치의과학석사 학위논문

Oral Candidal Infection of the Patients with Oral Discomforts : A Retrospective Study

구강 내 불편감을 가진 환자에서 구강 캔디다 감염에 대한 후향적 연구

2015년 7월

서울대학교 대학원

치의과학과 구강내과 · 진단학 전공

최 은 혜

Oral candidal infection

of the patients with oral discomforts

: a retrospective study

구강 내 불편감을 가진 환자에서
구강 캔디다 감염에 대한 후향적 연구

지도교수 박 희 경

이 논문을 치의과학석사 학위논문으로 제출함.

2015년 7월

서울대학교 대학원
치의과학과 구강내과 · 진단학 전공
최 은 혜

최은혜의 치의과학석사 학위논문을 인준함.

2015년 7월

위 원 장 _____ (인)
부위원장 _____ (인)
위 원 _____ (인)

- ABSTRACT -

Oral candidal infection of the patients with oral discomforts : a retrospective study

Eun-Hye Choi, D.D.S.

Program in Oral Medicine and Oral Diagnosis

Dept. of Dental Science,

Graduate School, Seoul National University

(*Directed by Associate Professor* **Hee-Kyung Park**, D.D.S., M.S.D., Ph.D.)

The aims of the study were to evaluate the value of Dentocult® CA compared to Sabouraud' dextrose agar (SDA) culture, to compare the candida culture positive rate, symptom improved rate after antifungal therapy among the patients with oral discomfort retrospectively, and to provide a clinical guideline for diagnosis and treatment in oral candidal infection.

The 328 dental records of patients who complained various oral discomforts at Seoul National University Dental Hospital from January 2012 to September 2013

were reviewed. Candida culture data were obtained from both Dentocult®CA and SDA and analyzed with McNemer test. Based on initial clinical diagnosis, patients' records were categorized as group 1 (glossodynia without objective sign, 58 patients), group 2 (oral mucosal lesion, 101 patients), group 3 (hyposalivation, 31 patients) and group 4 (oral candidiasis, 38 patients). 100 patients were excluded because of inconsistency with initial diagnosis (e.g., squamous cell carcinoma, epithelial dysplasia, leukoplakia) and failure to follow-up after initial examination or antifungal therapy. Candida culture positive percents were compared with Chi-square test among group 1, 2, and 3. The correlation between Candida culture positive and systemic diseases was analyzed with Chi-square and Fisher's extract test. After antifungal therapy, the mean Candida colony count was compared between symptom-improved and symptom-sustained with Student's t-test.

The result suggested that the relative validity of Dentocult®CA approximately equals that of SDA (p=0.066). Dentocult®CA showed 89.1% sensitivity and 85.3% specificity. *Candida* culture positive was found in 21 patients (43.1%), 40 patients (39.6%) and 22 patients (72.0%) in three groups, respectively. There was a significant difference among three groups (p=0.003). In addition, gastrointestinal disease (p=0.030) and genitourinary disease (p=0.012) had significant correlations with the *Candida* culture positive rate. Mean *Candida* colony count showed no significant differences between symptom-improved and

symptom-sustained group.

Oral C*andida* carriage is significantly related to hyposalivation. Antifungal medication can be considered initially or in the process of main symptom treatment, especially in hyposalivation group.

Keywords: oral candidiasis, Dentocult^{R}CA, *Candida* culture, oral discomforts

Student Number **:** 2013-23545

Oral candidal infection

of the patients with oral discomforts

: a retrospective study

Eun-Hye Choi, D.D.S.

Program in Oral Medicine and Oral Diagnosis

Dept. of Dental Science,

Graduate School, Seoul National University

(*Directed by Associate Professor* **Hee-Kyung Park**, D.D.S., M.S.D., Ph.D.)

- CONTENTS-

I. INTRODUCTION

Candida species *(Candida spp.)* are the most common fungal pathogens isolated from the oral cavity. It has been reported that the prevalence of oral *Candida* carriage in healthy adults ranges from 1.9% to 62.3%.[1] Oral candidiasis is considered as an opportunistic fungal infection occurring with underlying predisposing conditions and is caused by several *Candida spp.* which inhabit the oral cavity commensally. The growing incidence of *Candida* infection is relative with the use of broad spectrum antibiotics, growth of HIV infection, and immunosuppressive therapy.[2-4] While oral candidiasis has previously been considered to be a disease mainly of the elderly and very young, its occurrence throughout the general population is now recognized.[5] Since virulence factors and sensitivity to antifungal agents differ among *Candida spp.*, the requirement to identify isolates in species level has been emphasized.[6]

To diagnose oropharyngeal candidiasis, European Society of Clinical Microbiology 2012 guideline recommends to take a swab from the lesion.[7] In a clinical setting, diagnosis is usually based on typical clinical manifestations such as acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis, and angular cheilitis.[8] Although infections by *Candida spp.* are most frequently localized and superficial, candidiasis can be systemic and associated with high mortality in severely immunocompromised patients.

