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PRIKAZ SLUČAJA
CASE REPORT

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Dorzalna površina jezika i halitoza: morfološki aspekti

Dorsal Lingual Surface and Halitosis: a Morphological Point of View

Odsjek za prirodne i zdravstvene znanosti te znanost o okolišu Sveučilišta L'Aquila, L'Aquila, Italija
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Sažetak

Svrha rada: Zanimanje za proučavanje prostora oko jezičnih papila i s time povezanog biofilma porastao je posljednjih nekoliko godina jer su one identificirane kao potencijalni izvor parodontopatogenih mikroorganizama i uzrok halitoze. Građa dorzuma jezika pogoduje stvaranju složenoga bakterijskog biofilma u kojemu se često nalaze patogeni mikroroganizmi. Svrha ovoga preliminarnog istraživanja bila je na temelju novoga kliničkog protokola pronaći povezanost između morfologije jezičnih papila i biofilma koji uzrokuje halitozu. **Materijali i metode:** U istraživanje je bio uključen jedan ispitanik pogođen halitozom. Fotografiran je dorzum jezika kako bi se uočila područja u kojima su bile vidljive naslage. Zatim je dvovremenskom tehnikom uzet otisak dorzuma jezika te je podijeljen i oštricom razrezan na šest dijelova prema Winkelovu Tongue-Coated-indeksu. Njihova topografija pregledana je pod stereomikroskopom LEICA LED 2000 i analizirana softverom ImageJ. **Rezultati:** Pokazalo se da je dubina papila bila povezana s prisutnošću vidljivoga biofilma na jeziku, a posredno i s halitozom kod pacijenta. **Zaključak:** Morfologija papila na dorzalnoj površini jezika utječe na prisutnost biofilma. Prikazani protokol može se dalje razmatrati za kliničku primjenu u dijagnostici i u personaliziranom liječenju halitoze.

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Ključne riječi
halitoza; jezik; prianjanje bakterija; Winkel indeks jezičnih naslaga

Uvod

U normalnim uvjetima dorzalna površina jezika ružičaste je boje ili je prekrivena tankim bijelim naslagama. Površinu kolonizira mnoštvo bakterija, uglavnom u području brazdi, fisura i oko visokih jezičnih papila. Ta anatomska skrovišta stvaraju pogodne uvjete za razvoj mikroorganizama jer su slinom zaštićena od ispiranja. Niska razina kisika pogoduje razvoju anaerobnih mikroorganizama (1). Jezične naslage sastoje se od vidljivoga bijelo-smeđeg sloja vezanog za površinu dorzuma jezika, a čine ga odljuštene epitelne stanice, krvne stanice, metaboliti, hranjive tvari i bakterije. Pronađeno je da je na jednu epitelnu stanicu na dorzumu jezika vezano više od 100 bakterija, a samo oko 25 bakterija adherira na jednu stanicu u svim ostalim dijelovima usne šupljine (2). Razvijeni su različiti indeksi za kvantificiranje jezičnih naslaga: Miyazaki i suradnici (3) jezične naslage klasificiraju kao prisutne ili odsutne u trima područjima, no nema pokazatelja koji kvantificiraju debljinu. Winkel i njegovi kolege (4) jezik su podijelili na šest područja te su svakom području neovisno dali ocjenu – 0 (nema naslaga), 1 (tanki sloj) i 2 (debeli sloj). Konačna vrijednost Winkelova Tongue Coating-indeksa dobiva se zbrajanjem svih šest ocjena.

Jezik zbog velike površine ima bogatu mikrofloru. Pokazalo se da je dorzalno-posteriorna površina jezika utočište velikom broju mikroorganizama (10^9 ili 10^{10} CFU cm^2) (5).

