ABSTRACT

Introduction: Patients with advanced bone resorption, non-resilient mucosa or presence of exostoses, require specific methods of prosthetic treatment with complete or partial dentures, namely the use of soft materials for lining. Their porous structure is a prerequisite for bacterial and fungal colonization, that creates conditions for the occurrence of denture stomatitis.

The purpose of the present in vivo study is to explore the species and quantity of Candida spp. in the saliva of patients with complete dentures lined with two types of silicone-based elastic materials after a six-month period of observation.

Material and methods: The research was conducted upon 45 completely edentulous patients at the age from 48 to 90 (67.9 ± 9.99 years on the average). They were divided into three groups (A, B, C). For the identification and quantity assessment of the presence of Candida spp. in saliva, the researchers used Chrom agar Candida (BioMerieux) and MALDI TOF spectrophotometric analysis – VITEK MS (BioMerieux).

Results: The screening of the patients before denture delivery demonstrated absence of Candida spp. in the saliva of 44.4% and Candida albicans – 24.4%. After denture delivery, on the third and the sixth month, we found an increased quantity of C. tropicalis in the patients of groups A and B. In the saliva of the patients with denture stomatitis, the predominantly present species was C. tropicalis (54%) isolated in quantities over 10^4 CFU/ml.

Conclusion: The presence of C. tropicalis in the saliva related to this type of treatment is a prerequisite for the development of hard-to-treat candidoses.

Keywords: soft denture liner, resilient liner, Candida spp., saliva, denture stomatitis

INTRODUCTION:

Completely edentulous patients with advanced bone resorption and presence of painful neurogenic spots, thin, non-resilient mucosa and exostoses, require specific methods of prosthetic treatment, namely the making of two-layer complete dentures (acrylic resin complete dentures lined with elastic material). Dentures of this type distribute uniformly masticatory pressure and reduce the trauma of the mucosa. Better denture retention and stability is achieved, due to the improved marginal seal and engagement of additional ridge undercuts [1]. Despite their advantages, elastic lining materials demonstrate some shortcomings that are well systematized by R. Basker& J. Devenport [2].

The high humidity and temperature of the oral cavity, the individual standard of oral hygiene, as well as the qualitative and quantitative composition of the saliva are factors for bacterial and fungal colonization (predominantly of Candida spp.) of removable denture and oral cavity [3, 4]. In a research on 465 healthy persons, 65 and more years of age, wearing removable dentures, Budtz-Jorgensen [5] isolates Candida in 86% of the patients. The results of this study demonstrate most frequent presence of C. albicans (65%), C. glabrata (15%), C. tropicalis (9 %), C. mycoderma (4 %) and other species (7%). Similar data is provided and by other authors [6]. Luo&Samaranayake [7] observe that C. albicans and C. glabrata have better adhesion to the denture surface, due to their hydrophobicity.

The porous structure of the elastic lining materials as well as the lower final hardness increases the probability of bacterial and fungal colonization on their surfaces. Materials with higher final hardness retain fewer microorganisms. P. Wright [8] reports the 9-year success of relining denture with Molloplast B. Some authors prove the fungicidal effect of the elastic lining materials that should be attributed to their property to regulate the pH of the oral environment [9]. That effect can be reduced by the aging of the materials or the use of inappropriate denture cleaners [10]. According to Valentini et al. [3], a larger quantity of biofilm is formed on the silicone-based elastic materials and it is containing Candida spp. different from Candida albicans that are hard to treat.

One of the most frequent diseases of the oral mucosa in complete or partial denture wearers is denture stomatitis. It is present in 15-70% of the patients and most fre-
quent asymptomatic usually diagnosed at random clinical examinations. Denture stomatitis has various types that are well systematized by Newton in 1962 [11]. A number of authors determine Candida albicans as the main strain for denture stomatitis, followed by C. glabrata and C. tropicalis [7, 12, 13, 14, 15, 16, 17], but most of the researchers find fungi on the mucosa and the denture surface. Pereira-Cenci [18] reports that in the recent years a change of the Candida species, specific for this medical condition is observed – the shift is from C. albicans to C. glabrata, C. tropicalis, C. krusei and other non-albicans species. On the other hand, Altarawnehet, et al. [19] report absence non-albicans species in the saliva of patients with denture stomatitis.

