11/09

УНИВЕРЗИТЕТ У БЕОГРАДУ

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Београд,24.9.2013. год.
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На основу чл. 123. став 4. Закона о високом образовању ("Службени гласник РС", број 76/05, 100/07-аутентично тумачење, 97/08 и 44/10), чл. 46. ст. 5. тач. 3. Статута Универзитета у Београду - пречишћен текст ("Гласник Универзитета у Београду", број 162/11,167/12 и 172/13) и чл. 14. – 21. Правилника о већима научних области на Универзитету у Београду ("Гласник Универзитета у Београду", број 134/07, 150/9 и 158/11, а на захтев Стоматолошког факултета број 3/64 од 25.6.2013. године,

Веће научних области медицинских наука, на XXII седници одржаној дана 24. септембра 2013. године, донело је

О Д Л У К У

ДАЈЕ СЕ сагласност на предлог теме докторске дисертације:

Кандидат:

Сања Матић

Преложени назив теме: „Утицај полиморфизама гена за инфламаторне цитокине и њихове рецепторе на ниво циркулишућих цитокина и клиничке параметре код пацијената са хроничном пародонтопатијом и шећерном болести типа 2".

Одобрени назив теме: „Утицај полиморфизама гена за инфламаторне цитокине и њихове рецепторе на ниво циркулишућих цитокина и клиничке параметре код пацијената са хроничном пародонтопатијом и дијабетес мелитусом типа 2".

Председник Већа

Проф. др Весна Спасојевић-Калимановска

Доставити:
- Факултету
- секретару Већа
- архиви Универзитета

УНИВЕРЗИТЕТ У БЕОГРАДУ
СТОМАТОЛОШКИ ФАКУЛТЕТ
СЕКРЕТАРИЈАТ

ПРИМЉЕНО: 07-10-2013

Орг. јед.	Број	Прилог	Вредност
03	1316/3		

2005/4011

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Centralna biblioteka

P O T V R D A

U Centralnoj biblioteci Stomatološkog fakulteta urađena je kategorizacija objavljenih radova SANJE MATIĆ-PETROVIĆ, indeksiranih u Web of Science bazi.

Na osnovu Pravilnika o postupku i načinu vrednovanja, i kvantitativnom iskazivanju naučnoistraživačkih rezultata istraživača ("Službeni glasnik RS", br. 38/08) utvrđeno je da je Sanja Matić-Petrović publikovala dva rada u časopisima sa impakt faktorom (*Journal Citation Report* lista).

1. **Matić Petrović Sanja**, Cimbaljević Milena, Radunović Milena, Kuzmanović Pfićer Jovana, Jotić Aleksandra, Pucar Ana (2015). Detection and sampling methods for isolation of Candida spp. from oral cavities in diabetics and non-diabetics. BRAZILIAN ORAL RESEARCH, volumen 29, broj 1, str. 1-7 (Kategorija M23)

2. **Matić Petrović Sanja**, Zelić Ksenija, Milašin Jelena, Popović Branka, Pucar Ana, Zelić Obrad (2014). Detection of herpes simplex virus type 1 in gingival crevicular fluid of gingival sulcus/periodontal pocket using polymerase chain reaction. SRPSKI ARHIV ZA CELOKUPNO LEKARSTVO, volumen 142, broj (5-6), str. 296-300. (Kategorija M23)

Bibliotekar
Centralne biblioteke

Ružica Petrović

U Beogradu, 3.11.2015. godine

NASTAVNO-NAUČNOM VEĆU
STOMATOLOŠKOG FAKULTETA
UNIVERZITETA U BEOGRADU

PREDMET:

Izveštaj komisije za ocenu i odbranu doktorske disertacije
kandidata dr Sanje Matić

Na IV redovnoj sednici Nastavno-naučnog veća Stomatološkog fakulteta u školskoj 2015/2016. godini, održanoj15.03.2016.. godine, doneta je odluka o imenovanju Komisije za ocenu i odbranu završne doktorske disertacije dr Sanje Matić, pod nazivom **„Uticaj polimorfizama gena za inflamatorne citokine i njihove receptore na nivo cirkulišućih citokina i kliničke parametre kod pacijenata sa hroničnom parodontopatijom i dijabetes melitusom tipa 2"**.

U Komisiju su imenovani:

Prof. dr Saša Čakić, Klinika za parodontologiju i oralnu medicinu, Stomatološki fakultet, Univerzitet u Beogradu;

Prof. dr Zoran Aleksić, Klinika za parodontologiju i oralnu medicinu, Stomatološki fakultet, Univerzitet u Beogradu;

Prof. dr Branka Popović, Institut za humanu genetiku, Stomatološki fakultet, Univerzitet u Beogradu

Prof Dr Nebojša Lalić, Klinika za endokrinologiju i bolesti metabolizma, Kliničkog centra Srbije, Medicinski fakultet, Univerzitet u Beogradu

Nakon razmatranja priloženog materijala, komisija podnosi Nastavno-naučnom veću sledeći

IZVEŠTAJ

BIBLIOGRAFSKI PODACI O KANDIDATU

Doktor stomatologije Sanja Matić je rođena 16. februara 1984. godine u Negotinu. Stomatološki fakultet Univerziteta u Beogradu upisala je 2003. godine, a diplomirala 2009. godine sa prosečnom ocenom 9,64. U toku studija bavila se studentskim naučno-istraživačkim radom i kao autor ili koautor učestvovala na sedam studentskih kongresa. Akademske 2008/2009.godine učestvovala u nastavi kao demonstrator na Klinici za bolesti zuba. Nakon obavljenog pripravničkog staža na klinikama Stomatološkog fakulteta u Beogradu i Doma zdravlja Vračar u Beogradu, stručni ispit za doktore stomatologije je položila 28. aprila 2010. godine.

Doktorske studije upisala je akademske 2009/2010. godine iz naučne oblasti Oralna medicina i parodontologija. Položila je sve ispite predviđene planom i programom sa prosečnom ocenom 9,69. Od upisa doktorksih studija do januara 2011. godine bila je stipendista Fondacije za razvoj naučnog i umetničkog podmlatka i stipendista Ministarstva nauke i tehnološkog razvoja (angažovana na projektu broj 145042). Januara 2011. godine angažovana je kao pripravnik na projektu Ministarstva za nauku i tehnološki razvoj republike Srbije pod nazivom „Interakcija etiopatogenetskih mehanizama parodontopatije i periimplantitisa sa sistemskim bolestima današnjice“ broj 41008. Kao student doktorskih studija, od 2011. godine volonterski je učestvovala u praktičnoj nastavi studenata IV i V godine studija na Klinici za oralnu medicinu i parodontologiju.

Dr Sanja Matić je autor ili koautor deset radova saopštenih na domaćim i međunarodnim studentkim kongresima i sedam radova saopštenih na međunarodnim naučnim skupovima (M34). Autor je dva rada objavljena časopisima indeksiranim u bazi SCI liste (M23).

PREGLED DOKTORSKE DISERTACIJE

Nastavno-naučno veće Stomatološkog fakulteta, Univerziteta u Beogradu je 2016. godine prihvatilo predlog teme i odredilo mentore, prof. dr Anu Pucar i prof. dr Jelenu Milašin za izradu ove doktorske disertacije.

Doktorska disertacija dr Sanja Matić je izložena na 155 strana, raspoređenih u sedam poglavlja: Uvod, Ciljevi, Materijal i metode, Rezultati, Diskusija, Zaključci i Literatura. Tekst je dokumentovan sa 9 slika, 61 tabelom i 16 grafikona.