Introduction

The usual appearance of the dorsal surface of the tongue is either pinkish or with a thin white coating. This surface is colonized by large amounts of bacteria, mostly in the presence of fissures, crypts and high mucosal papillae. These anatomical niches create an environmental condition where microorganisms are embedded and well-protected from the flushing action of the saliva. Also, oxygen levels in such environment are low, thus promoting the development of anaerobic microbiota (1). The coating of the tongue consists of a visible white-brownish layer adhering to the dorsum of the tongue and embedding desquamated epithelial cells, blood cells and metabolites, nutrients and bacteria. Indeed, more than 100 bacterial species were found attached to a single epithelial cell on the dorsum of the tongue, whereas only about 25 bacteria adhere to each cell in other areas of the oral cavity (2). Different indexes were developed for quantifying the degree of tongue coating: Miyazaki et al.(3) reported that tongue coating is present or absent in three areas, while no indication of thickness was recorded. Winkel at al.(4) divided the tongue in six areas, scoring each one independently from 0 (no coating) to 1 (light coating) and 2 (heavy coating). The final value of the Winkel Tongue Coating Index has been obtained by adding all six scores. Therefore, the tongue microflora offering a large surface area represents a unique ecological niche in

Neugodan zadah iz usta čest je problem koji pogađa velik postotak odrasle populacije (6, 7), pri čemu se hlapljivi sumporovi spojevi smatraju najvažnijom komponentom. Smatra se da je jezični biofilm glavni izvor hlapljivih sumporovih spojeva s udjelom od 60 do 70 posto, a ostatak potječe od plaka (2, 8).

Istraživanja pokazuju da bakterije koje žive na jeziku dominantno pridonose halitozi (9, 10).

Površinu jezika opisao je Maeda (11) i to kao površinu koja se sastoji od okomito položenih papila. Hesse je izradio trodimenzionalni model supstrata i biofilma – prvi sloj sastoji se od vezivnoga tkiva i papila, drugi od tankoga biofilma, a posljednji od debeloga biofilma. Pretpostavlja se da je površinski sloj biofilma zbog doticaja sa zrakom aeroban, a u dubljim slojevima uvjeti su anaerobni (12). Hesse (13) je istaknuo da se u slučaju primjene sile na papile, kao pri struganju jezika, one malo saviju i štite biofilm koji ostaje u intersticijskom prostoru. To pokazuje znatan utjecaj strukture supstrata na stabilnost jezičnoga biofilma u slučaju mehaničkog naprezanja. Pojedinačna papila ne može se promatrati samo kao jednostavan izdanak koji viri s površine jezika, nego kao nakupina vlakana, kao što su to istaknuli Kobayashi i suradnici (14). Duboke brazde na jeziku povezane su s dvostruko većim ukupnim brojem bakterija i znatno snažnijim zadahom iz usta (15), iako drugi autori nisu potvrdili povezanost većeg broja bakterija i povećane hrapavosti površine jezika (16, 17).

Svrha ovog istraživanja bila je preliminarnim protokolom pronaći povezanost između hrapavosti jezika i prisutnosti biofilma u jezičnim naslagama kod pacijenta s halitozom.

Materijali i metode

U istraživanje je bio uključen jedan ispitanik pogođen halitozom. Fotografiran mu je dorzum jezika kako bi se uočila područja u kojima su bile vidljive naslage (slika 1.).

Uzet je prvi otisak u alginatu da bi se dobio sadreni model na osnovi kojega je izrađena individualna žlica od kitastoga silikonskog materijala. Zatim je uzet drugi otisak kombiniranjem žlice od kitastoga silikona s rijetkim silikonom (primjenom dvovremenske tehnike) (slike 2. i 3.).

Dobiveni otisak podijeljen je i oštricom razrezan na šest dijelova u skladu s Winkelovim Tongue Coating-indeksom (18), te je topografija promatrana pod stereomikroskopom LEICA LED 2000. Slike su analizirane u programu Image J (19), a parametar koji se analizirao bila je dubina prostora između papila.

Dobiveni podatci obrađeni su deskriptivnom statističkom analizom (srednja vrijednost, standardna devijacija, Shapiro-Wilkov test normalnosti, t-test) u programu XLSTAT 2015.4.1.

the oral cavity. The dorso-posterior surface of the tongue was reported to harbor a high quantity of attached microbes (109 or 1010 CFU cm^2) (5). Oral malodor is a common problem affecting a large percentage of the adult population (6,7) and volatile sulfur compounds (VSC) are thought to be the most important volatile components.