The quantity of Candida spp. in the saliva, where some types of denture stomatitis are observed, are controversial. Some authors claim that in levels over 400 (4x10^2) CFU/ml of yeast, the development of denture stomatitis can be expected [20], while according to others, 40% of the patients are carriers of Candida spp. with quantity around 800 (8x10^2) CFU/ml and without any clinical symptoms. The same authors register clinical manifestation of oral candidiasis for levels above 20000 (2x10^4) CFU/ml in saliva [13].

The purpose of the present in vivo study is to explore the species and quantity of Candida spp. in the saliva of patients with complete dentures lined with two types of silicone-based elastic materials after a six-month period of observation.

MATERIAL AND METHODS: Selection and Distribution of the Patients

In the Faculty of Dental Medicine – Sofia 45 patients were prosthetically rehabilitated with dentures (14 men and 31 women), they were between the age of 48 and 90 (67.9 ± 9.99 years on the average) and were distributed in three groups according to the material their dentures were made of.

- First group (control group, group C) – patients treated with conventional complete dentures (made only of heat-polymerized rigid acrylic resin) in both upper and lower jaw, (n=15).
- Second group (group A) – patients treated with complete dentures – conventional denture on the upper jaw, and on the lower jaw – denture lined with heat-polymerized silicone-based elastic material [Molloplast B (Detax, Germany)], (n=15).
- Third group (group B) – patients treated with complete dentures - conventional denture on the upper jaw, and on the lower jaw – denture lined with auto-polymerized silicone-based elastic material [Megabase (Dreve, Germany)], (n=15).

All patients signed informed consent form for participation in the present research. The scientific research was approved by the Research Ethics Commission “KENIMUS” (Statement No. 21/2016).

Before denture delivery, samples were taken from the three experimental groups (n=45), of unstimulated whole saliva, to be excluded any increased levels of Candida spp. The patients were observed for a period of six months. On the third month, we tested 43 patients. In the course of the research two patients refused to be examined. Samples were taken of unstimulated whole saliva for species identification and semi-quantitative estimation of Candida. To the moment of the writing of this article, for a time of six months, 35 patients were observed. For the rest of them (n=8) the six month period ends at the end of 2017.

Before denture delivery and on the third and sixth months after prosthetic treatment, the patients were clinically diagnosed for the presence or lack of denture stomatitis, and its type was determined after the established classification by Newton. Type I – localized erythemic areas (petechiae) usually located on the palate, early stage of the disease. Type II – diffuse erythema covering the whole mucosa under the denture structure of a part of it. Type III – inflammatory nodular/papillary hyperplasia on the mucosa of the palate and the alveolar ridge. On the sixth month, the observed patients were divided into two new groups - a group with denture stomatitis and another one without denture stomatitis.

Selection of patients – criteria for elimination

In the research were not included patients with systemic diseases (asthma, diabetes Type I, uncontrolled diabetes Type II, Sjögren’s Syndrome, conditions of immuno-deficiency) and ones diagnosed with denture stomatitis, as a result of wearing old removable dentures. Patients who had used antibiotics in the previous 3 months and patients who had undergone radiotherapy or chemotherapy in the previous six months were also excluded from this study.

Collection of saliva

During the collecting of saliva, the patients was seated comfortably on the dental chair with his (her) head tilted slightly forward. The samples were taken always in the morning between 09:00 and 12:00 and the patients had been preliminary instructed not to eat before the collecting, not to consume liquids, not to smoked and not to rinse their mouths with antiseptic solutions in the last 2 hours. The necessary quantity of saliva (around 0.5 ml) was collected in sterile containers by the method of spitting (the patients gathered saliva at the bottom of their oral cavity and spitted it at intervals of 60 s), whereas the first portion (the so called dead saliva) was discarded. The samples were stored in a refrigerator at 4°C and carried to the microbiological laboratory in a cooler bag.

