teeth to allow measurements of the mesiodistal crown dimensions; regularly poured study models; the nonexistence of mesiodistal and occlusal abrasion, caries lesions and Class II fillings; the casts without any prosthetic or composite restorations and any tooth anomalies.

The dental casts processing was performed out using a digital caliper with precision of 0.01 mm (Model No. CD6 GS, Mitoyoto Co, Tokyo-Japan). Ten models were randomly selected to be measured twice at an interval of ten days and measured by an experienced examiner (GF). In order to calculate the true interpremolar and intermolar widths - IPW(t) and IMW(t), as well as Pont's estimation for interpremolar and intermolar widths - IPW(e) and IMW(e), mesiodistal crown diameters of the four maxillary incisors, maxillary interpremolar width, maxillary first premolars distal pits and maxillary intermolar width and maxillary first molars central fossae, were measured.

Statistical analysis

Statistical software IBM SPSS 20 was used for data analysis. Average values, standard deviation and variation coefficient were calculated separately for male and female gender. Using Pont's formulae, incisal and arch widths were estimated and Pearson correlation coefficients between the values of calculated and measured arch width were determined. The independent samples t-test

Studentov t-test korišćen je da se odredi signifikantnost razlika u veličini zuba i širine zubnih lukova izmedju polova. Korišćen je Wilcoxon test za određivanje sistemske greške. P vrednosti manje / ili jednako 0,05 se smatraju signifikantnim.

Rezultati

Statističko poređenje pokazalo je da nema značajnih razlika između prvog i drugog premeravanja za meziodistalni promer zuba, maksilarnu interpremolarnu i intermolarnu širinu luka. Rezultati ispitivanja pokazali su da su vrednosti za interpremolarnu i za intermolarnu širinu luka izmerene na studijskim modelima pacijenata kod oba pola manje, nego vrednosti koje su dobijene primenom Pontove formule ($p<0,001$) (Tabela 1 i Tabela 2). Pearsonov koeficijent korelacije poka-zuje da postoji signifikantna korelacija između vrednosti interpremolarne i inter-molarne širine luka, izmerenih direktno na studijskim modelima pacijenata, i vrednosti dobijenih primenom Pontove formule, na nivou značajnosti $p<0,001$ (Tabela 3). U Tabeli 4. prikazana je procentualna zastu-pljenost osoba kod kojih su izmerene vrednosti ispod/iznad ili jednake vredno-stima predviđenim PI (/+ 1 mm). Vrednosti stvarne interpremolarne širine, kod 71,3% osoba muškog pola i kod 66% osoba ženskog pola manje su od očekivanih vrednosti po Pontovoj formuli.

Vrednosti stvarne intermolarne širine, kod 68,3% osoba muškog pola i 66% osoba ženskog pola manje su od očekivanih vrednosti po Pontovoj formuli. Samo kod 12,5% osoba muškog pola i 7,7% osoba ženskog pola (inter-premolarna širina), i 18,8% osoba muškog pola i 20,5% osoba ženskog pola (intermolarna širina) nema signifikantne razlike (−1 mm/+1 mm) u odnosu na očekivane vrednosti.

was employed to detect if there was a significant difference between tooth and/or arch width values for both sexes. Wilcoxon statistical test was applied to determine the systematic error. P values less than/or equal to 0.05 were considered to be significant.

Results

The statistical comparison showed that the differences between the first and second measurements for mesiodistal diameters of teeth and maxillary interpremolar arch width and maxillary intermolar arch width were insignificant. Mean values of interpremolar arch width for both sexes are presented in Table 1 and for the intermolar arch width in Table 2. The results showed that the true interpremolar and intermolar arch widths measured on the casts for both male and female groups were generally lower than the interpremolar and intermolar arch widths estimated by Pont's formula ($p<0.001$). Pearson's correlation coefficient revealed that significant correlations exists between interpremolar and intermolar arch widths determined directly on the dental casts and those estimated by Pont's Index (Table 3; $p<0.001$). Table 4 contains the percentage of subjects whose measured values were under/over or equal to Pont's prediction (/+ 1 mm).

The values of measured interpremolar width in 71% of male subjects and 66% of female subjects were lower than the expected values based on Pont's formula. The values of measured intermolar width in 68.3% of male subjects and 66% of female subjects were lower than the expected values based on Pont's formula.

Only in 12.5% of male subjects and 7.7% of female subjects (interpremolar width) and 18.8% of male subjects and 20.5% of female subjects (intermolar width) there was no significant difference (-1mm/+1mm) between the measured and expected values.

Tabela 1. Deskriptivna analiza za interpremolarnu širinu (mm)
Table 1. Descriptive analysis for interpremolar width (mm)

	Gender					
	Male	(N=101)		Female	(N=100)	
	Mean	(±) SD	CV	Mean	(±) SD	CV
IPW(t)	37.75	1.94*	5.14	36.81	2.15	5.83
IPW(e)	39.93	2.36** [a]	5.92	38.60	2.56	6.63
SI	31.94	1.89**	5.92	30.88	2.05	6.63
SI/ IPW(t)	0.85	0.05	5.76	0.84	0.05	6.50

*p < 0.01, **p < 0.001, a- Pont's estimation vs. true value (p < 0.001); IPW(t) - True interpremolar width as measured on the casts, IPW(e) - Pont's estimation for interpremolar width, SD - Standard Deviation.

Tabela 2. Deskriptivna analiza za intermolarnu širinu (mm)
Table 2. Descriptive analysis for intermolar width (mm)

	Gender					
	Male	(N=101)		Female	(N=100)	
	Mean	(±) SD	CV	Mean	(±) SD	CV
IMW(t)	47.27	2.66**	5.63	45.97	2.24	4.88
IMW(e)	49.91	2.95** [a]	5.92	48.25	3.20	6.63
SI	31.94	1.89**	5.92	30.88	2.05	6.63
SI/IMW(t)	0.68	0.04	6.57	0.67	0.04	5.98

**p < 0.001, a - Pont's estimation vs. true (p < 0.001); IMW(t) - True intermolar width as measured on the casts, IMW(e) - Pont's estimation for intermolar width, SD - Standard deviation.

Tabela 3. Koeficijent korelacije *(r)* između izmerenih i vrednosti širine luka prema Pontovoj formuli
Table 3. Correlation coefficient (*r*) between measured and arch width values according to Pont's formulae

	Males (*n* = 101)		Females (*n* = 100)	
	r	*p*	*r*	*p*
IPW(e)	0.46	< 0.001	0.45	< 0.001
IMW(e)	0.33	< 0.001	0.49	< 0.001

IPW(e) - Pont's estimation for interpremolar width, IMW(e) - Pont's estimation for intermolar width.

Tabela 4. Broj i procenat ispitanika kod kojih su izmerene vrednosti ispod, iznad ili po Pontovim predviđanjima ± 1mm
Table 4. Number and percentage of subjects who have measured values under, over or Pont's prediction ± 1mm

		Under Pont's prediction	Over Pont's prediction	Pont's prediction ±1 mm
IPW	Males (*n*=101)	72 (71.3%)	9 (8.9%)	20 (19.8%)
	Females (*n*=100)	66 (66.0%)	13 (13.0%)	21 (21.0%)
IMW	Males (*n*=101)	69 (68.3%)	12 (11.9%)	20 (19.8%)
	Females (*n*=100)	66 (66.0%)	13 (13.0%)	21 (21.0%)

IMW - Intermolar width

Diskusija

Predvidjanje idealne veličine zubnog luka i postizanje stabilnih rezultata po završetku ortodontske terapije često su tema ispitivanja[12,21]. Jedna od metoda opisana je još 1909. godine od strane Ponta, koji je ispitivanje sproveo na francuskoj populaciji, pri čemu tačan broj ispitanika nije poznat, pa je i sam autor predložio neophodnost provere indeksa na drugim populacijama[13]. Poznata je činjenica da se veličina zuba razlikuje između polova i populacija[1,6-9,22]. Zbog toga je klinička primena Pontovog indeksa bila tema brojnih studija sa namerom da se odredi da li se može koristiti u predviđanju idealne širine zubnog luka kod pripadnika različitih naroda[15-18,20,23-25]. Rezultati ispitivanja sprovedenog na ortodontskim pacijentima u srpskoj populaciji pokazuju da su interpremolarna i intermolarna širina zubnog luka, koje su izmerene na modelu, generalno manje od interpremolarne i intermolarne širine predvidjene Pontovom formulom, p<0,001. Ovi rezultati poklapaju se sa rezultatima dobijenim prethodnim ispitivanjima[14,23]. Medjutim, Nimkarn i sar.[19] opisuju da su predvidjene vrednosti precenjene u odnosu na vrednosti izmerene na studijskim modelima, zato dobijene rezultate treba razmatrati s oprezom.

Primenom Pontovog indeksa kod ispitanika Indijske populacije, Gupta i sar. dobili su signifikantno značajnu zavisnost izmedju veličine širine inciziva i veličine zubnog luka[16].

Discussion

In order to produce more stable results and to predict an ideal arch width, up to now, different clinical studies have been performed[12,21]. One of these was described in 1909 by Pont who acquired the data from French population, but without the exact number of subjects included in the study[13]. Pont also proposed the necessity of testing the index on other populations as well[13]. It is a well known fact that the teeth size varies between sexes and populations[1,6-9,22]. Having in mind these facts and using different criteria, the clinical application of Pont's Index has been extensively investigated in numerous studies[15-18,20,23-25] in order to determine whether the Pont's Index could be used in prediction of an ideal arch width for different populations[23-25].

The results of our study based on Serbian population showed that the values of measured interpremolar arch width and intermolar arch width were generally lower than Pont's prediction (Table 1). These findings were consistent with those found for other populations[14,23]. However, Nimkarn et al.[19] discovered an overestimation of arch widths relative to actual arch width measured on the dental casts. Thus the obtained results should be taken with caution.