Like oral candidiasis, burning mouth syndrome (BMS), hyposalivation, Sjögren syndrome, oral lichen planus (OLP), and oral recurrent aphthae sometimes shows similar oral discomfort. BMS is characterized by a burning sensation in the tongue, in the

absence of clinical and laboratory findings. Microbiological oral culture is necessary to exclude possible bacterial or fungal invasions before diagnosis of the BMS.[9] Also, it is crucial to discriminate between asymptomatic carriage and pathogen. OLP is a chronic inflammatory disease of unknown etiology affecting the oral mucosa. It has been reported that *Candida* infection ranges from 37 % to 50% in OLP.[10] In certain cases of imposition with OLP[11] or insufficient clinical manifestations pointing within oral mucosa, the lesion may be underdiagnosed or incorrectly diagnosed so that proper intervention with antifungal agents can be delayed with prolonged patients' discomfort.

The aims of the study were to evaluate the value of Dentocult® CA compared to Sabouraud' dextrose agar (SDA) culture, to compare the Candida culture positive rate, symptom improved rate after antifungal therapy among the patients with oral discomfort retrospectively, and to provide an clinical guideline for diagnosis and treatment in oral candidal infection.

II. MATERIALS AND METHODS

Ethical approval

This retrospective study was approved by the institutional review board at Seoul National University Dental School (SNUDS), South Korea (IRB number, S-D20130023 and S-D20130024). The dental records of the patients with oral discomfort at the Department of Oral Medicine, Seoul National University of Dental Hospital (SNUDH), Seoul, from January 2012 to September 2013 were reviewed.

Inclusion and exclusion criteria

All patients showed various oral mucosa discomforts and pain such as burning, tingling, throbbing, dysgeusia / hypogeusia, hyposalivation / xerostomia and numb feeling with or without oral lesion.

Patients who diagnosed as BMS, OLP (either clinically or biopsy confirmed), oral lichenoid mucositis, chronic oral mucositis, recurrent oral aphthae, hyposalivation, and Sjögren syndrome were included. Squamous cell carcinoma, epithelial dysplasia and leukoplakia were excluded. Patients who failed to visit the clinic more than twice or impossible to check the antifungal responses were also excluded.

Collection of oral *Candida* samples and culture, Identification of oral yeast samples

For yeast culture with SDA and identification by VITEK 2 ID-yeast system (API 32-C

System bioMériux yeast identification programme, Lyon, France), commercialization kit containing a pair of sterile cottons in preservation media, the transport media (Transystem^{TM}), was used to collect samples in the suspected oral mucosa region. After swabbing, the kit was sent to laboratory medicine for yeast culture and identification in Seoul National University Hospital (SNUH). Samples were cultured to SDA and incubated at 30℃ for 3 weeks with rubbing to three different directions. After yeast culture, only one colony was isolated to be identified. Results were obtained between 15-18 hours in fully automated VITEK 2 system. Usually, the VITEK 2 ID-YST system for the identification and susceptibility testing of microorganisms is used for invasive candidiasis or candidemia in SNUH.[12]

In order to examine with Dentocult^{R}CA (Orion Diagnostica, Helsinki, Finland), material was collected for cultivation on that kit from the oral regions where patient felt discomfort regardless of clinical manifestations, using a sterile cotton swab according to the manufacturer's recommendation. After rotating the swab on both side of the dip-slide, incubation performed in Nickerson's medium[13] modified by Orion Diagnostica for 2 days at 39 degree Celsius. One examiner recorded all samples on average from both sides by using the manufacturer's scale according to BUDTZ-JöRGENSEN[14]: i: no growth, ii: 1-20 colonies, iii: 21-50 colonies, iv: >50 colonies or confluent growth (mean values of both sides of the dip-slide). According to reply from Orion Diagnostica, dipslide techinique itself was compared to the standard loop method on Petri dish, showing an excellent correlation. Also the model chart of Dentocult^{R}CA is based partly on that work where they have prepared suspensions of microbes with known concentrations and recorded the corresponding colony densities as the model chart. However, they admit that

colony densities should rather be interpreted not as direct references to microbial cell concentrations but as relative amounts of yeast in the oral cavity when sampling is carried out by swabbing the mucous membrane or lesions.

Semi-quantitative test for Dentocult®CA

By using McFarland turbidity standard, four *candida spp.* (*C. glabrata, C. tropicalis, C. lusitaniae and C. parapsilosis*) were chosen to evaluate the semi-quantitative value of the Dentocult®CA. The turbidity of the Mcfarland 0.5 standard is equal to 1-2 x 10^8 CFU/㎖. Four different *Candida spp.* stock solutions were diluted 1:10, 1:100 and 1:1000 respectively. After 48 hours, colony counts on Dentocult®CA were compared based on the concentration. (Fig 1)

Measurement of unstimulated whole salivary flow rate (UWSFR) and stimulated whole salivary flow rate (SWSFR)

UWSFR and SWSFR were measured between 9:00 AM and 4:30 PM. Patients were seated on a chair in a "coach-man" position comfortably and requested to spit (without swallowing) for 10 continuous minutes into a graduated 10ml Falcon tube. Then, patients requested to chew sugar-free gum to stimulate their salivary gland for 2 minutes without spitting. After that, SWSFR were measured for 5 minutes during chewing gums. UWSFR and SWSFR were recorded in milliliters per minute (㎖/min). Hyposalivation was defined as UWSFR < 0.1 ㎖/min.