Detection, identification and semi-quantitative estimation of Candida spp. in saliva

1. Inoculation of the material:

All collected samples were inoculated in chromogenic agar for the isolation and identification of Candida spp. Chrom agar Candida (BioMerieux).

1. Three inoculations were made: inoculation of 10 µl of the material without dilution, inoculation of diluted saliva in saline in proportion 1:10 (50 µl saliva + 450 µl saline) and in saline in proportion 1:1000 (10 µl from the 1:10 solution + 990 µl saline).
2. The inoculated materials were cultivated for 48 hours at a temperature of 35°C in aerobic conditions.

3. Identification of the isolated species was conducted by:
   - Direct identification of Candida albicans – recognizing is done directly from the chromatogenic environment by the green color of the colonies.
   - All the rest of the Candida species were identified by using MALDI TOF spectrophotometric analysis – VITEK MS (Biomerieux).

Semi-quantitative estimation of the isolated fungi was conducted [21]. The identified species of Candida were written in the result in CFU/ml (Colony forming units/ml – colonies formed on one ml, reflecting the number of living fungi).

For achieving standardization of the hygiene factor for the dentures, all patients included in the present research were freely provided with one and the same tablets for cleaning dentures (Protefix, Germany) for the whole period of observation (6 months).

For the statistical analysis, the researchers used a computer configuration SPSS version 19. For representing the presence of relation between categorical (nonparametric) variables, the researchers used crosstabulation, χ² criteria (chi-square) of Pearson and correlational analysis after Spearman, with confidence interval 95%.

Table 1. Presence of Candida spp. in saliva (count patients)

<table>
<thead>
<tr>
<th>Candida spp. by groups</th>
<th>Group I</th>
<th>Group I</th>
<th>Group II</th>
<th>Group II</th>
<th>Group III</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>control</td>
<td>Molloplast</td>
<td>Molloplast</td>
<td>Megabase</td>
<td>Megabase</td>
</tr>
<tr>
<td>With/ Without in time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before prosthetic treatment</td>
<td>With Candida spp.</td>
<td>66.7% (10/15)</td>
<td>33.3% (5/15)</td>
<td>46.7% (7/15)</td>
<td>53.3% (8/15)</td>
<td>53.3% (8/15)</td>
</tr>
<tr>
<td></td>
<td>Without Candida spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months after prosth. treatment</td>
<td>53.3% (8/15)</td>
<td>46.7% (7/15)</td>
<td>73.3% (11/15)</td>
<td>26.7% (4/15)</td>
<td>84.6% (11/13)</td>
<td>15.4% (2/13)</td>
</tr>
<tr>
<td>6 months after prosth. treatment</td>
<td>63.6% (7/11)</td>
<td>36.4% (4/11)</td>
<td>78.6% (11/14)</td>
<td>21.4% (3/14)</td>
<td>100% (10/10)</td>
<td>0% (0/10)</td>
</tr>
<tr>
<td>p values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p (with, without– before)</td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p (with, without– 3 m)</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p (with, without– 6 m)</td>
<td>0.009*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p (with before, 3 m, 6m)</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS:

In the group of patients researched by us, 88.9% (40/45) had old inconvenient dentures, and 11.1% (5/45) had no dentures. The screening research found that 44.4% (20/45) of the patients had no Candida spp. in their saliva. In the rest of them, the predominant species were C. albicans – 24.4% (11/45) and C. glabrata – 8.9% (4/45).