By applying Pont's Index on Indian population, Gupta et al. showed significant relationship between the arch widths sum and incisor widths sum[16]. Pont's Index suitability in a sample consisted of Navajo-Indians and American dental medicine students was studied, where the authors

Primena Pontovog indeksa kod pripadnika Navaho indijanaca i studenata stomatologije u USA nije prikladna i autori predlažu revidiranje koje bi omogućilo kliničku primenu[18]. Slične rezultate objavili su Nimkarn i sar. u Indiji[19]. Podaci za populaciju Australijskih aboridžina, Indonežana i Australijanaca bele rase, takodje potvrdjuju da Pontov indeks nije prikladan za upotrebu u kliničkoj praksi[14]. Utvrdjeno je takodje da ovaj indeks ne treba koristiti u ovom obliku u analizi idealnog zubnog luka kod pripadnika turskog naroda[20].

U ispitivanju, Pearsonov koeficijent korelacije pokazao je da postoji značajna korelacija između vrednosti inter-premolarne i intermolarne širine koje su izmerene na studijskim modelima i vrednosti dobijenih matematičkim izračunavanjem. Ovi nalazi su u saglasnosti sa nekim od prethodnih studija[16], a razlikuju se od ispitivanja većeg broja autora koji su objavili slabu korelaciju između očekivane širine i stvarne širine zubnog luka[14,19,23]. Iako postoji statistički značajna pozitivna korelacija izmerenih vrednosti i po formuli sračunatih vrednosti IPŠ i IMŠ, ipak između ovih vrednosti postoji i statistički značajna razlika na maksimalnom nivou statističke značajnosti ($p<0,001$). Dobijeni rezultati navode na zaključak da Pontov indeks u ovom obiku nije odgovarajući za srpsku populaciju. Brojni autori su objavili slične rezultate na osnovu kojih zaključuju da su potrebni populacioni specifični indeksi [14,18,19, 20, 24].

U uzorku zubni lukovi pacijenata su uži u poređenju sa vrednostima dobijenim izračunavanjem, pa se može reći da ova formula precenjuje potrebnu širinu zubnog luka da bi se izbeglo stanje teskobnosti zuba[25].

Zaključak

Pontov indeks koji se koristi u ortodonciji za određivanje potrebne širine zubnog luka za smeštaj zuba u pravilan niz, bez teskobe, nije primenjljiv u postojećem obliku kod pacijenata srpskog porekla, mada treba uzeti ove zaključke s oprezom, s obzirom na to da su doneseni na osnovu analize relativno malog broja studijskih modela (200 modela). Neophodno je sprovesti ispitivanja na velikom uzorku da bi se odredili populaciono specifični standardi za srpsku populaciju.

reported that the Pont's Index usage in clinical practice should be furthermore reviewed[18]. Also, the similar results were presented by Nimkarn et al.[19] The data from Australian Aborigines, Indonesians and white Australian populations, confirmed that the Pont's Index is not applicable in clinical practice[14]. Additionally, it was established that Pont's Index should not be used during the analysis of the ideal arch within Turkish individuals[20]. The previously discussed investigations showed that Pont's Index was uncertain clinical parameter to be used for dental arch width prediction.

In study, calculation of Pearson's correlation coefficient revealed the existence of significant correlations between interpremolar and intermolar arch widths calculated directly on the dental casts and those estimated by Pont's Index. The present findings are in agreement with some of the previous studies[16], however there is still a great number of them which presented opposite results i.e. poor correlation between the anticipated arch width estimated by Pont's Index and the actual arch width calculated directly from the casts[14,19,23]. Although there is statistically significant positive correlation between values, there is also a statistically significant difference between values obtained from formulas for inter-premolar and -molar width ($p<0.001$). The obtained results sugest that Ponts Index in this form is not suitable for Serbian population. Numerous authors also published simmilar findings, concluding that there is a need for population specific indexes[14,18,19,20,24].

In our study, sample arches are narrower compared to Pont sample which indicates that Pont's index inclines to overestimation of the arch width required to unloose crowding[25].

Conclusion

According to the limitations of the study, in a sample of 200 subjects, it could be concluded that Pont's index which is used in orthodontics to determine the necessary width of the dental arch for normal occlusion without crowding is not applicable for the Serbian population in its current form. Thus, it is essential to establish new specific standards for prediction of ideal dental arch based on a larger sample in order to use it in everyday clinical practice.

Koflikt interesa

Autori nemaju nikakvu finansijsku korist ili sukob interesa.

Conflict of Interest

The authors have declared that no COI exists.

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OSLOBAĐANJE POTENCIJALNO TOKSIČNIH KOMPONENTI IZ AKRILATA ZA MEKO PODLAGANJE PROTEZA

A RELEASE OF POTENTIALLY TOXIC COMPONENTS FROM THE ACRYLIC RESINS FOR SOFT RELINING DENTURES

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Sažetak

Uvod: *Meki akrilati pripadaju grupi hladno polimerizovanih materijala koji se aplikuju na bazalnu površinu zubne proteze kako bi se eliminisale mehaničke iritacije, omogućilo ozdravljenje oštećene ili inflamirane sluzokože ili se oralno tkivo pripremilo (kondicioniralo) za prihvatanje nove nadoknade.*
Cilj *istraživanja bio je ispitivanje ritma oslobađanja komponenti mekih akrilata u tri različita modela veštačke pljuvačke, u toku tridesetodnevnog opservacionog perioda.*
Materijal i metode: *U ispitivanju su korišćena dva meka akrilata:poli (etil metakrilat)/ n-butil metakrilat i poli (etil metakrilat) / metil metakrilat, koji su odlagani u tri različita modela veštačke pljuvačke, u okviru tri opservaciona perioda: jedan, sedam i trideset dana. Ispitivanje je obuhvatilo detekciju metil metakrilata, etil metakrilata, butil metakrilata, di butil ftalata i benzoil peroksida tečnom hromatografijom pod visokim pritiskom.*
Rezultati: *Količina oslobođenih komponenti srazmerno se povećava sa porastom trajanja opservacionog perioda, bez obzira na model veštačke pljuvačke.Najviše vrednosti svih ispitivanih parametara uočene su nakon tridesetodnevnog opservacionog perioda. Model veštačke pljuvačke nije uticao na ritam oslobađanja komponenti, što znači da se one nesmetano oslobađaju u usnu duplju bez obzira na sastav pljuvačke pacijenta.*
Zaključak: *Sa porastom dužine opservacionog perioda došlo je i do očvršavanja materijala, čime se završava njihova upotrebna vrednost.*

Ključne reči: *mekiakrilati, potencijalnotoksičnesupstance, HPLC*

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Abstract

Introduction: *Soft acrylic resins belong to a group of cold curing materials that are applied to the basal surface of the dental prosthesis in order to eliminate mechanical irritation, and to allow for the recovery of damaged or inflamed mucous membranes, and prepare the oral tissue to accept a new compensation.*
The aim *of the study was to examine the rhythm of the release of soft acrylic resin components in three different models of artificial saliva, during a thirty-day observation period.*
Material and Methods. *Two soft acrylic resins were used in the study: poly (ethyl methacrylate)/n-butyl methacrylate and poly (ethyl methacrylate)/methyl methacrylate, which were deposited in three different models of artificial saliva within the three observation periods: one, seven and thirty days. The test involved the detection of methyl methacrylate, ethyl methacrylate, butyl methacrylate, dibutyl phthalate and benzoyl peroxide under high-pressure liquid chromatography.*
Results: *The amount of released components increased proportionately with the increase in the duration of the observation period, regardless of the artificial saliva model. The highest values of all tested parameters were detected after a thirty-day observation period. The artificial saliva model did not affect the release of the components, which means they are freely released into the oral cavity regardless of the saliva composition of the patient.*
Conclusion:*As the length of the observation period increased, the material was solidified, thus ending their use value.*

Key words: *soft acrylic resins, potentially toxic substances, HPLC*

Uvod

Meki akrilati pripadaju grupi hladno polimerizovanih materijala koji se aplikuju na bazalnu površinu zubne proteze kako bi se eliminisale mehaničke iritacije, omogućilo ozdravljenje oštećene ili inflamirane sluzokože ili se oralno tkivo pripremilo (kondicioniralo) za prihvatanje nove nadoknade. Meki akrilati ostaju rezilijentni određeni vremenski period i zahvaljujući svojim viskoelastičnim svojstvima obezbeđuju podjednaku raspodelu mastikatornih sila na potporna tkiva, te na taj način uklanjaju bol i tegobe kod nosioca proteza sa veoma redukovanim, oštrim i podminiranim alveolarnim grebenima i preosetljivom ili pokretnom sluzokožom[1,2].

Osnovni polimer (prah) dvokomponentnog sistema mekih akrilata najčešće je poli(etil-metakrilat) (PEMA) ili poli (metil-metakrilat) (PMMA). Tečnost predstavlja mešavinu estarskih plastifikatora (dibutil-ftalat (dBuFt), butil-glikolat) koji čine 30-60% i etanola, kao rastvarača, čiji sadžaj iznosi 4-60% [2,3]. Plastifikatori, koji se dodaju akrilatnim smolama, čine ove materijale mekim na temperaturi tela[4]. Plastifikatori su rastvorljivi u oralnim tečnostima, a njihov postepeni gubitak vremenom vodi ka otvrdnjavanju materijala u ustima pacijenta[5]. Aktivator hladne polimerizacije je benzoil peroksid (BP).

Pri mešanju praha i tečnosti čestice polimera apsorbuju etanol. Čestice PEMA manje molekulske mase omogućavaju bržu i obimniju penetraciju alkohola unutar polimera u odnosu na klasični PMMA. Sa druge strane, dejstvom međumolekulskih Van der Valsovih sila lanci polimera se separiraju, pa se veliki molekuli plastifikatora interponiraju između njih. Nakon homogenizacije cela masa dobija želatinoznu formu zbog lanaca polimera zarobljenih unutar matriksa sastavljenog od etanola i plastifikatora[1].

