Actual problem
UDC: 616.31-006:616-002.828

Presence of *Candida albicans* in potentially malignant oral mucosal lesions

Nada VUČKOVIĆ[1]
Marija BOKOR-BRATIĆ[2]
Dejan VUČKOVIĆ[3]
Ivana PIĆURIĆ[2]

Recently, an interest in the study of oral candidiasis has markedly increased mainly because of its association with viral infections due to human immunodeficiency, but also because of its relation with potentially malignant lesions of oral mucosa. These lesions belong to the wide group of leukoplakia. Leukoplakia is a clinical term used to describe a range of nonspecific white lesions, whose appearance does not generally correlate well with histopathologic changes; therefore, biopsy should be performed in all cases to determine which are precancerous or potentially malignant ones. In order to study the association of Candida albicans and the types of mucosal lesions, we took 30 consecutive biopsies of oral mucosa and the smears for microbiologic examination from the changed surface of mucosa and from the rest of oral cavity. The study group consisted of 30 patients, 21 women and 9 men, with the average age of 50.23 years (range, 25-77 years). In 6 cases Candida was diagnosed in mucosal biopsy. In the smear from the lesion, it was present in 3 cases, and 2 cases were found in the smear from an unchanged oral mucosa. In 9/30 cases (30%) Candida was positive regardless of the smear area or mode of diagnosis. The most common lesion is leukoplakia, diagnosed in 12/30 cases (40%), in 6 female and 6 male patients. The average age of those patients was 52.42 years. The lesions were located as follows: cheek mucosa - 5 cases; gingival mucosa - 2; lower lip - 2; floor of the mouth - 2; soft palate - 1; Candida was present in 3/12 cases. The lesion with the second highest incidence is lichen planus (9 cases), with positive Candida infection in 4/9 (44.44%). Epithelial dysplasia, although diagnosed in a very small number of cases (1/30 or 3.3%) with leukoplakia, was associated with a Candida infection. Generally, Candida is present in potentially malignant oral mucosal lesions (in 3/12 or 25% of leukoplakia cases, in 4/9 or 44.44% of lichen planus cases, and 1/1 squamous papilloma), with an increasing incidence in lesions with serious dysplastic epithelial changes.

[1]INSTITUTE OF PATHOLOGY AND HISTOLOGY, CLINICAL CENTER, NOVI SAD, SERBIA AND MONTENEGRO
[2]CLINIC FOR STOMATOLOGY, MEDICAL FACULTY, NOVI SAD, DEPARTMENT FOR ORAL MEDICINE AND PARODONTOLOGY, SERBIA AND MONTENEGRO
[3]DEPARTMENT OF PATHOLOGY, INSTITUTE FOR LUNG DISEASES, SREMSKA KAMENICA, SERBIA AND MONTENEGRO

KEY WORDS: *Candidiasis, Oral; Mouth Mucosa; Mouth Diseases; Mouth Neoplasms; Precancerous Conditions*

INTRODUCTION

Recently, interest in the study of oral candidiasis has markedly increased mainly because of its association with human immunodeficiency virus (HIV) infection, but also due to its relation with potentially malignant lesions of oral mucosal. Many authors have studied the characteristics of oral mucosa in immunocompromised patients in order to find the differences in immunologic reactions to the development of candidiasis. There are a number of oral lesions that are clearly associated, more often than others, with either candidial infestation or frank invasion (1).

The yeast-like fungus *Candida albicans* and a few other *Candida* species are capable of producing skin, mucous membrane, and internal infections. The organism lives with the normal flora of the mouth, vaginal tract, and gut, so the results of culture analysis must be interpreted carefully. Pregnancy, oral contraception, antibiotic therapy, diabetes, skin maceration, topical steroid therapy, certain endocrinopathies, and factors related to depression of cell-mediated immunity may allow the yeast to become pathogenic and produce budding spores and elongated cells (pseudohyphae) or true hyphae with septate walls. The yeast infects only the outer layers of the epithelium of mucous membrane and skin (the stratum corneum). The primary lesion is a pustule, the contents of which dissects horizontally under the stratum corneum

Address correspondence to:
Prof. Dr. Nada Vučković, Institute of Pathology and Histology, Clinical Center, Novi Sad, Hajduk Veljkova 3 , 21000 Novi Sad, Serbia and Montengero

The manuscript was received: 09.07.2003
Provisionally accepted: 19.09.2003
Accepted for publication: 11.12.2003

and peels it away. Clinically, this process results in a red, denuded, glistening surface with a long, cigarette paper-like, scaling, advancing border. The infected mucous membranes of the mouth accumulate scale and inflammatory cells that develop into characteristic white or white-yellow, curdy material (2-4).

