Candida spp. in oral cancer and oral precancerous lesions

Francesca Gallè[1], Giuseppe Colella[2], Valeria Di Onofrio[1], Raffaele Rossiello[3], Italo Francesco Angelillo[4], Giorgio Liguori[1]

[1]Chair of Hygiene and Epidemiology, Department of Studies of Institutions and Territorial Systems, University "Parthenope", Naples, Italy;
[2]Department of Head and Neck Pathology, Second University of Naples, Naples, Italy;
[3]Department of Human Pathology, Second University of Naples, Naples, Italy;
[4]Department of Public, Clinical and Preventive Medicine, Second University of Naples, Naples, Italy

_____ SUMMARY _____

To assess the presence of *Candida* spp. in lesions of the oral cavity in a sample of patients with precancer or cancer of the mouth and evaluate the limitations and advantages of microbiological and histological methods, 103 subjects with precancerous or cancerous lesions and not treated were observed between 2007 and 2009. The presence of *Candida* in the lesions was analyzed by microbiological and histological methods. Cohen's k statistic was used to assess the agreement between culture method and staining techniques. Forty-eight (47%) patients had cancer and 55 (53%) patients had precancerous lesions. *Candida* spp. were isolated from 31 (30%) patients with cancerous lesions and 33 (32%) with precancerous lesions. *C. albicans* was the most frequent species isolated in the lesions. The k value showed a fair overall agreement for comparisons between culture method and PAS (0.2825) or GMS (0.3112). This study supports the frequent presence of *Candida* spp. in cancer and precancerous lesions of the oral cavity. Both microbiological investigations and histological techniques were reliable for detection of *Candida* spp. It would be desirable for the two techniques to be considered complementary in the detection of yeast infections in these types of lesions.

KEY WORDS: Candida spp., Oral precancer and cancer, Microbiological and histological diagnosis.

INTRODUCTION

With more than 90% of malignancies being squamous cell carcinomas originating from the oral mucosa, oral cancer is one of the ten most prevalent cancers worldwide (Reichart, 2001; Chen and Myers, 2001; Rosenquist *et al.*, 2005; Hooper *et al.*, 2007; Al-Rawi and Talabani, 2008).

Candida species are common members of the oral microflora and are generally regarded as being commensals. However, they are able to cause a range of opportunistic infections, referred to as candidoses (Williams *et al.*, 2001; Morace and Borghi, 2010).

The prevalence of diseases caused by *Candida* spp. has increased in recent years, mainly due to the increasing number of immunocompromised patients. *Candida albicans* is still the predominant species isolated, and it has the potential to infect virtually any tissue within the body. However, it is predominantly found on the oral and vaginal mucosa (de Araújo Navas *et al.*, 2009; Maninder and Usha, 2008; McCullough *et al.*, 2002).

The possible association between *Candida* spp. and oral neoplasia was first reported in the 1960s (Cawson, 1969; Williamson, 1969), with later reports suggesting a link between the presence of *C. albicans* in the oral cavity and the development of oral squamous cell carcinoma (OSCC) (Daftary *et al.*, 1972; Bastiaan and Reade, 1982; Rodríguez *et al.*, 2007).

Corresponding author
Giorgio Liguori
Department of Studies of Institutions
and Territorial Systems
University "Parthenope"
Via Medina 40 - 80133 Naples, Italy
E-mail: giorgio.liguori@uniparthenope.it

Candida infection has been associated with malignant development in the oral cavity ever since it was found to cause candidal oral leukoplakia and to correlate with oral epithelial dysplasia (McCullough *et al.*, 2002; Cawson, 1969; Sitheeque and Samaranayake, 2003; Farah *et al.*, 2010; Wu *et al.*, 2012). The fact that epithelial dysplasia improves after elimination of *Candida* spp. from infected tissue also supports a causal link (Williams *et al.*, 2001). Candida-infected leukoplakia appears to have a higher rate of malignant transformation than other types (Reibel, 2003). Most patients have chronic oral candidosis since early childhood and also exhibit a highly increased risk for developing oral carcinoma at a young age (Rautemaa *et al.*, 2007).

Several studies have investigated the pathogenic mechanism of *Candida* involved in carcinogenesis, and have highlighted the ability of some species to convert nitrite and nitrate in nitrosamines and other substances to produce acetaldehyde (Sitheeque and Samaranayake, 2003; Rossano *et al.*, 1993; Scardina *et al.*, 2009). Few investigations have verified the prevalence of *Candida* spp. colonization and the site of lesions, and have been concerned less with the evaluation of diagnostic techniques (McCullough *et al.*, 2002; Barrett *et al.*, 1998; Dwivedi *et al.*, 2009).

At present, traditional diagnostic techniques for these types of lesions are based on histological staining of tissue sections, and microbiological methods, which allows the growth of the yeasts on culture media.