In 2.2% (1/45) we found C. parapsilosis, in 2.2% (1/45) - C. kefir, in 2.2% (1/45) – C. lucitaniaeand in 2.2% (1/45) – saccharomyces. In the rest of the patients we observed the following combinations of Candida spp: C. albicans + C. glabrata – 4.4% (2/45), C. guillermondi + C. tropicalis – 2.2% (1/45), C. krusei + C. kefir – 2.2% (1/45), C. albicans + C. inopsicus – 2.2% (1/45) and C. dubtiensis + C. tropicalis – 2.2% (1/45). The distribution of the numbers of the patients in groups is presented in Table 1. In the patients, in whose saliva we found Candida, the quantitative data were within norm (≤10³ CFU/ml) in 22.2% (10/45), in 17.8% (8/45) there was light (≥10³ ≤10⁴ CFU/ml) presence, and in 15.6% (7/45) – moderate presence (≥10⁴ ≤10⁵ CFU/ml). The quantitative distribution of the patients in groups is presented in Table 2. Before denture delivery, none of them had been diagnosed with denture stomatitis.
We did not find statistically significant correlation between the quantity and the species of *Candida* spp., and the presence or lack of old dentures ($\chi^2 = 12.939; p = 0.29$; $r_s(\text{species}) = 0.003; r_s(\text{quantity}) = 0.006$).

*C. glabrata* was present in three patients with old dentures and in one without dentures. In one of the patients without dentures, we found *C. parapsilosis* and in another such patient – saccharomyces yeast.

On the third and the sixth months after the prosthetic treatment, in the control group, it was discovered mainly *C. albicans*. On the third month – 26.7% (4/15), and on the sixth month – 27.3% (3/11). In the rest of the patients on the third and the sixth months non-albicans spp. or a combination of *C. albicans* with non-albicans *Candida* ($p < 0.05$) were found, Diagram 1. In 46.7% (7/15) on the third month and 36.4% (4/11) on the sixth month, no *Candida* in the saliva were isolated, Table 1. The quantitative indicators according to groups are presented in Table 2. The increase of the number of patients with *Candida* spp. on the third month did not show any statistical significance ($\chi^2 = 31.071, p = 0.41, r_s = 0.46$).

### Table 2. Quantity of *Candida* spp. in saliva by groups (count patients)

<table>
<thead>
<tr>
<th>Group</th>
<th>Quantity of <em>Candida</em> spp.</th>
<th>Time</th>
<th>Without <em>Candida</em> spp.</th>
<th>$&lt;10^3$ CFU/ml (norm)</th>
<th>$\geq10^3$ &lt; $10^4$ CFU/ml (light)</th>
<th>$\geq10^4$ $&lt;10^5$ CFU/ml (moderate)</th>
<th>$\geq10^5$ CFU/ml (heavy)</th>
<th>$p$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Without prosthet. treatment</td>
<td>After 3 months</td>
<td>After 6 months</td>
<td>After 3 months</td>
<td>After 6 months</td>
<td>After 3 months</td>
</tr>
<tr>
<td>Group I</td>
<td>control</td>
<td>33.3% (5/15)</td>
<td>46.7% (7/15)</td>
<td>36.4% (4/11)</td>
<td>53.3% (8/15)</td>
<td>26.7% (4/15)</td>
<td>21.4% (3/14)</td>
<td>46.7% (7/15)</td>
</tr>
<tr>
<td>Group II</td>
<td>Mollopl.</td>
<td>20.0% (3/15)</td>
<td>13.3% (2/15)</td>
<td>9.1% (1/11)</td>
<td>26.7% (4/15)</td>
<td>6.7% (1/15)</td>
<td>-</td>
<td>20.0% (3/15)</td>
</tr>
<tr>
<td>Group III</td>
<td>Megabase</td>
<td>26.7% (4/15)</td>
<td>26.7% (4/15)</td>
<td>9.1% (1/11)</td>
<td>20.0% (3/15)</td>
<td>46.7% (7/15)</td>
<td>21.4% (3/14)</td>
<td>6.7% (1/15)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Megabase</td>
<td>20.0% (3/15)</td>
<td>-</td>
<td>27.3% (3/11)</td>
<td>-</td>
<td>13.3% (2/15)</td>
<td>28.6% (4/14)</td>
<td>26.7% (4/15)</td>
</tr>
<tr>
<td>Group V</td>
<td>Megabase</td>
<td>-</td>
<td>13.3% (2/15)</td>
<td>18.2% (3/17)</td>
<td>-</td>
<td>6.7% (1/15)</td>
<td>28.6% (4/14)</td>
<td>-</td>
</tr>
<tr>
<td>Group VI</td>
<td>Megabase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$P$ (before) = 0.19

