The stomato-oncological significance of the lesions of oral mucosa of microbiological origins and the aspects of their prevention

Ph.D. thesis

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1.Introduction

There are billions of viruses, bacteria, archaea, fungi and protozoa living in the oral cavity and this symbiosis is called oral microbiom. Most of the microorganisms in this unique ecosystem are harmless and some of them are necessary for the human body. However, certain pathogens may cause diseases of the tissues of the oral cavity. The most common oral diseases having a microbiological origin are dental caries and periodontal disease, which are considered endemic diseases. Apart from them other defects may occur such as infections of the soft tissues, inflammation and there are certain pathogens that may take part in the formation of benign and malignant oral tumors. It is crucial to study these pathogens and to prevent and treat the diseases caused by these pathogens as the number of oral tumor cases tends to increase all over the world. Furthermore, Hungary is in the first place among European countries regarding the incidence and mortality of these diseases. According to the statistics conducted in 2018, Hungary was the third country regarding incidence and the fourth country regarding mortality in the world. The etiology of oral tumors is multicausal, however, there are classical, primary etiological factors (e.g. smoking and immoderate alcohol consumption) and there are cocarcinogenic factors, which increase the risk of the formation of tumors. The number of smokers tends to decrease in the world because of several anti smoking campaigns but the number of patients having oral tumor either remains

constant or shows a small increase. There may be several etiological factors, such as the carcinogenic effect of microbiological agents.

Human papillomavirus (HPV) causes the most common sexually transmitted infection in the world; there are 6 million new cases registered worldwide each year. The mechanical contact necessary for the transmission of the pathogen is a micro injury. The most common form of spreading the disease is sexual transmission and the consequence of this infection may involve the mucus membrane of the genitals, of the anus and of the oral cavity and the surrounding areas and the skin of the fingers. The oncogene genotypes of HPV, such as HPV16 and HPV18 cause 99% of cervical cancer infections. HPV may enter the oral cavity due to the changes in sexual behavior and it may cause transient or persistent infections that may cause the formation of benign or malignant tumors. The occurrence of the HPV16 genotype increases the risk of the formation of tumors in the oral cavity 13 times. HPV screening is important to avoid cervical cancer as it effectively reduces the incidence and the mortality of this illness. As the incidence rate of oral cancer types due to HPV positive increases steadily we need to implement oral HPV screening. Although there is a sample collecting protocol in gynecology, there is no such protocol in stomatology, as a unanimous position has not developed yet but there are very dynamic studies in this respect. A universally accepted screening protocol could be a significant preventive means of oral HPV infections, so it

could be an effective prevention of oral malignant tumors, which are caused by HPV.

Apart from HPV studies, the second most popular works that scrutinize the relationship of microbiological agents and oral cancer is related to the carcinogenic role of *Candida* strains. Candidiasis of the oral cavity is a common opportunistic infection of the mouth mucosa caused by the overgrowth of several *Candida* species, the most common being *C. albicans*. The most common occurrence of oral candidiasis is denture stomatitis related to *Candida*. This form of candidiasis is a chronic illness, characterized by atrophy, inflammation, erythema and edema, and this illness occurs in 65% of patients who wear dentures. The key factors that enable this deformity is the wearing of laminar dental prosthesis that do not fit properly, not adequate oral hygiene and denture cleaning, and *Candida* colonization. Adequate oral hygiene and the mechanic cleaning of teeth or of the dentil are very important for the therapy of denture stomatitis. The patients' efforts can be hindered by their impeded manuality (e.g.: when they have arthritis or they are through serious operations or a stroke) therefore the additional usage of antiseptic and or other chemical agents is important. Chlorhexidine digluconate (CHX) is considered the most important antiseptic agent in the treatment of oral biofilms. Locally used sustained-release varnishes (SRV) and gels have appeared recently and by applying them carcinogenic and periodontopathogenic bacteria can be eliminated, however, the antifungal effect of these materials were not thoroughly tested. The role of *Candida* in carcinogenesis is not completely clear but it

