



## Prevalence and possible predictors of the occurrence of denture stomatitis in patients older than 60 years

Prevalencija i mogući prediktori nastanka protetskog stomatitisa kod pacijenata starijih od 60 godina

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### Abstract

**Background/Aim.** Denture stomatitis (DS) is one of the most common oral health problems among elderly population with removable dentures. Despite the high prevalence, etiology of the disease is not completely understood. It appears to be multifactorial, with a predominance of local factors. The aim of the study was to determine the prevalence and risk factors that contribute to the development of DS in upper removable denture wearers. **Methods.** This clinical study comprised three groups of subjects with upper removable dentures: the DS group, and the positive and negative control groups. Swab samples were taken from the tongue and palatal mucosa for microbiological examination. Data of denture age, dentures night wearing, unstimulated salivary flow rate (USFR) and saliva pH values were evaluated for all the participants. **Results.** The prevalence of DS was found to be 26.5%. Significantly higher values of overnight wearing ( $p = 0.000$ ) and the mean age of dentures ( $p = 0.022$ ) were found in the DS group compared to the controls. In relation to the positive mycological finding, a borderline significance difference among the groups was confirmed ( $p = 0.053$ ). No significant association was found between DS and gender, age, the type of dentures, USFR, pH of saliva and bacteria findings. The patients who wore dentures at night had 26 times more chances to get DS compared to the patients who did not wear them overnight. **Conclusion.** This study confirms similar characteristics of DS prevalence in elderly population of Vojvodina compared to European. Continuous (overnight) wearing of dentures is considered to be major direct risk factor for DS development, while secondary role is attributed to denture age and oral *Candida* infection.

### Key words:

dentures; stomatitis, denture; prevalence; candidiasis, oral; oral hygiene; preventive dentistry.

### Apstrakt

**Uvod/Cilj.** Protetski stomatitis (PS) jedno je od najčešćih oboljenja usne duplje kod starijih osoba sa mobilnim protezama. Uprkos velikoj rasprostranjenosti, etiologija oboljenja nije u potpunosti razjašnjena. Ona je verovatno multifaktorijalna, sa predominacijom lokalnih faktora. Cilj rada bio je da se utvrde prevalencija i faktori rizika koji doprinose nastanku PS kod nosilaca gornjih mobilnih proteza. **Metode.** Studija je obuhvatila tri grupe ispitanika sa gornjim mobilnim protezama: grupu sa PS, te pozitivnu i negativnu kontrolnu grupu. Brisevi jezika i nepca uzeti su za mikrobiološko ispitivanje. Za sve ispitanike utvrđivani su podaci o starosti proteza, noćnom nošenju proteza, sijalometriji i pH vrednosti pljuvačke. **Rezultati.** Prevalencija PS iznosila je 26,5%. Utvrđena je statistički značajna razlika između grupe sa PS i kontrolnih grupa u odnosu na noćno nošenje proteza ( $p = 0,000$ ) i prosečne starosti proteza ( $p = 0,022$ ). Pozitivan mikološki nalaz u granicama statističke značajnosti ( $p = 0,053$ ) utvrđen je kod pacijenata sa PS. Nije utvrđena značajna povezanost između protetskog stomatitisa i pola, uzrasta, tipa proteza, sijalometrijskog nalaza, pH vrednosti pljuvačke i bakteriološkog nalaza. Pacijenti koji nose proteze noću imali su 26 puta veću šansu da obole od PS u poređenju sa pacijentima koji ih skidaju preko noći. **Zaključak.** Ova studija je potvrdila slične karakteristike prevalencije PS između populacije starih osoba Vojvodine i Evrope. Kontinuirano (noćno) nošenje proteze smatra se glavnim direktnim faktorom rizika od nastanka PS, dok se favorizujuća uloga pripisuje starosti proteze i oralnoj kandidijazi.

### Ključne reči:

zubna proteza; stomatitis; prevalenca; kandidijaza, oralna; usta, higijena; stomatologija, preventivna.

## Introduction

The most common oral health problem among the elderly population in Vojvodina is tooth loss and dentures wearing. Denture stomatitis (DS) is the clinical diagnosis of the disease that occurs in adults with removable dentures. Edema and inflammation of the mucosa covered by denture base are objective signs of the disease<sup>1, 2</sup>. Subjective symptoms as pain, itching and burning sensation are described, but in most patients with DS are asymptomatic<sup>3</sup>. Systematic review of numerous observational and experimental studies analyzing an association between mucosal lesions and wearing of removable dentures has shown that the DS prevalence ranges from 1.1% to over 36.7%<sup>4</sup>. According to Gendreau and Loewy<sup>5</sup>, denture-related mucosal lesions are present in 15–75% of edentulous patients, mostly female. Moreover, some studies suggest that DS is present in two thirds of the patients using removable dentures<sup>6</sup>. Regardless of the large number of studies published there are controversial conclusions in relation to DS prevalence, mainly due to heterogeneity and variations in research methodology<sup>1, 7, 8</sup>.

