Identification of *Candida albicans* using different culture media and its association in leukoplakia and oral squamous cell carcinoma

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**Abstract**

**Context:** *Candida*, leukoplakia and oral squamous cell carcinoma.

**Aims:** The aim of the present study has assessed the association of *Candida albicans* with normal control group, leukoplakia and oral squamous cell carcinoma lesions of the oral cavity by using cornmeal broth +5% milk and milk serum liquid culture media.

**Settings and Design:** The present study comprised of total 80 patients, which included, 30 patients of Leukoplakia, 30 patients of oral squamous cell carcinoma and normal control group comprised of 20 healthy individuals who were not having any relevant medical, dental and habit history.

**Subjects and Methods:** Saliva and Biopsy was taken from clinically suspected leukoplakia and oral squamous cell carcinoma lesions for the confirmation of histopathological diagnosis. Saliva samples were inoculated for fungal growth in Sabouraud Dextrose Agar, and culture-positive samples had undergone for the germ tube test. Germ tube-positive samples were further taken for chlamydospore production in milk serum and cornmeal broth +5% milk media separately at 8 and 16 h.

**Statistical Analysis Used:** Chi-square test, Fischer extract test.

**Results:** In the normal control group, no fungus growth was found; however, leukoplakia and oral squamous cell carcinoma showed fungus growth with positive germ tube test and chlamydospore formation; the result also showed rapid and quantitatively more chlamydospore formation in cornmeal broth +5% milk in comparison to serum milk culture media. There was no growth of *Candida* in mild and moderate form of leukoplakia. Whereas in a severe form of leukoplakia, there was more quantitative chlamydospore growth in cornmeal broth +5% milk liquid media.

**Conclusions:** In this study, we have tried to compare the efficacy of cornmeal broth +5% milk and milk serum for the identification of *C. albicans*. Both the culture media were able to promote the growth of chlamydospore in *C. albicans*. Among different grades of leukoplakia, the growth of *C. albicans* was seen in severe dysplastic patient only while mild and moderate dysplasia showed no Candida growth.

**Keywords:** Candida albicans, chlamydospore, liquid media

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INTRODUCTION

The production of chlamydospore is a diagnostic tool used to identify C. albicans. The time required to produce them with standard methods is 48–72 h in rice meal agar. This time can be shortened using liquid media such as cornmeal broth and dairy supplements.[1]

The presence of Candida in the mouth together with epithelial changes may predispose individuals to Candidal infection. Epithelial changes of the oral mucosa, such as atrophy, hyperplasia and dysplasia, may compromise the mucosal barrier and facilitate Candidal invasion such as C. albicans or Candida tropicalis.[2]

The presence of Candida in mouth together with epithelial changes may predispose to Candidal infection which together with other cofactors may also induce epithelial dysplasia leading to malignant change.[3]

In general, the detection of Candida in the oral cavity is performed with different objectives, i.e., fungus growth, germ tube test and chlamydospore using different techniques. The most frequently used primary isolation medium for Candida is Sabouraud Dextrose Agar (SDA).[4] however, it is not a differential medium; hence, different species cannot be distinguished easily from one another.[5]

With the increasing importance of candidiasis in the Oral cavity, there is a need for a practical method for identification of fungus. Recently, a new culture media that utilize cornmeal broth +5% milk has been developed and has shown to be suitable for the rapid production of C. albicans chlamydospores under varying conditions, and in the preliminary assessment, it has been shown to be relatively superior as compared to the other media.

With this background, the present study was planned with an aim to identify Candida albicans using different culture media with a focus on evaluating and comparing the efficacy of cornmeal broth +5% milk and milk serum in oral leukoplakia while at the same time to evaluate its association with oral leukoplakia and oral squamous cell carcinoma.

SUBJECTS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology, Career Postgraduate Institute of Dental Sciences and Hospital, Lucknow. The study participants included 20 normal healthy volunteers, 30 patients with clinical and histopathological diagnosis of leukoplakia and 30 patients with clinical and histopathological diagnosis of oral squamous cell carcinoma, after ethical approval (Ethical No. CPGIDSH/1285/16).