Potentially malignant oral mucosal lesions belong to the wide group of leukoplakia, and as a clinical term is used to describe a range of nonspecific white slightly raised, white, translucent patches to dense, white, opaque lesions, usually well-defined plaques that show little tendency to extend peripherally, with or without ulceration on the vermilion border of the lips or any part of oral mucosa. The most common sites of oral leukoplakia are commissures and buccal mucosa. Smoking is the most common cause of oral lesions, but chronic irritation from carious teeth or malaligned dentures is also a cause. Histologic changes occur, varying from mild scaling and epidermal thickening with minimal inflammation to varying degrees of dysplasia or carcinoma in situ (5-8).

The clinical appearance of leukoplakia does not generally correlate well with the histopathologic change; therefore, biopsy should be performed for all cases. Small lesions may be removed and simply followed if the histology is benign. Plaques that histologically exhibit atypical or even malignant features should be properly treated. Many lesions clear spontaneously when cigarette or pipe smoking is stopped. Long-term follow-up is desirable to check for recurrences (7,9).

The hypothesis is that cancer (CA) is commonly associated with different oral mucosal lesions regardless of the patient's sex and the anatomical part of oral mucosa, this association being more expressed in potentially malignant diseases of oral mucosa. The aim of the study is to establish if there is any significant association of *Candida albicans* infection with oral mucosal lesions; also, if there exists such an association, to determine the type of lesion that is most frequently, connected with it.

MATERIAL AND METHODS

In order to study the association of *Candida albicans* and the types of mucosal lesions we took 30 consecutive biopsies of oral mucosa obtained from the Clinic for Stomatology of the Medical Faculty in Novi Sad. All biopsy samples were processed according to the standardized laboratory method in the Institute of Pathology and Histology, Clinical Center, Novi Sad. At the same time, with obtaining the biopsy, the smear for microbiologic examination was taken from the lesion and from the rest of unchanged mucosa of oral cavity. At least four histological sections stained with the HE method and four stained with the PAS method, for the visualization of fungi, were examined per case. Although the epithelial hyperplasia, hyperkeratosis, superficially located microabscessus and chronic inflammatory infiltrate in the lamina propria of mucosa pointed to the infection with *Candida*, the reliable evidence were PAS-positive hyphae, to visualize glucan, manan and other polysaccharides in fungal hyphae. Smears were prepared and stained according to standardized microbiological laboratory method.

RESULTS

The study group consisted of 30 patients, 21 women and 9 men, with the average age of 50.23 years (range, 25-77 years). The group of female patients had the average age of 52.24 years (range, 25-77 years). The group of male patients had the average age of 45.56 years (range, 30-55 years). Biopsy sites are presented in Table 1.

Table 1. Biopsy site in relation to gender

Site of biopsy	Women	Men	Total
Cheek	11	5	16
Gingival	3	2	5
Lower lip	3	1	4
Soft palate	2	0	2
Floor of mouth	1	1	2
Tongue	1	0	1

Histopathologic diagnoses from the biopsies of lesions are listed in Table 2. In Table 3, the type of lesions and their localization are listed.

Table 2. Histopathologic diagnosis in relation to gender

Diagnosis	Women	Men	Total
Leukoplakia	6	6	12
Lichen planus	9	0	9
Fibroma	4	2	6
Papilloma	1	0	1
Pemphigus vulg.	0	1	1
Cheilitis actinica	1	0	1

Table 3. Histopathologic diagnosis and the site of biopsy

Diagnosis	Cheek	Lower lip	Gingiva	Floor of mouth	Soft palate	Tongue	Total
Leukoplakia	5	2	2	2	1		12
Lichen planus	8	1					9
Fibroma	2		3			1	6
Actinic cheilitis		1					1
Papilloma					1		1
Pemph. vulg.	1						1
Total	16	4	5	2	2	1	30

The distribution of lesions has no statistical significance concerning the site of lesion, nor the patients' gender nor the type of histopathologic diagnosis. In one leukoplakia case, a mild dysplasia (grade I) was diagnosed in a female patient of 77 years, with a lesion in the oral cavity floor and with the simultaneous presence of *Candida* in the smear from the lesion and in the smear from the rest of oral mucosa.