Oslobađanje etanola iz polimerizovanog kondicionera u oralnu sredinu započinje neposredno nakon predaje proteze pacijentu, što se kompenzuje apsorpcijom vode[2,3]. Kako se gubitak etanola i apsorpcija vode ne odigravaju istovremeno i istom brzinom, fizička svojstva materijala se vremenom menjaju. Nakon okončavanja apsorpcionog postupka, materijal potpuno očvrsne, pa ga je potrebno zameniti ili protezu koristiti kao definitivnu[6,7].

Introduction

Soft acrylic resins belong to a group of cold curing materials that are applied to the basal surface of the dental prosthesis in order to eliminate mechanical irritation, and to allow for the recovery of damaged or inflamed mucous membranes, and prepare the oral tissue to accept a new compensation. Soft acrylic resins remain resistant for a certain period of time and, thanks to their viscoelastic properties, provide an equal distribution of the masticatory forces to the supporting tissues, thus removing the pain and distress of the prosthesis carrier with very reduced, sharp and submerged alveolar ridge and hypersensitive or movable mucous membrane[1,2].

The basic polymer (powder) of a two-component soft acrylate system is usually poly (ethyl methacrylate) (PEMA) or poly (methyl methacrylate) (PMMA). The liquid is a mixture of ester plasticizers (dibutyl-phthalate (DBP), butyl glycolate) consisting of 30-60% of ethanol as a solvent, which content is about 4-60%[2,3]. Plasticizers, which are added to acrylic resins, make these materials soft at body temperature [4]. Plasticizers are soluble in oral fluids, and their gradual loss in time leads to the hardening of the material in the mouth of the patient[5]. The cold curing agent is benzoyl peroxide (BP).

Ethanol is absorbed by the polymer particles when mixing the powder with the liquid. The particles of the PEMA with smaller molecular weight allow for faster and more extensive penetration of alcohol within the polymer than the conventional PMMA. On the other hand, by the action of intermolecular Van der Waals forces, the chains of the polymer are separated, so that the large molecules of the plasticizer interpenetrate between them. After homogenization, the whole mass is obtained by gelatinous form due to the chains of polymers trapped inside the matrix composed of ethanol and plasticizers[1].

The release of ethanol from the polymerized conditioner into the oral environment begins immediately after giving the dentures to the patient, which is compensated by water absorption[2,3]. Since the loss of ethanol and water absorption do not occur simultaneously and at the same speed, the physical properties of the material change over time. After the end of the absorption process, the material is fully hardened, so it should be replaced or the dentures used as a definitive one[6,7].

Komponente koje ulaze u sastav mekih akrilata u literaturi mogu biti toksične za oralna tkiva ukoliko se u potpunosti ne neutralizuju u procesu polimerizacije[8-11]. Kako ne postoji apsolutno vezivanje materijala u toku njegove pripreme i izrade zubnih proteza, u istraživanju se krenulo od pretpostavke da će u vremenu korišćenja mekih akrilata kao lajnera doći i do oslobađanja komponenti u okolni tečni medijum.

Cilj istraživanja bio je ispitivanje ritma oslobađanja komponenti mekih akrilata u tri različita modela veštačke pljuvačke, u toku tridesetodnevnog opservacionog perioda.

Materijal i metode

U ispitivanju su korišćena dva meka akrilata: *Lang Flexacryl* (PEMA/ n-butil metakrilat) i *Lang Immediate*(PEMA/ metil metakrilat) (Lang Dental MFG.Co., SAD).
Od oba materijala napravljeno je po 27 uzoraka dimenzija 10 x 10 x 2 mmpolimerizovnih prema uputstvu proizvođača, u kalupu od kondenzacionog silikona. Nakon izrade uzorci su pokazivali očekivanu rezilijentnost.

Uzorci su podeljeni u tri eksperimentalne grupe od po 3 uzoraka, koji su potapani u tri različita modela veštačke pljuvačke (tabela 1), u toku tri opservaciona perioda:jedan, sedam i trideset dana (*n*=27).

According to the literature, the components included in the structure of soft acrylic resins may be toxic to oral tissues if they are not completely neutralized in the polymerization process[8-11]. Since there is no absolute bonding of material during its preparation and development of dental prostheses, the research started from the assumption that at the time of using soft acrylates as a liner, the components will be released into the surrounding liquid medium.

The aim of the study was to examine the rhythm of the release of soft acrylate components in three different models of artificial saliva, during a thirty-day observation period.

Material and methods

Two soft acrylic resins that were used during the study are *Lang Flexacryl* (PEMA/n-butyl methacrylate) and *Lang Immediate* (PEMA/methyl methacrylate) (Lang Dental MFG.Co., USA).

27 samples were made of both materials (dimension: 10x10x2 mm) according to the manufacturer's instructions, in a condensation silicone mold. After making the samples, they showed the expected resilience.

Samples were divided into three experimental groups consisting of three samples, submerged in three different models of artificial saliva (Table 1), during three observation periods: one, seven and thirty days (*n = 27*).

Table 1. Modeli veštačke pljuvačke
Table 1. Models of artificial saliva

Model 1 [12]		Model 2		Model 3 [13]	
g komponenti / l dejonizovane vode g components / l deionized water					
ksantan guma xanthan gum	0.18	ksantan guma xanthan gum	0.18	natrijum hidrogen karbonat sodium hydrogen carbonate	4.2
kalijum hlorid potassium chloride	1.20	kalijum hlorid Potassium chloride	1.20	natrijum hlorid sodium chloride	0.5
natrijum hlorid sodium chloride	0.85	natrijum hlorid sodium chloride	0.85	kalijum karbonat potassium carbonate	0.2
magnezijum hlorid magnesium chloride	0.05	magnezijum hlorid magnesium chloride	0.05		
kalcijum hlorid calcium chloride	0.13	kalcijum hlorid calcium chloride	0.13		
di kalijum hidrogen ortofosfat\ di potassium hydrogen orthophosphate	0.13	di kalijum hidrogen ortofosfat di potassium hydrogen orthophosphate	0.13		
metil p-hidroksibenzoat methyl p-hydroxybenzoate	0.35	metil p-hidroksibenzoat methyl p-hydroxybenzoate	0.35		
		α amilaza α-amylase	0.20		

Model 1 i 2 međusobno se razlikuju u sadržaju enzima, α amilaze. Inkubacija uzoraka u veštačkoj pljuvačci obavljena je u zatvorenim plastičnim posudama, u vodenom kupatilu, na temperaturi 37±10°C. Odnos mase uzoraka i zapremine veštačke pljuvačke iznosio je 0,1 g materijala / 1ml veštačke pljuvačke (ISO 10993-5: 1999)[14-16].

Količina oslobođenih supstanci ispitivana je u modelu pljuvačke nakon uklanjanja uzorka posle svakog od navedenih perioda. Ispitivanje je obuhvatilo detekciju metil metakrilata (MMA), etil metakrilata (EMA), butil metakrilata (BuMA), di butil ftalata (dBuFt) i benzoil peroksida (BP).

Korišćeni uređaj za tečnu hromatografiju pod visokim pritiskom (HPLC) je Agilent 1100 Series (SAD), sa DAD 1200 detektorom i analitičkom kolonom SUPELCO Discovery HS C18 250 × 4,6 mm, 5 μm, Sigma-Aldrich, SAD. Kao eluent poslužio je metanol. Protok mobilne faze iznosio je 1 cm^3/min, a zapremina injektiranja uzorka 20 μl. Kolona je termostatirana na 25°C. Talasna dužina detekcije bila je 205 nm.

Uzorci rastvora tri modela veštačke pljuvačke korišćeni su za HPLC analizu bez prethodne posebne obrade. Uzorci su filtrirani na ekono-filteru prečnika pora 0,45 μm, nakon čega je po 20 μl injektirano u HPLC uređaj.

Kalibracione krive su izrađivane od serije rastvora svake od ispitivanih supstanci u metanolu. Početna koncentracija ispitivanog jedinjenja bila je oko 1 mg/cm^3, od koje je, zatim, razblaživanjem metanolom pravljena serija rastvora manjih koncentracija. Iz dobijenih hromatograma očitavani su potrebni podaci: retenciono vreme svakog jedinjenja (Rt) i površina pika (A). Uređaj očitava UV/VIS spektar u svakoj tački pika na hromatogramu, tako da se iz spektara može odrediti i vrednost λ_{max}, tj. talasna dužina na kojoj jedinjenje ima maksimalnu apsorbancu.

Koncentracija ispitivanih komponenti u modelima veštačke pljuvačke predstavljena kao μg supstance/cm^3 rastvora pljuvačke. Koncentracije su sagledavane kroz srednju vrednost sa standardnim devijacijama. Vršena je komparacija dobijenih vrednosti koncentracija unutar iste grupe materijala u zavisnosti od dužine inkubacionog perioda, kao i između dva ispitivana materijala.

Model 1 and 2 differ in the content of the enzyme, α amylase. Incubation of samples in artificial saliva was performed in closed plastic containers, in a water bath, at a temperature of 37 ± 10°C. The mass ratio of samples and volume of artificial saliva was 0.1 g of material/1 ml artificial saliva (ISO 10993-5:1999)[14-16].

A quantity of released substances was examined in the saliva model after removal of the sample after each of the mentioned periods. The study involved the detection of methyl methacrylate (MMA), ethyl methacrylate (EMA), butyl methacrylate (BMA), dibutyl phthalate (dBuFt) and benzoyl peroxide (BP).

Agilent 1100 Series (USA), with the DAD 1200 detector and the analytical column SUPELCO Discovery HS C18 250× 4.6 μm, 5 mm, Sigma-Aldrich, USA was used as a high-pressure liquid chromatography device (HPLC). The methanol was used as an eluent. The flow rate of the mobile phase was 1 cm^3/min, and the sample injection volume was 20 ml. The column is thermostated at 25°C. The wavelength of detection was 205 nm.