P (after 3 m.) $< 0.0001$

P (after 6 m.) $< 0.0001$

$P$ values

$p$ without (bef., 3 m., 6 m.) $= 0.26$

$p$ norm (bef., 3 m., 6 m.) $= 0.11$

$p$ light (bef., 3 m., 6 m.) $= 0.006^*$

$p$ moderate (bef., 3 m., 6 m.) $= 0.31$

$p$ heavy (bef., 3 m., 6 m.) $= 0.37$

$p$ without (bef., 3 m., 6 m.) $= 0.0002^*$

$p$ norm (bef., 3 m., 6 m.) $= 0.0006^*$

$p$ light (bef., 3 m., 6 m.) $= 0.0003^*$

$p$ moderate (bef., 3 m., 6 m.) $= 0.02^*$

$p$ heavy (bef., 3 m., 6 m.) $= 0.0002^*$

$p$ without (bef., 3 m., 6 m.) $= 0.0001^*$

$p$ norm (bef., 3 m., 6 m.) $= 0.05^*$

$p$ light (bef., 3 m., 6 m.) $< 0.0001^*$

$p$ moderate (bef., 3 m., 6 m.) $= 0.002^*$

$p$ heavy (bef., 3 m., 6 m.) $= 0.65$

$P$ (before) $< 0.0001$

$P$ (after 3 m.) $< 0.0001^*$

$P$ (after 6 m.) $= 0.46$

$P$ (after 6 m.) $< 0.0001^*$
The quantitative change also wasn’t significant (\(p = 0.08\)). Both the species and the quantitative changes were significant on the sixth months (\(\chi^2 = 24.750, p = 0.05\)). Positive correlation was estimated both for the species and for the quantity of *Candida* spp. (Spearman’s correlation \(r_s(spp.) = 0.54, r_s(amount) = 0.64\)).

The presence of *Candida* spp. in the saliva of group A, as well as in that of group B, was statistically significant as compared to group C (\(p < 0.05\)), and that applied both to the third and to the sixth months (Table 1). On the third month in 26.7% (4/15) of the patients in group A we found *C. albicans*, in 26.7% (4/15) – *C. tropicalis*, and in 26.7% (4/15) we found no *Candida* (\(p \leq 0.05\)). On the sixth month, we estimated a decrease of the number of the patients with *C. albicans* – 21.4% (3/13) and an increase in the number of the patients with *C. tropicalis* – 35.7% (5/13), Diagram 2.

There it was also both the species and the quantity that were not statistically significant on the third month (\(\chi^2 = 19.688, p = 0.18\)). On the other hand, the differences between the sixth and the third months were significant (\(\chi^2(spp.) = 49.875, p = 0.000, r_s = 0.96 / \chi^2(amount) = 21.194, p = 0.05, r_s = 0.76\)).

In the patients from group B the same statistical dependences were observed. The change in the species and the quantity of Candida on the third month was not significant (\(\chi^2(spp.) = 16.429, p = 0.66, r_s = 0.26 / \chi^2(amount) = 15.179, p = 0.35, r_s = -0.05\), Diagram 3.
Diagram 3. Distribution of patients with Candida spp. in time – group B

On the third month C. albicans was found in 38.5% (5/13), and C. tropicalis in 30.8% (4/13). In 15.4% (2/13) we found C. parapsilosis, and in 15.4% (2/13) no Candida spp. were registered (p ≤ 0.05). Here, on the sixth month, an increase of the number of patients with C. tropicalis – 50% (5/10) was also estimated.