Application of antifungal medication and its response

Nystatin, fluconazole or itraconazole were prescribed to relieve patient's symptom topically or systemically. Based on manufacturer's recommendation, counts under 20 colonies for side indicated the patient to be a carrier with the spatula method. Subjective symptom improvement by visual analogue score (VAS) or objective lesion improvement was considered to evaluate response of the antifungal treatment after 2 weeks.

Statistical analysis

Statistical analysis was performed using a software program (SPSS Version 21, Chicago, IL, USA). McNemar test was used to compare SDA culture and Dentocult^{R}CA. Characteristics of the subjects were summarized with the use of descriptive statistics. Level of significance between the groups was assessed using overall exact chi-square test, as well as an odds-ratio calculation with a 95% confidence interval. P values <0.05 were considered statistically significant. The correlation between *Candida* culture positive and systemic diseases was analyzed with Chi-square and Fisher's extract test. Student's t-test was used to compare the mean *Candida* colony count between symptom-improved and symptom-sustained after antifungal therapy.

III. RESULTS

Evaluation of Dentocult®CA for Oral Candidiasis

Dentocult®CA showed 89.1% sensitivity and 85.3% specificity. (Table 1) One hundred and fifty-one of the 328 patients (46.0%) showed positive results with Dentocult®CA. Positive cultures in culture method with SDA were obtained from 138 patients (42.1%). The detection rates between two methods were not significant by the McNemar test.

Characteristics of the study populations

The subjects were 328 patients visiting the Department of Oral medicine. They consisted of 251 females and 83 males, and their average age was 61.98.

Based on initial clinical diagnosis, patients' records were divided into 4 groups; glossodynia without objective sign, oral mucosal lesion, hyposalivation and oral candidiasis. The age of patients range from 29 to 84 years (median 63 years) in patients with glossodynia without objective sign, from 20 to 90 (median 59.2) with oral mucosal lesion, from 50 to 84 (median 68.0) with hyposalivation and from 28 to 88 with oral candidiasis. (Table 2)

Prevalence of *Candida* culture positive

Candida culture positive were found in 21 patients (43.1%), 40 patients (39.6%) and 22 patients (72.0%) in three groups, respectively. There was a significant difference among three groups (p=0.003). (Table 3)

Correlations between *Candida* culture positive and systemic disease

Gastrointestinal disease (p=0.030) and genitourinary disease (p=0.012) had significant correlations with the candida culture positive rate. (Table 4)

Identification of *Candida* species in four groups

C. albicans, C. glabrata C. tropicalis, *C. lusitaniae*, *C. rugose*, *C. sphaerica*, and *C. dubliniensis* are identified. *C. albicans,* is the most frequently isolated and *C. glabrata* is followed in all groups. The descriptive results are shown in Table 5.

Mean colony account between symptom-improved and symptom-sustained group after antifungal treatment

In a experimental study by using McFarland turbidity standard, four *candida spp.* (*C. glabrata, C. tropicalis, C. lusitaniae, and C. parapsilosis*) were chosen to evaluate the semi-quantative value of the Dentocult®CA. The turbidity of the Mcfarland 0.5 standard is equal to 1-2 x 10^8 CFU/㎖. Four different *Candida spp.* stock solutions were diluted 1:10, 1:100 and 1:1000 respectively. After 48 hours, colony counts on Dentocult®CA were compared based on the concentration. (Fig 1) Dentocult®CA was proved that it's useful to measure semi-quantitatively and to be used widely including some non-*candida albican* species culture.

Mean candida colony count showed no significant differences between symptom-improved and symptom-sustained group. (Table 6, Fig 2)

IV. DISCUSSION

Hyposalivation group (UWSFR less than 0.1mL/min) correlated with high oral *candida* carriage rate significantly in the present study. It corresponds with the earlier studies, an inverse correlation between salivary flow rate and *Candida* counts in saliva.[15, 16] A study of 601 patients reported that xerostomia possibility is likely to increase with the age and medication. The xerostomia prevalence increased with increasing number of medications used.[17] In a cross-sectional study of unmedicated adults, there was no significant influence of age on the stimulated salivary flow rate or pH.[18] Decreased salivary flow rate and secretory immunoglobulin A level were associated with psuedomembranous oral candidiasis in HIV positive individuls.[19] In addition, cardiovascular and genitourinary disease showed significant relationship with oral *candida* culture positive (p<0.05). It could be explained not those diseases by themselves but the medications which could make synergistic anticholinergic effect such as antihypertensives (e.g., diuretics, b-blockers, and angiotensin-converting enzyme inhibitors), antihistamines, muscarinic receptor, α-receptor antagonists, bronchodilators, and skeletal muscle relaxants.[20] Positive yeast growth from 50% to 58.6% (overall 57.3%) was reported in patients with gastroesophageal reflux disease.[21] However, the sample size is too small to conclude the relationship between the candida culture positive and genitourinary disease.