In 6 out of 30 (20%) cases, *Candida* presence in mucosal biopsy was diagnosed. In the smear from the lesion, it was present in 3 cases, and 2 cases were found in the smear from an unchanged oral mucosa. The presence of *Candida* in the biopsy material, in

the smear from the lesion and from the surrounding mucosa, in relation to the site of the lesion and the patients' gender is listed in Table 4. In 9 out of 30 cases (30%), *Candida* was positive regardless of the smear area or mode of diagnosis. In two cases, *Candida* was positive in two diagnostic procedures (biopsy and smear).

Table 4. *Candida* presence in biopsy, from lesion smear, and from unchanged mucosa, in relation to lesion type, localization, and gender

Diagnosis	Localization	Gender	*C.albicans* + biopsy	*C.albicans* + lesion smear	*C.albicans* + smear
Leukoplakia	Lower lip	Female	+		
Leukoplakia	Soft palate	Female	+		
Lichen planus	Cheek	Female	+		+
Lichen planus	Cheek	Female	+		
Papilloma	Soft palate	Female	+		
Fibroma	Gingiva	Male	+		
Leukoplakia	Floor of mouth	Female		+	
Lichen planus	Lower lip	Female		+	+
Lichen planus	Cheek	Female		+	

DISCUSSION

The exact pathologic significance of fungal, mainly the candidial infection is still unknown, but leukoplakia could be a potentially malignant oral mucosal lesion, and the *Candida* infection is generally accepted as the main etiologic factor for the occurrence of oral epithelial neoplasia. The infection usually accompanies denture stomatitis, with immunosuppressed states, including HIV and diabetes (10).

Noninvasive hyphae and fungi could be diagnosed in the smears and in the culture, but could be lost during the laboratory handling of the biopsy specimen, leading to a negative result in histopathologic examination. Cawson reported negative *Candida* test results in 20 healthy people, as well as in 31 smears from unchanged oral mucosa areas in leukoplakia biopsy proven patients (11). This is well in opposition to the results of other authors who showed that 25% to 90% of healthy population is *Candida* carriers (12). In our material, *Candida* was diagnosed in 9 out of 30 cases (30%) at least by one diagnostic procedure.

The most common biopsy site is cheek mucosa (16/30, 53.33%), and in this area lichen planus is the most common lesion (8/9, 88.89%). At the same time, this region is the most frequently infected with *Candida*, which was diagnosed in 3/9 cases (33.33% of all cases, but in 2/6 biopsy material and in 2/3 smears from the lesion and in the smear from unchanged mucosa). Other authors report similar incidence of cheek Candidiasis (13,14).

The most common lesion in our material was leukoplakia, which was diagnosed in 12/30 cases (40%), in 6 women and 6 men. The distribution of lesions is presented in Table 3. *Candida* was present in 3/12 cases. In only one case mild dysplasia was present (1/12, 8.3% of leukoplakia cases or 3.3% of all cases). Squamous cell carcinoma develops in up to 17% of all leukoplakia patients. In a study of 500 patients with oral leukoplakia, there was squamous cell carcinoma in 9.6% of the cases and dysplasia in an additional 24%. Leukoplakia on the floor of mouth and the ventral surface of tongue is associated with the highest risk of cancer. The development into carcinoma takes 1 to 20 years (9,11,15). In our material, carcinoma in situ or invasive carcinoma was not diagnosed in the close proximity to any of potentially malignant oral mucosa lesions.

The lesion with the second highest incidence in our material was lichen planus (9 cases, with positive *Candida* infection in 4 of them, i.e. 44.44%). The onset before middle age is rare; the mean age of the onset is in the sixth decade, although in our material the average age of those patients was 51.56 years. Usually, women outnumber men by more than 2:1, but in our study it is 9:0 (probably because the overall ratio was 21:9). Oral lichen planus can occur without a cutaneous disease. The mucous membrane involvement was observed in more than 50% of patients with cutaneous lichen planus. The lesions may be located on the tongue and lips, but the most common site is the buccal mucosa. In our cases, the lesions were found on the cheek mucosa in 8 cases and on the lower lip in 1 case. *Candida* infection was established in 17% to 25% of ulcerated and nonulcerated cases of lichen planus. Malignant transformation occurred in 1.2% of patients (16-18).

Epithelial dysplasia, although diagnosed in a very small number (1/30 or 3.3%), is associated with leukoplakia and with *Candida* infection diagnosed both in the smear from the lesion and elsewhere. Generally, *Candida* is present in potentially malignant oral mucosa lesions (3/12 or 25% of leukoplakia cases, in 4/9 or 44.44% of lichen planus cases, and 1/1 squamous papilloma). This result is in agreement with others (10,15).