The tongue biofilm is considered to be the principle site for the generation of VSC accounting for 60–70% of the total, with plaque contributing the rest (2,8).

The studies show that bacteria residing on the tongue make the dominant contribution to oral malodor (9,10).

The surface of the tongue has been described by Maeda (11) as a surface consisting of papillae oriented perpendicular to the tongue plane. Hesse modelled a three dimensional structure of the substratum and biofilm with the first layer consisting of the connective tissue core of the papillae, the second layer having a thin biofilm cover and the last one consisting of a thick biofilm cover. The uppermost layer of the biofilm is considered to be aerated and hence aerobic, whereas deeper layers of the biofilm are anaerobic (12). Hesse (13) reported that in case the forces are applied to the papillae, for example, when scraping the tongue, the papillae bend slightly and protect the biofilm, thus remaining in the interstitial volume. This demonstrates the significant influence of the substratum structure on the stability of the tongue biofilm under mechanical stress. Each papilla itself cannot be seen just as a simple stud sticking out from the surface of the tongue but a cluster of individual strands, as showed by Kobayashi et al. (14). The presence of deep fissures has been related to twice the total counts of bacteria and to significantly higher mouth and tongue odor scores (15), although other authors have failed to confirm the association of higher bacterial counts with increased surface roughness of the tongue (16,17).

The aim of this research was to correlate the roughness of the tongue with the presence of the tongue coating biofilm in a halitosis patient. A preliminary protocol was designed for this purpose.

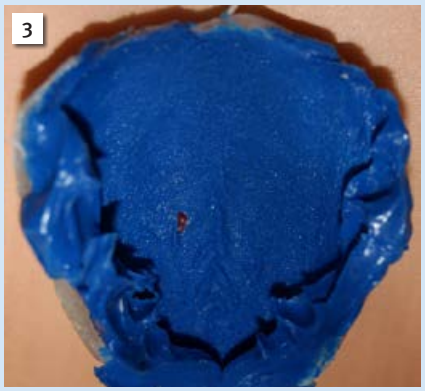
Material and Methods

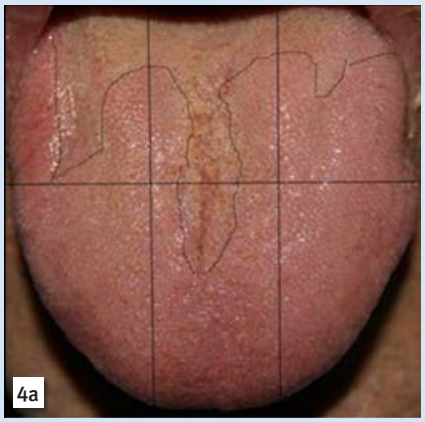
One subject with halitosis was selected and included in the study. A picture of the lingual dorsum was taken to spot the areas where the coating was visible (Figure 1).

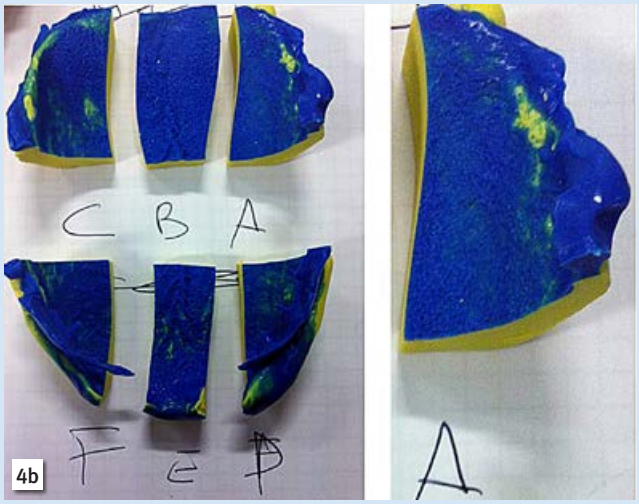
The first impression was taken by alginate obtaining an exact replica in plaster and an impression tray was modelled utilizing silicone putty impression material. Subsequently, the second impression was taken combining the tray in silicone putty with a silicone material, thus having a very low-light density (using the 2-step double-mix impression technique) (Figures 2-3).