U **Uvodu** kadnidat opisuje kliničke karakteristike hronične parodontopatije, dijabetesa melitusa tipa 2 i dvosmernu vezu ovih oboljenja. Zatim opisuje dosadašnja saznanja i nedoumice u vezi sa etiologjim ova dva oboljenja, odnosno govori o velikoj, ali nedovoljno ispitanoj ulozi nasledne osnove za oba pomenuta oboljenja. Prikazano je da prisustvo određenih polimorfizama može uticati na povećanu/smanjenu predispoziciju za razvoj oboljenja i odgovor na terapiju. Opisana je uloga ispitivanih citokina i receptora u patogenezi hronične parodontopatije, dijabetesa melitusa tipa 2, kao i uticaj polimorfizama na ekspresiju ili funkciju ispitivanih molekula. S obzirom da sami polimorfizmi nisu dovoljno ispitivani kod oba oboljenja istovremeno, kandidat ističe potrebu za ispitivanjem ovih genskih varijacija u okviru pomenutih oboljenja.

U sledećem poglavlju jasno su definisani **Ciljevi** studije:

- Odrediti da li ispitivani polimorfizmi predstavljaju faktore rizika za oboljevanje od parodontopatije i dijabetesa i da li su povezani sa kliničkim parametrima parodontopatije i dijabetesa
- Određivanje odnosa sistemskog nivoa citokina i kliničkih parodontoloških parametara kod grupa ispitanika sa dijagnostikovanom hroničnom parodontopatijom
- Odrediti distrubuciju genotipova i alela za polimorfizme proinflamatornih citokina (TNF-α i LT-A) i njihovih receptora (TNFR1 i TNFR2) kod sistemski zdravih ispitanika bez kliničkih znakova parodontopatije, sistemski zdravih ispitanika sa hroničnom parodontopatijom i ispitanika sa parodontopatijom i dijabetes melitusom tip2
- Određivanje serumskih koncetracija ispitivanih citokina (TNF-α i LT-A) i njihovih receptora (TNFR1 i TNFR2).
- Određivanje povezanosti polimorfizama sa sistemskim nivoima proinflamatornih citokina

U poglavlju **Ispitanici, Materijal i metode** adekvatno i precizno je prezentovana metodologija rada. Istraživanje predstavlja studiju preseka koja je obuhvatila 180 ispitanika raspoređenih u tri grupe: kontrolnu, grupu ispitanika sa hroničnom parodontopatijom i ispitanike sa parodonotpatijom i dijabetes melitusom tipa 2. Kandidat detaljno opisuje kriterijume za odabir pacijenata u studiju, anamnestički karton kojim su praćeni činioci koji mogu uticati na kliničko stanje parodontopatije ili dijabetesa, kao i dijagnostički protokol za određivanje hronične parodontopatije i dijabetesa. Za molekularno genetičke analize uzimani su uzorci brisa bukalne sluzokože iz kojih je izolovana DNK a zatim metodom PCR/RFLP (lančana reakcija polimeraze i restrikciona analiza) vršeno detektovanje polimorfizama. Biohemijske analize, određivanje serumskih koncentracija citokina i receptora vršene su iz seruma dobijenih iz periferne venske krvi ELISA metodom. Statistička obrada podataka vršena je pomoću SPSS 18.0 (SPSS Inc, Chicago, IL, USA) paketa za obradu podataka.

U poglavlju **Rezultati** prvo su prikazani demografski podaci, zatim klinički parodontološki, podaci o dijabetesu, analize hematoloških i biohemijskih rezultata. Zatim su prikazani rezultati učestalosti genotipova i alela za svaki ispitivani polimorfizam kao i povezanost ovih varijacija sa kliničkim parametrima parodontopatije i dijabetesa. Kandidat je predstavio rezultate biohemijskih analiza kao i povezanost ovih analiza sa ispitivanim polimorfizmima. Regresionim modelima određivani su zavisni i nezavisni faktori koji opisuju promenlijvost parametara destrukcije parodoncijuma i koncentracije citokina, kao i faktori koji doprinose većoj ili manjoj predispoziciji za oboljevanje od parodontopatije ili dijabetesa.

U poglavlju **Diskusija** kadnidat izlaže saznanja i činjenice drugih relevenatih radova i upoređuje sa dobijenim rezultatima. Takođe, za razliku od većine drugih studija, diskutuje o svim faktorima koji su praćeni kroz anamnestičke kartone, klinička i biohemijska merenja, koji mogu uticati na određene ishodišne varijable, a obično su zanemarena. Kandidat je literaturi pronašao mali broj radova koji se bavi ispitivanjem uloge ovih polimorfizama kod ispitanika sa dijabetesom i parodontopatijom, pa tako rezultati studije dr Sanje Matić doprinose ovoj nedovoljno ispitanoj oblasti.

Zaključci su jasno formulisani i napisani u skladu sa ciljevima disertacije. Kandidat navodi da određeni genotipovi i aleli nose veći rizik za oboljevanje od hronične parodontopatije. Klinički parodontološki parametri kod sistemski zdravih ispitanika sa parodontopatijom su pod uticajem nekih od ispitivanih genskih varijacija, dok se ti uticaji nisu izdvojili kao značajni kod ispitanika sa parodontopatijom i dijabetesom, što kandidata navodi na razmišljanje o različitim etiološkim i patogentksim dešavanjima u parodoncijumu kod sistemski zdravih i oboleih od dijabetesa. Koncentracija određenih parodontoloških parametara negativno korelira sa serumskim koncentracijama receptora u parodontopatiji, dok su u dijabetesu te korelacije pozitivne, što navodi na razmišljanje da su sinteza receptora i njegova korelacija sa parodontalnom destrukcijom u dijabetesu izmenjeni.

Literatura obuhvata 292 bibliografske jedinice iz stranih i domaćih naučnih i stručnih publikacija i knjiga koje se bave ovom problematikom, a koje pokrivaju najvažnije aspekte ovog istraživanja.

KONAČNA OCENA DOKTORSKE DISERTACIJE

Doktorska disertacija dr Sanje Matić je dobro dokumentovana studija koja daje doprinos u nedovoljno istraživanoj oblasti uticaja genskih varijacija na dijabetes i parodontopatiju. Povezivanje ovih polimorfizama sa dijabetesom i parodontopatijom, kao i sa sistemskim nivoom citokina/receptora nedovoljno je obrađeno u literaturi, dok u domaćoj nije proučavano. S obzirom da su genske varijacije karakteristika određenih populacija, ova disertacija daje značajan doprinos proširivanju znanja iz ove oblasti za ispitanike naše populacije. Genski polimorfizmi smatraju se faktorima predispozicije za oboljevanje od oboljenja, kao i činiocima koji mogu uticati na razvoj bolesti i terapijski odgovor. S toga je poznavanje uticaja određenih polimorfizama u patogenzi jednog ili više oboljenja istovremeno bitan faktor koji bi mogao pomoći prevenciji, ali i usmeriti terapiju ovih oboljenja.

Rezultati prikazani u ovoj disertaciji predstavljaju dobru osnovu za buduća istraživanja koja će se ispitivati genske uticaje na jedno ili kombinaciju više oboljenja.

Doktorska disertacija ispunjava sve kriterijume propisane Zakonom o Univerzitetu i statusima Univerziteta i Stomatološkog fakulteta u Beogradu

Na osnovu iznetog, komisija predlaže Naučno-nastavnom veću Stomatološkog fakulteta Univerziteta u Beogradu da prihvati pozitivan izveštaj i kandidatu dr Sanji Matić odobri javnu odbranu doktorske disertacije pod nazivom **„Uticaj polimorfizama gena za inflamatorne citokine i njihove receptore na nivo cirkulišućih citokina i kliničke parametre kod pacijenata sa hroničnom parodontopatijim i dijabetes melitusom tipa 2"**.

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Prof. dr Saša Čakić
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Univerzitet u Beogradu

U Beogradu, mart 2016.

Objavljeni radovi:

Matić Petrović S, Cimbaljević M, Radunović M, Kuzmanović Pfićer J, Jotić A, Pucar A. Detection and sampling methods for isolation of Candida spp from oral cavities in diabetics and non-diabetics. Bra Oral Res. 2015;29(1)1-7.