The aim of the present study was to assess the presence of *Candida* spp. in lesions of the oral cavity in a sample of patients with precancer or cancer of the mouth recruited over a period of three years in Naples, Italy. We evaluated the limitations and advantages of microbiological and histological methods in detecting yeast infection in this category of subjects.

MATERIALS AND METHODS

One hundred and three subjects (41 females and 62 males, mean age: 61.07 years, range: 22-84 years), who underwent biopsy, with a histopathological diagnosis of precancerous lesions or OSCC, and not previously treated with chemo/radiotherapy or antifungal agents, were observed between 2007 and 2009 in the Department of Head and Neck Surgery, Second University of Naples, Italy. This study was approved by the Institutional Review Board and all patients recruited on the study were duly informed by the operator, who had them sign the specific informed consent form.

Before the day of sampling, each patient was recommended not to eat or brush their teeth from the previous evening. For microbiological analysis, samples were collected from lesions with sterile swabs, immediately placed in transport medium, and processed for the detection of fungi on plates that contained Sabouraud–dextrose agar (SDA) and chloramphenicol. After 48 h at 37°C, isolates were identified through the presumptive germ tube test and the API 20 C AUX system (bioMérieux Italia, S.p.A.).

At the same time, a biopsy was also performed on each lesion. Specimens were fixed in formalin and embedded in paraffin, and then processed. The lesions were classified according to the World Health Organization Classification of Tumours (World Health Organization, 2005; Warnakulasuriya *et al.*, 2007). Periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) stains, suitable to demonstrate the presence of fungal elements within tissues, were used (Sitheeque and Samaranayake, 2003). The observation of hyphae and/or blastospores in the PAS- and GMS-stained sections was expressed as the presence or absence of yeasts (Barrett *et al.*, 1998).

Cohen's k statistic was used to assess the agreement between the culture method and staining techniques for detecting infections. The Landis and Koch classification for the interpretation of k was used. At the time of sampling, information on the presence or absence of predisposing factors such as smoking, alcohol, oral hygiene, and dentures was also collected for each patient. The role of risk factors was analyzed using the χ^2 test for comparison between infected and non-infected patients.

RESULTS

On the basis of histopathological diagnosis, among 103 subjects included in our study, 48 (47%) patients (17 female and 31 male) had cancer and 55 (53%) patients (24 female and 31

male) had precancerous lesions. The most frequent cancer site was the floor of the mouth (14%), while for precancerous lesions it was the cheek (31%). The predisposing factors considered for the two groups studied were distributed as shown in Figure 1.

In the comparison between the two groups, significant differences (χ^2 test) were found only among infected and non-infected patients who used prostheses (p=0.02). No differences were seen among patients who wore fixed or removable dentures.

Candida spp. were isolated through culture methods from 64 patients: 31 (30%) from cancerous lesions and 33 (32%) from precancerous lesions. *C. albicans* was the most frequent species isolated, and accounted for 21 (20.4%) and 29 (28.2%) of the yeasts from cancerous and precancerous lesions, respectively, followed by *Candida glabrata* and *Candida tropicalis*. Other species of *Candida* (*Candida inconspicua*, *Candida famata* and *Candida kefyr*) and *Saccharomyces cerevisiae* were only isolated in patients with cancer (Table 1).

PAS staining disclosed fungi in 18 (17.5%) cancerous lesions and 15 (14.6%) precancerous lesions. GMS staining disclosed fungi in 17 (16.5%) cancerous lesions and 17 (16.5%) precancerous lesions (Table 2). All the samples negative to cultural method were also negative to histological techniques (data not shown).

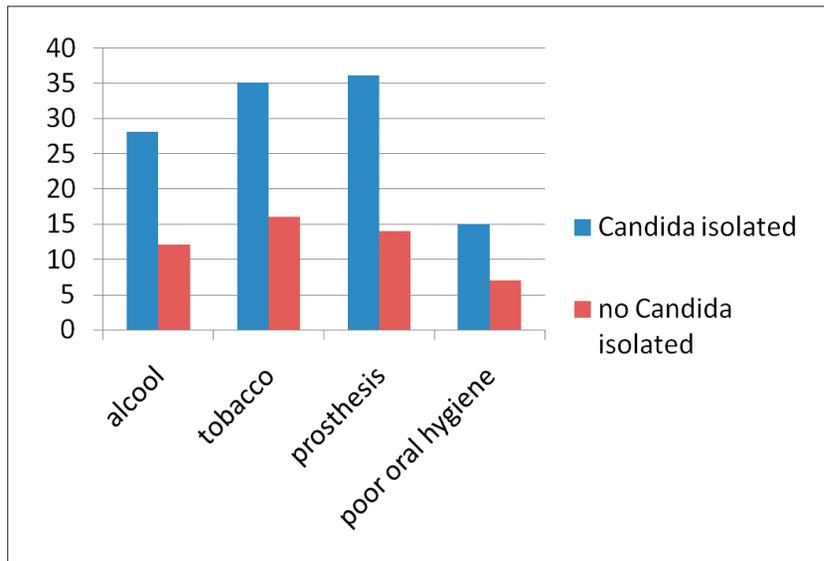The k value was 0.2825 (95% CI: 0.1197–0.4453)