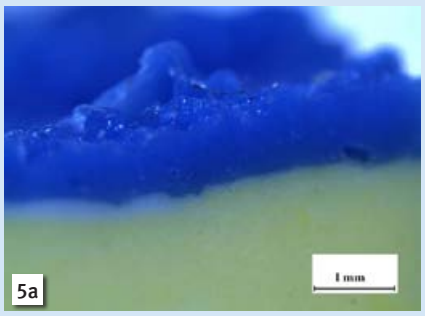
The obtained impression was divided and cut with a blade in six parts, according to Winkel Tongue Coated Index (18), and their contours were observed with the stereo-microscope LEICA LED2000. The images were analyzed by the Image J software (19), and the depths among papillae were considered to be parameters.

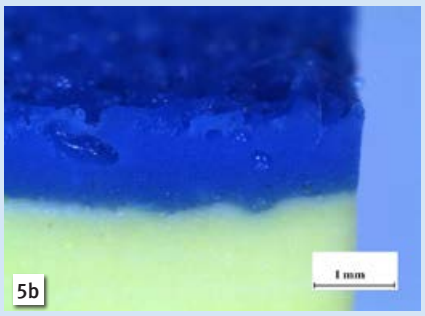
The obtained data were processed by descriptive statistical analysis (Mean, SD, Shapiro-Wilk Normality, and T-Student) using the XLSTAT software (2015.4.01. version).











Slika 1. Jezik s vidljivim biofilmom
Figure 1 The tongue with visible biofilm
Slika 2. Sadreni model
Figure 2 The plaster model
Slika 3. Dvovremenski otisak
Figure 3 2-step double-mix impression
Slika 4. Rezanje otiska prema Winkelovu Coating-indeksu
Figure 4 Impression cut according to the Winkel Index Coating
Slika 5. Stereomikroskopske slike povećane 2x
Figure 5 Stereomicroscope pictures at 2X magnification

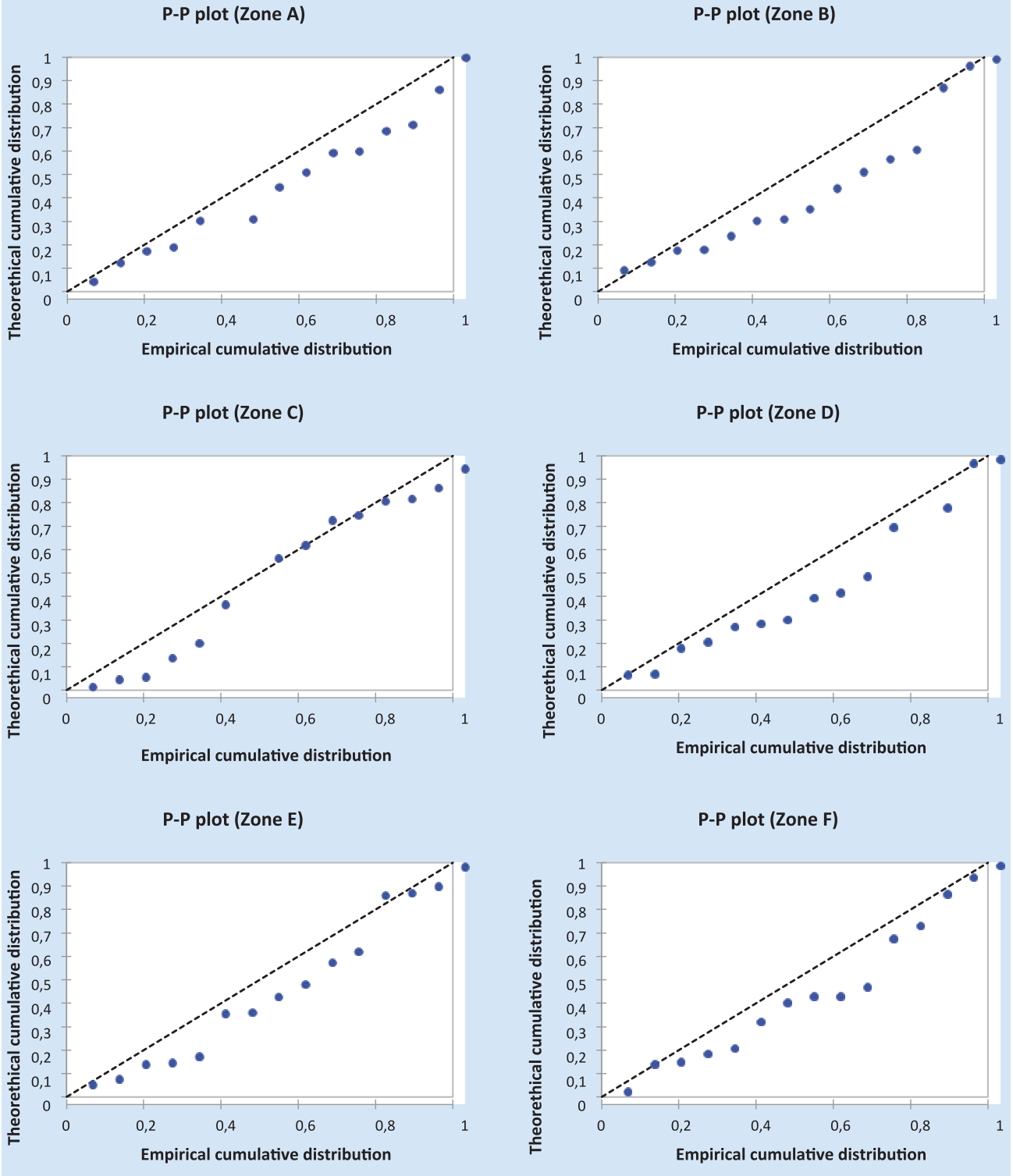
Tablica 1. Shapiro-Wilkov test normalnosti
Table 1 Shapiro-Wilk Normality test

