Article

Diagnosis of oral mycotic infections using a fluorescence detection device (VELscope®)

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ABSTRACT

BACKGROUND: Candida albicans is a commensal in the oral cavity of the 40-65% of the adult human population. In healthy individuals this colonization remains benign, though in many casese an infection can occur. Diagnosis of oral candidiasis is usually based on clinical aspects, patients' symptoms and risk factors. Presence of Candida hyphae can be microbiologically evaluated. Specimens from the oral mucosa are cultured or collected on glass plates and stained (i.e. by the periodic acid-Schiff (PAS)). The culture method has proved to be less specific than the PAS method, studied suggested that oral candidiasis may be incorrectly diagnosed if based upon results obtained with the culture methods. Therefore, tests results must be compared to clinical findings and symptoms. VELscope® reveals natural tissues fluorescence produced by fluorophores of the oral mucosa: FAD+, collagen cross-links, keratin, porphyrin, fibrin. There are as well tissue components that decrease autofluorescence, that is, hemoglobin and melanin. Any biochemical variation of these elements will also produce an alteration of tissue fluorescence. For this reason, fluorescence is used for the screening of oral cancer.

AIM: The aim of this study is to determine sensitivity and specificity of VELscope® in the diagnosis of oral mycosis.

MATERIALS AND METHODS: Oral cavity of 29 patients has been inspected evaluating the mucosa loss of fluorescence with VELscope®. Positive outcomes of VELscope® was compared to the positive outcomes of the microbiological oral swab and the cytological smear on slides. The study was approved by the Padua Hospital Ethical Committee. The Siemel method was used in the statistical analysis.

RESULTS: VELscope® sensitivity was equal to 0.9167, CI95% = (0.6152 - 0.9979), its specificity was equal to 0.1667, CI95% = (0.0209-0.4841). The likelihood positive ratio (LR+) was of 1.1 CI95% = (0.811 - 1.493) and LR- of 0.5 CI95% = (0.052 - 4.807).

CONCLUSIONS: Decreased blood flow, increased cells catabolism and collagen degeneration cause an alteration of tissues fluorescence, which is captured by VELscope®. Oral infections of C. albicans occurs in patients under immunodeficiency conditions, therefore the examination with VELscope® has high probability to yields false negative findings. For this reason, the VELscope® device is characterized by a higher sensibility (0.9167) than specificity (0.1667).

VELscope® sensitivity resulted higher than its specificity. High false positive ratio is probably due to the presence of autoimmune disease on the oral cavity together with the mycotic infection. Although, due to the small sample size, wide confidence intervals and low LR+ and LR- do not show a conclusive statistical value.

KEYWORDS: Oral mycosis; VELscope®; Candidiasis; Candida albicans; Oral disease; Oral pathology; Oral medicine

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AIM

The aim of this study is to determine sensitivity and specificity of VELscope® in the diagnosis of oral mycosis.

BACKGROUND

Mycotic infections of the oral cavity are caused by Candida albicans (80% of cases) and by C. tropicalis, C. parapsilosis, C. krusei, C. guillermondii, C. glabrata e C. dubliniensis. The clinical aspect of psudomembranes is known from ancient Greece, though the fungus was at first detected by Langenbeck in 1839 (1).

Candida albicans is a commensal in the oral cavity of the 40-65% of the adult human population (2). In healthy individuals this colonization remains benign, though under any conditions, even immunodeficiency, an infection can occur.

The pathogenicity of this dimorphic fungus is due to host's immunity system, oral microenvironment-modifying factors and pathogenetic properties of Candida.

Immunity status known as risk factors are newborns, AIDS, cancer, neutropenia, agammaglobulinemia, leukemia, lymphoma, aplastic anemia, immunosuppressant pharmacological therapy, xerostomia, radiotherapy, diabetes mellitus, pregnancy, renal failure, hyperthyroidism (3). When a cells invasion occurs, monocytes, neutrophils and macrophages present the antigen to the lymphocytes who recognize it as a non-self, fighting the infections. This process does not enhance during an immunity system imbalance, thus an oral infection occurs, described as oral candidiasis.

Oral microenvironment-modifying factors are poor fitting dentures, loss of vertical dimension, poor oral hygiene, smoking, alcohol. C. albicans is also found in angular cheilitis, denture-associated erythematous, median rhomboid glossitis.

Pathogenetic properties of Candida is its polymorphism from yeast to hypha, adhesins and invasins expression, biofilm organization, hydrolytic enzymes secretion, rapid adaptation to pH changing, thigmotropism.

Candida is a rounded and oval-shaped yeast of 3-30 µm diameter. Clinical aspects of oral candidiasis are pseudomembranous, erythematous, hyperplastic chronic candidiasis, angle cheilitis, prosthetic stomatitis, median rhomboid glossitis, mucocutaneous candidiasis.