Despite the prevalence, the etiology of the disease is not completely understood. It appears to be multifactorial, with the predominance of local factors. Colonization of *Candida* genus yeasts, bacteria, denture trauma, poor oral and denture hygiene, continual and night-time wearing of removable dentures, denture age, reduced salivation, low pH values of mucosa and saliva are described as possible factors that contribute to DS occurrence<sup>5, 9–12</sup>. In most of the above mentioned studies, the differences between the groups with and those without DS were analyzed with univariate tests. Additionally, risk factors for DS should be studied with multivariable statistical techniques because most of the factors are interrelated<sup>13</sup>.

There is a lack of evidence concerning the DS prevalence among elderly population in Vojvodina. Also, it remains unclear what are the major risk factors for denture-related mucosal lesions. The aim of this study was to determine the prevalence and risk factors for the development of DS in upper removable denture wearers.

## Methods

This prospective clinical study included all the patients with upper removable dentures (complete dentures, acrylic partial dentures, cast partial dentures), treated at the Department of Oral Medicine of the Clinic for Dentistry of Vojvodina, Faculty of Medicine, Novi Sad, Serbia in the period from March 2010 to January 2014. The sample comprised 159 patients (132 females and 27 males, ranged 60–85 years, average age being 65.84 years) and 30 denture patients (25 females and 5 males of average age of 65.30 years, ranged 60–76 years) who were visiting the Clinic for Dentistry for regular check-ups. Exclusion criteria from the study were: relatively new dentures (aged less than one year), uncompleted medical documentation, oral mucosa lesions (except DS) and the presence of systemic disease (immune, hematologic, neoplastic, infectious and endocrine).

Based on the above mentioned criteria 39 patients were excluded from the study and three groups of patients created: the DS group, and the positive and negative control. The DS group comprised 50 patients following the diagnosis made by the oral medicine specialist according to the Newton classification<sup>3</sup>. Lesions were classified as DS if there were visible inflammatory changes under the denture and discomfort. The positive control group comprised 70 patients with removable dentures which were treated at the Department of Oral Medicine because of subjective symptoms (*glossodynia*, *glossopyrosis* and *dysgensia*) without the presence of oral lesions. Thirty patients with no subjective symptoms and oral mucosa lesions participated as the negative control group. The study was approved by the Local Ethical Committee of the Dental Clinic in Novi Sad. Each patient signed a written informed consent form prior to enrolment in the study.

Age, gender, prosthetic restoration information (prosthesis type, denture age, previous prosthesis, dentures night wearing), regular intake of medications, unstimulated salivary flow rate (USFR), saliva pH values and microbiological findings were collected in a questionnaire structured for the purposes of this study.

Swab samples from the tongue and palate (each lasting 10–15 sec) for mycological and bacteriological examination were taken in all the groups. All samples were taken in the morning (from 8 a.m. to 10 a.m.), and reached the laboratory within 2 h. All swab samples were taken by one investigator. Swab samples were plated on Sabouraud dextrose agar and incubated under aerobic conditions at 37°C for 48 h for yeasts. Colonies were subcultured and their identification included germ tube formation in bovine serum, chlamyospore formation in corn meal agar, ability to grow at 45°C and assimilation test (API 20C Aux, bioMerieux, Marcy l'Étoile, France). Bacteria inoculation was performed on blood agar, MacConkey's agar, and tioglikol media with dextrose (Difco, Detroit, MI, USA) in aerobic conditions at 37°C for 24/48 h. Inoculated culture media was observed after 48 h in order to detect the presence of clinically significant species of bacteria and yeasts in the examined material. Microbiological finding from the tongue or palatal swab sample for each patient was considered positive if yeasts or bacteria growths were detected with more than 5 colonies.

Whole saliva specimens were collected between 8 a.m. and 10 a.m. after swabs were taken. The patients were instructed not to eat, drink, smoke and perform oral hygiene at least 2 h before saliva collection. The patients were comfortably seated and trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they made for 10 min into a sterile graduated glass tube through a glass funnel. Only the liquid component (not the foam) of saliva was measured. Saliva volume and the collection time were used to calculate USFR. The normal value of secreted unstimulated saliva was > 0.1 mL/min.