Zona • Zone	Srednja vrijednost (mm) • Mean Value (mm)	SD	Shapiro-Wilkov test normalnosti (p) • Shapiro-Wilk Normality test (P value)
Zona A • Zone A	0.25	0.05	> 0 .05
Zona B • Zone B	0.46	0.23	> 0 .05
Zona C • Zone C	0.17	0.03	> 0 .05
Zona D • Zone D	0.20	0.07	> 0 .05
Zona E • Zone E	0.28	0.12	> 0 .05
Zona F • Zone F	0.14	0.05	> 0 .05

Tablica 2. Shapiro-Wilkov test normalnosti
Table 2 Shapiro-Wilk Normality test

Izrazito vidljiva prisutnost biofilma (A-B-C) srednja vrijednost u mm • High visible presence of biofilm (A-B-C) mean values mm	Slabo vidljiva prisutnost biofilma (D-E-F) srednja vrijednost u mm • Low visible presence of biofilm (D-E-F) mean values mm	p* • P value *
0.3 ± 0.18	0.21 ± 0.1	< 0.05

* Studentov t-test • T-Student test



Slika 6. P-P grafovi testa normalnosti: točka ispod dijagonalne linije upućuje na normalnu distribuciju
Figure 6 Normality test P-P plot: the spot under the diagonal line shows a normal distribution

Rezultati

Promatranjem pod stereomikroskopom dobiveni su zanimljivi rezultati. Parametar koji se analizirao bio je dubina fisura s duge i kratke strane svake zone (slike 4. i 5.) te su srednje vrijednosti uspoređene između šest zona. Šest zona podijeljeno je u dvije skupine na temelju vidljivosti biofilma na snimljenim fotografijama.

Svih šest zona pratilo je normalnu distribuciju podataka (slika 6.).

Srednje vrijednosti u zonama A, B i C iznosile su 0,25 + 0,05 mm, 0,46 + 0,23 mm i 0,17 + 0,03 mm. Te tri zone imale su vidljiv biofilm na snimljenoj fotografiji.