Unlike group A, in group B on the sixth month, we did not find patients without Candida spp. in their saliva (Tables 1 and 2). On the sixth month C. albicans was registered in 30% (3/10), C. parapsilosis was estimated in 10% (1/10), and in 10% (1/10) – a combination of C. parapsilosis and C. tropicalis. In this group the change of species and the increase of the quantity of Candida on the sixth month was also statistically significant as compared to the third month ($\chi^2_{(spp.)} = 16.444, \ p = 0.06, \ r_s = 0.65 / \chi^2_{(am.)} = 16.444, \ p = 0.05, \ r_s = 0.58$).

On the sixth month in 37.1% (13/35) of the patients denture stomatitis was diagnosed. In one female patient, it was of the Type II according to Newton and had spread both under the upper and the lower denture. In the rest of the patients, we observed the condition mainly under the upper denture ant it was of Type I according to Newton, Pictures 1, 2, 3.

Fig. 1. Patient P.A. with denture stomatitis Type I according to Newton;

In more than half of the patients, 53.8% (7/13) the pathogen was C. tropicalis. In 30.8% (4/13) the denture stomatitis was caused by C. albicans, in 7.7% (1/13) – by C. glabrata, and in 7.7% (1/13) – by a combination of C. albicans and C. kefir (Tables 3 and 4).
In the control group, denture stomatitis was diagnosed in 27.3% (3/11) of the patients. In one patient, in the saliva was found \textit{C. glabrata} in quantities above \(10^8\) CFU/ml. In another patient what was found was a combination of \textit{C. albicans} and \textit{C. kefir} also in quantity \(\geq 10^8\) for \textit{C. kefir} and \(\geq 10^3 - \leq 10^4\) for \textit{C. albicans}, and in the third one – \textit{C. tropicalis} and \textit{C. dubliensis} in quantity \(\geq 10^4 - \leq 10^5\) CFU/ml, Table 4.

In this group, there was a significant difference between the two groups (those with and those without stomatitis) regarding \textit{Candida} spp. (\(p = 0.002^*\), \(r_s = 0.80\)), but in the group with denture stomatitis, there were no statistically significant differences regarding \textit{Candida} spp. (\(p = 1.0\)).

In group A denture stomatitis was diagnosed in 42.9% (6/14). In this group, there was no significant difference between the two groups (with and without stomatitis) regarding \textit{Candida} spp. (\(p = 0.15\), \(r_s = -0.03\)), but in the larger part of the patients with denture stomatitis, the species found was \textit{C. tropicalis} (\(p=0.0007^*\)) (Table 4). In 50.0% (3/6) the quantity of \textit{C. spp.} was above \(10^5\), where two of the patients had \textit{C. tropicalis} (above \(10^5\) and \(10^6\), and one had \textit{C. albicans} (\(\geq 10^5 - \leq 10^6\)). In 33.3% (2/6) the quantitative data were \(\geq 10^4 - \leq 10^5\) where one of the patients was with \textit{C. tropicalis} and another one – with \textit{C. albicans}. In 16.7% (1/6) \textit{C. tropicalis} was found in quantity \(\geq 10^3 - \leq 10^4\).

In group B we diagnosed denture stomatitis in 40% (4/10) of the patients. In this group, there were no significant differences both between the two groups (\(p = 0.52\), \(r_s = -0.46\)) and inside the group with denture stomatitis with regard to \textit{Candida} spp. (\(p = 1.0\)). In two patients the condition was caused by \textit{C. tropicalis}, where the quantity in one of them was \(10^5\), and in the other \(\geq 10^4 - \leq 10^5\). In the other two patients the cause for denture stomatitis was \textit{C. albicans} in quantity \(\geq 10^3 - \leq 10^4\) for one of them, and \(\geq 10^4 - \leq 10^5\) for the other, Table 4.
DISCUSSION

Although the research of saliva gained large popularity in the recent years, the scientific literature provides relatively few publications quoting data of the species and quantity of Candida in the saliva in cases of denture stomatitis. The publications on the elastic lining materials and their relation to the development of this condition are also few.