Usually, the main laboratory diagnostic tests include visualization of fungi in tissue through direct microscopy or histopathology, culture method using a glucose peptone agar (Sabouraud's agar) and detection of antibody, fungal antigens, or DNA with assimilation of genetic tests such as PCR-based methods into routine diagnosis. Tony

Axell et al have indicated that there is a statistically significant relationship between Oricult-N, previous commercial name of Dentocult®CA and periodic acid-Schiff's methods(PAS).[22] Dentocult®CA showed the validity corresponding to SDA culture in determining oral *Candida* carriage with good sensitivity and specificity in this study. Dentocult®CA has some benefits; faster results within 48 hours, semi-quantitative determination, reasonable cost for patients, easy to handle for clinicians, no requirements for auxiliary culture kits longer expired period. If the symptom persists with other medicines, biopsy might be considered. In addition to empirical therapy, biopsy is recommended to rule out more serious lesion such as squamous cell carcinoma in acute atrophic candidiasis and hyperplastic candidiasis.[8] AW Barrett et al have reported that epithelia dysplasia and histologically-determined fungal infection have a significant association, and they recommend considering antifungal therapy to manage oral dysplasia lesions.[23]

Quantitative determination of yeasts in the oral cavity has been used as one of the markers of infection tentatively. It has been believed that increasing candidal load [colony-forming units (CFU) per ml of saliva] and infection had proportional. The diagnosis of oral candidiasis may be established when a culture form saliva is higher than 400 CFU per ml.[24] However, normal candidal carriage is about 300-500 CFU per ml of saliva in about 50% of the healthy population.[25-29] In contrast to those results, candidal load ranges from 4000 to 2000 CFU per ml in infected individuals.[26, 30, 31] Candidiasis without objective manifestations may induce tongue pain with hyposalivation.[32] Manfredi et al conclude that there is no single cut-off value to clearly establish whether the patient is simply a carrier of *Candida spp.* or is developing or affected by an

infection.[33] In accordance with this, there is no significant quantitative difference between anti-fungal respond and *Candida* colony count. The implication of this study is that antifungal medicine could be considered to relieve major symptoms in *Candida* carriage patients when main symptoms persist in spite of proper medications.

In the present study, 34 patients (39.5 %) showed positive among total of 86 OLP patients. This is accordance with findings of other previous studies.[10, 34, 35] These differences among prevalence rates of *Candida* could be explained on the basis of the different culture methods used by different investigators. Antifungal therapy in OLP is controversial. Among the 34 patients in the study group 15 (44.1%) showed positive *Candida* culture on SDA medium and none of the 34 histological sections of OLP showed candidal hyphae on PAS staining. No significant association of positive candidal culture with respect to symptoms and patterns of lichen planus were found.[36] In some of *Candida*-positive patients, antifungal medications seems to be useful to relieve both subjective symptoms and objective signs.[10, 37] Beta defensins expression was correlated with epithelial integrity in OLP. Loss of epithelial cells due to erosion resulted in concomitant decrease in beta defensins and increased *Candida* biofilms in vitro.[38] Therapeutic aspect of OLP is context to accompanying candidal infection. [39]

Similar and adverse results were reported in *candida* species distribution. Yuki Takagi et al reported that *C. albicans* composed the majority of the isolated from both healthy and oral candidiasis groups, and *C. glabrata* was identified in 16.7% of the healthy control and 22% of the oral candidiasis patients.[40] This study revealed that 77.08% of culture positive group has *C. albicans* and 13.89% of them had *C. glabrata.* Although denture-wearing, immunosuppressions, antibiotic therapy, and aging were known to risk factors

for oral colonization or infection with *C. glabrata.*[40] There was no significant relationship between systemic disease and *Candida spp.*

This is the first study that statistically compared among different oral discomforts groups related to oral candida infections. Present study has several limitations. First, this was a retrospective observational study. Some of the trends observed in this study could have reached statistical significance if drop-out rates of the patients had been included. Second, objective scales to evaluate antifungal therapy were insufficient. Further researches will be required to ascertain the relationship that is actually present from commensalism to parasitism in *Candida spp.* transition.