Srednje vrijednosti u zonama D, E i F iznosile su 0,20 + 0,07 mm, 0,28 + 0,12 mm i 0,14 + 0,05 mm. Biofilm u tim trima zonama bio je slabo vidljiv na fotografiji (slika 7.). U prvoj skupini (A – B – C) dubine fisura bile su veće u odnosu prema drugoj skupini (D – E – F). Razlika između tih dviju skupina bila je statistički značajna – p vrijednost iznosila je < 0,05 (tablice 1. i 2.).

Rasprava

Papilarna struktura dorzuma jezika jedinstven je milje u usnoj šupljini s velikom površinom koja pogoduje nakupljanju naslaga i mikroorganizama. Osim toga, položaj jezika na granici između usne šupljine i ždrijela osigurava pristup mnogim vrstama hranjivih tvari, metabolita i bakterija (20).

Poznato je da se dorzalna površina ljudskog jezika sastoji od četiriju različitih vrsta papila: filiformnih, raspoređenih na dorzumu, fungiformnih koje se nalaze anteriorno, folijatnih koje su smještene straga bočno i cirkumvalatnih koje se nalaze uz sulkuse (21).

Površina jezika opisuje se kao skup različitih papila i okusnih pupoljaka. Svaka papila može se promatrati kao nakupina okružena matricom i slinom te izložena oralnim plinovima. To rezultira određenom oksigenacijom biofilma na površini: u dubini papila razina kisika je vrlo niska, a na površini je viša (13).

Takvi uvjeti pogoduju različitim vrstama bakterija, a hrapavost površine omogućuje prianjanje i formiranje biofilma u kojemu bakterije koegzistiraju (22).

Različiti čimbenici mogu promijeniti površinsku hrapavost, uključujući dob, spol, izlučivanje sline, imunosni odgovor i gastrointestinalne poremećaje (21).

Atrofični jezik mogu uzrokovati prehrambeni deficiti, kandidijaza i Sjögrenov sindrom (23, 24).

Murayama i Kobayashi pokazali su da se izbočine filiformnih papila i pore fungiformnih papila mogu uspješno reproducirati niskoviskoznim silikonom za otiske (25).

Uemori i suradnici u svojem su se radu 2012. bavili pouzdanošću otiska jezika pri utvrđivanju hrapavosti dorzuma i mogućnošću njegova korištenja kao dijagnostičkog sredstva za kvantifikaciju stupnja atrofije te za prikaz morfologije jezičnih papila (26).

U ovom radu kvantitativno se analizira dubina papila i fisura te njihova povezanost s biofilmom u jezičnim naslagama kod osobe s halitozom. Čini se da je korištena metoda ispra-

Results

The stereomicroscope observation revealed some interesting outcomes. The parameter considered was the depth of the fissures on the long and on the short side of each zone (Figures 4-5), and such mean values were compared between the 6 zones. The 6 zones were divided in two groups, based on the visibility of the biofilm on the taken picture.

All of the six zones resulted by following a normal distribution (Figures 6).

The zones A, B and C mean values resulted respectively as 0.25 $\pm$ 0.05 mm, 0.46 $\pm$ 0.23 mm and 0.17 $\pm$ 0.03 mm. These three zones produced those with high visible presence of biofilm on the taken picture.

The zones D, E and F mean values resulted respectively as 0.20 $\pm$ 0.07 mm, 0.28 $\pm$ 0.12 mm and 0.14 $\pm$ 0.05 mm. These three zones produced those with a low visible presence of biofilm on the taken picture (Figure 7). The first group (A-B-C) showed higher values of depth fissures compared to the second group (D-E-F). The difference between the two groups was statistically significant with a p value < 0.05 (Tables 1-2).

Discussion

The papillary structure of the dorsum represents a unique ecological niche in the oral cavity, offering a large surface area that favors the accumulation of oral debris and microorganisms. In addition, its location as a crossroad between the oral cavity and the pharynges provides access to many different types of nutrients, products and bacteria (20).

It is a well-known fact that dorsal surface of the human tongue includes four distinct types of papillae: filiform, distributed over the dorsum; fungiform, located anteriorly; foliate found in the lateral posterior regions and circumvallate located along the sulcus (21) .