Saliva pH values were determined by using the standard pH paper indicator with the sensitivity of 0.5 (Neutralit, Merck, Darmstadt, Germany). Strips of paper indicator were placed into the glass tube with previously collected saliva. After 15 sec the values of pH were determined by comparing

the change in color of the paper strips in relation to the attached scale. The pH values from 6.5 to 7.0 were considered normal. According to the measured saliva pH value, the patients were classified into two groups: the group with acid pH value (pH < 6.5) and the group with normal and alkaline pH value (pH ≥ 6.5).

Data were described as frequency distribution, mean ± standard deviation (SD). One-way Anova for continuous variables and  $\chi^2$  test for attributive characteristics were applied for testing the differences between the groups. When normal distribution of numeric features was absent, Kruskal-Wallis test was used. If univariate analyses showed a *p*-value < 0.05 for the group difference, the variable was selected for multivariable logistic regression. Direct logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI). Statistical analysis was performed using SPSS, version 17 (WinWrap Basic, Nikiski, AK, USA).

## Results

Among a total of 189 participants evaluated, DS was confirmed in 26.5%. The prevalence of DS associated with upper removable dentures was analyzed according to age, gender, the type of dentures and night wearing of dentures. In relation to age, the mean value of 65.14 ± 5.19 years was determined in the DS group (Table 1).

The study included 150 subjects (24 male and 126 female), the average age 65.66 ± 4.8 years (range 60–85 years). In relation to the type of denture, a total of 99 (66%) were using complete dentures, 35 (23.3%) partial acrylic dentures and 16 (10.7%) cast partial dentures. According to the Newton classification, in the DS group localized slight hyperemia was diagnosed in 24 (48%) of the patients, diffuse hyperemia in 24 (24%) subjects, while 14 (28%) patients had papillary hyperplasia.

Significantly higher values of overnight wearing (*p* = 0.000) and the mean age of dentures (*p* = 0.022) were present in the group with DS compared to the control groups. In relation to the night wearing, 57 (81.4%) of the patients from the positive control group and 11 (36.7%) from the negative control group did not wear dentures at night, while in the patients with palatal lesions this factor was present in 41 subjects (82%).

The average age of dentures in the positive and negative control groups was 6.87 and 7.5 years, respectively and 9.58 years in the DS group. A positive microbiological finding on yeasts was more often confirmed in patients with DS than in the control groups. However, the significance of this difference was at borderline (*p* = 0.053). Only the mean rank saliva pH value in the negative controls showed significant differences compared with the mean rank saliva pH value in the DS and the positive control groups (*p* = 0.041). No signifi-

Table 1

Univariate tests of potential risk factors among the groups of patients				
Risk factors	Positive control (n = 70)	DS group (n = 50)	Negative control (n = 30)	<i>p</i> -value
Age (years), $\bar{x} \pm SD$	66.19 ± 10.11	65.14 ± 10.19	65.30 ± 8.72	0.05 <sup>3</sup>
Medications intake, n (%)	51 (73.3)	41 (81.4)	21 (70.0)	0.05 <sup>2</sup>
USFR (mL/min), $\bar{x} \pm SD$	0.26 ± 0.23	0.26 ± 0.15	0.27 ± 0.13	0.05 <sup>1</sup>
≤ 0.1, n (%)	3 (3.4)	1 (2)	0 (0%)	
0.11–0.3, n (%)	54 (77.1)	37 (74)	26 (86.7)	0.05 <sup>2</sup>
> 0.31, n (%)	13 (18.6)	12 (24)	4 (13.3)	
Saliva (pH), $\bar{x} \pm SD$	6.34 ± 0.68	6.34 ± 0.65	6.70 ± 0.65	0.05 <sup>1</sup>
< 6.5, n (%)	39 (55.7)	27 (54)	12 (40)	0.05 <sup>2</sup>
≥ 6.5, n (%)	31 (44.3)	23(46)	18 (60)	
Denture age (years), $\bar{x} \pm SD$	6.87 ± 7.27	9.58 ± 7.27	7.50 ± 6.76	0.05 <sup>1</sup>
Gender, n (%)				
male	10 (14.3)	9 (18)	5 (16.7)	
female	60 (85.7)	41 (82)	25 (83.3)	0.05 <sup>2</sup>
Type of denture, n (%)				
complete	47 (67.2)	34 (68)	18 (60)	0.05 <sup>2</sup>
partial acrylic	16 (22.8)	11 (22)	8 (26.7)	
cast partial	7 (10)	5 (10)	4 (13.3)	
Overnight wearing of dentures, n (%)				
no	57 (81.4)	9 (18%)	11 (36.7)	0.05 <sup>2</sup>
yes	13 (18.6)	41(82%)	19 (63.3)	
Yeasts swab, n (%)				
positive	33 (47.2)	32 (64%)	12 (40)	
negative	37 (52.8)	18 (36%)	18 (60)	0.05 <sup>2</sup>
Bacteria swab, n (%)				
positive	16 (22.8)	8 (16%)	3 (10)	
negative	54 (77.2)	42 (84%)	27 (90)	0.05 <sup>2</sup>