Matić Petrović S, Zelić K, Milašin J, Popović B, Pucar A, Zelić O. Detection of Herpes Simplex Virus Tzpe 1 in Gingival Crevicular Fluid/Periodontal Pocket using Polymerase Chain Reaction. Srp Arh Celok Lek.2014;142(5-6)296-300.

Original Research
Periodontics

Detection and sampling methods for isolation of *Candida* spp. from oral cavities in diabetics and non-diabetics

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

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Abstract: The purpose of this study was to detect *Candida* spp. on the tongue and in the subgingival sites in healthy and type 2 diabetes (T2D) patients with chronic periodontitis (CP), and to compare the accuracy of sampling methods. This study included 131 patients divided into four groups: healthy control (group A), nondiabetics + CP (Group B), diabetics with good metabolic control + CP (group C) and diabetics with poor glycoregulation + CP (Group D). Cotton swab samples from tongue and subgingival samples were obtained from each patient with help of sterile paper points and a sterile curette. Swab cultures were made on Sabouraud dextrose agar. The number of CFUs was counted. The sampling methods for subgingival plaque were compared by Receiving Operator Curve (ROC). The presence of *Candida* spp. on the tongue was statistically significant among groups (group D *vs*. others three groups: χ^2: $p < 0.005$ for each group). Positive findings of subgingival *Candida* spp. did not differ among the groups. There were no significant differences in the quantification of *Candida* spp., neither on the tongue, nor in the subgingival samples. 17.2% of diabetic patients revealed the presence of *Candida* spp. in the subgingival samples, with negative finding on tongue. There was a significant difference in the sampling methods for subgingival plaque ($p = 0.000$). *Candida* spp. is more prevalent on the tongue of diabetics. The sampling of subgingival plaque by a sterile curette is more accurate than with paper points. Subgingival plaque may represent a reservoir of commensals. It is necessary to standardize the sampling of subgingival plaque.

Keywords: Diabetes Mellitus, Type 2; Chronic Periodontitis; Candida; Periodontal Pocket.

Introduction

Type 2 diabetes mellitus (T2D) is a chronic metabolic disorder that leads to progressive defects in insulin secretion based on insulin resistance.[1] For thirty years periodontitis has been acknowledged as the sixth chronic complication of diabetes.[2,3] *Candida* infections are chronic opportunistic infections related to diabetic patients. The presence of *Candida* spp. in oral cavities of diabetics varies between 50-80%.[4,5,6,7] Yeasts commonly inhabit tongue, palate and buccal mucosa, and it has recently been found in the subgingival sites.[8] The periodontium may represent a reservoir of opportunistic microorganism, especially in *immunocompromised*

patients.[9] The presence of yeasts in subgingival sites was examined in relationship to general health and periodontal status. It varies between 30-50% in the case of diabetics.[4,7,10] Yeasts[11] and viruses[12] could have a significant role in the pathogenesis of periodontal diseases. The immunological response around hyphae of *Candida* spp. is similar to the response to periopathogens of bacterial origin, and consists of chronic mononuclear inflammatory cells with sporadic neutrophil leucocytes.[11] The potential role of yeasts in the pathogenesis of periodontitis is especially important for diabetic patients, because antibiotics are commonly used in the treatment of periodontitis.

The purpose of this study was to detect *Candida* spp. on the tongue and in subgingival sites in healthy and T2D patients with chronic periodontitis (CP), and to compare the accuracy of sampling methods.

Methodology

Subjects, Ethical approval

This cross-sectional study was approved by the Ethical Committee of the School of Dentistry, University of Belgrade (Ethics Approval no. 36/8). It included 131 patients divided into four groups. Group A (n = 35) consisted of healthy volunteers without clinical signs of CP. Group B (n = 30) consisted of healthy subjects diagnosed with CP. Group C (n = 26) included T2D patients with good glycoregulation and diagnosed CP and group D (n = 40) consisted of T2D patients with poor metabolic control and diagnosed CP.

Inclusion and exclusion criteria

The exclusion criteria were: presence of any disease except T2D, aggressive periodontitis, usage of medication that might affect the periodontium, *e.g.* corticosteroids, antibiotics, antiseptics, history of oral candidiosis treatment, pregnancy, lactation and periodontal treatment in the last 1.5 year.

Anamnesis data and biochemical/hematological analysis

Self-reported information about blood type, everyday intake of sweets and smoking habits were recorded. According to the blood type, patients were divided into O *vs.* A+B+AB blood type.[13] Patients were classified according to their smoking status as "non-smokers" and "smokers". Fasting plasma glucose levels (FPG), glycated hemoglobin (HbA_1c), hematological parameters (RBC, Hgb, HCT, MCV, MCH, MCHC, RDW) and sedimentation rate were measured.

Diagnosis of CP and T2D

T2D was diagnosed according to the criteria of the American Diabetes Association[1] by measuring glycaemia during 2 h 75 g oral glucose tolerance test (OGTT), as well as HbA_1c values. Non-diabetics exhibited normal parameters on OGTT and $HbA_1c < 6.5\%$. Glycoregulation was classified, according to HbA_1c, as satisfactory ($HbA_1c \leq 7.5\%$) and as with poor metabolic control ($HbA_1c > 7.5\%$).

Full mouth clinical examinations were performed at six sites per tooth and evaluated on each tooth in order to access periodontal parameters: plaque index-Silness Loe (PI), dichotomous bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL). Two calibrated doctors performed examinations using periodontal probe (XP 23/UNC-15, Hu-Friedy, Chicago, USA). Periodontitis was diagnosed if subject exhibited CAL > 1 mm and PPD > 3 mm at least at three sites in two quadrants.[14,15] Patients without clinical signs of periodontitis exhibited PPD < 3 mm and CAL = 0 mm.

Sample collection and cultivation

Samples were collected a day after periodontal examination. Oral swabs were collected by swabbing ten times from the dorsum of the tongue with the help of a dry sterile cotton stick. Swab cultures were immediately inoculated on Sabouraud dextrose agar (SDA) (Oxoid, Basingstoke, UK). The tooth with deepest PPD was isolated by means of sterile cotton rolls and the supragingival plaque was removed by using sterile gauze and a curette. Two sterile paper points with a size of 30 were placed into the pocket for 30s until a mild resistance appeaed. Paper points contaminated by blood were not included in the analysis. Subgingival samples were obtained from the same pocket by means of a sterile curette (S4L/4R SS G.Hartzell&Son, Concord, California). Both subgingival samples were

inoculated in sterile plastic tubes containing 1 mL of Sabouraud dextrose broth (Oxoid, Basingstoke, UK). The plastic tubes were vortexed for 1 min and 20ml of suspended broth was streaked on SDA. Samples were inoculated at 37°C for 48 h. The cultural and microscopic qualities of the yeasts were examined, and the germ-tube production test, as well as the carbohydrate and potassium nitrate assimilation tests, were performed when needed. After incubation, one calibrated microbiologist counted the growth density and number of Colony Forming Units (CFUs). The yeast growth density from tongue samples was defined as rare, medium or dense. The number of CFUs was measured for samples taken by means of paper points. Depending on the CFU/ml there were defined three groups: 1: < 500, 2: 500 - 2500 and 3: > 2500.

Statistical analysis

Statistical analyses were carried out by using SPSS 18.0 software package for Windows (SPSS inc., Chicago, USA) and MedCalc for Windows, version 13.3.30 (MedCalc Software, Mariakerke, Belgium) for the Receiving Operators Curve (ROC) analysis.

Descriptive data were presented as Mean ± SD or the percentage for discrete measures. t-test and One Way ANOVA were used for normally distributed data. Non-parametric data were analyzed using by using the Kruskall-Wallis and Mann-Whitney test. Categorical variables were compared using the Chi Square Test (χ^2). The relationship between CFU and clinical parameters was determined by Spearman's correlation coefficient. The linear regression model was used to determine predictors of the presence of *Candida* spp. ROC analysis was carried out in order to compare sampling methods for subgingival plaque collection for isolation of *Candida* spp. Differences were considered significant when p-value was < 0.05.

