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 (Reichert, 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).
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; Sithheeque 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. 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 chemoradiotherapy 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.

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 ($\chi^2$ 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](image.png)

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

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

<table>
<thead>
<tr>
<th>Lesions</th>
<th>N.</th>
<th>Candida spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neg.</td>
</tr>
<tr>
<td>Cancer</td>
<td>48</td>
<td>17</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Erythroplakia</td>
<td>1</td>
<td>-</td>
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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).

Regarding 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.

REFERENCES