seems to be substantiated in several points. Furthermore, it is shown that *C. albicans* is able to synthesize carcinogenic nitrosamines under *in vitro* conditions and it synthesizes the transformation of alcohol into acetaldehyde, which is a toxic, mutagenic chemical. Chronic *Candida* infection can start inflammatory reactions thus playing a role in carcinogenesis. It was also shown that if leukoplakia is caused by *Candida*, it is more susceptible to become malignant compared to other types of leukoplakia. According to several studies the formation of a *Candida* biofilm reservoir on the surface of the teeth leads to a higher risk of oral HPV infection. Micro traumas and inflammation may cause the formation of benign or malignant oral tumors, the etiology of which is often oral HPV infection.
The avoidance or the treatment of *Candida* infection can be an effective means in the elimination of a potential carcinogenic risk among patients who wear dentures therefore it can contribute to the prevention of cancer of the oral cavity.

2.Aims

The aims of my research were inspired by the dramatic stomato-oncological epidemiological situation mentioned earlier and also by the fact that non classical etiological factors came into view. The main focus of my research is the study of the prevention of the most common and most significant microbiological agents -as potential causative factors - in the aspect of stomato-oncology. Therefore my aims are the following:

1. The suitability study of sample collection by oral brush biopsy from representative areas of the oral cavity in screening oral HPV infection.
2. The suitability study of sample collection by oral brush biopsy from representative areas of the oral cavity for HPV screening depending on the latest meta-analytic data.
3. The examination of oral HPV infection of female patients who have been infected by HPV and who have not had tumor in the genital area and the examination of their sexual partners.
4. The study of genital HPV cross contamination depending on sexual transmission.
5. The study of the effect of varnish that contains chlorhexidine and thymol in the change of biofilms that are formed by *Candida albicans, Candida tropicalis, Candida parapsilosis* and *Candida glabrata*.
6. The study of the effect of varnish that contains chlorhexidine and thymol in the formation of biofilms that are formed by *Candida albicans, Candida tropicalis, Candida parapsilosis* and *Candida glabrata*.

3.Materials and methods

3.1Studying the relationship between genital and oral HPV infection by examining the patients

There were a number of patients who were volunteers from Budapest and the scope of this study is based on patients selected in order to suit the criteria of our research. Patients who volunteered to undergo cervical cancer screening were given clinical and cytological examinations and an HPV screening, as well. After these

initial examinations the patients requested an oral cancer screening and HPV screening and if they had a sexual partner they requested that their partners should be examined for oral cancer, genital (glans penis, corona glandis, external urethral meatus) and oral HPVscreening. In the case of female patients, the data of the test results of the following cases were not processed: those patients who had gynecological malignant tumor; those patients who had previously had chemotherapy or radiotherapy, those patients whose immune system had previously been suppressed, those patients who had received aspecific immune strengthening therapy (Inosine Pranobex) and those patients who had received a vaccination against HPV. In the case of male patients the data of the test results of the following cases were not processed: those patients who had genital malignant tumor; those who had previously had chemotherapy or radiotherapy, those whose immune system had previously been suppressed, those who had received aspecific immune strengthening therapy (Inosine Pranobex) and those patients who had received a vaccination against HPV. We processed the data of 34 female patients who lived in a partner relationship and the data of 14 female patients who did not live in a partner relationship. In the case of couples, the average age of women were 30,3 years (19-60 years), the average age of men were 35,7 years (21-66 years) and in the case of female patients who did not live in a partner relationship their average age was 28,9 years (22-40 years). The patients were informed about the possibility of the oro-genital transmission of HPV and about the procedure of the examination and they signed a consent declaration form. (Ethics license code: SE RKEB:

131/2018). The genital examination and sampling, the oral cancer screening and HPV sampling was always done by the same doctor.

There was a stomato-oncological screening done by the naked eye of the examining doctor during the oral cavity examination, followed by brush biopsy sampling of the representative areas of the oral cavity in the following order: right cheek, upper lip, upper buccal gums, left cheek, lower lip, lower buccal gums, hard palate, back of the tongue, sides of the tongue, tip of the tongue, lower surface of the tongue, bottom of the tongue, lower lingual gums, dorsal and lateral parts of the tongue, soft palate, and the arches of the pharynx. Exfoliated cells collected with the cytological brush were put into 1 mL of Phosphate buffered saline transport medium, then the samples were stored at -20°C. Collection of the genital and oral samples patients and their partners were usually done at the same time, or in the case of partners the sampling was done with a time difference of maximum 1 week.
HPV specific sequences were detected by the Medical Microbiological Institute of the Faculty of General Medicine of the University of Debrecen. The preparation of the exfoliated cells (for the purpose of nucleic acid isolation) took place in the following way: the 1 mL cell suspension was centrifuged at 500 g for 5 minutes at room temperature, and the cell sediment was suspended in 200 µl PBS. The isolation of the DNA was done with innuPrep Viral RNA/DNA kit (Analytik Jena, Germany) according to the recommendation of the producer of the equipment.