Results

Demographic, clinical and biochemical data are presented in Table 1. Groups were matched by age, gender and smoking status.

Differences between groups C and D were observed for HbA1c (Mann-Whitney, p = 0.000) and FPG (Mann-Whitney, p = 0.000). Hematological parameters did not differ between groups. Groups were matched according to their blood type (χ^2, p = 0.541).

PI differed between groups (Table 2). BOP was different between group A and other groups (Bonferroni: B *vs.* C = B *vs.* D = C *vs.* D: p = 1.000). PPD did not differ significantly between groups B, C and D (Bonferroni: B *vs.* C = B *vs.* D = C *vs.* D, p = 1.000).

Positive finding of *Candida* spp. on tongue were found in 38/131 (27.3%) patients. The presence of *Candida* spp. on tongue was significantly higher in group D (χ^2: group A *vs.* D: p = 0.033, B *vs.* D: p = 0.007, C *vs.* D: p = 0.046) (Figure 1). When comparing *Candida* spp. findings on

Table 1. Demographic and biochemical data of patients.

	Group A	Group B	Group C	Group D	p-value
Age	43.57 ± 3.389	47.07 ± 10.869	48.31 ± 6.851	47.55 ± 7.527	[1]0.056
Sex (m/f)	14 (28.6%) / 21(71.4%)	14(40%) /16(60%)	14(53.8%) / 12(46.2%)	26(65%) /14(35%)	[2]0.164
Smokers N(%)/ Nonsmokers N(%)	8(22.9%) / 27(77.1%)	11(36.7%) / 19(63.3%)	8(30.8%) / 18(69.2%)	10(25.0%) / 30(75.0%)	[2]0.606
FPG (mmol/l)	4.65 ± .539	4.73 ± 0.624	7.49 ± 1.875	11.247 ± 4.061	[3]0.000
HbA1c (%)	4.81 ± 0.623	4.86 ± 0.635	7.09 ± 0.578	10.84 ± 1.366	[3]0.000
RBC (x10^{12})	4.53 ± .467	4.68 ± .703	4.58 ± .363	4.69 ± .601	[1]0.587
Hgb	137.50 ± 10.276	138.43 ± 12.263	138.88 ± 11.669	137.19 ± 14.731	[3]0.883
HCT (l/l)	0.41 ± .0538	0.43 ± .709	0.41 ± .032	0.42 ± .064	[3]0.423
MCV (fl)	91.52 ± 7.516	91.72 ± 7.755	89.22 ± 3.520	89.28 ± 10.486	[1]0.077
MCH (pg)	30.71 ± 2.243	30.30 ± 2.613	29.58 ± 3.667	28.98 ± 3.062	[3]0.060
MCHC (g/l)	333.88 ± 29.170	329.66 ± 28.846	339.01 ± 14.495	327.49 ± 22.803	[1]0.196
RDW(%)	14.26 ± 1.501	14.28 ± 1.64	13.79 ± 1.546	14.68 ± 1.789	[1]0.170

All values are presented as Mean ± SD.
[1]One Way ANOVA; [2]Pearson Chi Square Test; [3]Kruskal-Wallis; [4]Independent Sample t-test.

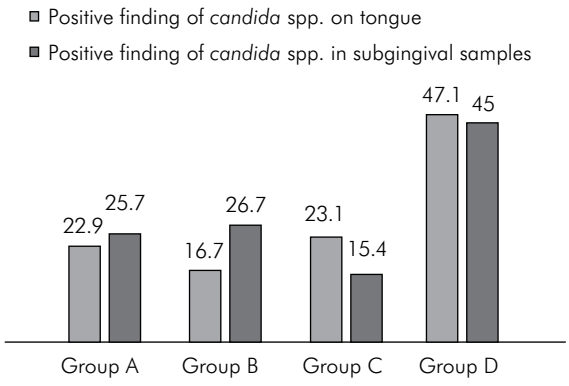

Figure 1. Presence of *Candida* spp. on tongue and in subgingival samples.

tongue of non-diabetics (20.3%) and diabetics (37.9%), a statistical difference was observed (χ^2, p = 0.028).

The quantification of *Candida* spp. on the tongue did not differ between groups. The univariate logistic regression model was applied, in order to identify parameters that could predict positive finding of *Candida* spp. on tongue. Age, gender, blood type, everyday intake of sugars, smoking habits, number of teeth, diabetes duration, treatment mode, FPG level, HbA1c, RBC, Hgb, MCV, HCT, MCH, MCHC and RDW were analyzed as potential predictors. There was found no predictor for the positive finding of yeasts on tongue.

The subgingival findings of *Candida* spp. were positive in 41/131 (29.50%). There was no difference in the presence of yeast in subgingival sites between groups (χ^2, p = 0.060) (Figure 1).

There was no relation between presence of subgingival *Candida* spp. and clinical periodontal parameters. The quantification of subgingival *Candida* spp. was not different between groups.

In the case of diabetic patients, there was a positive correlation between the presence of subgingival *Candida* spp. and HbA1c (Spearman correlation coefficient, r = 0.276, p = 0.025).

Logistic regression analysis did not identify any parameter that could predict the presence of *Candida* spp. in subgingival samples.

18/131 (12.9%) patients presented negative *Candida* spp. findings on the tongue and positive findings in subgingival samples.

There was a statistical difference regarding sampling methods for subgingival plaque collection and yeast detection (Table 3). The ROC curve was used to compare the diagnostic techniques of both collecting methods. The referent sampling method was by means of a sterile curette. There was a difference between methods (p = 0.000). Sensitivity was 0.576 and specificity was 0.919. The area under the curve was 0.747. Asymptomatic 95% Confidence Interval was 0.638-0.857 (Figure 2).

Discussion

The proposed microbiological etiologies of the periodontal disease have been changing for decades. There is increasing evidence about the involvement of microorganisms other than bacteria (*e.g.* viruses[11] and yeasts[8,12]) in the pathogenesis of periodontal disease.

Candida spp. is a common oral saprophyte. Yeast may form biofilm, which is an essential strategy for their survival in oral milieu.[16,15] Beside biofilm formation, this genus is able to produce exoenzimes, proteinases and metabolites in order to adhere to epithelial cells and inhibit the function of polymorphonuclears.[17,18,19] It can be isolated in about 50% of healthy population without clinical signs of infection.[13] In the case of diabetics, this prevalence is higher.

The prevalence and quantification of *Candida* spp. on tongue and subgingival samples were examined in diabetic and non-diabetic patients. To the best of our knowledge, this is the first study that examined patients with a clinically healthy periodontium, subjects diagnosed with periodontitis and diabetics. In our study, the overall prevalence of *Candida* on the tongue was 27.3%. The most frequent finding was in a group of poorly controlled diabetics. Differences in the prevalence of *Candida* spp. on tongue in the case of diabetic patients is in accordance with other studies,[6,20,21] but the percentage (37.9%) was lower compared to other studies, where findings of *Candida* on tongue varied between 59-77%.[4,5,7] The quantification of yeast growth on tongue did not differ between groups, which is contrary to other studies[6,22] probably because denture wearers were included and sampling methods were different.

Candida spp. was a commonly occurring microorganism in samples from subgingival sites.

Table 2. Clinical periodontal parameters.

Clinical parameter	Group A	Group B	Group C	Group D	p-value
PI	0.82 ± 0.423	1.72 ± 0.767	2.65 ± 0.458	2.23 ± 2.32	[1]0.000
BOP (%)	39.61 ± 19.273	62.057 ± 24.288	64.24 ± 24.043	64.41 ± 28.86	[2]0.000
PPD (mm)	2.02 ± 0.524	2.89 ± 0.944	2.85 ± 0.932	2.69 ± 0.756	[2]0.000
CAL (mm)	0	3.56 ± 2.142	3.98 ± 1.947	4.12 ± 2.104	[1]0.661

All values are presented as Mean ± SD.
[1]Kruskal-Wallis Test; [2]One Way ANOVA.