Pseudomembranous candidiasis appears especially in the oropharyngeal region, check mucosa and lateral surfaces of the tongue. It is characterized by white-yellow plaques that detach upon rasping leaving an erythematous zone. It is typically observed in immune depressed individuals, elderly patients and infants. Symptoms are rarely a burning and itching sensation (4).

Erythematous candidiasis is most located on the palate and tongue, with disappearing of filiform papillae and smoothening atrophy of the dorsal surface of the tongue. Symptoms can be mild burning and itching sensation. Hyperplastic chronic candidiasis is characterized by nodular aspect, eventually covered by whitish and not detaching plaques.

Angular cheilitis appears at the lip commissures with fissures and or cracks that evolves in crusts and is usually painful. Angular cheilitis is often colonized by different streptococci and other bacteria such us Staphylococcus Aureus.

Prosthetic stomatitis is a denture-associated erythematous candidiasis that manifests on the base of poor fitting prosthetics.

Median rhomboid glossitis locates on the midline of the dorsal posterior region of the tongue, with an asymptomatic rhomboid plaque of firm consistency and red or pink color. Atrophy of filiform papillae is observed and its limits are clear.

The diagnosis of oral candidiasis is usually based on clinical aspects, patients' symptoms and risk factors.

Presence of Candida hyphae can be microbiologically evaluated directly, isolated in cultured or examined on cytological smears. Biopsy is indicated only to diagnose hyperplastic candidiasis.

Direct microscope exam is a rapid and inexpensive examination often necessary in case of diagnosis doubts. Specimens are collected with mouthwash or oral swabs. After being pretreated with 10% potassium hydroxide (KOH), samples can be colored with calcofluor or gram color and observed under microscope light or evidenced by fluorescine-calcofluor.

The most used culture medium is Sbouraud dextrose agar (SDA) added by chloramphenicol 0.05g/l or gentamicin 0.5 g/l to inhibit bacterial growth. After 24-48 hours incubation the presence of germ tubes or filamentation is macroscopically observed for a positive outcome (5).

For the cytological examination, smears are stained by periodic-acid Schiff (PAS) or gram color (6)(Figures 1,2).

Bioptic samples are colored by PAS or Grocott-Gomori's Methenamine Silver (GMS).

Being Candida a commensal, evaluation of this fungus in cultures or samples does not imply an

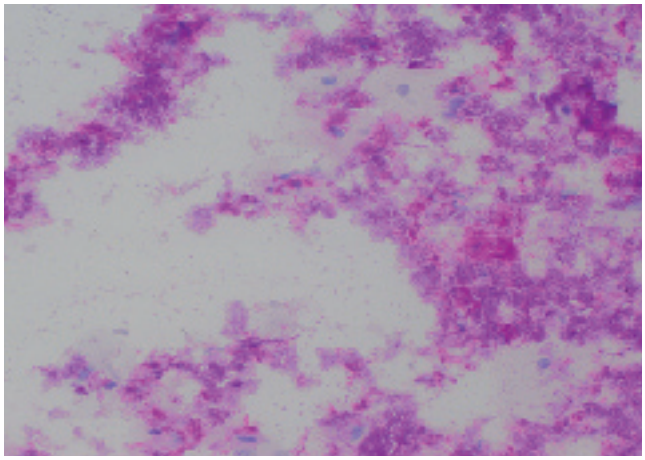

Figure 1. Microscopic view of the cytology of the oral cavity. Presence of several bacterial aggregates, epithelial cells and Candida's hifae. PAS stain. Original magnification 200x.

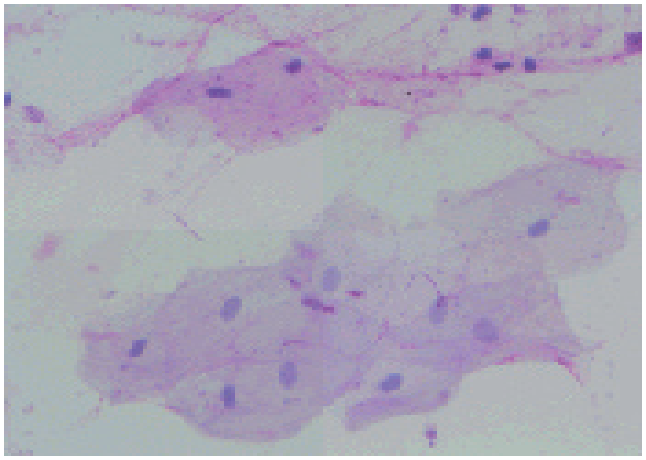

Figure 2. Microscopic view of a cytology of the oral cavity. Presence of bacterial aggregates, epithelial cells and Candida's hyfae. PAS stain. Original magnification 320x.

actual infection, thus the culture method has proved to be less specific than the cytological exam (7).

Immunological and genetic techniques are necessary to diagnose an infection of C. dublinensis which is typical of HIV patients (8).

Treatment of oral mycotic infections is based upon three factors: early diagnosis, correction of risks factors, usage of more efficient drugs.