The samples of the solution of three models of artificial saliva were used for HPLC analysis without prior processing. Then, the samples were filtered on a 0.45 mm pore diameter filter, which was followed by an injection of 20 μl into an HPLC device.

The calibration curves were made from a series of solutions of each of the tested substances in methanol. The initial concentration of the tested compound was about 1 mg / cm^3, from which, after dilution with the methanol, a series of solutions of lower concentrations were made. The required data from the obtained chromatograms were read out: the retention time of each compound (Rt) and the surface of the peak (A). The device reads the UV / VIS spectrum at each peak point on the chromatogram, so that the λ_{max} value can be determined from the spectrum i e., the wavelength at which the compound has a maximum absorbance.

The concentration of the tested components in artificial saliva models is presented as μg of the substance/cm^3 of the saliva solution. Concentrations were viewed through a mean value with standard deviations. A comparison was made of the obtained concentration values within the same group of materials, depending on the length of the incubation period, as well as between the two tested materials.

Rezultati

U Tabelama 2 i 3 prikazane su dobijene vrednosti oslobođenih komponenti ispitivanih materijala u tri modela veštačke pljuvačke HPLC metodom, nakon tri opservaciona perioda.

Količina oslobođenih komponenti srazmerno se povećava sa porastom trajanja opservacionog perioda, bez obzira na model veštačke pljuvačke. Veća koncentracija oslobođenih komponenti uočena je kod materijala *Lang Immediate*.

U tumačenju rezultata treba naglasiti i činjenicu da su uzorci sa porastom dužine opservacionog perioda menjali svoju konzistenciju, te su nakon trideset dana postali čvrsti, izgubivši rezilijentnost.

Results

Table 2 and table 3 show the obtained values of the released components of the tested materials in three models of artificial saliva using HPLC method after three observation periods.

The amount of released components increases proportionally with the increase in the duration of the observation period, regardless of the artificial saliva model. A higher concentration of released components was detected in *Lang Immediate* material.

In interpreting the results, it should be emphasized that the samples with the increase in the length of the observation period changed their consistency, and after thirty days they became firm, losing their resilience.

Tabla 2. Srednje vrednosti i standardne devijacije količina oslobođenih komponenti u uzorcima različitih modela veštačke pljuvačke nakon ekstrakcije uzoraka *Lang Flexacryl*

Table 2. Mean values and standard deviations of the quantities of released components in samples of various models of artificial saliva after the extraction of *Lang Flexacryl* samples

Veštačka pljuvačka/Atrificial saliva	Potencionalno toksična supstanca/ potentially toxic substances	Koncentracija jedinjenja u veštačkoj pljuvačci, μg/cm^3 Compound concentration in artificial saliva, μg/cm^3		
		1 dan/day	7 dana/days	30 dana/days
Model 1	EMA	31.207 ± 1.543	51.884 ± 2.156	76.334 ± 3.897
	BuMA	0.650 ± 0.032	7.670 ± 0.426	28.,667 ± 1.924
	BP	0.930 ± 0.066	3.896 ± 0.444	9.721 ± 0.844
	dBuFt	1.137 ± 0.073	2.770 ± 0.281	9.572 ± 0.612
Model 2	EMA	31.913 ± 1.438	55.807 ± 2.789	84.177 ± 4.112
	BuMA	0.540 ± 0.028	6.297 ± 0.513	26.502 ± 1.623
	BP	0.643 ± 0.051	2.613 ± 0.352	7.879 ± 0.917
	dBuFt	1.364 ± 0.079	2.304 ± 0.243	9.026 ± 0.761
Model 3	EMA	29.634 ± 1.728	51.704 ± 3.395	86.292 ± 4.643
	BuMA	1.135 ± 0.086	7.394 ± 0.450	30.651 ± 1.750
	BP	0.443 ± 0.048	3.691 ± 0.390	10.165 ± 0.755
	dBuFt	1.303 ± 0.086	2.149 ± 0.195	11.644 ± 0.853

Tabla 3. Srednje vrednosti i standardne devijacije količina oslobođenih komponenti u uzorcima različitih modela veštačke pljuvačke nakon ekstrakcije uzoraka *Lang Immediate /*

Table 3. Mean values and standard deviations of the quantities of released components in samples of various models of artificial saliva after extraction of *Lang Immediate* samples

Veštačka pljuvačka/Atrificial saliva	Potencionalno toksična supstanca/ potentially toxic substances	Koncentracija jedinjenja u veštačkoj pljuvačci, μg/cm^3 Compound concentration in artificial saliva, μg/cm^3		
		1 dan/day	7 dana/days	30 dana/days
Model 1	MMA	46.400 ± 3.564	73.796 ± 4.615	116.444 ± 6.552
	EMA	13.154 ± 0.612	24.125 ± 1.442	34.261 ± 2.720
	BP	1.274 ± 0.095	5.774 ± 0.331	10.548 ± 0.784
	dBuFt	1.813 ± 0.122	4.997 ± 0.222	7.234 ± 0.,533
Model 2	MMA	63.868 ± 3.942	74.878 ± 5.120	118.274 ± 5.988
	EMA	19.894 ± 0.541	25.968 ± 1.314	34.485 ± 3.672
	BP	0.637 ± 0.081	5.317 ± 0.452	11.630 ± 0.699
	dBuFt	2.149 ± 0.114	5.359 ± 0.198	7.159 ± 0.488
Model 3	MMA	48.319 ± 3.177	75.850 ± 4.016	119.777 ± 7.146
	EMA	16.433 ± 0.565	21.994 ± 1.845	32.652 ± 3.349
	BP	0.460 ± 0.068	3.106 ± 0.299	11.247 ± 0.841
	dBuFt	1.981 ± 0.106	5.730 ± 0.241	7.527 ± 0.621

Diskusija

Ispitivanje je obuhvatilo analizu tri različita rastvora veštačke pljuvačke u smislu detekcije traženih komponenti HPLC metodom. Srazmerno sa vremenom odlaganja došlo je do porasta koncentracije oslobođenih komponenti u modelima pljuvačke, što ukazuje i na njihovo smanjenje u samim uzorcima, te i povećanja njihove biokompatibilnosti. Model veštačke pljuvačke nije uticao na trend dobijenih rezultata, kao ni dodatak enzima α amilaze. U skladu sa dobijenim rezultatima može se zaključiti da sastav pljuvačke ne utiče na oslovađanje nevezanih komponenti iz mekih akrilata.

Dosadašnja ispitivanja ukazala su na potencijalno toksični efekat pojedinih nevezanih supstanci iz akrilatnih materijala [11,16-18]. Istraživanje Kostić i sar. ukazalo je na smanjenje količine ovih komponenti u uzorcima materijala nakon njihovog potapanja u vodenu sredinu[19] i postpolimerizacionih procedura[20].

Nevezani ili rezidualni mononomer, MMA ili EMA, može imati alergijski ili iratabilni efekat. Teško je predvideti individualni tolerantni nivo rezidualnog monomera. Količina zaostalog MMA treba da je u rasponu od 1 do 3%[21]. Prema standardu (ISO 1567:1999) maksimalno dozvoljena količina rezidualnog MMA za toplopolimerizovane akrilate iznosi 2,2%, a za hladno-polimerizovane 4,5%[22].

Discussion

This study involved the analysis of three different artificial saliva solutions in terms of detection of the required components by HPLC method.

In proportion to the time of disposal, there has been an increase in the concentration of released components in saliva models, which also indicates their decrease in the samples itself, and also the increase in their biocompatibility. The artificial saliva model did not affect the trend of the obtained results, nor the addition of the α amylase enzyme. In accordance with the obtained results, it can be concluded that the composition of the saliva does not affect the release of unbound components from the soft acrylic resins.

Previous studies have indicated the potentially toxic effect of certain unbound substances from acrylic materials[11,16-18]. Research by Kostic et al. indicated a decrease in the amount of these components in the samples of the material after their immersion in the aqueous environment[19] and the post-polymerization procedures[20].

An unbound or residual monomer, MMA or EMA may have an allergic or irreversible effect. It is difficult to predict the individual tolerant level of the residual monomer. The amount of residual MMA should range from 1 to 3%[21]. According to the standard (ISO 1567:1999), the maximum permissible amount of residual MMA for

BP se koristi u dermatologiji, gde postoje opisani slučajevi kontaktnog dermatita u oko 1% slučajeva[23-25]. Oyama i Imai su ukazali na citotoksični efekat BP[26]. Kompletna količina BP se ne utroši u postupku pokretanja polimerizacije akrilata, mada se on prahu akrilatnog materijala dodaje u vrlo niskim koncentracijama 0,2 i 1,28%[23,25]. BP nije pokazao značajnije varijacije u količini oslobođenoj iz uzoraka *Lang Immediate* i *Lang Flexacryl*. Uočeno je ravnomerno oslobađanje iz uzoraka akrilata, a najveća koncentracija u pljuvačci bila je nakon trideset dana inkubacije uzoraka.

Dokazana je i citotoksičnost ftalata, plastifikatora akrilatnim materijalima[26-30]. Vrednosti oslobođene količine plastifikatora (dBuFt) nakon prvog dana potapanja, u uzorcima mekih akrilata, takođe su slične. U slučaju *Lang Immediate*, količina dBuFt ravnomerno raste sa porastom dužine inkubacije. Dobijene vrednosti su, nakon trideset dana, manje u odnosu na *Lang Flexacryl*. Kod *Lang Flexacryl* nagli porast koncentracije plastifikatora odvija se između sedmog i tridesetog dana potapanja. Sa oslobađanjem plastifikatora u rastvor veštačke pljuvačke, evidentirane su promene fizičkih karakteristika mekih akrilatnih materijala.

U cilju poboljšanja biokompatibilnosti mekih akrilata sintetisani su i kondicioneri bez ftalata, čiji je nedostatak brz gubitak viskoelastičnosti. Sintetisan je i oralni kondicioner bez etanola, dobijen kombinacijom vinil estara i PEMA[2].