The results we obtained do not confirm the statements published by Gusmão [6] and Budtz-Jørgensen [5], that over 80% of the patients with dentures have presence of Candida spp. in their saliva. According to the screening research we made, in 44.4% of the patients, there was not registered any Candida spp. in the saliva, provided that 89.9% of them had old dentures. Moreover we did not find a correlation between the presence of complete or partial dentures and the species and quantity of Candida in the saliva (p(chi-square ) = 0.29; r(species) = 0.003; r(quantity) = 0.006). In our research, we registered C. albicans only in 24.4% of the patients, while in 31.2% we found non-albicans spp. and in the largest number of patients - C. glabrata. These results of ours can confirm the above quoted data published by the authors mentioned [5, 6] referring to the predominance of C. albicans over the other non-albicans spp. although the in Budtz-Jørgensen [5] the research is limited to denture surfaces only.

The predominant presence of C. albicans after denture delivery remained in the control group. In this group, unlike the other two groups, a slight increase of the patients with no Candida spp. in their saliva was observed. The increase had no statistical significance both on the third and on the sixth months, a fact also refuting the data of the above cited authors [5, 6], Diagrams 1, 2, 3. The porous structure of the elastic lining materials for complete dentures and the species and quantity of Candida in the saliva (p(chi-square ) = 0.29; r(species) = 0.003; r(quantity) = 0.006). In our research, we registered C. albicans only in 24.4% of the patients, while in 31.2% we found non-albicans spp. and in the largest number of patients - C. glabrata. These results of ours can confirm the above quoted data published by the authors mentioned [5, 6] referring to the predominance of C. albicans over the other non-albicans spp. although the in Budtz-Jørgensen [5] the research is limited to denture surfaces only.

In our research, in 27.3% of the patients from the control group, 42.9% from group A and 40% from group B denture stomatitis was diagnosed (Types I and II according to the classification of A. Newton). In the control group the cause for denture stomatitis in 1/3 of the patients was C. glabrata, and in the remaining 2/3 of the patients – combinations of non-albicans spp. or C. albicans with non-albicans spp. (Table 4). In the other two groups (group A and group B) the main pathogenic causes were C. albicans and C. tropicalis, while C. tropicalis predominated in the patients from group A. In the control group (group C) there was a statistically significant difference between the Candida spp. in the two groups (the patients with and those without denture stomatitis) (p < 0.05), unlike the situation in groups A and B (p > 0.05), Table 4.

The quantity of Candida in the saliva that should be considered related to observable denture stomatitis is controversial. The quantity levels obtained by the estimations in our research are in confirmation with the data of both of the above quoted authors [13, 20]. In the examination of our patients we registered both quantities below $10^3$ and above $10^3$ and $10^4$, without data of denture stomatitis (Table 2), and on the other hand, denture stomatitis was diagnosed both in cases of quantity $10^4$ – $10^5$ and above $10^5$ and in cases with quantitative levels of $10^3$ – $10^5$. The quantitative values where denture stomatitis is diagnosed or is not diagnosed cannot be systematized. It becomes clear that the presence of Candida spp. in saliva, as well as its quantities, are not the only factor determining the emergence of this disease. What should also be taken into consideration is the influence of some additional factors like the surface characteristics of the materials, the protective factors of saliva, the degree of hygiene, the diet, the recommendations of the dental MD, etc. Two of our patients from the control group declared they slept with their dentures and that is pointed out by some authors [1, 2] as a predisposing factor for, the emergence of denture stomatitis.

The results we obtained in the course of this research support the view that denture stomatitis is an opportunistic infection related to imbalanced oral eubiosis or impaired specific cellular immunity.