In healthy individuals, topic medicament is sufficient, while for immunodeficient patients systemic therapy is needed. Most used antimycotic drugs are polyenes such as anfotericin b and nystatin, azaleas such as clotrimazole, miconazole, ketoconazole, fluconazole, itraconazole, echinocandins such as anidulafungin, caspofungin and micafungin (9). Nystatin has been the most used topic medicament in the past for its low price, though it has showed lack of adhesion to oral surfaces. Fluconazole has proved to have more efficiency then nystatin, inhibiting Cytochrome P450's enzyme sterol 14-demethylase, it increases permeability of the cells and inhibits fungus growth, exiting a fungistatic effect. Azoles has also shown less toxicity than other medicaments. In 2016, the Infectious Disease Society of America (IDSA) added on the guidelines for the treatment of oral candidiasis the medicament with miconazole mucoadhesive buccal tablets 50mg/die (10).

Tissues fluorescence is the property of a tissue to reflect a radiation when a radiation hits it and it is due to the presence of fluorophores. Fluorophores in the oral mucosa are flavin andenin dinucleotide in its oxidized form (FAD+), collagen cross-links, keratin, porphyrin, fibrin. Fluorescence of the oral mucosa is decreased by tissue components such us melanin and hemoglobin (11). The examination of tissues fluoresce has recently been used to detect early chemical variation of the structure of tissues, such us cancerous lesions, bacteria and fungus infections and to guide biopsy of precancerous lesions (12) .

VELscope® device permits to evidence tissues fluorescence lightening the tissues with blue LED light and filtering reflection lights.

MATERIALS AND METHODS

Traditional oral examination and anamnesis of oral cavity of 29 patients were collected, depending on patients symptoms and risk factors for oral mycosis, an oral swab and smears on 5 slides were collected. The oral mucosa has later been inspected with VELscope®, evaluating tissue's loss of fluorescence. Positive outcomes of VELscope® examination was compared to the positive outcomes of the microbiological and the cytological analysis. The study was approved by the Padua Hospital Ethical Committee. The Siemel method was used in the statistical analysis.

RESULTS

Total patients enrolled in the study was 29 adults, 22 females and 6 males, aged between 31 and 82 years old. Patients' symptoms were itching (25), xerostomia (2) and pain (1). 27 patients presented risk factors for oral mycosis, such us oral lichen planus (15), erosive lichen planus (3), burning mouth syndrome (4), lichenoid reaction (2), autoimmune diabetes (1), glossitis, mucositis, aftosis associated to lack of vitamin B12 (1), Graft Vs Host Disease (GVHD) (1), absence of symptoms (2).

Microbiological exam showed negative results in 28 cases. The positive outcome was found in a

GVHD patient with lichenoid lesions on the oral mucosa.

Cytology showed positive outcomes in 12 cases.

VELscope® exam resulted positive in 25 cases. All patients presented pathological or fisiological condition of fluorescence loss: oral lichen planus (15), erosive lichen planus (3), lichenoid reaction (2), scars (2), atrophic glossitis (2), mucositis (1), aftosis (1).

VELscope® sensitivity was equal to 0.9167, CI95%=(0.6152 - 0.9979), its specificity was equal to 0.1667, CI95%=(0.0209 - 0.4841).

The likelihood positive ratio (LR+) was of 1.1 CI95%=(0.811 - 1.493) and LR- of 0.5 CI95%=(0.052 - 4.807).

DISCUSSION

Being C. albicans a commensal in the oral cavity, both microbiological and cytological tests can interpret the presence of Candida as a physiological condition or underestimate an infection when a low number of hyphae is found. For this reason, the diagnosis of oral candidiasis is often based on clinical findings, patients' symptoms and risk factors (13).

VELscope® device is indicated for the screening of precancerous lesions, bacterial and mycotic infections of the oral cavity (14).

Decreased blood flow, increased cells catabolism and collagen degeneration cause an alteration of tissues fluorescence, which is captured by VELscope®. These conditions are found in patients under immunodeficiency conditions, therefore, in these cases the examination with VELscope® has high probability to yields false negative findings in the screening of oral mycosis, being immunodeficiency a risk factor for the infections of C. albicans.

Sensibility of the VELscope® device resulted close to 1 (0.9167), defining the VELscope® exam a sensible test. Its specificity resulted lower (0.1667), due to environmental features of the oral cavity of susceptible population.

CONCLUSION

Finding hyphae of Candida in the laboratory tests of the oral cavity is not conclusive for a diagnosis of oral candidiasis (15).

Fluorophores in the oral mucosa are the same cells that alter under autoimmune diseases, thus leading to false positive findings. Therefore, VELscope® sensitivity is higher than its specificity.

Although, the sample size was small, thus wide confidence intervals and low LR+ and LR- do not lead to a conclusive statistical value.

AUTHOR CONTRIBUTIONS

All authors contributed to the concept, acquisition, and analysis of data, drafting of the article, and critical revision for important intellectual content.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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