Inclusion criteria
Age was 25–60 years irrespective of gender. In Group I, healthy volunteers having no oral lesion and not having the habit of tobacco or alcohol use were included; in Group II, patients with the confirmed diagnosis of leukoplakia were included and in Group III, patients with the confirmed diagnosis of oral squamous cell carcinoma were included in the study.

Exclusion criteria
Cases with a known history of systemic disorder were excluded; Also immunocompromised patient and patient suffering from mental illness were excluded and treated cases of leukoplakia and oral squamous cell carcinoma were excluded from the study.

Sample collection
The participants were explained in detail about the procedure, and a signed consent form was taken from them. All the participants were asked to rinse the mouth with distilled water thoroughly to remove any food or debris. After 10 min, the phosphate buffer solution was used as an oral rinse method in saliva collection. Samples were obtained by requesting participants to keep and swirl the solution for 1 min and then expectorate all saliva into presterilized containers without swallowing. After collection of saliva, the biopsy was performed only from those who were suspected as leukoplakia and squamous cell carcinoma of the oral cavity for histopathological diagnosis.

Processing and Preparation of samples and culture media for fungus growth.

The culture tubes were incubated at 37°C for 48 h under aerobic conditions. After 48 h, the plates were observed for the growth of Candida-like colonies. Culture tubes with no growth were further incubated for 7 days before declaring them negative for Candida growth.

The culture tubes showing the growth of Candida-like colonies and morphology-like Candida were stored at 4°C for further testing.

C. albicans (ATCC 10231) was used as a standard strain.

For germ tube test
Serum human venous blood was collected and allowed to clot. The specimen was placed in a test tube rack to speed
up the clotting action. After clotting, the specimen was placed in a centrifuge machine at 3000 rpm for 10 min.

Serum was separated and used for germ tube test.

Once the growth of the fungus was obtained in the culture tubes, a very small isolated Candida-like colony was picked with a sterile loop and was suspended in a test tube containing 0.5-ml human serum. This mixture was incubated at 42°C for 2–3 h. A drop of the mixture was placed on a clean glass slide and covered with a clean coverslip. This was first examined under a low-power objective to locate the group of cells, and later, the presence of germ tube was confirmed under the high-power objective of the microscope (×40).

C. albicans (ATCC 10231) was used as positive control and C. tropicalis (ATCC 13803) was used as negative control.

**For chlamydospores**

Cornmeal broth +5% milk-cornmeal agar media were modified to prepare the broth. It was mixed with cold distilled water, stirred at 6°C and stored at 6°C. Next day, insoluble components were removed by filtration, then 5% pasteurized milk was added. The prepared media were autoclaved at 121°C for 20 min. It was allowed to cool and then poured into sterile test tubes.

Milk serum was prepared from nonpasteurized cow’s milk. Sulfuric acid was added drop by drop in milk until it forms a curd. The supernatant was removed, and the leftover was filtered with filter paper until it became clear, then autoclaved at 121°C for 20 min. It was allowed to cool and then poured into the test tubes.

**Method of inoculation**

A small inoculum from an isolated Candida colony was picked up with a sterile inoculating loop and was suspended in a test tube containing cornmeal broth +5% milk culture media and milk serum separately by rubbing the inoculated loop against the wall of the test tube. This helps in diluting the pasty colonies by giving the media turbid appearance. The two different test tubes containing cornmeal broth +5% milk and serum milk were inoculated. The mixture was incubated at 45°C for 8 h and 16 h, respectively.

Chlamydospores were observed under wet mounts. A drop of inoculated broth media was placed onto the slide; over it, a drop of lactophenol cotton blue stain was added, and the presence of chlamydospores was confirmed under high-power magnification.

**Comparison of culture media for rapid and quantitatively more production of chlamydospores**

Only germ tube-positive samples proceeded for further study, and positive samples were transferred into both cornmeal broth +5% milk culture media and milk serum to compare the rapid production of chlamydospores.