According to data from scientific literature, stomatitis occurs in 15-70% of people wearing dentures, and as main pathogen causing it most authors point out C. albicans [12, 13, 14, 16, 17]. And yet these claims are founded most often upon testing the mucosal and denture surfaces. Presence of C. albicans and absence of non-albicans spp. in the saliva of patients with denture stomatitis is reported by S. Altarawneh et al. [19]. On the other hand, T. Pereira-Cenci et al. [18] report that in recent years there is an observable shift in the species of Candida in cases of denture stomatitis - from C. albicans to C. glabrata, C. tropicalis and C. krusei. Our data are in contradiction to the statements of Altarawneh et al. [19] and are in confirmation of the data reported by T. Pereira-Cenci et al. [18]. In our research, in 27.3% of the patients from the control group, 42.9% from group A and 40% from group B denture stomatitis was diagnosed (Types I and II according to the classification of A. Newton). In the control group the cause for denture stomatitis in 1/3 of the patients was C. glabrata, and in the remaining 2/3 of the patients – combinations of non-albicans spp. or C. albicans with non-albicans spp. (Table 4). In the other two groups (group A and group B) the main pathogenic causes were C. albicans and C. tropicalis, while C. tropicalis predominated in the patients from group A. In the control group (group C) there was a statistically significant difference between the Candida spp. in the two groups (the patients with and those without denture stomatitis) (p < 0.05), unlike the situation in groups A and B (p > 0.05), Table 4.

The quantity of Candida in the saliva that should be considered related to observable denture stomatitis is controversial. The quantity levels obtained by the estimations in our research are in confirmation with the data of both of the above quoted authors [13, 20]. In the examination of our patients we registered both quantities below $10^3$ and above $10^3$ and $10^4$, without data of denture stomatitis (Table 2), and on the other hand, denture stomatitis was diagnosed both in cases of quantity $10^4$ – $10^5$ and above $10^5$ and in cases with quantitative levels of $10^3$ – $10^5$. The quantitative values where denture stomatitis is diagnosed or is not diagnosed cannot be systematized. It becomes clear that the presence of Candida spp. in saliva, as well as its quantities, are not the only factor determining the emergence of this disease. What should also be taken into consideration is the influence of some additional factors like the surface characteristics of the materials, the protective factors of saliva, the degree of hygiene, the diet, the recommendations of the dental MD, etc. Two of our patients from the control group declared they slept with their dentures and that is pointed out by some authors [1, 2] as a predisposing factor for, the emergence of denture stomatitis.

The results we obtained in the course of this research support the view that denture stomatitis is an opportunistic infection related to imbalanced oral eubiosis or impaired specific cellular immunity.
CONCLUSION
Prosthetic treatment with complete dentures lined with silicone-based elastic materials requires careful planning, taking into consideration the general medical profile of the patient and the individual specifics of his (her) oral environment.

The presence of C. tropicalis in the saliva accompanying this type of denture treatment creates a prerequisite for the development of hard-to-treat candidiases that may spread through other parts of the digestive system.

Auto-polymerized elastic materials create conditions for the significant increase in the quantity of Candida in the saliva both at the end of the third and at the end of the sixth month. It is recommendable for the dentures lined with auto polymerized elastic materials to be used for shorter periods of time, two to four months, and if necessary, the elastic material to be replaced with new one.

Acknowledgements
This study is financially supported through Research Project No 5085/2016 from the Medical University – Sofia, Bulgaria.

The authors thank Dr Boyanka Pavlova, the Medical-Diagnostic Laboratory Cibalab Ltd., Department of Microbiology for the assistance provided in the research.

The authors thank Queisser Pharma, Germany for the free of charge denture tablets for all patients throughout the study.

REFERENCES:
7. Luo G, Samaranayake LP. Candida glabrata, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with Candida albicans. APMIS. 2002 Sep;110(9):601-10. [PubMed] [CrossRef]


---

*Please cite this article as:* Yankova M, Yordanov B, Baykuchev R, Raykova V, Mitov I. Presence of Candida spp. in the saliva of patients with complete dentures, lined with silicone-based elastic materials. *J of IMAB*. 2017 Oct-Dec;23(4):1813-1822. DOI: https://doi.org/10.5272/jimab.2017234.1813

Received: 09/09/2017; Published online: 15/12/2017

---

**Address for correspondence**

Mariana Yankova,
Department of Prosthetic Dental Medicine, Faculty of Dental Medicine, Medical University, Sofia,
1, St. Georgi Sofiiski blvd., Sofia, Bulgaria.
E-mail: marianayankova13@gmail.com, m.jankova@abv.bg