USFR – Unstimulated saliva flow rate; <sup>1</sup> Kruskal Wallis test; <sup>2</sup>  $\chi^2$ -test; <sup>3</sup> Anova test;  $\bar{x}$  – mean values; SD – standard deviation.

cant differences were found between the groups in relation to gender, age of patients, medications intake, USFR mean and range values, type of dentures, as well as of microbiological bacteria findings (Table 1).

The results of univariate analysis presented in Table 1, indicate that overnight wearing, age of the dentures, positive oral swab for *Candida* and acid pH value of saliva were significantly associated with the occurrence of DS.

However, the results of logistic regression analysis indicate that only night wearing of dentures (OR = 26.16) was significantly associated with DS. The coefficient of determination from 0.495 showed a relatively good explanation of the dependent variable (DS) over the other independent variables. The *p*-value of Hosmer Lemeshow test showed that the model described well the original data (0.768 > 0.05). The overall success of prediction was 82.5%. According to the Wald criterion, night wearing of dentures significantly contributed to the prediction of disease (*p* < 0.01). Assuming that other variables remained unchanged, the ratio of the probability, which was 26.16, showed that the patients who wore dentures at night had 26 times more chances to manifest DS compared to the patients who removed them overnight (Table 2).

## Discussion

The etiology of DS is multifactorial. Factors associated with the development of DS are: local and systemic<sup>2</sup>. Pressure caused by dentures, continual wearing, reduced salivation, poor denture hygiene, type of dentures material and denture age are described as important factors causing the disease<sup>5</sup>. In relation to systemic impact of DS, diabetes mellitus, smoking, long-term use of antibiotics and corticosteroids, radio(chemo)therapy, nutritional factors – lack of vitamin B12, folate and iron are considered as most common factors for inducing DS<sup>14</sup>.

Our results confirmed that the prevalence of DS in Vojvodina was 26.5%. Previous epidemiological studies showed that the prevalence of DS in patients who wore removable dentures ranged from 15% to 75%<sup>5</sup>. Furthermore, data obtained from studies in South America<sup>15</sup> and Finland<sup>16</sup> were similar to the results of our study. The outcome prevalence is significantly affected by the size of the study. In studies that included 100 or less subjects the prevalence ranged from 45% to 77.5%<sup>17</sup>, while in those with 200 or more, outcome prevalence varied between 17% and 55.5%<sup>13</sup>. More than 80% of our sample consisted of older female subjects; in previous studies<sup>8,18</sup> it was shown that the prevalence of DS was higher in older female adults. However, in our study, no statistically significant difference between the genders was noticed.

Our results indicate that continuous (overnight) dentures wearing are major predictor for DS occurrence. Kossioni<sup>11</sup> and Jeganathan et al.<sup>19</sup> also came to that conclusion. Furthermore, Barbeau et al.<sup>9</sup> found that night wearing is considerably more present in subjects with extended inflammation compared to those with restricted inflammation. In our study, the overnight or continuous denture wearing was observed in more than 80% of the patients with DS. The presence of dentures certainly caused changes in microenvironment between palatal mucosa and denture base, but continuous wearing further favored the development of DS.

The negative impact of night wearing was pronounced due to the absence of mechanical effects of saliva and its antimicrobial compounds (lysozyme, lactoferrin, salivary peroxidase, immunoglobulin A), which are its integral part<sup>20</sup>. It should be noted that reduced flow of saliva in the space between denture base and palatal mucosa, which is caused by good sealing of denture, possibly leads to dynamic changes in pH values and mechanical cleaning capability.

Kossioni<sup>11</sup> points out the relationship between the increased age of the current denture and pinpoint hyperemia.