**RESULTS**

None of the specimens in normal controls had positive growth. In leukoplakia group and oral squamous cell carcinoma group, positive growth was observed in 36.7% and 46.7% of cases, respectively [Figure 1]. Statistically, the intergroup difference in the pattern of growth was significant [Table 1]. Subsequent assessment for C. albicans was done using the germ tube test among the cases resulting in a positive culture. On germ tube test, a total of 36.4% specimens were positive in leukoplakia and 78.6% in oral squamous cell carcinoma groups. Statistically, the difference in positivity rate between the two groups was significant too \((P=0.048)\) [Table 2]. The specimen positive for germ tube test was subsequently subjected to further assessment for chlamydospore formation with serum milk and corn milk broth, respectively [Figure 2].

In both leukoplakia and oral squamous cell carcinoma groups, at 8 h, none of the specimens showed chlamydospore formation. However, at 16 h, in both the groups, chlamydospore formation was observed in milk serum [Figures 3 and 4] of all the samples [Table 3].

In corn milk broth, chlamydospore formation was seen in 50% and 36.4% of leukoplakia and oral squamous cell carcinoma cases, respectively. However, no chlamydospore formation was observed in milk serum in oral squamous cell carcinoma cases.

**Table 1: Candida with Sabouraud’s agar media in normal control, leukoplakia and oral squamous cell carcinoma cases**

| Growth          | Normal control | Leukoplakia (%) | OSCC (%) |
|-----------------|----------------|-----------------|---------|
| Negative growth | 20             | 19 (63.3)       | 16 (53.3) |
| Positive growth | 0              | 11 (36.7)       | 14 (46.7) |
| Total           | 20             | 30 (100)        | 30 (100) |

\(\chi^2=12.8\) (df=2); \(P=0.002\). Candida albicans ATCC 10231 was taken as standard strain, \(P\) value is significant and candidal growth in Sabouraud’s agar media is maximum in OSCC as compared to the rest.

OSCC: Oral squamous cell carcinoma

**Table 2: Germ tube test with serum in leukoplakia and oral squamous cell carcinoma cases**

| Result     | Standard strain     | Leukoplakia \((n=11)\), \(n\) (%) | OSCC \((n=14)\), \(n\) (%) |
|------------|---------------------|-----------------------------------|-----------------------------|
| Positive   | Candida albicans ATCC 10231 | 4 (36.4) | 11 (78.6) |
| Negative   | Candida tropicalis ATCC 13803 | 7 (63.6) | 3 (21.4) |
| Total      |                      | 11 (100) | 14 (100) |

\(P=0.048\) (Fisher’s exact test). \(P\) value is significant with more of germ tube growth in human serum within OSCC patient. OSCC: Oral squamous cell carcinoma, \(n\)=Number of patient
carcinoma samples at 8 h and all the samples in both the groups at 16 h. Statistically, the difference in the proportion of patients showing chlamydospore formation was not significant at both the time intervals ($P = 1$) [Table 4].

For both leukoplakia as well as oral squamous cell carcinoma groups, the number of total chlamydospores formed was higher for cornmeal broth +5% milk as compared to serum milk [Table 5]. For both serum milk as well as cornmeal broth +5% milk media, number of total chlamydospores formed was higher for oral squamous cell carcinoma as compared to leukoplakia group [Table 5].

All the cases in the mild group showed a negative growth; in moderate group, 54.5% showed positive growth, and in severe group, 75% showed positive growth. Statistically, the difference in positive growth rate among different grades of leukoplakia was significant ($P = 0.003$) [Table 6].

Germ tube positivity was higher in severe grade as compared to moderate grade, yet this difference was not significant statistically ($P = 0.061$) [Table 7]. Number of chlamydospores was 25 for serum milk and 181 for cornmeal broth +5% milk medium [Table 8]. No positive growth was observed in mild and moderate leukoplakia groups whereas in a severe group, it was positive in 50% of cases. Statistically, this difference was significant ($P = 0.002$) [Table 9].