FIGURE 1 - *Distribution of predisposing factors in infected and non-infected patients.*

TABLE 1 - *Candida spp. isolated in cancerous and precancerous lesions.*

Lesions	N.	Candida spp.				
		Neg.	C. albicans	C. glabrata	C. tropicalis	Other species of Candida (C. famata, C. kefyr, C. incospicua, S. cerevisiae)
Cancer	48	17	21	4	2	4
Lichen planus	22	12	9	1	-	-
Leukoplakia	32	10	20	1	1	-
Erythroplakia	1	-	1	-	-	-

TABLE 2 - *Candida spp. disclosed by histological and microbiological methods in patients with cancer and precancer.*

Diagnostic assays	Candida isolated		No Candida isolated	
	Cancer (%)	Precancer (%)	Cancer (%)	Precancer (%)
Culture method	31 (30)	33 (32)	17 (16.5)	22 (21.5)
PAS	18 (17.5)	15 (14.6)	30 (29.1)	40 (38.8)
GMS	17 (16.5)	17 (16.5)	28 (27.2)	41 (39.8)

for comparison between culture method and PAS, and 0.3112 (95% CI: 0.1395–0.483) in the second case (culture method *vs*. GMS), which represents a fair overall agreement of the methods according to the Landis and Koch classification (Landis and Koch, 1977).

DISCUSSION

Today, laboratory diagnosis of oral candidosis is not always performed, and a presumptive diagnosis is often the only one made, based on the patient's history, clinical presentation, and response to antifungal treatment rather than on cultural and histological methods (Gonsalves *et al.*, 2008). However, especially in critical patients, the characterization of yeast infection is important when choosing the appropriate therapy. Therefore, in precancer and cancer patients, the diagnosis of oral candidosis should always be performed: in precancer patients, epithelial dysplasia could improve after elimination of *Candida* spp., and in precancer patients, resolution of infection can prevent more aggressive candidosis after radio-chemotherapy.

Our results support the frequent presence of *Candida* spp. in the cancerous and precancerous lesions of the oral cavity (McCullough *et al.*, 2002; Barrett *et al.*, 1998; Liguori *et al.*, 2007; Liguori *et al.*, 2009). As reported in other studies, *C. albicans* comprises 78% of isolated yeasts, which makes it the most frequent species in cancerous and precancerous lesions. As previously suggested, certain strains of *C. albicans* probably have properties that are important in the development of pathological conditions and premalignant changes (McCullough *et al.*, 2002; Rindum *et al.*, 1994).

With regard to the non-albicans species, *C. glabrata* and *C. tropicalis* were isolated from patients with precancerous and cancerous lesions, while *C. incospicua, C. famata, C. kefyr* and *S. cerevisiae* were found only in some cancerous lesions (Belazi *et al.*, 2004; Henry *et al.*, 2004).

With regard to the oral site mainly involved, our results agree with other authors (Mashberg *et al.*, 1989; Neville and Day, 2002; Roblyer *et al.*, 2009). Among the risk factors normally considered, our study found significant differences only in subjects colonized by *Candida* spp. who use prostheses (p=0.02) (Salaspuro, 2007; Gonsalves *et al.*, 2008). Cohen's k statistic showed a fair overall agreement between the two types of methods, so both microbiological and histological techniques were reproducible enough to detect *Candida* in patients with oral cancer or precancer lesions. However, in our opinion, it is advisable not to use only one technique to detect *Candida* spp. in these types of lesions.

The culture method allows the isolation of yeasts to give a precise indication of species and to test their drug susceptibility, which is especially important when deciding on a targeted therapy. Microbiological analysis is a reliable method to assess the presence of *Candida* spp. in advance and possibly establish a treatment for precancerous lesions on the basis of the antifungal susceptibility patterns shown by isolated strains. It is also increasingly sensitive, specific and rapid thanks to molecular systems, which can shorten the time needed for diagnosis. However, the culture method is limited because it may highlight occasional fungi in the oral cavity that are not responsible for infection.

Histological methods, by contrast, may disclose hyphae and blastospores in tissue specimens,

which may indicate that the yeast have invaded the tissue, although they are not as sensitive as cultural methods, as reported in the present and previous studies (McCullough *et al.*, 2002; Barrett *et al.*, 1998; Brand 2012).

Therefore, our findings suggest that, where possible, a correct therapeutic-clinical approach in patients with oral cancerous/precancerous lesions should use both methods, that could be considered complementary.

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