The dorsal surface of the tongue is described as a set of various papillae and taste buds. Each papilla itself can be seen as a cluster embedded in a matrix, and the saliva, which in turn is exposed to the mouth gaseous air. This implies a particular oxygenation of the biofilm that is created on this surface: the oxygen level is very low in deep surfaces of the papilla and it is consumed by the metabolisms of the bacteria. Conversely, the level of oxygen is higher at the top of the surface (13).

This structure and its ecological layout favors the harbor of different species of bacteria, and its roughness allows the adhesion and the formation of a biofilm where the bacteria coexist (22).

The surface roughness degree can change due to several factors including age, sex salivary secretion, immunological defense, gastrointestinal disorders (21).

An atrophic tongue can be caused by nutritional deficiencies, as well as candidiasis or by Sjogren syndrome (23) (24).

Murayama and Kobayashi reported that the protrusions of filiform papillae and taste pores of fungiform papillae were successfully reproduced by using a low viscous silicone impression material (25).

In 2012, Uemori et al. carried out a study on reliability of the tongue impression in order to determine the roughness

na, što potvrđuju citirani autori. To nam je omogućilo istraživanje morfologije papila i povezivanje nalaza s prisutnošću vidljivoga biofilma.

Naše istraživanje potvrdilo je da je morfologija dorzuma jezika jedinstveni milje za prianjanje i zaštitu biofilma. Stoga je analiza hrapavosti i morfologije papilarnih struktura temeljno važna za dijagnozu i personalizirano liječenje patoloških stanja koja zahvaćaju taj organ.

of the tongue dorsum and to exploit it as diagnostic tool to quantify the degree of atrophy as well as morphology of the lingual papillae (26).

The results obtained in the present study reveal that the depths of papillae and fissures were expressed quantitatively. Also, the relationship between the coated biofilm found in a halitosis-affected subject and the abovementioned depths was analyzed. The method used in this study appeared to be correct, as confirmed by the cited authors. It allowed us to investigate morphologically the papillary structure and to associate it with a visible biofilm presence.

Our study confirmed the fact that the tongue dorsum morphology offers a unique environmental niche for biofilm coating and adhesion. Thus, the study of its roughness and its papillary structure is fundamental for diagnosis and personalized treatments of pathologies affecting this organ.

Sukob interesa

Autori izjavljuju da nisu u sukobu interesa.

Zahvala

Preliminarni podatci iz ovog istraživanja predstavljeni su kao poster na 67. nacionalnom kongresu Talijanskoga društva za anatomiju i histologiju.

Conflict of interest

Authors declare they have no conflict of interest.

Acknowledgement

Preliminary data regarding this study were presented as poster at the 67th National Congress of Italian Society of Anatomy and Histology

Abstract

Objective: The interest in the study of the tongue papillary niches and the related biofilm has increased in recent years because they form a suitable source of periodontal microorganisms and are associated with development of halitosis. Tongue dorsum structure represents a factor favoring a particular and complex bacterial biofilm where periodontal pathogens are frequently found. The aim of this preliminary study was to associate the tongue papillary structure with the biofilm causing halitosis by means of a new clinical protocol. **Material and Methods:** In this study, one subject affected by oral malodor was selected and included. A photograph of lingual dorsum was taken to spot the areas with visible lingual coating. A tongue dorsum impression was obtained, divided and cut with a blade in six parts, according to Winkel Tongue Coated Index by means of the 2-step double-mix impression technique. The contours of the six parts were observed by the stereomicroscope LEICA LED2000 and analyzed by ImageJ software. **Results :** The results showed that the depth of papillae was associated with visible presence of the tongue biofilm and indirectly correlated with halitosis in patients.. **Conclusions:** The morphological papillary structure of the tongue dorsal surface influences the presence of the tongue biofilm. The presented protocol can be further considered in clinical application for a correct diagnosis and a personalized treatment of halitosis.

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Key words
Halitosis; Tongue; Bacterial Adhesion; Winkel Tongue Coated Index; Clinical Protocols

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