Table 3. Comparison of sampling methods for subgingival plaque collection.

Paper point method	Sterile curette method		Total	p-value
	Negative finding	Positive finding		
Negative finding	90 (68.7%)	14 (10.7%)	104 (79.4%)	[1]p < 0.000*
Positive finding	8 (6.1%)	19 (14.5%)	27 (20.6%)	
Total	98 (74.8%)	33 (25.2%)	131 (100%)	

All values are presented as N (%).
[1]Pearson Chi Square Test.

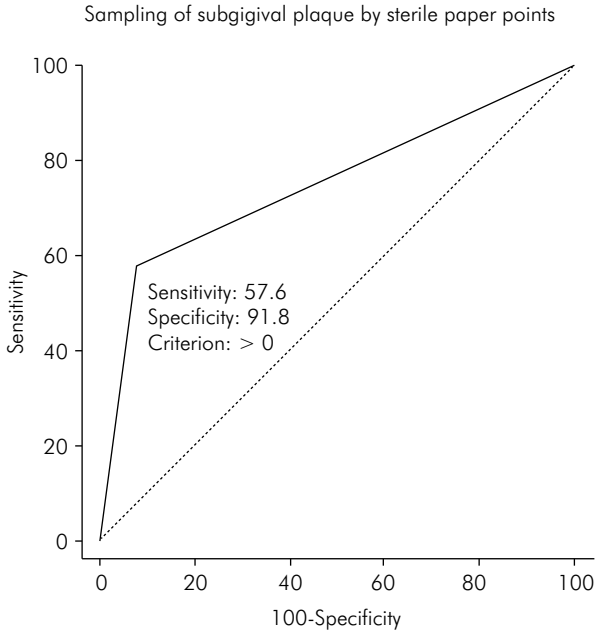

Figure 2. ROC curve for sampling methods of subgingival plaque by sterile curette and by sterile paper points.

Statistical differences of yast presence were not observed between groups or between diabetics *vs.* non-diabetics. Sardi *et al.*[20] found differences in subgingival findings of yeasts between well-controlled insulin dependent T2D patients and control group of healthy CP patients. When comparing the prevalence of subgingival positive findings in good *vs.* poorly controlled diabetics, Melton *et al.*[4] found no significant differences. Studies examining the prevalence of subgingival yeasts in healthy patients according to their periodontal status, demonstrated the impact of the periodontal probing depth on the presence of subgingival yeasts[8] which was not the case in our study. *Candida* spp. is an opportunistic pathogen, and it is considered as marker of immunocompromised patients. Periodontitis itself has been recognized as a state of disturbed cellular and humoral immune local response[23] and patients with diagnosed diabetes are also considered immunocompromised. Our results, which show a similar prevalence of subgingival yeasts in healthy patients and healthy patients with diagnosed CP, are in contrast with these facts. We examined only the presence of yeasts which does not always lead to clinical infection. Some authors indicate that the presence of yeasts in subgingival sites is transient.[24] The reaction of host immunity around yeasts was not a subject of investigation or the exact species of *Candida* genus. *Candida* spp. is capable of adhering to epithelial cells and inducing inflammation.[25]

There is an increasing number of studies investigating subgingival prevalence of *Candida* spp. Some studies used a sterile curette as sampling method,[7,24] while others used sterile paper points.[8,9] To the best of our knowledge, there is no explanation in any of these studies about the choice of the sampling method. In an attempt to answer this question, we used both methods in the same pocket and ROC analysis was carried out. A sterile

curette method was defined as the "golden standard". Specificity, which represents true negative results, was excellent (0.919) but sensitivity, which represents the probability that test results will be positive when the disease is present, was 0.576. The area under the curve shows a fair accuracy of the test. According to this ROC analysis, it may be concluded that subgingival plaque sampling by means of a sterile curette is more accurate than sampling by means of sterile paper points. This is in agreement with the fact that *Candida* spp. forms its colonies on the surface of the epithelial cells[26], *i.e.* it is necessary to "scratch" with the help of a curette in order to ensure the accuracy of the results. On the other hand, sampling by sterile paper points is more appropriate if it is necessary to quantify *Candida* spp. Sampling with paper point can be standardized in terms of paper point size, duration of the presence of the paper point in the pocket and paper point pressure in the pocket. In different studies, there are differences in paper point size and insertion duration when sampling the subgingival plaque. Considering that numerous studies use paper points as sampling method, methodology should be standardized in order to compare results.

It has already been proven that subgingival sites may be a reservoir of *Candida* spp.[9] In our study 12.9% of all patients harbored yeast in subgingival sites with no presence on tongue. The subgingival area is beneficial for *Candida* growth.[16]

The detection of yeast prevalence and their quantification, as well as the recognition of different species, virulence factors and drug resistance in subgingival biofilm are important because of the emerging usage of antibiotics as adjuvant periodontitis therapy. The usage of a broad spectrum antibiotics may lead to *Candida* opportunistic infections and periodontal destruction. Some authors indicate that the presence of yeasts in the subgingival area is transient,[24] *i.e.* the study to be carried out should be rather longitudinal than cross-sectional.

Conclusions

Candida spp. is more prevalent on tongue in the case of diabetics than in the healthy control group, regardless of periodontal status. In addition to that, diabetics with poor glycoregulation exhibited more yeast than patients with good metabolic control. The subgingival area may represent reservoir of commensals. However, longitudinal studies are needed to confirm these results. Correspondingly, it is necessary to standardize sampling methods for the collection of subgingival plaque.

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Detection of Herpes Simplex Virus Type 1 in Gingival Crevicular Fluid of Gingival Sulcus/Periodontal Pocket Using Polymerase Chain Reaction

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SUMMARY

Introduction Pathogenesis and some characteristics of periodontitis cannot be fully explained by bacterial etiology alone. Herpes viruses may bridge the gap between clinical characteristics and molecular understanding of periodontal destruction.

Objective The aim of this study was to investigate the prevalence of herpes simplex virus type 1 (HSV-1) in gingival crevicular fluid (GCF) of healthy and damaged periodontium in Serbian population and to explore potential correlation between the presence of this virus and the level of periodontal destruction.

Methods Samples were collected from gingival sulcus/periodontal pockets by sterile paper points and the presence of viral DNA in gingival crevicular fluid was assessed by PCR.

Results There was no statistically significant difference in HSV-1 in presence between periodontitis patients (PG=38.9%) and healthy controls (HC=32.3%), (Chi-square test, with Yates' correction p=0.7574). However, HSV-1 positive patients showed significantly higher values of parameters of periodontal destruction (PPD=7.11±2.52, CAL=5.46±2.34) than periodontitis patients without HSV-1 in gingival crevicular fluid (PPD=4.70±1.79, CAL=3.39±2.65) (p values respectively, p=0.002 and p=0.023, Independent Samples T-Test). HSV-1 occurred more often in deeper (PPD≥6 mm) (69.2%) than in shallow pockets (3 mm<PPD<6 mm) (18.2%) (Chi-square test, with Yates' correction, p=0.008). Plaque index was lower in the HSV-1 positive group (0.84±0.69 vs. 1.43±0.76, p=0.023, Independent Samples T-Test).

Conclusion This study demonstrated that the presence of HSV-1 in the gingival crevicular fluid coincides with a higher degree of tissue destruction in patients with periodontitis.

Keywords: periodontitis; herpes simplex; gingival crevicular fluid; periodontal pocket

INTRODUCTION

Plaque-associated periodontal diseases are chronic infections caused by a mixed microbial flora, resulting in an inflammatory process that leads to periodontal attachment loss and ultimately tooth loss [1]. Although bacteria of dental biofilm are known to be the most important etiological factor for periodontal disease, a susceptible host is also needed. Immune-inflammatory reaction that develops in periodontal tissues in response to chronic bacterial presence results in the destruction of structural components of the periodontium [2].