The prevalence as well as number of chlamydospores was higher in oral squamous cell carcinoma as compared to leukoplakia, and cornmeal broth had a better ability to discriminate between the two. No chlamydospores were observed in normal controls and mild and moderate grades of leukoplakia, thus signifying that chlamydial growth is a characteristic finding of oral squamous cell carcinoma and severe grade of leukoplakia only.
**DISCUSSION**

The oral cavity harbors hundreds of different microbial species, and *C. albicans* is the most common fungal pathogen in humans. It exists as a commensal inhabitant of mucosal surfaces in most healthy individuals. However, alterations of host or environment can lead to overgrowth of fungus and infection to the host. Hence, the present study was undertaken to assess and identify the association of *C. albicans* in normal control group, leukoplakia and oral squamous cell carcinoma by using different liquid culture media.

We have compared mainly liquid media; cornmeal broth +5% milk and milk serum, and as a result, we have concluded that the incidence of *C. albicans* is more in oral squamous cell carcinoma as compared to leukoplakia. Furthermore, cornmeal broth +5% milk is a better media than milk serum for the production of chlamydospore and for the specific test of *C. albicans*.

The sample size distribution is shown in Table 1. Groups 2 and 3 included 30 patients of leukoplakia and oral squamous cell carcinoma each, while in Group 1, 20 normal healthy controls were taken that did not have clinical evidence of any abnormalities.

*Candida*-like growth was seen in leukoplakia and oral squamous cell carcinoma while normal control does not show any growth as shown in Table 2. Numerous researchers have found the same finding. In a study by Saigal et al., it was reported that in a normal control group, no fungus growth was found; however, potentially malignant and malignant cases showed fungal growth. However, the difference between leukoplakia and oral squamous cell carcinoma groups was not statistically significant (P > 0.05). In this study, more growth of *Candida* was seen in oral squamous cell carcinoma than leukoplakia. This result is in accordance with research of Vuckovik et al. They
have examined the presence of *C. albicans* in potentially malignant oral mucosal lesions. They have shown that among 30 patients of leukoplakia, nine cases were proven positive for *Candida*. In another study by Canković and Bokor-Bratić, it was shown that the *Candida* was found in higher number of cases in oral cancer patients as compared to normal control group. They have found *Candida* on 9 of the 30 cancer surfaces. In all of the above studies, it was assessed that no fungal growth was seen in control groups while increased fungal growth is seen in leukoplakia and oral squamous cell carcinoma.[9]

This *Candida*-like growth was further evaluated using germ tube test with human serum in leukoplakia and oral squamous cell carcinoma cases. After the germ tube test according to Table 3 the fungus was observed within 8 hours in milk serum media that showed negative growth for chlamydospore in Leukoplakia and Oral Squamous Cell Carcinoma patients. While after 16 hours all the susceptible cases of Leukoplakia and Oral Squamous Cell Carcinoma showed 100% growth. Statistically, the difference in positivity rate between the two groups was significant (*P* = 0.048).

Positive germ tube test was used for further evaluation of *C. albicans*. This was in accordance with studies of Meru *et al*. They studied 60 patients with leukoplakia and 30 patients with oral lichen planus. They isolated the fungus by inoculating the swab in SDA. Further identification of *Candida* species was done using the germ tube test. They found a positive germ tube test on 42 patients. The reason of such findings could be attributed to *Candida* growth on many preexisting pathological processes as reported by Mccarthy *et al*. While similar observations were assessed by Maiback. They suggested that defective epithelium may act as a predisposing factor for the growth of *C. albicans*. Arendorf *et al*. suggested that tobacco smoking might lead to localized epithelial alteration which allows colonization by *Candida*. Further, *Candida* species have inducible enzyme system that allows them to replicate using polycyclic aromatic hydrocarbon as their source of energy.[8]

After the germ tube test, according to Table 4, the fungus was observed within 8 h in milk serum media that showed negative growth for chlamydospore in leukoplakia and oral squamous cell carcinoma patients. While after 16 h, all the susceptible cases of leukoplakia and oral squamous cell carcinoma showed 100% growth. Christian *et al.*, in the year 2003, showed the efficacy of casein agar as useful media for the production of chlamydospore. They showed *C. albicans* growth can be obtained in 48 hrs., for this is the most probable reason was that this is due to the fungus candida that decomposes casein for the production of chlamydospore. As it is a well-proven fact about milk that it helps in the formation of *C. albicans* chlamydospore in combination with corn meal broth.[9]