Table 2

Multivariable logistic regression of potential risk factors								
Risk factors	B	S.E.	Wald	df	<i>p</i>	EXP (B)	95% CI for EXP (B)	
							lower	upper
Age	0.018	0.025	0.530	1	0.466	1.018	0.970	1.069
Medications intake	0.618	0.665	0.832	1	0.655	1.754	0.358	2.554
USFR	-1.452	1.361	1.139	1	0.286	0.234	0.016	3.370
Saliva pH	0.317	0.408	0.605	1	0.437	1.373	0.617	3.054
Denture age	0.013	0.034	0.137	1	0.711	1.013	0.947	1.083
<b>Overnight wearing</b>	<b>3.264</b>	<b>0.571</b>	<b>32.633</b>	<b>1</b>	<b>0.000</b>	<b>26.161</b>	<b>8.536</b>	<b>80.177</b>
Yeasts swab	0.241	0.544	0.196	1	0.658	1.272	0.438	3.697
Bacteria swab	-0.273	0.644	0.180	1	0.672	0.761	0.216	2.689
Gender	0.627	0.689	0.828	1	0.363	1.872	0.485	7.226
Complete dentures	-0.280	0.887	0.099	1	0.753	0.756	0.133	4.304
Partial dentures	-0.224	0.980	0.052	1	0.820	0.800	0.117	5.460
Constant	-5,243	3.473	2.279	1	0.131	0.005		

USFR – Unstimulated saliva flow rate; *p* – significance; EXP (B) – Odd ratio; CI – confidence interval.

The age of dentures plays an important role for the occurrence of the disease; thus, in long-term wearing, maintenance of hygiene is difficult and there is tendency towards porosity of denture base. Roughness of denture surface contributes to the increased adhesion of microorganisms and reduced removing of denture plaque after a thorough cleaning<sup>21</sup>. Such a denture is considered as inappropriate and could lead to mucosal trauma and inflammation. In our study, the average age of dentures among the patients with palatal inflammation was significantly higher than that in the control groups. However, observing the influence of all risk factors, denture age did not substantially contribute to the prediction of the disease.

The salivary flow rate and saliva pH values showed no significant influence as DS development factor in this study, although hyposalivation ( $\leq 0.1$  mL/min) was registered in 2% of DS patients. In contrast to that, Sakki et al.<sup>10</sup> suggest that reduced production of saliva is one of the DS risk factors. Olsen and Birkeland<sup>22</sup> point out the difference between the groups with and without DS in relation to the pH values of denture biofilms. However, the same authors did not confirm positive correlation between increased number of yeasts and acidity<sup>23</sup>. It should be noted that pH values in those studies were measured on denture biofilm using microelectrodes, and the yeasts were isolated from denture biofilm.

The prevalence of *Candida* genus yeasts in clinically normal mouths of healthy patients ranges from 25% to 50%. The increased number, up to 88%, is reported in denture wearers. Nevertheless, yeasts are also found in 52% among subjects without removable dentures<sup>24</sup>. It is well-known that dentures itself are most important predisposing factors in the development of oral candidiasis<sup>7,25</sup>. *Candida* has been reported frequently by most of authors as a most common causative agent of DS<sup>5,13,26,27</sup>. Despite this fact, we did not find *Candida* growth as a major risk factor in the occurrence of DS, which is also confirmed in our previous study<sup>28</sup>. Although the prevalence of positive *Candida* swab findings in subjects with DS showed borderline signi-

ficant difference compared to the groups, logistic regression results did not extricate *Candida* as a meaningful predictor. According to some authors, the high prevalence of *Candida* oral colonization is explained by the fact that old age is associated with the development of systemic disease, changes in eating habits and hygiene, as well as the composition of the saliva<sup>20,29</sup>. In some previous studies<sup>26,27,30</sup> colonization of *Candida* has been observed, but we evaluated the presence of oral *Candida* infection only at the current site (palatal lesion and dorsal surface of the tongue). Etiological factors, such as poor denture hygiene, continual and nighttime wearing of removable dentures, accumulation of denture plaque and poor-fitting dentures appear to increase the ability of *Candida albicans* to colonize both the denture and oral mucosal surfaces, where it acts as an opportunistic pathogen<sup>5</sup>.

Several previous studies have pointed out the pronounced impact of poor denture hygiene in the development of palatal inflammation<sup>7,8,12</sup>. Even though we did not analyze this factor in the present study, our previous results emphasized the importance of maintaining proper denture hygiene<sup>28</sup>.

### Conclusion

The present study shows similar characteristics of DS prevalence between the population of Vojvodina and Europe. Continuous (overnight) wearing of dentures is considered to be the major direct factor in DS development, while secondary role is attributed to denture age and oral *Candida* infection. Local factors as gender, age, type of dentures, US-FR, the pH of saliva, and microbiological findings show no significant influence. Nowadays, it seems that dentists insufficiently participate in raising patients' awareness of DS prevention. Patients should be educated that removing dentures at night, replacing worn-out dentures after 5 years, as well as periodical visiting dentist are essential in prevention of oral mucosa lesions and DS.

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