V. CONCLUSIONS

In summary, the present study concludes the following:

1. Approximately equal validity of Dentocult®CA compared to that of SDA, with good sensitivity and specificity that of SDA.
2. A significant relationship of the *Candida* culture positive with hyposalivation.
3. A significant relationship of the *Candida* culture positive with gastrointestinal and genitourinary diseases.
4. *C. albicans* is the most frequently isolated and *C. glabrata* is followed in all groups.
5. No significant differences between symptom-improved and symptom-continued group after antifungal medication, no mean colony count between two groups.

VI. REFERENCES

1. Scully C, Ei-Kabir M, Samaranayake LP. Candida and oral candidosis: a review. *Critical Reviews in Oral Biology & Medicine.* 1994;5:125-157.

2. Crist AE, Jr., Johnson LM, Burke PJ. Evaluation of the Microbial Identification System for identification of clinically isolated yeasts. *Journal of clinical microbiology.* 1996;34:2408-2410.

3. Heelan JS, Sotomayor E, Coon K, D'Arezzo JB. Comparison of the rapid yeast plus panel with the API20C yeast system for identification of clinically significant isolates of Candida species. *Journal of clinical microbiology.* 1998;36:1443-1445.

4. Latouche GN, Daniel HM, Lee OC, Mitchell TG, Sorrell TC, Meyer W. Comparison of use of phenotypic and genotypic characteristics for identification of species of the anamorph genus Candida and related teleomorph yeast species. *Journal of clinical microbiology.* 1997;35:3171-3180.

5. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. *Journal of oral microbiology.* 2011;3.

6. Williams D, Lewis M. Oral Microbiology: Isolation and identification of candida from the oral cavity. *Oral diseases.* 2000;6:3-11.

7. Cuenca-Estrella M, Verweij PE, Arendrup MC, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. *Clinical Microbiology and Infection.* 2012;18:9-18.

8. Akpan A, Morgan R. Oral candidiasis. *Postgraduate medical journal.* 2002;78:455-459.

9. Scala A, Checchi L, Montevecchi M, Marini I, Giamberardino MA. Update on burning mouth syndrome: overview and patient management. *Critical Reviews in Oral Biology & Medicine.* 2003;14:275-291.

10. Lundström IM, Anneroth GB, Holmberg K. Candida in patients with oral lichen planus. *International journal of oral surgery.* 1984;13:226-238.

11. Mehdipour M, Taghavi Zenouz A, Hekmatfar S, Adibpour M, Bahramian A, Khorshidi R. Prevalence of Candida species in erosive oral lichen planus. *Journal of dental research, dental clinics, dental prospects.* 2010;4:14-16.

12. Graf B, Adam T, Zill E, Gobel UB. Evaluation of the VITEK 2 system for rapid

identification of yeasts and yeast-like organisms. *Journal of clinical microbiology.* 2000;38:1782-1785.

13. Nickerson WJ, Mankowski Z. A polysaccharide medium of known composition favoring chlamydospore formation in Candida albicans. *The Journal of infectious diseases.* 1953;92:20-25.

14. Budtz-Jorgensen E. Evaluation of a dehydrated test strip,Microstix -Candida for detection of Candida-induced denture stomatitis. *Scandinavian journal of dental research.* 1976;84:229-233.

15. Torres SR, Peixoto CB, Caldas DM, et al. Relationship between salivary flow rates and Candida counts in subjects with xerostomia. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 2002;93:149-154.

16. Navazesh M, Wood GJ, Brightman VJ. Relationship between salivary flow rates and Candida albicans counts. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 1995;80:284-288.

17. Villa A, Abati S. Risk factors and symptoms associated with xerostomia: a cross-sectional study. *Australian dental journal.* 2011;56:290-295.

18. Parvinen T, Larmas M. Age dependency of stimulated salivary flow rate, pH, and lactobacillus and yeast concentrations. *Journal of dental research.* 1982;61:1052-1055.

19. Mahajan B, Bagul N, Desai R, et al. Pseudomembranous Type of Oral Candidiasis is Associated with Decreased Salivary Flow Rate and Secretory Immunoglobulin A Levels. *Mycopathologia.* 2015:1-6.

20. Scully C, Bagan J-V. Adverse drug reactions in the orofacial region. *Critical Reviews in Oral Biology & Medicine.* 2004;15:221-239.

21. Meurman JH, Toskala J, Nuutinen P, Klemetti E. Oral and dental manifestations in gastroesophageal reflux disease. *Oral surgery, oral medicine, oral pathology.* 1994;78:583-589.

22. Axell T, Simonsson T, Birkhed D, Rosenborg J, Edwardsson S. Evaluation of a simplified diagnostic aid (Oricult-N) for detection of oral candidoses. *Scandinavian journal of dental research.* 1985;93:52-55.

23. Barrett A, Kingsmill V, Speight P. The frequency of fungal infection in biopsies of oral mucosal lesions. *Oral diseases.* 1998;4:26-31.

24. Epstein J, Pearsall N, Truelove E. Quantitative relationships between Candida albicans in saliva and the clinical status of human subjects. *Journal of clinical microbiology.* 1980;12:475-476.