Bacterial etiology has not been able to explain rapid periodontal tissue breakdown in cases with minimal plaque, or low levels of common risk factors [3]. Other aspects of periodontitis that cannot be fully explained by bacterial etiology are disease remission and reactivation [4], periodontitis site specificity [5], progression of periodontal destruction in some patients and not in others [6], evolution of gingivitis to periodontitis, or stable to disease-active periodontitis [7].

Herpes viruses and their biology may provide some answers for better understanding of mechanisms involved in the degradation of periodontal tissues.

Herpes viruses have been found in periodontal tissues and in gingival crevicular fluid in chronic [8], advanced [9] and aggressive [10] periodontitis as well as in the periodontium of HIV patients [11] and patients with the following syndromes: Papillon-Lefévre [12], Down [13] and Kostmann [14].

Currently, it is believed that the pathogenesis of some types of periodontitis is a multistep process, involving a complex interaction between the host, bacteria, viruses, and a variety of environmental factors.

OBJECTIVE

The aim of this study was to investigate the prevalence of HSV-1 in gingival crevicular fluid of healthy and damaged periodontium in Serbian population and to explore whether there is a correlation between the presence of this virus and the level of periodontal destruction.

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METHODS

The study, approved by the Ethical Committee of the School of Dentistry, included 67 subjects (25 men and 42 women, age 18-76). The group of patients with periodontitis (periodontitis group -PG) consisted of 36 patients (18 men and 18 women, age 23-76) who had clinical signs of periodontitis and were treated at the Clinic for Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade. Healthy control group (HC) consisted of 31 volunteers (7 men and 24 women, age 18-33) without clinical signs of periodontitis. Exclusion criteria for the study were: 1) known systemic diseases (cardiovascular, respiratory, renal, malignancy, etc.), 2) presence or history of any severe infections, 3) systemic antibiotic or immunomodulatory treatment in the previous 3 months, 4) long-term treatment with any medication suspected to affect the periodontium (e.g. non-steroidal anti-inflammatory drugs), 5) pregnant or lactating women and 6) less than 20 teeth present, 7) less than 3 teeth from Ramfjord examination model, (8) any therapy of periodontitis 1.5 year period prior to the study.

Anamnestic data included history of oral manifestation of recurrent herpes infection and information regarding smoking.

Clinical examinations included the determination of Plaque index – PI (Silness-Löe) [15], Gingival index – GI (Löe-Silness) [16], bleeding on probing – BOP (Mühlemann-Son) [17], clinical attachment loss – CAL and probing pocket depth – PPD. The probings were done by Williams probe calibrated in millimeters and were assessed on six Ramfjord's teeth [18, 19]. Subjects were assigned to the periodontitis group (PG) if they had at least three sites with probing pocket depth ≥3 mm and a clinical attachment loss ≥2 mm in at least three quadrants. The PG group was divided into two subgroups according to PPD values. The first subgroup included patients with PPD 3-6 mm and the second patients with PPD ≥6 mm. Subjects were assigned to the control group (HC) if they had PPD ≤3 mm, CAL=0 and did not have bleeding on probing.

The samples were collected 24 hours after the periodontal examination in order to avoid blood contamination of the samples. All samples were collected from the gingival sulcus/deepest periodontal pocket. The sample site was isolated from saliva with cotton rolls and gently air dried. The supragingival plaque was removed by sterile cotton pellets. Two paper points were inserted in each gingival sulcus/periodontal pocket until a mild resistance for 30 seconds. Paper points contaminated with blood were not used in the analysis. Those points were placed in sterile plastic tubes containing saline. All samples were stored at -70°C until further analysis.

The PCR procedure was carried out at the Laboratory for Molecular Biology, School of Dental Medicine, University of Belgrade. After thawing, the DNA was isolated by boiling at 100°C for 10 minutes.

HSV-1 type-specific oligonucleotide primers (forward 5'- ATA CCG ACG ATA TGC GAC CT and reverse 5'- TTA TTG CCG TCA TAG CGC GG) were used to amplify the 110bp region of thyimidine kinase gene, unique for HSV-1.

The PCR was performed in the total volume of 25 μl containing 2.5 μl of 10X PCR buffer (MBI Fermentas, Lithuania), 1.5 μl of $MgCl_2$, 0.2 mM dNTPs, 0.375 μM of each primer, 1 unit of Taq DNA polymerase (MBI Fermentas, Lithuania), 3 μl of biological sample and water to the final volume of 25 μl.

The PCR amplification was performed in a thermal cycler (PCR Express, Hybaid, USA). After the initial incubation at 94°C for 10 minutes, the PCR procedure included a 35-round amplification process that was performed in three steps covering denaturation (at 94°C for 1 minute), annealing (at 52°C for 1 minute) and extension (at 72°C for 3 minutes), followed by a final extension at 72°C for 7 minutes.

The PCR products were loaded onto 8% polyacrilamide gels and stained with 0.5 μg/ml of ethidium bromide after electrophoresis. The gels were analyzed and photographed under UV rays on transilluminator (Power Station 300plus, Labnet International, INC, USA). A one-kb DNA ladder digest (MBI, Fermentas, Lithuania) was used as a molecular size marker.

Each gel contained a negative and a positive control; for the negative control, samples were replaced with water while DNA samples obtained from patients with herpes labialis were used as positive controls.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 15. The Kolmogorov-Smirnov test allowed for normal evaluation of data distribution. The Independent Samples T-test was used for comparing continuous variables. Statistical differences between frequencies were tested with Chi-square test with Yates' correction. In all analyses the significance level was set at 0.05.

After training and calibration, the samples were collected by the first and second author. For the evaluation of intra- and inter- reliability, 10% of randomly selected subjects were re-examined 2 weeks after the first examination. Reliability was tested by applying the Cohen-Kappa test (performed in SPSS for Windows). The Cohen's Kappa score was determined for each periodontal index in order to test the intra-and inter-observer agreement.

RESULTS

The Kappa scores were 0.5-0.7, representing a very good agreement [20].

The age and sex distribution of study subjects are shown in Table 1. HC and PG subjects were matched for smoking, but not for age. Clinical parameters for both groups are presented in Table 2.

There were no statistically significant differences in the presence of HSV-1 between PG (38.9%) and HC (32.3%) groups (Chi-square test, with Yates' correction p=0.7574). The difference in mean age was not found neither between

Table 1. Age and sex distributions of patients

Variable	Healthy control group	Periodontitis group
Number of patients	31	36
Age, years (mean±SD)	18-33 (24.81±4.79)	23-76 (45.83±14.49)
Woman (%)	24 (77.4)	18 (50.0)
Smokers (%)	5 (16.1)	10 (27.8)

Table 2. Clinical parameters of Healthy control (HC) and Periodontitis group (PG)

Parameter	Mean±SD		p*
	HC	PG	
PI	0.74±0.77	1.20±0.78	0.018
GI	1.27±0.76	2.19±0.62	0.000
BOP	1.29±0.80	2.45±1.22	0.000
PPD	2.00±0.00	4.19±2.70	0.000
CAL	0.00±0.00	5.64±2.36	0.000

* p-values determined by Independent-Samples T Test

PI – Plaque Index (Silness-Löe); GI – Gingival Index (Löe-Silness); BOP – bleeding on probing (Mühlemann-Son); PPD – probing pocket depth; CAL – clinical attachment loss

Table 3. Clinical parameters of HSV-1 positive and HSV-1 negative patients with clinical signs of periodontitis

Parameter	HSV-1 patients		p*
	Positive	Negative	
PI	0.84±0.69	1.43±0.76	0.023
GI	2.31±0.41	2.10±0.73	0.333
BOP	2.59±0.60	2.36±1.49	0.524
PPD	7.11±2.52	4.70±1.79	0.020
CAL	5.46±2.34	3.39±2.36	0.023