The quality of the isolated DNA was verified by polymerase chain reaction (PCR) specific for the human ß-globin gene. We detected the HPV-specific sequences (to detect mucosal infectious HPV genotypes) by consensus nested PCR, and the PCR was specific to their L1 gene. The determination of HOV genotypes was done by restriction fragment length polymorphism analysis (RFLP), or by sequencing GP amplimers (Macrogen, Amsterdam, Netherlands). When we suspected a mixed infection of patients, if the sample proved to be positive during the MY PCR test we determined the genotypes by using the GenoFlow HPV array test kit with the recommendation of the producer (DiagCor, Kowloon Bay, Hong Kong). In the case of the genotypes HPV6, HPV11, HPV 16, HPV18, HPV31 and HPV 33 we confirmed the test results of genotype determination by type specific PCR tests. In some cases - due to the fact that the copy amount of the virus was too low - our attempt to detect the genotype was not successful and then we classified the HPV samples into the slightly positive, NA category, which means that the genotype cannot be determined.

3.2Examination of the effect of varnish with chlorhexidine+a thymol agent on various Candida biofilms (therapeutic efficacy study)

In order to test the therapeutic efficacy of the Cervitec Plus® varnish we bred several *Candida* species in a breeding vessel with 24 holes for 48 hours. There was a 6 mm diameter and 1 mm thick acrylate disc in 0.5 mL Sabouraud medium with 8% glucose presence in each

hole. We examined an isolate of one of the species of *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata*. These isolates were from the samples of the Clinical Microbiological Laboratory of Semmelweis University and had proved to be having a biofilm making capability in previous studies. After 48 hours of incubation at 37 ° C, the acrylate discs were washed with sterile saline solution and a Corsodyl® (0.2% chlorhexidine) solution and then we applied a Cervitec Plus® (1% chlorhexidine + 1% thymol) varnish on the biofilm-coated discs. We applied this method in the case of every discs for 5 minutes. We used Nystatin (3% suspension) as a positive control and sterile physiological saline solution as a negative control. We measured the metabolical activity of the biofilms after the application of the substances mentioned above by using the 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) test. The XTT test examines the viability of the cells by the tetrazolium colorimetric process. XTT is broken down into a water-soluble colored formazan during the cellular metabolic activity, which can easily be measured in the cellular supernatant, so that sensitivity to the drug can be tested without destroying the biofilm. On the other hand, this method also allows the examination of the intact biofilm. We added 100 µL of 0.5 mg / mL XTT to each hole that contained the disk and we could measure optical density (OD) at 450 nm for 3 hours after incubation at 37 ° C. The study was carried out at the Clinical Microbiology Laboratory of Semmelweis University.

3.3Testing the effect of Chlorhexidine + Thymol on Various Candida Biofilms (testing prevention efficiency)

We treated the acrylate discs first with the products and then incubated them
in solutions of *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata* for 1 hour at 37 ° C in order to evaluate the preventive effect of antifungal agents. After one hour, the discs were washed with physiological saline solution to remove non-adherent fungal cells. The discs were then incubated in 0.5 mL Sabouraud medium containing 8% glucose. We washed the discs 48 hours later and the metabolic activity of the biofilm was measured by the XTT method, and optical density (OD) was recorded at 450 nm. The OD of each sample was compared to the negative control OD (physiological saline) in order to determine the percentage reduction in the number of living cells in the therapeutic and preventive samples. The study was carried out at the Clinical Microbiology Laboratory of Semmelweis University.

We measured the metabolical activity of the same number of individual organisms among *C. albicans, C. parapsilosis, C. tropicalis* and *C. glabrata* in order to calibrate the XTT probe according to the protocol prescribed by Kuhn: there were no significant differences found in the cases of several *Candida* species in our study.