25. Williamson J. Diurnal variation of Candida albicans counts in saliva. *Australian dental journal.* 1972;17:54-60.

26. Arendorf T, Walker D. The prevalence and intra-oral distribution of Candida albicans in man. *Archives of Oral Biology.* 1980;25:1-10.

27. BUDTZ-JÖRGENSEN E, THEILADE E, THEILADE J, ZANDER HA. Method for studying the development, structure and microflora of denture plaque. *European Journal of Oral Sciences.* 1981;89:149-156.

28. Bastiaan RJ, Reade PC. The prevalence of Candida albicans in the mouths of tobacco smokers with and without oral mucous membrane keratoses. *Oral Surgery, Oral Medicine, Oral Pathology.* 1982;53:148-151.

29. Oliver Dt, Shillitoe E. Effects of smoking on the prevalence and intraoral distribution of Candida albicans. *Journal of Oral Pathology & Medicine.* 1984;13:265-270.

30. Farah CS, Ashman RB, Challacombe SJ. Oral candidosis. *Clinics in dermatology.* 2000;18:553-562.

31. Farah C, Lynch N, McCullough M. Oral fungal infections: an update for the general practitioner. *Australian dental journal.* 2010;55:48-54.

32. Osaki T, Yoneda K, Yamamoto T, Ueta E, Kimura T. Candidiasis may induce glossodynia without objective manifestation. *The American journal of the medical sciences.* 2000;319:100.

33. Manfredi M, Polonelli L, Aguirre-Urizar J, Carrozzo M, McCullough M. Urban legends series: oral candidosis. *Oral diseases.* 2013;19:245-261.

34. Hatchuel DA, Peters E, Lemmer J, Hille JJ, McGaw WT. Candidal infection in oral lichen planus. *Oral Surgery, Oral Medicine, Oral Pathology.* 1990;70:172-175.

35. Vučković N, Bokor-Bratić M, Vučković D, Pićurić I. Presence of Candida albicans in potentially malignant oral mucosal lesions. *Archive of Oncology.* 2004;12:51-54.

36. Shivanandappa SG, Ali I, Sabarigirinathan C, Mushannavar LS. Candida in Oral Lichen Planus. *Journal of Indian Academy of Oral Medicine and Radiology.* 2012;24:182-185.

37. Silverman S, Gorsky M, Lozada-Nur F. A prospective follow-up study of 570 patients with oral lichen planus: persistence, remission, and malignant association. *Oral Surgery, Oral Medicine, Oral Pathology.* 1985;60:30-34.

38. Augustine J, Maity C, Kumar P, Gupta S, Ghosh D, Verma M. Epithelial Loss Correlated with Decreased Beta Defensins and Increased Risk of Candida Infections in Oral Lichen Planus. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.* 2015;3:e111.

39. Sarode SC, Sarode GS, Patil A. Therapeutic aspect of oral lichen planus in context to accompanying candidal infection. *Oral oncology.* 2014;50:e34.

40. Takagi Y, Fukano H, Shimozato K, et al. Genotypes of Candida albicans isolated from healthy individuals and their distribution in patients with oral candidiasis. *Journal of Infection and Chemotherapy.* 2013;19:1072-1079.

Table 1. Comparison of *Candida* culture results between Dentocult®CA and SDA culture

SDA culture	Dentocult®CA		
	Positive	Negative	total
Positive	123	15	138
Negative	28	162	190
Total	151	177	328
Sensitivity	89.13 (83.94-94.32)		
Specificity	85.26 (80.22-90.30)		
False positive rate	14.74 (9.70-19.78)		
Diagnostic accuracy	86.89 (83.24-90.54)		
Positive predictive value	81.46 (75.26-87.66)		
Negative predictive value	91.53 (87.42-95.63)		

The detection rates between two methods were not significant by McNemar test (p=0.066)

Table 2. Clinical profile of study populations

	Groups											
	Glossodynia without objective sign (n=58)			Oral mucosal lesion (n=101)			Hyposalivation (n=31)			Oral candidiasis (n=38)		
	F	M	Total	F	M	Total	F	M	Total	F	M	Total
Number	52	6	58	70	31	101	26	5	31	27	11	38
Mean age	61.0	63.2	61.2	59.7	61.6	60.3	68.3	68.8	68.4	66.2	71.6	67.8
(Median)	(63.0)	(61.5)	(63.0)	(57.6)	(65.0)	(59.2)	(67.3)	(71.0)	(68.0)	(67.0)	(76.0)	(69.8)
Range	29-84	45-80	29-84	20-90	37-86	20-90	50-84	55-78	50-84	28-88	32-81	28-88

n, number of patients; F, female; M, male.