* p-values determined by Independent-Samples T Test

Table 4. HSV-1 in Periodontitis group (PG) of patients according to the pocket depth (PPD)

PG patients	PPD		p*
	3–6 mm (N=22)	>6 mm (N=13)	
HSV+ (%)	4 (18.2)	9 (69.2)	0.008

* p-values determined by using Chi-Square Test with Yates' correction exact test

the patients with or without HSV-1 nor between genders. Sixteen female (38.1%) and 8 male (32.0%) patients were positive for HSV-1 in gingival crevicular fluid samples (Chi-square test with Yates' correction, p=0.8105). Furthermore, the distribution of HSV-1 was similar in smokers (40.0%) and non-smokers (34.6%) (Chi-square test with Yates' correction, p=0.938). We also tried to establish a correlation between the presence of HSV-1 and the recurrence of oral herpes infections. Seventeen patients could not recall if they ever had a recurrent herpes infection. For the rest of them, 14 patients noted at least one episode of herpes labialis, and 36 denied it. There was no statistically significant difference in the presence of HSV-1 between patients who had recurrent HSV infection in the oral region (50.0% were HSV positive) and patients who had not (38.9% were HSV positive) (Chi-square test, p=0.6924).

However, within the PG group, several clinical parameters differed significantly depending on the presence of the virus (Table 3). Probing pocket depth and clinical attachment loss revealed higher values in the patients with HSV, while plaque index was lower in the HSV-1 positive group than in the HSV-1 negative group. In addition, HSV-1 occurred more often in deeper pockets (Table 4), and three out of four deepest pockets measuring 11 mm harbored HSV1.

DISCUSSION

As previously mentioned, the etiopathogenesis of the periodontal disease is not completely clarified. The initial event in the development of periodontitis is the formation of dental biofilm followed by gingivitis. T lymphocytes, B lymphocytes and monocytes/macrophages infiltrate can lead to the accumulation of herpes viruses in the periodontal tissue, as these cells are considered to be the source of viruses [21, 22]. Reactivation of herpes viruses can decrease the local host resistance and lead to the overgrowth of periodontal pathogenic bacteria, as *Porphyromonas gingivalis* [7].

Herpes viruses may contribute to the progression of periodontitis through a number of mechanisms. It is assumed that these viruses are able to express cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation and tissue tropism [23]. They can infect or alter structural cells and host defense cells in the periodontium, and thereby reduce the ability of periodontal tissues to resist bacterial insults [22].

In the multitude of studies dealing with the presence of viruses from the Herpesviridae family in the periodontium, the majority focused on EBV-1 and HCMV [14, 24, 25, 26]. HSV was investigated to a lesser extent [8, 27, 28]. To the best of our knowledge, this is the first study conducted in Serbian population regarding HSV-1 detection in the gingival sulci in subjects with a healthy periodontium as well as in the periodontal pockets of periodontitis patients.

We decided to analyze the presence of this particular virus because it is most common of all viruses from the Herpesviridae family, and causes well-known and frequent oral pathologies – herpetic stomatitis and recurrent herpetic infections most usually manifested as herpes labialis. Our results showed a high prevalence of HSV-1 in GCF (35.8%), which is in agreement with some other authors who reported a high prevalence of this virus in specimens taken with paper points from gingival crevicular fluid/periodontal pockets [8, 9, 27]. Contrary to our results, Nibali et al. [28] found a low prevalence of all investigated herpes viruses, especially HSV-1 in both patients with periodontitis and healthy controls.

In the present study the hypothesis that the presence of HSV-1 is in correlation with the development of periodontitis could not be confirmed because we did not detect any difference in the presence of this virus between the control group and patients with chronic periodontitis. Although, the subjects with a healthy periodontium were much younger than those with periodontitis, this discrepancy should not have an impact on our results as the peak of the primary herpetic infection occurs until the age of five [29]. However, we can assume that periodontal disease did not develop in younger individuals, or did not lead to clinically

noticeable tissue destruction yet. Consequently, it would be valuable to conduct a follow-up of young patients with HSV-1 detected in their periodontium and periodically make clinical examinations.

As for the different number of male and female individuals in the healthy control group, gender itself is not considered as the predilection factor for periodontal destruction [30]. On the other hand, lactation, pregnancy, oral contraceptives, menstrual period may have an impact on periodontal tissues, which is why we excluded females with any of the mentioned conditions. Regarding the influence of gender on HSV-1 prevalence, no gender differences were found in the study performed in Romania from 2004-2005 [31]. As the prevalence of HSV-1 infection varies among different geographic regions, and Romania borders with Serbia, we consider findings of this study relevant in regard to our population.

Contreras and Slots [27] also failed to detect differences in the presence of HSV-1 between PG and HC groups. On the other hand, Grenier et al. [8] reported a higher prevalence of HSV-1 in subjects with periodontitis than in healthy controls. Parra and Slots [9] also found statistically higher prevalence of HSV-1 in patients with chronic periodontitis than in patients with mild gingivitis. The same results were reached by Contreras et al. [22] in gingival tissue specimens. Surprisingly, Bilichodmath et al. [32] found higher prevalence of HSV-1 in patients with chronic periodontitis than in patients with the aggressive form of the disease, but they explained the results as the influence of their patients' age.

The most important result in our study is the relationship between the presence of HSV-1 and pocket depth. Our results showed a significantly higher prevalence of HSV-1 in deeper pockets than in shallower ones; clinical parameters (CAL, PPD) also showed significantly higher values in HSV+ periodontitis patients than in HSV-, which is in agreement with the results of Slots et al. [7]. Other authors did not find correlation between the depth of periodontal pockets and HSV-1 presence. [8]. Our results also showed lower values for the plaque index in PG HSV+ patients, which speaks in favor of HSV-1 influence on periodontal tissue destruction and confirms the hypothesis that viruses might have influence on periodontitis progression in patients with good oral hygiene [3]. Kamma et al. [33] detected significantly higher frequencies of HCMV, EBV-1 and HSV in active and progressive periodontitis sites than in stable sites.

Herpes viruses and in particular HSV-1 are considered to have a potential role in the pathogenesis of some oral diseases. There is evidence of a higher presence of HCMV, EBV-1 and HSV in Nigerian malnourished children with acute necrotizing ulcerative gingivitis (ANUG) [34]. The hypothesis is that herpes viruses can affect the host's immune system, facilitating the development of secondary bacterial infections. Sabeti et al. [35] found a clear relationship between symptomatic periapical lesions and the presence of HCMV and EBV. They presume that viral infections contribute to immune impairment, which in turn creates a fertile ground for endodontopathogenic bacterial infections. This model of pathogenesis could be potentially applied to the shifting of gingivitis toward periodontitis. Furthermore, phases of remission and reactivation of periodontitis might coincide with the latency and the reactivation of viruses [36], whilst viral tissue tropism could explain the site-specificity of periodontal destruction in some patients [37].

CONCLUSION

In the present study, we demonstrated that the presence of HSV-1 in the GCF is related to the degree of tissue destruction in the patients with periodontitis. The confirmation of the role of HSV-1 in the pathogenesis of periodontitis will require a larger sample along with a prospective study that would detect the presence of HSV in the periodontium before the onset, at the time of periodontitis initiation, and periodically during its development. Also, future studies demonstrating the role of HSV infection in the pathogenesis of periodontitis should prove that eradication of viral infection can prevent the progression of periodontal destruction.

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Откривање вируса *herpes simplex* тип 1 у гингивалној течности сулкуса или пародонталног џепа ланчаном реакцијом полимеразе

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КРАТАК САДРЖАЈ

Увод Патогенеза и неке клиничке одлике пародонтопатије не могу се до краја објаснити бактеријском етиологијом. Херпесвируси би могли да премосте јаз који постоји између клиничких особина и познавања патогенетских механизама пародонтопатије на молекуларном нивоу.