Although the significance of fungal infection is sometimes underrated, its clear association with a moderate to severe epithelial dysplasia was established, and the dysplastic lesions infected with fungi had a three times greater chance to aggravate their degree. The higher incidence of fungal infection with potentially malignant oral mucosal lesions was found, as compared with those without malignant potential (7,15).

CONCLUSION

In our study group, the most common lesion was leukoplakia (12/30, 40%), and the second one was lichen planus (9/30, 30%). The most common site of the lesions was cheek mucosa (16/30, 53.33%). Positive test results to *Candida* were found in 9 cases (30%). Epithelial dysplasia was diagnosed in only one case (3.3%), with a positive test results in the smear of the lesion area, as well as in the surrounding unchanged mucosa. Probably in our

study group, as a highly selected one from a specialized clinic for stomatology, and in a relatively small number of cases, it is not possible to prove the frank association of potentially malignant oral mucosal lesions with the candida infection.

It is assumed that a small number of epithelial dysplasia cases is due to a poor knowledge of the population about the potential malignancy of oral mucosal lesions and, consequently, inattention to minor changes. Another reason could be only sporadic biopsy sampling of oral mucosa from discrete lesions. Both conclusions could be drawn based on a large number of invasive carcinoma in this region, which are diagnosed from year to year.

Acknowledgement:

This study is a part of the research project No. 1490, which is in part financially supported by the Ministry of Science, Technology and Development of Serbia, Belgrade.

REFERENCES

1. Reichart PA, Samaranayake LP, Philipsen HP. Pathology and clinical correlates in oral candidiasis and its variants: a review. Oral Diseases 2000;6:85-91.

2. Shafer WG, Hine MK, Levy BM. A Textbook of Oral Pathology. 4th ed. Philadelphia: Saunders Company; 1983. p. 917.

3. Samaranayake LP. Essental Microbiology for Dentistry. Edinburgh: Churchill Livingstone; 2002. p. 142-7.

4. McCullough MJ, Ross BC, Reade PC. Candida albicans: a review of its history, taxonomy, epidemiology, virulence attributes, and methods of strain differentiation. Int J Oral Maxillofac Surg 1996;25:136-44.

5. Bokor-Bratić M, Vučković N. Cigarette smoking as a risk factor associated with oral leukoplakia. Arch Oncol 2002;10:67-70.

6. Bokor-Bratić M. Oralna medicina. Novi Sad: Praktikum; 2002. p.107.

7. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and Maxillofacial Pathology. Philadelphia: Saunders Company; 1995. p. 711.

8. Toussaint S, Kamino H. Noninfectious Erythematous, Papular and Squamous Diseases. In: Elder D et al, editors. Lever's Histopathology of the Skin, 8th ed. Philadelphia: Lippincott- Raven Press Publishers; 1997. p.151-84.

9. Bokor-Bratić M. The prevalence of precancerous oral lesions. Oral leukoplakia. Arch Oncol 2000;8:169-70.

10. Barrett AW, Kingsmill VJ, Speight PM. The frequency of fungal infection in biopsies of oral mucosal lesions. Oral Diseases 1998;4:26-31.

11. Cawson RA, Odell EW. Essentials of Oral Pathology and Oral Medicine. 6th ed. Edinburgh: Churchill Livingstone;1998. p. 372.

12. Lynch D.P. Oral candidiasis. Oral Surg Oral Med Oral Pathol 1994;78:189-93.

13. Ghezzi MC, Trancassini M, Cipriant P, Mancici C, Brenciaglia MI. Comparison between adherence of C. albicans and Candida spp. to human epithelial cells. Boll Ist Sieroter, Milan 1986;65:436-9.

14. Ghannoum M, Asu-Elteen K. Correlative relationship between proteinase production, adherence and pathogenicity of various strains of Candida albicans. J Med Vet Mycol 1986:24:407-13.

15. McCullough MM, Jaber M, Barrett AW, Bain L, Speigh PM, Porter SR. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncol 2002;38:3391-3.

16. Hatchuel DA, Peters E, Lemmer J, Hille JJ, McGraw WT. Candidal infection in oral lichen planus. Oral Surg Oral Med Oral Pathol 1990;70:172-5.

17. Silverman S Jr. Oral Lichen Planus: A Potentially premalignant Lesion. J Oral Maxillofac Surg 2000;58:1286-8.

18. Eisenberg E. Oral Lichen Planus: A Benign Lesion. J Oral Maxillofac Surg 2000;58: 278-85.