3.4Statistical analysis

We used IBM SPSS 23 software (IBM Corporation, Armonk, New York, United States) for the statistical evaluation of our results. When we examined the relationship between genital and oral HPV infections we demonstrated the genital and oral HPV involvement among female and male patients by using frequency charts during the statistical calculations and when we examined genital and oral HPV involvement among couples we analyzed the data of HPV occurrence of the partners, as well. We compared the ratio of certain groups by using the chi square test and Fischer's exact test. The significant differences that appeared next to the significance level of 95% are marked with an asterisk (*) in the charts. When we grouped HPV infection on the basis of sex and localization we used Bonferroni correction in order to compare the genotype distribution and other distributions. The Significance level is: $p<0.005$. When we examined the metabolic activity of Candida species in the in vitro study, it was characterized by the mean percentage reduction of OD and standard deviation (SD). In order to determine the differences between the efficacy of the different solutions, we used Kruskal-Wallis and Tukey post hoc tests for each *Candida* species and the statistical significance limit was drawn at $p <0.05$.

4. Results

4.1Results of the study of the relationship between genital and oral HPV infection

There were no cases of precancerous or malignant lesions in the stomato-oncological screening of 82 patients.
We conducted the genital HPV screening of 34 couples, and it means that we handled 136 samples. Furthermore, we also conducted the oral and genital HPV screening of 14 female patients who did not live in a relationship, and it means that we handled 28 samples thus, a total of 164 samples were typed. 76 of these samples were detected to contain HPV DNA (46.30%). When we typed the genital samples of those female patients who lived in a partner relationship HPV DNA was detected in 28 cases (82,40 %) and when we typed the samples of those female patients who did not live in a partnership relationship HPV DNA positivity was detected in 10 cases (71,40%) therefore HPV DNA positivity was detected in 79,20% in all of the female genital samples. When we screened 34 male patients for genital HPV we detected HPV DNA in 50% (17/34) of the cases. The gender distribution of genital HPV infection varies significantly (79.2% vs. 50%, $p = 0.006$).
When we analyzed the oral samples we detected HPV DNA in 21 cases (25,60%), when we analyzed the samples of female patients who lived in a partnership relationship we detected HPV DNA in 9 cases (26,50%), when we analyzed the samples of single female patients we detected HPV DNA in 2 cases (14,30%) therefore HPV DNA positivity was detected in 11 cases in all of the female patients (22,90%) and it was detected in 10

cases in all of the male patients (29,40%). Genital HPV infection was detected in a higher rate among both female and male patients compared to oral HPV infection. This difference is statistically significant in the case of female patients (79,2% vs. 22,9%, $p<0,001$), but it was not statistically significant in the case of male patients (50% vs. 29,4%, $p=0,08$).

It can be observed that oral HPV infection is more common among those female and male patients who are infected with genital HPV compared to those female and male patients who are not infected with genital HPV. This characteristic is 35,50% vs. 23,50% ($p= 0,328$) among male patients and 32,14% vs. 0% ($p=0,162$) among female patients.

When we examined orogenital transmission we could observe that the partners of genital HPV infected men have a higher oral HPV infection rate than the partners of male patients who did not have genital HPV infection (35,30% vs. 17,60%, $p=0,438$). However, when we examined female patients, the partners of those patients who had genital HPV negativity had a higher rate of oral HPV infection than the partners of female patients who had genital HPV infection (50% vs. 25%, $p= 0,328$). However, these differences are not significant either in the case of men or women.

When we examined genital transmission we did not detect any difference between the genital HPV infection of the patient and his or her partner. There were no difference between HPV positive and negative female patients (50% vs. 50%) and there were no difference between HPV positive and negative male patients (82,40% vs. 82,40%).