Nije moguće precizno odrediti standardne vrednosti minimalnih količina analiziranih komponenti akrilatnih materijala koje bi mogle izazvati toksičnu ili alergijsku reakciju oralnog tkiva. Sa druge strane, u koncentrovanom obliku oni pokazuju jak toksični efekat. Obzirom na dokazano toksično dejstvo, treba težiti njihovom maksimalnom smanjenju strogim poštovanjem odnosa praha i tečnosti, polimerizacionog postupka propisanog od strane proizvođača i odlaganjem nadoknada od hladno polimerizovanih akrilata u vodu nekoliko dana pre predaje pacijentu[31-33].

thermally curing acrylates is 2.2% and for cold curing 4.5%[22].

BP is used in dermatology, where there are described cases of contact dermatitis in about 1% of cases[23-25]. Oyama and Imai pointed to the cytotoxic effect of BP[26]. The total amount of BP is not consumed in the starting process of the polymerization of acrylic resins, although it is added to the powder of acrylic material at very low concentrations of 0.2 and 1.28%[23,25]. BP did not show significant variations in the amount released from the *Lang Immediate* and *Lang Flexacryl* samples. A uniform release from acrylate samples was observed, and the highest concentration in saliva was after thirty days of sample incubation.

Cytotoxicity of phthalate, a plasticizer of acrylate materials was also demonstrated [26-30]. The values of the released amount of plasticizer (dBuFt) after the first day of immersion, in soft acrylate samples, are also similar. In *Lang Immediate* case, the amount of dBuFt increases uniformly with the increase of the incubation period. The values obtained after thirty days were less compared to the *Lang Flexacryl* model. In *Lang Flexacryl* case, a sudden increase in the concentration of the plasticizer occurs between the seventh and thirtieth day of immersion. With the release of the plasticizer into artificial saliva, changes in the physical characteristics of soft acrylic materials were recorded.

In order to improve the biocompatibility of soft acrylic resins, the conditioners without phthalate were synthesized, whose deficiency is a rapid loss of viscoelasticity. Oral conditioning without ethanol is also synthesized, obtained by the combination of vinyl esters and PEMA[2].

It is not possible to accurately determine the standard values of the minimum quantities of analyzed components of acrylic materials that could cause a toxic or allergic reaction of the oral tissue. On the other hand, in a concentrated form, they show a strong toxic effect.

Given the proven toxic effect, the maximum reduction by strict observance of the powder and liquid ratio is very important. It is done through the polymerization procedure prescribed by the manufacturer and the disposal of prosthetics made of cold curing acrylics in water for several days before handing over to the patient[31-33].

Zaključak

Ispitivane potencijalno toksične supstance ravnomerno su se oslobađale u vodenu sredinu, srazmerno sa dužinom inkubacije. Najviše vrednosti svih ispitivanih parametara uočene su nakon tridesetodnevnog opservacionog perioda. Model veštačke pljuvačke nije uticao na ritam oslobađanja komponenti, što znači da se one nesmetano oslobađaju u usnu duplju bez obzira na sastav pljuvačke pacijenta. Veća koncentracija oslobođenih komponenti uočena je kod materijala *Lang Immediate*.

Sa porastom dužine opservacionog perioda došlo je i do očvršavanja materijala, čie se završava njihova upotrebna vrednost. Inflamatorni efekat komponenti mekih akrilata na oralnu sluzokožu biće predmet budućih istraživanja.

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Conclusion

Tested potentially toxic substances were uniformly released into the aquatic environment, in proportion to the incubation length. The highest values of all tested parameters were detected after a thirty-day observation period. The artificial saliva model did not affect the release of the components, which means they are freely released into the oral cavity regardless of the composition of the patient's saliva. A higher concentration of released components was detected in the *Lang Immediate* material.

As the length of the observation period increased, the material was solidified, thus ending their use value.

The inflammatory effect of the soft acrylic components on the oral mucosa will be the subject of future research.

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SEKRETORNI LEUKOCITNI INHIBITOR PROTEAZE I NJEGOVA ULOGA U NASTANKU KARCINOMA GLAVE I VRATA

SECRETORY LEUKOCYTE PROTEASE INHIBITOR AND ITS ROLE IN VIRUS INDUCED HEAD AND NECK CANCERS

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Sažetak

Uvod: *Oralni karcinom skvamoznih ćelija (OSCC) je najčešći zloćudni tumor i čini preko 90% oralnih karcinoma. Iako je konzumiranje duvana u raznim oblicima glavni etiološki agens, danas je učestalost karcinoma izazvanih virusom u porastu.*
Humani sekretorni inhibitor leukocitne proteaze (SLPI) je član (urođenih) proteina povezanih sa imunitetom. Ima raznovrsna dejstva uključujući antiinflamatorno, antibakterijsko, antifungalno i antivirusno. SLPI je identifikovan kao jedini najsnažniji faktor u sprečavanju prenosa HIV-1 kroz oralnu sekreciju. Studije su nedavno pokazale njegov efekat protiv virusom izazvanih oralnih karcinoma. Najčešće proučavani virusi u oralnoj onkogenezi uključuju Humani Papilloma virus, Epstein Barr virus i Herpes simplex virus. SLPI je pokazao inverznu korelaciju sa pojavom HPV-pozitivnih tumora. U prisustvu HSV infekcije postoji smanjena regulacija SLPI. Smanjen SLPI je zabeležen u Epstein Barr virus povezanom nazofaringealnom karcinomu. Ovaj informativnii članak razmatra različite efekte SLPI sa posebnim fokusom na njegove efekte na virus humane imunodeficijencije-HIV, Humani Papilloma virus, Herpes simplex virus i Epstein Barr virus.
Zaključak: *Povećana SLPI ima zaštitnu ulogu u virusnoj onkogenezi, dok smanjeni nivoi povećavaju verovatnoću virusne infekcije.*

Ključne reči: *sekretorni inhibitor leukocitne proteaze, Humani Papilloma virus, Epstein Barr virus, Herpes simplex virus, oralni karcinom*

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Abstract

Introduction: *Oral squamous cell carcinoma (OSCC) is the commonest oral malignancy and accounts for over 90% of oral cancers. Though tobacco consumption in its various forms is the main etiological agent, nowadays, the incidence of virus induced cancers is increasing.*
Human Secretory Leukocyte Protease Inhibitor (SLPI) is a member of the innate immunity-associated proteins. It has a variety of actions including anti-inflammatory, antibacterial antifungal and antiviral effects. SLPI has been identified as the single most potent factor in preventing the transmission of HIV-1 through oral secretions. Studies have recently demonstrated an effect against virus induced oral cancers. The most commonly studied viruses in oral oncogenesis include Human Papilloma Virus, Epstein Barr Virus and Herpes simplex virus. SLPI has shown an inverse correlation with the occurrence of HPV-positive tumours. In the presence of HSV infection, there is a down regulation of SLPI. Decreased SLPI has been noted in Epstein Barr Virus associated nasopharyngeal carcinoma. The present review discusses the diverse effects of SLPI with special focus on its effects on Human Immunodeficiency Virus, Human Papilloma Virus, Herpes Simplex Virus and Epstein Barr Virus.
Conclusion: *Increased SLPI has a protective role in viral oncogenesis while decreased levels increase the probability of viral infection.*

Key words: *Secretory leukocyte protease inhibitor, Human Papilloma Virus, Epstein Barr virus, Herpes simplex virus, Oral cancer*

Uvod

Oralni karcinom skvamoznih ćelija (OSCC) je najčešći zloćudni tumor i čini preko 90% oralnih karcinoma[1]. Ima multifaktorijallnu etiologiju sa genetskim i ekološkim faktorima koji igraju ulogu u njegovom nastanku. Iako je konzumiranje duvana u raznim oblicima glavni etiološki agens, danas je učestalost karcinoma izazvanih virusom u porastu[1]. Najčešće proučavani virusi u oralnoj onkogenezi uključuju *Humani Papilloma virus, Epstein Barr virus i Herpes simplex virus*[1].

Humani sekretorni inhibitor leukocitne proteaze (SLPI) je katjonski protein povezan sa urođenim imunitetom. Gen SLPI je lokalizovan na hromozomu 20q12-13.2[2]. Ima različite imunološke sposobnosti koje uključuju njegovu antiinflamatornu i antimikrobnu aktivnost[3]. Zbog svoje antiproteazne aktivnosti, može inhibirati virusne infekcije[4]. SLPI je takođe proučavan kod mnogih vrsta karcinoma kao što su rak pluća, dojke, jajnika, jetre, itd. Ima ulogu u metastaziranju tumora[5]. Ovaj informativni članak se bavi različitim funkcijama SLPI i njegovom mogućom ulogom u karcinomima usne duplje izazvanih virusima.

Humani sekretorni inhibitor leukocitne proteaze (SLPI)

SLPI je prvi put identifikovan u sekretima pacijenata sa hroničnom opstruktivnom plućnom bolešću (COPD) i cističnom fibrozom pa se smatra inhibitorom antielastaze[2]. Nastaje iz različitih ćelija tela, uključujući neutrofile, makrofage, β-ćelije pankreasa, epitel bubrežnih tubula, acinusne ćelije velikih pljuvačnih i submukoznih žlezda kao i sluzokožu respiratornog i gastro-intestinalnog trakta[2,3]. U usnoj duplji je prvi put identifikovan u parotidnim sekretima, ali je od tada izolovan iz gotovo svih humanih izlučevina uključujući semenu, cervikalnu, cerebrospinalnu, sinovijalnu, nazalnu, bronhijalnu kao i iz majčinog mleka i suza[2,3].