Циљ рада Циљ ове студије био је одређивање преваленције вируса *herpes simplex* тип 1 (*HSV-1*) у здравом и оболелом пародонцијуму особа у Србији, као и утврђивање могуће корелације између постојања ових вируса и степена оштећења пародонцијума.

Методе рада Узорци су узимани папирним поенима из гингивалних сулкуса или пародонталних џепова, а вирус је откриван реакцијом ланчаног умножавања молекула ДНК (енгл. *polymerase chain reaction – PCR*).

Резултати Није утврђена статистички значајна разлика у преваленцији *HSV-1* између особа с пародонтопатијом (32,3%) и здравим пародонцијумом (38,9%); χ^2-тест са Јејтсовом (*Yates*) корекцијом: p=0,7574. У групи испитаника с пародонтопатијом параметри који означавају степен оштећења (дубина пародонталног џепа – ДПЏ; ниво припојног епитела – НПЕ) били су значајно већи код оних с откривеним вирусом (ДПЏ: 7,11±2,52 *mm*; НПЕ: 5,46±2,34), него код испитаника без вируса у узорцима гингивалне течности (ДПЏ=4,70±1,79 *mm*; НПЕ=3,39±2,65); Студентов *t*-тест за невезане узорке: p=0,002, односно p=0,023. У дубљим пародонталним џеповима (ДПЏ≥6 *mm*) *HSV-1* је откривен статистички значајно чешће (69,2%) него у плићим џеповима (ДПЏ=3–6 *mm*) (18,2%); χ^2-тест са Јејтсовом корекцијом: p=0,008. Просечне вредности плак-индекса биле су ниже код испитаника са *HSV-1* (0,84±0,69) у поређењу са испитаницима код којих овај вирус није откривен (1,43±0,76); Студентов *t*-тест за невезане узорке: p=0,023.

Закључак Приказана студија показала је да је постојање *HSV-1* повезано са нивоом оштећења ткива код особа с пародонтопатијом.

Кључне речи: пародонтопатија; *herpes simplex*; гингивална течност; пародонтални џеп

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Na osnovu člana 49. Statuta Stomatološkog fakulteta Univerziteta u Beogradu, Nastavno naučno veće Stomatološkog fakulteta, na VI redovnoj sednici u školskoj 2015/16. godini, održanoj 07.06.2016. godine, donelo je sledeću

O D L U K U

Usvaja se pozitivan izveštaj Komisije za ocenu završene doktorske disertacije **dr Sanje Matić**, pod nazivom „UTICAJ POLIMORFIZAMA GENA ZA INFLAMATORNE CITOKINE I NJIHOVE RECEPTORE NA NIVO CIRKULIŠUĆIH CITOKINA I KLINIČKE PARAMETRE KOD PACIJENATA SA HRONIČNOM PARODONTOPATIJOM I DIJABETES MELITUSOM TIPA 2“

Imenovani/a će javno braniti doktorsku disertaciju, ukoliko dobije pozitivno mišljenje Veća naučnih oblasti medicinskih nauka Univerziteta u Beogradu, pred komisijom u sastavu:

1. prof. dr Saša Čakić
2. prof. dr Zoran Aleksić
3. prof. dr Branka Popović
4. prof. dr Nebojša Lalić, Medicinski fakultet u Beogradu

O b r a z l o ž e nj e

Veće naučnih oblasti medicinskih nauka, na sednici od 24.09.2013. godine, dalo je saglasnost na predlog teme doktorske disertacije dr Sanje Matić, pod nazivom „UTICAJ POLIMORFIZAMA GENA ZA INFLAMATORNE CITOKINE I NJIHOVE RECEPTORE NA NIVO CIRKULIŠUĆIH CITOKINA I KLINIČKE PARAMETRE KOD PACIJENATA SA HRONIČNOM PARODONTOPATIJOM I DIJABETES MELITUSOM TIPA 2“

Imenovani/a je objavio 2 rada:

- u časopisu „Brazilian Oral Research“, rad pod nazivom: „Detection and sampling methods for isolation of Candida spp. from oral cavities in diabetics and non-diabetics“ (2015)

- u časopisu „Srpski arhiv za celokupno lekarstvo“, rad pod nazivom: „Detection of herpes simplex virus type 1 in gingival crevicular fluid of gingival sulcus/periodontal pocket using polymerase chain reaction“ (2014)

Imajući u vidu napred navedeno, Nastavno naučno veće Stomatološkog fakulteta Univerziteta u Beogradu, rešilo je kao u dispozitivu.

Odluku dostaviti: Imenovanom/oj, Univerzitetu u Beogradu, Odseku za nastavu, Veću, Komisiji (4) i Pisarnici.

Referent kadrovskog odseka
Violeta Rastović

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Prof. dr Miroslav Vukadinović

Obrazac 1.

Fakultet STOMATOLOŠKI

UNIVERZITET U BEOGRADU

Stručno veće za medicinske nauke

Broj zahteva

(naziv stručnog veća kome se zahtev upućuje , shodno čl.6 Statuta Univerziteta u Beogradu i čl. 7. st.1 ovog pravilnika)

..god.

(Datum)

ZAHTEV

za davanje saglasnosti na izveštaj o urađenoj doktorskoj disertaciji

Molimo da, shodno članu 68. st.3. Zakona o univerzitetu ("Službeni glasnik RS" br. 20/98), date saglasnost na

izveštaj o urađenoj doktorskoj disertaciji kandidata SANJE DRAGO MATIĆ
(ime, ime jednog od roditelja i prezime)

KANDIDAT SANJA DRAGO MATIĆ prijavilo je doktorsku disertaciju pod nazivom
(ime, ime jednog od roditelja i prezime)

„UTICAJ POLIMORFIZAMA GENA ZA INFLAMATORNE CITOKINE I NJIHOVE RECEPTORE NA NIVO CIRKULIŠUĆIH CITOKINA I KLINIČKE PARAMETRE KOD PACIJENATA SA HRONIČNOM PARODONTOPATIJOM I DIJABETES MELITUSOM TIPA 2“

Univerzitet je dana 24.09.2013 svojim aktom pod br. 61206-3512/2-2013 dao saglasnost na predlog teme

doktorske disertacije koja je glasila

„UTICAJ POLIMORFIZAMA GENA ZA INFLAMATORNE CITOKINE I NJIHOVE RECEPTORE NA NIVO CIRKULIŠUĆIH CITOKINA I KLINIČKE PARAMETRE KOD PACIJENATA SA HRONIČNOM PARODONTOPATIJOM I DIJABETES MELITUSOM TIPA 2“

Komisija za ocenu i odbranu doktorske disertacije kandidata SANJE DRAGO MATIĆ
(ime, ime jednog od roditelja i prezime)

obrazovana je na sednici održanoj 15.03.2016. odlukom fakulteta pod br. 3/10

u sastavu:

ime i prezime člana komisije:	zvanje:	naučna oblast:
SAŠA ČAKIĆ	PROFESOR	KLINIČKE STOM. NAUKE
ZORAN ALEKSIĆ	PROFESOR	KLINIČKE STOM. NAUKE
BRANKA POPOVIĆ	PROFESOR	BAZIČNE STOM. NAUKE
NEBOJŠA LALIĆ	PROFESOR	ENDOKRINOLOGIJA

Nastavno-naučno veće fakulteta prihvatilo je izveštaj Komisije za ocenu i odbranu doktorske disertacije na sednici održanoj dana 07.06.2016.

DEKAN FAKULTETA

Prof. dr Miroslav Vukadinović

Prilog:
1. **Izveštaj komisije sa predlogom**
2. **Akt Nastavno-naučnog veća fakulteta o usvajanju izveštaja**
3. **Primedbe date u toku stavljanja izveštaja na uvid javnosti, ukoliko je takvih primedbi bilo.**