Table 3. Prevalence of *Candida* culture positive in 3 groups

	Groups			
	Glossdynia without objective sign	Oral mucosal lesion	Hyposalivation	Overall *P* value
Candida culture				0.003
Negative, n (%)	37 (63.8)	61 (60.4)	9 (29.0)	
Positive, n (%)	21 (36.2)	40 (39.6)	22 (72.0)	
Total	58	101	31	

Table 4. Correlation between *Candida* culture positive and systemic disease

Systemic diseases		Dentocult®CA, n (%)		
		Negative	Positive	*P* value
Cardiovascular	Yes	38 (34.5)	54 (45.8)	0.084[a]
	No	72 (65.5)	64 (54.2)	
Pulmonary	Yes	6 (5.5)	11 (9.3)	0.267[a]
	No	104 (94.5)	107 (90.7)	
Gastrointestinal	Yes	9 (8.2)	20 (16.9)	0.047[a]
	No	101 (91.8)	98 (83.1)	
Genitourinary	Yes	5 (4.5)	17 (14.4)	0.012[a]
	No	105 (95.5)	101 (85.6)	
Endocrine, Metabolic	Yes	30 (27.3)	45 (38.1)	0.081[a]
	No	80 (72.7)	73 (61.9)	
Immunologic	Yes	1 (0.9)	3 (2.5)	0.336[b]
	No	109 (99.1)	115 (97.5)	
Hematologic, Oncologic	Yes	2 (1.8)	2 (1.7)	0.994[b]
	No	108 (98.2)	116 (98.3)	
Neurologic, behavior, psychiatric	Yes	11 (10.0)	20 (16.9)	0.126[a]
	No	99 (90.0)	98 (83.1)	
Osteoarthritis	Yes	12 (10.9)	9 (7.6)	0.392[a]
	No	98 (89.1)	109 (92.4)	

[a]Chi-squre test, [b]Fisher's extract test

Table 5. Prevalence of *Candida* species identified in 4 groups

	Glossodynia without objective sign (n=58)	Oral mucosal lesion (n=101)	Hyposalivation (n=31)	Oral candidiasis (n=38)
C. ablicans, n (%)	19 (86.4)	23 (79.3)	15 (71.4)	25 (78.1)
C. glabrata, n (%)	1 (4.5)	4 (13.8)	4 (19.0)	7 (21.9)
C. tropicalis, n (%)	1 (4.5)	0	1 (4.8)	0
C. lusitaniae, n (%)	1 (4.5)	0	0	0
C. rugose, n (%)	0	0	1 (4.8)	0
C. sphaerica, n (%)	0	1 (3.4)	0	0
C. dubliniensis, n (%)	0	1 (3.4)	0	0

Table 6. The results of symptom improvement after antifungal medications in *Candida* culture positive patients.

Total candida culture positive	Groups			
	Glossodynia without objective sign	Oral mucosal lesion	Hyposalivation	*P* value
No antifungal treatment	2	5	2	
Antifungal treatment	19	35	20	
Symptom improved, n (%)	14 (73.7)	26 (74.3)	14 (70.0)	0.939
Symptom continued, n (%)	5 (26.3)	9 (25.7)	6 (30.0)	
Total	21	40	22	

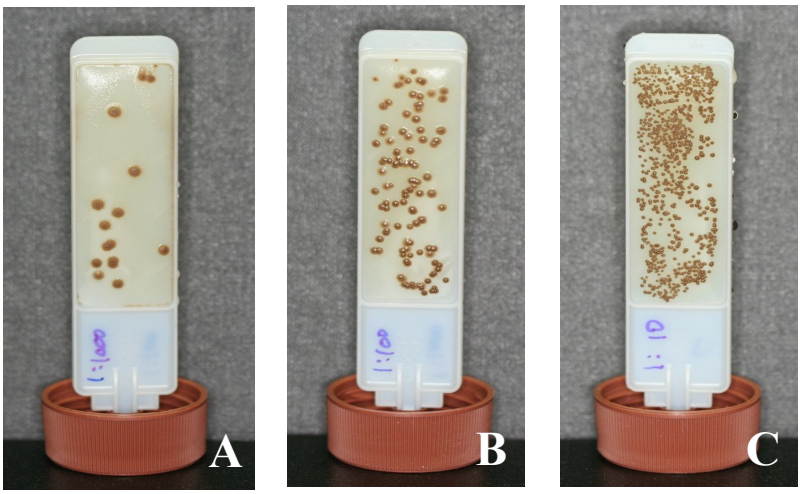

Figure 1. Semi-quantitative determination of the Dentocult®CA with *Candida tropicalis*.

A - 1:1000 dilution, B - 1:100 dilution, C – 1:10 dilution based on McFarland 0.5 standard.