Introduction

Oral squamous cell carcinoma (OSCC) is the commonest oral malignancy and accounts for over 90% of oral cancers[1]. It has a multifactorial etiology with genetic and environmental factors playing a role. Though tobacco consumption in its various forms is the main etiological agent, nowadays, the incidence of virus induced cancers is increasing[1]. The most commonly studied viruses in oral oncogenesis include *Human Papilloma Virus, Epstein Barr virus and Herpes simplex virus*[1].

Human Secretory leukocyte protease inhibitor (SLPI) is a cationic protein associated with innate immunity. The SLPI gene is localized on chromosome 20q12-13.2[2]. It has a variety of immunological capabilities, which include its anti-inflammatory and anti-microbial activity[3]. Due to its anti-protease activity, it can inhibit viral infections[4]. SLPI has also been studied in many cancers such as lung, breast, ovary, liver, etc. It has a role in metastasis of tumours[5]. The present review focuses on the various functions of SLPI and its possible role in virus-induced cancers of the oral cavity.

Secretory leukocyte protease inhibitor

SLPI was first identified in secretions from patients with chronic obstructive pulmonary disease (COPD) and cystic fibrosis and was considered as an antielastase inhibitor[2]. It is produced from varied cells of the body including neutrophils, macrophages, β-cells of the pancreas, epithelium of renal tubules, acinar cells of major salivary and submucosal glands as well as mucous membranes of respiratory and gastrointestinal tracts[2,3]. In the oral cavity, it was first identified in parotid secretions but since then has been isolated from almost all human secretions including seminal, cervical, cere-brospinal, synovial, nasal, bronchial as well as from breast milk and tears[2,3].

Antiinflamatorno dejstvo

Toksični proizvodi, kao što su serinske proteaze, koje oslobađaju neutrofili tokom inflamatornog procesa, uzrokuju veliko oštećenje tkiva . SLPI štiti tkivo od štetnih efekata upale uglavnom kroz njegovo delovanje na neutrofilnu elastazu. Deluje na makrofage i smanjuje oslobađanje infla-matornih citokina i azotnog oksida[2]. Uočeno je da gubitak SLPI dovodi do povećanog oštećenja tkiva . Takođe, inhibira i hemotaksu povezanu sa C5a.

Zarastanje rana

Utvrđeno je da se SLPI povećava kao odgovor na oštećenje kože. Smanjeni SLPI je povezan sa produženim vremenom zarastanja. Ova funkcija SLPI u promovisanju zarastanja rana je verovatno posledica njegove anti-inflamatorne aktivnosti koja dovodi do brže formacije matriksa u ranama[3,6].

Antibiotsko dejstvo

Studije su pokazale da SLPI može imati antibakterijska i antifungalna svojstva. To je baktericidno dejstvo protiv Gram-pozitivnih organizama kao što je *S. aureus* i Gram-negativnih organizama, kao što je *E. coli.* Ova baktericidna aktivnost može biti posledica vezivanja SLPI za mRNA ili DNK bakterije. Pored toga, može inhibirati razaranje opsonina i receptora za fagocitozu. On, takođe, može modifikovati obradu antimikrobnih peptida kao što su katelicidini[2,7].

SLPI je fungicidan za patogene gljive kao što su *Aspergillus fumigatus i Candida albicans.* Povećani nivo SLPI salivarnih ćelija produkuje se kao odgovor na oralnu kandidozu kod osoba zaraženih HIV-om. Koncentracije salivarnih SLPI smanjuju se sa godinama, koje mogu biti uzrok povećanih oralnih glji - vičnih infekcija kod starijih osoba [2].

Antivirusno dejstvo

Pošto mnogi virusi zahtevaju aktivnost proteaze za izazivanje infekcije, anti-proteaza, kao što je SLPI, može inhibirati virusne infekcije kao što je HIV. Ova anti-HIV aktivnost je nezavisna od njegove anti-proteazne aktivnosti. SLPI inhibira interakciju HIV-a sa receptorom na površini ćelije koji je identifikovan kao Aneksin 2[4].

Anti inflammatory Effects

Toxic products such as serine proteases which are released by neutrophils during the inflammatory process cause extensive tissue damage. SLPI protects tissue against the detrimental effects of inflammation mainly through its action on neutrophil elastase. It acts on macrophages and decreases the release of inflammatory cytokines and nitric oxide[2]. It has been seen that loss of SLPI results in increased damage to body tissues. Further, it inhibits C5a related chemotaxis.

Wound Healing

It was found that SLPI increased in response to cutaneous damage. The decreased SLPI was associated with the increased time of healing. This function of SLPI in promoting wound healing is probably a consequence of its anti-inflammatory activity leading to faster matrix formation in wounds[3,6].

Antibiotic Activity

Studies have postulated that SLPI may have antibacterial and antifungal properties. It is bactericidal against Gram-positive organisms such as S. *aureus* and Gram-negative organisms such as E. *coli*. This bactericidal activity may be due to binding of SLPI to mRNA or DNA of the bacteria. In addition, it may inhibit the destruction of opsonins and receptors for phagocytosis. It may also modify the processing of antimicrobial peptides such as cathelicidins[2,7].

SLPI is fungicidal to pathogenic fungi such as *Aspergillus fumigatus and Candida albicans.* Increased salivary SLPI levels are produced in response to oral candidiasis in HIV-1 infected persons. Salivary SLPI concentrations diminish with age which may be the cause for increased oral fungal infections in older individuals[2].

Anti-Viral activity

Since many viruses require protease activity for causing infection, an antiprotease like SLPI can inhibit viral infections such as HIV. This anti HIV activity is independent of its antiprotease action. SLPI inhibits the interaction of HIV with a cell surface receptor identified as Annexin 2[4].

SLPI je najefikasniji anti-HIV-1 faktor među urođenim inhibitornim molekulima u pljuvački[4]. Veruje se da inhibira transmisiju HIV-1 putem oralnog sekreta.

McNeeli je 1995. godine pokazao da je HIV-1 infekcija u monocitima blokirana humanom pljuvačkom[2,8]. SLPI je bio jedini molekul u pljuvački koji je imao antiretrovirusnu aktivnost u fiziološkim koncentracijama[9]. Ova anti-HIV-1 aktivnost SLPI je takođe viđena u perifernim krvnim mononuklearnim ćelijama, T ćelijama i limfocitnim tumorskim ćelijskim linijama. Nedavno je pronađeno da izlaganje oralnim keratinocitima i epitelnim ćelijama HIV-1 uzrokuje povećani SLPI protein i mRNA, čak i u odsustvu infekcije. Tako povećanje SLPI-a virusom može imati zaštitnu ulogu protiv HIV infekcije usne šupljine[9,10].

SLPI je takođe uključen u inhibiciju prenosa HIV-1 kroz majčino mleko. Veći SLPI pljuvačke kod odojčadi bio je povezan sa smanjenom transmisijom HIV-a kroz majčino mleko[11]. Slično tome, veći SLPI u vaginalnoj tečnosti bio je povezan sa smanjenom perinatalnom transmisijom HIV-a[9].

Mehanizam inhibicije: Čini se da inhibicija HIV-a od strane SLPI uključuje inhibiciju internalizacije HIV-a vezivanjem za Aneksin 2, molekul ćelijske površine. Pošto se vezivanje HIV-a za Aneksin 2 može prekinuti od strane SLPI, predtretman sa SLPI teoretski može inhibirati infekciju. Do sada nije pokazan efekat SLPI protiv drugih retrovirusa[12].

SLPI u karcinomima

SLPI je prekomerno izražen u mnogim vrstama karcinoma, uključujući rak jajnika, pluća i dojke. On je uključen u progresiju tumora[13]. Devogoodt i sar.[14] su otkrili da je SLPI regulisan ginekološkim karcinomima i da može poslužiti kao marker. Kod tumora, proteaze imaju važnu ulogu u lokalnom razaranju tkiva i širenju tumora. To podrazumeva da antiproteaze poput SLPI treba da ograniče širenje tumora. Međutim, pronađeno je da je SLPI konstantno povišen kod tumora pluća, jetre i jajnika i da je povezan sa povećanom malignom aktivnošću. Pokazalo se da SLPI izaziva proliferaciju ćelija in vitro[15]. Međutim, ova promaligna aktivnost je rezultat uloge antiproteaze, a ne proliferacionih stimulativnih svojstava[15]. Sayers i sar.[16] su pokazali da se SLPI reguliše u visoko metastatskim ćelijama karcinoma dojke. SLPI sprečava degradaciju progranulina, enzima povezanog sa proliferacijom ćelija i povećane invazivnosti u ćelijama raka dojke[16].

SLPI is the most effective anti-HIV-1 factor among the innate inhibitory molecules in saliva[4]. It is believed to inhibit HIV-1 transmission through oral secretions.

In 1995, McNeely demonstrated that HIV-1 infection in monocytes was blocked by human saliva[2,8]. SLPI was the only molecule in saliva which had antiretroviral activety at physiological concentrations[9]. This anti-HIV-1 activity of SLPI has also been seen in peripheral blood mononuclear cells, T cells and a lymphocyte tumour cell line. Recently, it was found that exposure of oral keratinocytes and epithelial cells to HIV-1 caused increased SLPI protein and mRNA even in the absence of infection. Thus increase in SLPI by the virus may have a protective role against HIV infection of the oral cavity[9,10].

SLPI is also implicated in inhibiting transmission of HIV-1 through breast milk. Higher salivary SLPI in infants was correlated with reduced HIV transmission through breast milk[11]. Similarly, higher SLPI in vaginal fluid was associated with decreased perinatal HIV transmission[9].

Mechanism of Inhibition: The inhibition of HIV by SLPI appears to involve inhibition of HIV internalisation by binding to Annexin II, a cell surface molecule. Since binding of HIV to Annexin 2 can be disrupted by SLPI, pretreatment with SLPI could theoretically inhibit infection. So far, no SLPI effect has been demonstrated against other retroviruses[12].