However, there is a difference between male and female patients who have a genital HPV infection in the respect of the genital HPV infection of their partners: the genital infection of the partners of male patients is 82,4% but the genital HPV infection of the partners of female patients is 50% and this difference has a statistical significance (p=0,023). When we examined the oral samples we could observe that the female partners of those male patients who had genital HPV infection had a higher rate of oral HPV infection than the partners of those female patients who had a genital HPV infection, however, this difference was not significant (35,30% vs. 25%,p=0,461).
The analysis of the 76 HPV DNA samples gave the following results: We detected LR HPV (HPV11,53,57,61,81 genotypes) in 5 samples and we found HPV HR monoinfection in 27 cases. Out of these 27 monoinfection cases we detected the HPV16 genotype in 15 cases; the HPV56 genotype in 3 cases; the HPV66 genotype in 3 cases, and found the following 6 HR strains: HPV18, 31, 33, 45, 51, 58. We could detect coinfection in four cases, which were the following: HPV16/6, 45/68, 16/51, 31/39/45, and all of these contained an HR genotype. We could not detect the HPV genotype of 40 samples, hence we marked them as (HPV NA) in the chart.
When examining the genotypes we found the following data among female patients: We could detect HR HPV in 23 cases among the 38 patients who had genital HPV infection (60,50%), and among them we could detect HPV16 DNA in 10 cases (44%), and this was the most frequent one among all the genotypes. When we examined the genotypes we found the following data

among male patients: We could detect HR HPV DNA in 4 cases (23%) among those who had genital HPV infection, and among these 4 cases 3 of them were typed as HPV16. When we examined the 21 oral HPV positive samples we could detect HR HPV in 4 cases (5,25%), all of which was typed as HPV16. If we examine the distribution of HPV16 based on sex we could observe that 2 of them were men and 2 of them were women. LR HPV DNA was only detected in genital samples and this genotype was detected in 5 cases, 4 of them were present in female patients (10,5%) and 1 of them was present in a male patient (5,9%). We were unable to identify the genotype among 40 cases (52%) of the 76 HPV DNA positive samples (HPV NA). The smallest rate (29%) was among the samples of female patients who had genital HPV infection (n=11) and the greatest rate (81,8%) was among female patients who had oral HPV infection (n=9).

4.2Results of the *in vitro* therapeutic and preventive efficacy of the varnish that contains chlorhexidine + thymol:

When we examined the percentage decrease of the optical density of certain *Candida* species in the biofilm during the therapeutic efficacy test we came to the following results: the effectiveness of the solutions were higher than 49%, except in the case of Cervitec Plus® and *C. glabrata*, in which case the decrease of OD was only 13%. Corsodyl® was found to have the highest rate of decrease of OD among all of the *Candida* species examined, which was 95,24% and 97,54%. In the case of *Candida albicans* and *Candida parapsilosis*, Corsodyl®

was followed by Cervitec Plus® and Nystatin. We could observe significant difference between the efficacy of Corsodyl® and Nystatin in the case of these species. ($p \leq 0.01$). The effectiveness of Cervitec Plus® was slightly better than that of Nystatin in the cases of *C. albicans* and *C. parapsilosis* (there was a slightly greater rate of efficacy in the latter), but there was no statistically significant difference. This difference was not signifiant when Cervitec Plus® was compared to Corsodyl®, which was more effective. In the case of *C. tropicalis* and *C. glabrata* the effectiveness of Corsodyl® had a higher significance than the effectiveness of Cervitec Plus®, but there was no significant difference between the effectiveness of Nystatin and Cervitec Plus®. In the case of *C. tropicalis* and *C. glabrata* Corsodyl® was the first and it was followed by Nystatin and Cervitec Plus®; however, there was no statistically significant difference between Corsodyl® and Cervitec Plus® ($p \leq 0.05$).

The reduction in the number of cells of each Candida species in the biofilm during the prevention study was as follows: in each case, the OD decreased by more than 55%. For each *Candida* species tested, Nystatin was the most effective preventive agent followed by Cervitec Plus® and Corsodyl®. For Nystatin, the mean OD decrease was between 97.13% and 98.29%, which was similar to the OD reduction that was achieved with Cervitec Plus® and these data are 89,53 % and 96,01 % respectively. All *Candida* groups had significantly higher OD reduction with Nystatin than with Corsodyl® ($p \leq 0.05$). Cervitec Plus® showed nearly the same values as Nystatin, and the differences were not statistically significant.

5.Conclusion

Based on the results we may conclude the following: stomato-oncological screening that is supplemented with the brush-biopsy sampling procedure, while using PCR technique is a non-invasive procedure suitable for detecting or identifying HPV DNA in the oral cavity and it should be done routinely. The clinical benefit of this is the HPV screening of genitally HPV infected patients and their partners as risk patients, and in order to prevent HPV infection among sexually active patients, HPV screening should be done prior to HPV vaccination, which is of great importance.