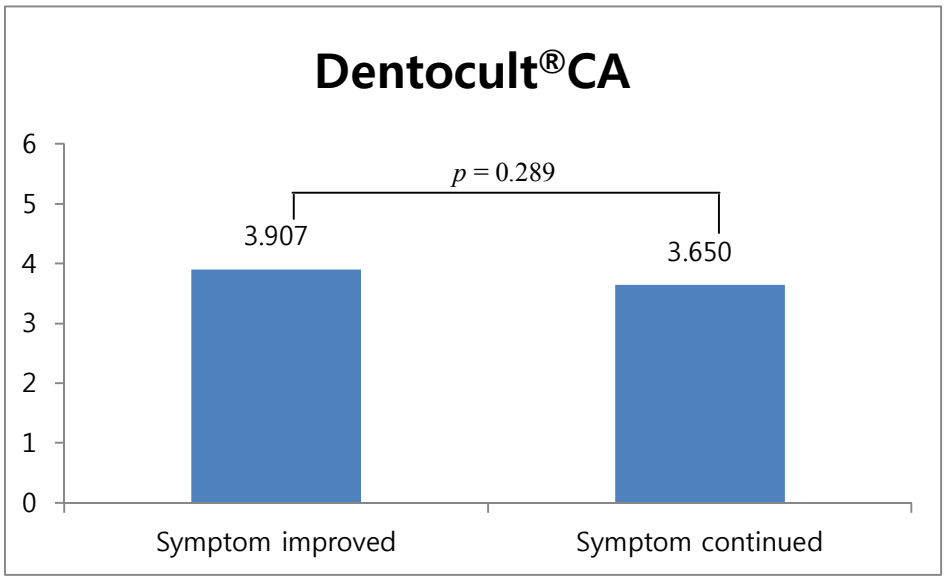

Figure 2. Mean colony count between symptom improved and symptom continued

국문초록

구강 내 불편감을 가진 환자에서 구강 캔디다 감염에 대한 후향적 연구

서울대학교 대학원 치의과학과 구강내과 · 진단학 전공

(지도교수 박 희 경)

최 은 혜

1. 목 적

본 연구의 목적은 구강 캔디다균 배양에서 통상의 Sabouraud's dextrose agar(SDA)를 이용한 배양법과 비교하여 Dentocult® CA 키트의 진단학적 유용성을 확인한다. 구강 내 불편감을 가지고 있는 환자를 대상으로 Dentocult® CA 키트를 이용하여 캔디다균 배양 양성율을 확인하고, VITEK 2 ID-yeast system 을 통해 캔디다균을 동정한다. 또한, 항진균 치료시의 증상 개선에 대한 후향적 연구를 실시하여 구강내 질환의 진단과 치료 시 구강 캔디다 감염에 대한 임상적 지표를 제시하는데 있다.

2. 방 법

서울대학교치과병원 구강내과에 2012년 1월부터 2013년 9월까지 구강 내 불편감을 주소로 내원한 환자들의 의무기록을 후향적으로 검토하여

Dentocult CA® 키트를 사용한 배양과 통상의 SDA 를 이용한 배양 결과를 McNemer test로 분석하였다. 임상진단에 따라 3가지 그룹으로 분류(1군-객관적 징후없는 설통, 2군-점막염증, 3군-타액분비저하군)하여 Chi-square test를 통해 각 군간 캔디다 배양검사 양성율을 분석하였다. 또한 Chi-square test와 Fisher's extract test를 통해 캔디다 배양검사 양성율과 전신질환과의 상관관계를 분석하였다. 항진균제 사용 후 구강 내 증상의 개선을 보인 군과 그렇지 않은 군간의 캔디다 집락 수의 평균을 Student's t-test를 통해 비교하였다.

3. 결 과

328 명의 환자들의 의무기록을 분석한 결과, Dentocult CA® 키트는 89.1%의 민감도, 85.3%의 특이도를 보이며, 통상의 SDA 진균 배양법과 통계적으로 유의한 차이를 보이지 않았다. 총 328 명의 환자 중, 초진시 임상진단에 따라 분류된 3 군에 해당하지 경우(편평세포암, 상피이형성증, 백반증 등)와 진균 배양 검사 이후 혹은 치료 약물 투약 후 내원하지 않아 치료 반응을 볼 수 없는 환자들(총 100 명)은 후향적 검토에서 제외하였다. 총 228 명의 환자 중 처음 내원시 구강 캔디다증으로 진단된 38 명을 제외한 1 군(58 명), 2 군(101 명), 3 군(31 명)에서 각각 21 명(36.2%), 40 명(39.6%), 22 명(72.0%) 캔디다균이 배양되었다. 세 군간의 캔디다 배양 양성율은 통계학적으로 유의미한 차이가 존재하였다(p=0.003). *C. albicans* 는 모든 군에서 가장 빈번하게 동정되었으며, *C. glabrata* 가 다음 순서로 검출되었다.

또한 캔디다 배양 양성율과 위장관 질환(p=0.030), 비뇨생식계 질환(p=0.012)은 통계학적으로 유의미한 관계가 있었다. 항진균제를 사용 후 구강내 증상의 개선을 보인 군과 그렇지 않은 군의 캔디다 집락수는 유의한 차이가 없었다.

주요어 : 구강 캔디다증, Dentocult CA®, 캔디다 배양, 구강점막질환

학 번 : 2013-23545