SLPI in cancers

SLPI is overexpressed in many cancers including ovary, lung and breast cancer. It has been implicated in tumour progression[13]. Devogoodt et al.[14] found that SLPI was upregulated in gynaecological cancers and could serve as a marker. In tumours, proteases have an important role in local tissue destruction and tumour spread. This implies that anti proteases like SLPI should restrict the spread of tumour. However, it has been found that SLPI is consistently elevated in tumours of the lung, liver and ovary and associated with increased malignant activity. SLPI has also been shown to cause cell proliferation in vitro[15]. However, this pro malignant activity is due to its role as an anti protease rather than its proliferation stimu-lating properties[15]. Sayers et al.[16] demonstrated that SLPI is upregulated in highly metastatic breast carcinoma cells.

Stepien i sar.[17] istraživali su serumski SLPI kod pacijenata kojima je dijagnostikovan papilarni karcinom tiroidne žlezde i multinodularna guša. Otkrili su da je SLPI značajno povećan kod ispitanika sa karcinomom, dok su slučajevi multinodularne gušavosti imali SLPI uporediv sa kontrolama. Povećana ekspresija SLPI navedenih karcinoma bila je povezana sa lošom prognozom[18].

Karcinomi povezani sa virusom humanog papiloma

Humani Papilloma virus (HPV) je uzročnik karcinoma grlića materice . Odnedavno se njegova uloga široko proučava u oralnim i orofaringealnim karcinomima[19]. Studije su otkrile da se karcinom krajnika i orofarinksa češće povezuje sa HPV -om nego sa oralnim karcinomom. Pušači i osobe koje žvaću duvan imali su manje šanse da imaju HPV pozitivne tumore nego nepušači i žvakači[19,20].

Pošto SLPI ima anti-HIV aktivnost, njena uloga je takođe istraživana u HPV pozitivnim tumorima. Istraživanja su pokazala da je SLPI značajno smanjen u metastazama u poređenju sa ne -metastatskim karcinomima skvamoznih ćelija glave i vrata (HNSCC). Dakle, viši SLPI korelira sa zaštitom od HPV infekcije. Takođe, povećana ekspresija receptora za Aneksin 2 zabeležena je kod visokorizičnih HPV pozitivnih HNSCC, što znači da bi povećan SLPI mogao da obezbedi zaštitu od HPV-a vezivanjem sa Aneksinom 2[21]. Ove studije pokazuju povezanost između SLPI i HPV u HNSCC. Pokazalo se da je SLPI inverzno povezan sa HPV pozitivnim tumorima. Inverzna povezanost HPV i SLPI sugeriše da povećani SLPI ima zaštitni efekat protiv HPV infekcije. In vitro studije su pokazale da blokiranje receptora aneksina A 2 sa SLPI uzrokuje smanjen ulazak HPV u ćelije[21].

Ustanovljeno je da pušenje povećava delovanje SLPI, što je verovatno razlog zašto je manje verovatno da će pušači razviti HPV pozitivne HNSCC[21]. Pierce Campbell i sar.[22] otkrili su da veći salivarni SLPI može pove -ćati rizik od HNSCC kod pušača

Hoffman i sar.[23] istraživali su vezu između ekspresije SLPI, HPV infekcije i bolesti limfnih čvorova kod karcinoma glave i vrata. Oni su takođe istraživali delovanje SLPI i navike vezane za duvan u normalnoj mukozi pacijenata bez HNSCC. Otkrili su da HPV pozitivni slučajevi imaju nisku ekspresiju SLPI.

SLPI prevents degradation of progranulin, an enzyme associated with cell proliferation and increased invasiveness in breast cancer cells [16] Stepien et al.[17] investigated the serum SLPI in patients diagnosed with papillary thyroid cancer and multinodular goitre. They found that SLPI was significantly increased in the subjects with cancer while multinodular goitre cases had SLPI comparable to controls. Increased SLPI expression the above cancers were associated with a poor prognosis[18].

Human Papilloma Virus Associated Cancers

Human Papilloma Virus (HPV) is the causative agent of cervical cancer. Recently, its role is being widely studied in oral and oropharyngeal carcinomas[19]. Studies have revealed that cancer of the tonsil and oropharynx is more commonly associated with HPV than oral cancers. Smokers and tobacco chewers were less likely to have HPV positive tumours than non-smokers and chewers[19,20].

Since SLPI has anti HIV activity, its role has also been investigated in HPV positive tumours. Studies have shown that SLPI was significantly decreased in metastatic as compared to non-metastatic head and neck squamous cell carcinomas (HNSCC). Thus, higher SLPI correlated with protection against HPV infection. Also, increased expression of Annexin 2 receptor was noted in high risk HPV positive HNSCC implying that increased SLPI could confer protection against HPV by binding with Annexin 2[21]. These studies demonstrate an association between SLPI and HPV in HNSCC. Further, SLPI has been shown to be inversely correlated with HPV positive tumours. An inverse association of HPV and SLPI suggests that increased SLPI has a protective effect against HPV-infection. In vitro studies have demonstrated that blocking of Annexin A2 receptor with SLPI causes reduced HPV entry into cells[21].

Smoking was found to increase SLPI expression which is the probable reason why smokers are less likely to develop HPV positive HNSCC[21] Pierce Campbell et al.[22] found that higher salivary SLPI might increase risk of HNSCC among smokers.

Hoffman et al.[23] investigated the relation between SLPI expression, HPV infection, lymph node disease in head and neck cancer.

Takođe su otkrili da je u jednom slučaju, kada je HPV bio pozitivan i da je SLPI bio visok, bila prisutna bolest čvorova. Pretpostavili su da se zaštitni efekat SLPI može izgubiti u HPV infekciji . Veća ekspresija SLPI kod pušača dovodi do smanjenja HPV infekcije i mogućeg kasnijeg pojavljivanja HNSCC u ve zi sa navikama.

Herpes Simplex virus (HSV)

Identifikovane su dve vrste HSV; HSV 1 koji uzrokuje orofaringealne infekcije i HSV 2 koji utiče na anogenitalne lokacije[1]. Do kraja 1970-ih smatralo se da je etiološki agens u cervikalnom i oralnom karcinomu Herpes Simplex virus. Iako je visokorizični HPV sada identifikovan kao uzrok karcinoma grlića mate - rice, HSV se smatra važnim kofaktorom u njegovoj pojavi[24]. Svetska prevalencaija OSCC-a, koja je bila pozitivna za HSV-1, bila je oko 15%; najveća prevalenca ija od 55% zabeležena je u Velikoj Britaniji. Industrijske zemlje imaju veći HSV-1 pozitivan OSCC u poređenju sa zemljama u razvoju[1].

Istraživanja su pokazala trostruko povećanje rizika od razvoja karcinoma grlića ma - terice kod pacijenata koji su seropozitivni za visokorizične HPV (hrHPV) i HSV-2 nasuprot hrHPV. Ispitanici pozitivni na HPV infekciju koji su takođe seropozitivni na HSV -1 imaju dvostruko veći rizik od razvoja karcinoma skvamoznih ćelija u poređenju sa HSV1-seronegativnim osobama sa sličnom izloženošću HPV-u. Ovi nalazi pokazuju da HSV i HPV mogu delovati sinergistički u razvoju karcinoma[24].

Infekcija HSV-2 povećava rizik od dobijanja HIV-a[9]. In vitro studije su pokazale da SLPI sprečava HSV infekciju vezivanjem za epitelne ćelije. Tačan mehanizam ove akcije još nije poznat[9]. Fakioglu i sar.[9] su dokazali da i HSV-1 i HSV-2 smanjuju SLPI i tako izbegavaju lokalni imuni sistem. HSV može uzrokovati ovo smanjenje bilo sprečavanjem oslobađanja SLPI, smanjenjem ekspresije gena ili izazivanjem njegove degradacije[9]. Uobičajeno prihvaćena metoda je regulacija SLPI mRNA i virusna degradacija[9].

HSV-1 i HSV-2 mogu uzrokovati smanjenje SLPI, čak i u prisustvu aciklovira koji zavisi od rane ekspresije HSV i nezavisno od replikacije virusa[24]. HSV-1 može smanjiti ekspresiju SLPI brže od HSV-2; tako HSV-1 infekcija anogenitalnog regiona može da promoviše hrHPV. Regulacija SLPI može takođe povećati stvaranje NF -Kb, što će dalje pro - movisati HSV infekciju. Smanjenje SLPI u prisustvu HSV infekcije može takođe promovisati sticanje HIV-a[9].

They also investigated SLPI expression and tobacco related habits in normal mucosa of patients without HNSCC. They found that HPV positive cases had low SLPI expression. They also found that in one case where HPV was positive and SLPI was high, nodal disease was present. They postulated that the protective effect of SLPI may be lost in HPV infection. Higher expression of SLPI in smokers leads to decreased HPV infection and probable later onset of habit associated HNSCC.

Herpes Simplex Virus (HSV)

Two types of HSV are identified; HSV 1 which causes oropharygeal infections and HSV 2 which affects anogenital sites[1]. Until the late 1970s, it was believed that the aetiological agent in cervical and oral cancers was herpes simplex virus. Though high risk HPV is now identified as the cause in cervical cancer, HSV is considered as an important cofactor in its occurrence[24].The worldwide prevalence of OSCC which were positive for HSV-1 was around 15%; the highest prevalence of 55% was seen in the United Kingdom. Industrialised nations have greater HSV-1 positive OSCC when compared to developing countries[1].

Studies have revealed threefold increase in the risk of developing cervical cancer in patients who are seropositive for high risk HPV (hrHPV) and HSV-2 versus hrHPV alone. Further, subjects testing positive for HPV infection who are also seropositive for HSV-1 have a twofold increased risk of developing oral squamous cell cancer on comparison with HSV-1-seronegative individuals with similar HPV exposure. These findings show that HSV and HPV may act synergistically in cancer development[24].