The brush-biopsy method, which was applied at representative areas of the oral cavity that we used in our study produced extraordinary oral HPV prevalence and HPV16 prevalence results compared to the meta-analytical studies conducted in 2017 and 2018. The higher oral HPV prevalence that we observed in our study confirms the validity of oral HPV screening, and the technique we applied may be a step forward in standardizing the oral HPV screening procedure. When we investigated the relationship between HPV genital and oral infections, we found that tumor-free genital HPV infection poses an increased risk of HPV infection in the same person for both sexes. Accordingly, in the clinical practice, HPV positive female patients who go for gynecological testing are also recommended to go to a routine screening for oral HPV infection, especially when they have HPV16 and HPV18 infections.

Our additional researches showed that the partners of men who were genital HPV positive are more likely to be infected with genital HPV and oral HPV than the partners of female patients who are genital HPV positive. The clinical significance of this is that in the case of genital HPV infection of men, the infection very rarely causes any physiological disorders therefore these male patients are so-called silent carriers. Therefore the routine screening of genital HPV for men is potentially a major means for preventing female genital and oral HPV infection. This result also plays an important role in interdisciplinary communication.

There is another result of our research. We found that there was a reduction in the biofilm of *C. albicans, C. tropicalis* and *C. parapsilosis in vitro* on the standardized acrylate surface of Cervitec Plus®, which is a varnish that contains CHX + thymol. This result suggests that Cervitec Plus® has the potential for therapeutic use for very common Candida-induced denture stomatitis in the cinical practice.

Using the same preparation on a standardized acrylate surface had the following result: the biofilms of *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata* were reduced on the treated surfaces after 48 hours, in vitro. The clinical significance of this is in the prevention of denture stomatitis, more specifically: Cervitec Plus® varnish can prevent the *Candida* infection of mechanically cleaned dentures or new dentures. Because of the unpleasant side effects of Nystatin (eg: oral irritation, diarrhea, nausea, vomiting, skin phenomena, allergy), Cervitec Plus® can be considered an alternative preventive agent for the tested *Candida* species and may

play an indirect role in preventing oral squamous cell carcinoma in patients with acrylate dental prosthesis.

New scientific findings:

Brush biopsy sampling procedure from representative areas of the oral cavity is suitable for oral HPV screening when the PCR technique is used.

The use of brush biopsy sampling procedures when it is applied with the PCR techniques in representative areas of the oral cavity also showed a higher proportion of oral HPV DNA for the overall HPV and HPV 16 prevalence, which were reported in the latest meta-analytical studies.

Genital HPV infection poses an increased risk of oral HPV infection in the case of patients who do not have genital cancer.

The partners of genital HPV positive male patients are at greater risk for genital and oral HPV infection compared to the partners of genital HPV positive female patients.

The varnish called Cervitec Plus that has an effect of the sustained release of CHX + thymol is in vitro suitable for the prevention and elimination of a *Candida* biofilm that can form on acrylate surfaces, and it is recommended for the prevention and treatment of denture stomatitis.

6. List of own publications

Publications related to the dissertation

Mensch K, Pongracz J, Nagy A, Kristof K, Bechir A, Pacurar M, Nagy G. (2017) Preventive and Therapeutic Effects of Chlorhexidine Containing Varnish on Candida Biofilm. Revista De Chimie, 68: 2808-2811. **IF: 1,412**

Mensch K, Szarka K, Mensch H, Dobai A, Magyar Z, Pacurar M, Vartolomei AC, Manuc D, Nagy CD. (2018) PCR Technique Assisting the Early Diagnosis of Human Papillomavirus A retrospective clinical study. Revista De Chimie, 69: 2781-2787. **IF: 1,412**

Publication not related to the dissertation

Mensch K, Simonffy L, Dombi C, Szabo BT, Varga J, Juhasz A, Dobo-Nagy C. (2017) Endodontic and microsurgical treatments of maxillary lateral incisor dens invaginatus in combination with cone-beam-computed tomography fusion imaging. Oral Radiology, 33: 147-152. **IF:0,466**

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