Infection with HSV-2 increases the risk of acquiring HIV[9]. In vitro studies have shown that SLPI prevents HSV infection by binding to epithelial cells. The exact mechanism of this action is not known yet [9]. Fakioglu et al[9] proved that both HSV-1 and HSV-2 decrease SLPI thereby evading the local immune system. HSV could cause this decrease either by preventing release of SLPI, decreasing gene expression or by causing its degradation[9]. The commonly accepted method is by downregulation of SLPI mRNA and by viral degradation[9].

HSV-1 and HSV-2 can cause decrease in SLPI even in the presence of acyclovir which is dependent on early gene expression of HSV

Epstein Barr Virus

Epstein Barr Virus (EBV) je bio prvi humani virus koji je identifikovan sa većim onkogenim potencijalom. EBV pogađa skoro 90% odraslih širom sveta. Povezan je sa oralnom vlasastom leukoplakijom, Burkittovim limfomom, nazofaringealnim karcinomom, karcinomom skvamoznih ćelija i infektivnom mononukleozom[25]. Pošto EBV klasično utiče na epitelne ćelije u usnoj duplji , nazofarinksu i pljuvačnim žlezdama, postoji sumnja da je uključen u premaligne lezije i kod oralnih skvamoznih karcinoma[1].

Međutim, EBV detekcija u OSCC-u je varijabilna, možda zbog etničkih i geografskih varijacija[1]. Ekspresija SLPI je ispitivana među EBV pozitivnim pacijentima koji imaju karcinom nazofarinksa (NPC). SLPI mRNA je značajno smanjena u NPC u poređenju sa normalnim epitelom, dok je ekspresija SLPI mRNA i proteina veća u EBV negativnim ćelijama. To podrazumeva zaštitnu ulogu SLPI u NPC-u povezanom sa EBV[18].

Kod NPC, lokalna upala je veoma važan uzročni faktor. SLPI ima jake antiinflamatorne sposobnosti, posebno inhibiranjem oslobađanja NF-Kb, oslobađanja histamina iz mastocita i C5a iz upaljenih pluća. Tako smanjenje SLPI može promovisati NPC[18].

and independent of replication of the virus[24]. HSV-1can decrease SLPI expression faster than HSV-2; thus HSV-1infection of the anogenital region can promote hrHPV. Downregulation of SLPI can also increase NF-Kb formation which will further promote HSV infection. The reduction of SLPI in the presence of HSV infection may also promote acquirement of HIV[9].

Epstein Barr Virus

Epstein Barr Virus (EBV) was the first human virus identified as having oncogenic potential. EBV affects almost 90% adults worldwide. It is associated with oral hairy leukoplakia, Burkitt's lymphoma, nasop-haryngeal cancer, oral squamous cell carcinoma and infectious mononucleosis[25]. Since EBV classically affects epithelial cells in the oral cavity, nasopharynx and salivary glands, there has been a suspicion that it is involved in premalignant lesions and in oral squamous cell carcinomas (OSCC)[1]. However, EBV detection in OSCC has been variable possibly due to ethnic and geo-graphical variations[1]. SLPI expression has been studied among EBV positive patients having nasopharyngeal carcinoma (NPC). SLPI mRNA was significantly downregulated in NPC when compared with normal epithelium while mRNA and protein expression of SLPI were higher in EBV negative cells. This implies a protective role of SLPI in EBV associated NPC[18].

In NPC, local inflammation is a very important causative factor. SLPI has strong anti-inflammatory abilities especially by inhibiting of release of NF-Kb, histamine release from mast cells and C5a from inflamed lung. Thus SLPI decrease can promote NPC[18].

Zaključak

HPV, HSV i EBV su virusi koji su uključeni u HNSCC. SLPI je antiproteaza sa antiinflamatornim, antibiotskim i antivirusnim svojstvima. Povećana SLPI ima zaštitnu ulogu u virusnoj onkogenezi, dok smanjeni nivoi povećavaju verovatnoću virusne infekcije. HSV infekcija smanjuje ekspresiju SLPI i može promovisati virusne infekcije. Prema tome, SLPI ima potencijal i kao biomarker u karcinomima, ali i kao potencijalni cilj za buduće terapije kako bi se sprečio karcinom izazvan virusom.

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Conclusion

HPV, HSV and EBV are viruses implicated in HNSCC. SLPI is an antiprotease with anti-inflammatory, antibiotic and antiviral properties. Increased SLPI has a protective role in viral oncogenesis while decreased levels increase the probability of viral infection. HSV infection decreases SLPI expression and can promote viral infections. Thus, SLPI has potential both as a biomarker in cancers as well as a potential target for future therapies to prevent virus induced cancers.

Conflict of Interest: Nil
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Radovi treba da budu napisani na A4 formatu sa duplim proredom, obezbeđujući 25 mm margine. Samo jedna kopija rada treba da sadrži prezime i prvo slovo autorovog imena u gornjem desnom uglu. Broj stranica rada počinje sa naslovnom stranom kao strana 1 i nastavlja se sa ređanjem.

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Poželjno je da se koriste reči prikladne za indeksiranje i pretraživanje. Ako takvih reči nema u naslovu, poželjno je da se naslovu doda podnaslov.

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APSTRAKT I KLJUČNE REČI

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Uvod: opisuje problem o kome se radi u radu

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Rezultati: opisuje primarno rezultate

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Strukturni apstrakti nisu potrebni kod uvodnika i pisma. Ispod apstrakta stoje ključne reči i to tri do pet. Ključne reči mogu biti uzete samo iz Medical Subjects Headings (MeSH).

Apstrakt treba da bude preveden i na engleski jezik (US style), sa naslovom, imenima autora, institucija i ključnim rečima.

TABELE I FIGURE

Svaka tabela sa jasnim naslovom na srpskom i engleskom treba da bude otkucana sa duplim proredom na odvojenom papiru. Obeležiti brojevima tabele jednu za drugom kako nailaze posle prvog navođenja u tekstu (obeležavaju se arapskim brojevima). Dati svakoj kolumni kratko ili skraćeno zaglavlje. Staviti objašnjenja u legendama svih nestandardnih skraćenica korišćenih u tabeli. Za jedinice i merenja vidi odeljak niže. Ne koristiti unutrašnje horizontalne i vertikalne linije. Staviti sve tabele na kraju vašeg fajla. Uvek odvojiti posebne kolumne upotrebom tabulatora, a ne upotrebom razmaknice, tabele moraju biti u tekst formatu.

Linijski prikazani dijagrami i ilustracije (fotografije, fotomikrografije itd.), trebaju biti osmišljene kao figure. Oni takođe treba da budu smešteni na odvojenom listu papira i numerisani jedan za drugim arapskim brojevima u saglasnosti sa prvim koji je citiran u tekstu. Figure treba da budu profesionalno nacrtane i fotografisane. Svaka figura treba da bude etiketirana pozadi ukazujući broj figure, prezime i prvo slovo imena autora, i vrh figure. Fotografije treba da se daju u dva primerka. Kolor fotografije ce se štampati samo u dogovoru sa urednikom ili ako autor sam snosi troškove. Fotomikrografije moraju imati obeleženu unutrašnju razmeru, i simbole, i strelice ili slova treba da su u kontrastu sa pozadinom. Na fotografijama pacijenata mora se sakriti identitet, osim ako se pacijenti u pismenoj formi slože sa objavljivanjem njihovih fotografija sa identitetom. Ukoliko ste pozajmili ili već publikovali negde fotografije priložite i pismenu dozvolu za reprodukovanje. Naslovi i detaljna objašnjenja fotografija treba da budu data u legendama. Ako su korišćeni simboli, strelice, brojevi ili slova za identifikaciju delova slike objasniti svaku jasno u legendi.

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ACKNOWLEDGEMENTS

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Journals:

1. Standard journal reference. (Note: list all authors if six or less; when seven or more, list only first three and add et al): Glass DA, Mellonig JT, Towle HJ. Histologic evaluation of bone inductive proteins complexed with coralline hydroxyapatite in an extraskeletal site of the rat. J Periodontol 1989;60:121–125.

2. Corporate author: Federation Dentaire Internationale. Technical Report No.28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. Int Dent J 1987;37:235.

3. No author given: Coffee drinking and cancer of the pancreas (editorial). BMJ 1981;283:628

4. Volume with supplement: Magni R, Rossoni G, Berti R, BN52021 protect guinea pig from heart anaphylaxis. Pharmacol Res Commun 1988; 20 Suppl 5:75–8.

Books or other monographs:

5. Personal author(s): Tullman JJ, Redding SW. Systemic Disease in Dental Treatment. St. Louis: The CV Mosby Company; 1983:1–5.

6. Chapter in a book: Rees TD. Dental management of the medically compromised patient. In: McDonald RE, Hurt WC, Gilmore HW, Middleton RA, eds. Current Therapy in Dentistry, vol. 7. St. Louis: The CV Mosby Company; 1980:3–7.

7. Dissertations and thesis: Teerakapong A. Langerhans Cells in human periodontally healthy and diseased gingiva. (Thesis). Houston, TX: University of Texas; 1987.92 p.

Other published material:

8. Newspaper article: Shaffer RA.Advances in chemistry are starting to unlock mysteries of the brain. The Washington Post 1989 Ang 7; Sect.A:2 (col. 5).

References – electronic quotations:

9. Online journals without volume and page information. Berlin JA , Antman EM. Advantages and limitations of metaanalytic regressions of clinical trials data. Online J Curr Clin Trials (serial online). June 4; doc 134. Accessed July 20, 2000.

10. Online journals with volume and page information. Fowler EB, Breault LG. Ridge augmentation with a folded acellular dermal matrix allograft: A case Report. J Contemp Dent Pract (serial online). 2001;2(3):31–40. Available from: Procter&Gamble Company, Cincinnat, OH. Accessed December 15, 2001.

11. World Wide Web.Centers for Disease Control and Prevention. Preventing emerging infectious diseases: Addressing the problem of antimicrobial resistance. Available at: http://www.cdc.gov/ncidod/emergplan/antiresist/. Accessed November 5, 2001.

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