In Table 5, chlamydospore formation in cornmeal broth was observed at the interval of 8 h and 16 h; it was observed that the growth was seen in both intervals, almost all *C. albicans* showed 100% growth after 16 h. A similar study was performed by Alicia *et al*. that showed good results, particularly in three of culture media cornmeal broth +5% milk, cornmeal broth +5% milk serum and cornmeal broth +tween-80. In the other two media, milk serum and milk serum +tween 80, no chlamydospore was formed under the established conditions.[3] The production of chlamydospores was most effective and was achieved at 16 h on the medium of cornmeal broth +5% milk. In Table 5, statistically, the difference in the proportion of patients showing chlamydospore formation was not significant at both time intervals (*P* = 1).

Tables 6 and 7 have shown the quantitative analysis of chlamydospore formation in leukoplakia and oral squamous cell carcinoma with milk serum and cornmeal broth; it showed that cornmeal broth was better media as compared to serum milk for the identification of *Candida*. Casal and Linares, in 1981, found that solid media containing complex carbohydrates, such as cornmeal and rice extract agar, are the best-known chlamydospore-inducing media for both *C. albicans* and *Candida dubliniensis*. Nakamoto, in the year 1998, demonstrated the rapid formation of *Candida* hyphae/germ tube in cornmeal broth and showed that cornmeal could replace serum as the use of cornmeal was more advantageous as compared to serum with regard to cold storage.[11]

Table 6 shows Candida like growth of different grades of Leukoplakia in sabouraud agar media. No growth was seen in mild whereas moderate and severe forms showed growth. Vuckovic *et al*. have found that time required to produce chlamydospore with standard method is 48–72 h in rice meal agar and tensioactive agent. This time can be reduced or sorted out using liquid media such as cornmeal broth and dairy supplements.[3] In Table 6 for leukoplakia, the difference was statistically significant (*P* = 0.019). Furthermore, for oral squamous cell carcinoma patients, statistically, the difference was very highly significant (*P* < 0.001).

According to Table 7, the comparison of number of chlamydospore is done and compared in milk serum and cornmeal broth +5% milk in oral squamous cell carcinoma and leukoplakia with more number of chlamydospore in oral squamous cell carcinoma.
Table 8 shows Candida-like growth of different grades of leukoplakia in SDA. No growth was seen in mild whereas moderate and severe forms showed the growth. This was in accordance with the studies of Dany et al. who concluded that 55% of the lesions with moderate dysplasia, 27% of the lesion with mild dysplasia and 18% of the lesion with no dysplasia showed the evidence of Candida whereas 15% of lesions in moderate dysplasia, 32% of lesions in mild dysplasia and 53% of lesion with no dysplasia showed no evidence of Candida. Similar results were obtained by Barrett et al. to determine the frequency of fungal infection in biopsies of oral mucosal lesions. They recorded 6.2% of lesions with mild epithelial dysplasia and 18% of lesions with moderate epithelial dysplasia showing the evidence of Candida. The association of moderate epithelial dysplasia with Candida infection was statistically significant with \( P < 0.01 \). In their follow-up study, they found that 21.9% dysplasias which were infected with fungi worsened in severity. They concluded that there was a significant association of fungal infection with moderate and severe epithelial dysplasia, and dysplastic lesions infected with fungus were almost three times more likely to worsen in histologic severity. McCullough et al. have also observed that \( \text{C. albicans} \) with very high potential to nitrosylate N-benzylmethylamine were more likely to be isolated from advanced, potentially malignant oral mucosal lesions. In addition to this, the author was able to show that the presence of Candida was more common in lesions with moderate dysplasia than in lesions with mild dysplasia or no dysplasia.

In Table 8, on between-grade comparison, both moderate and severe grades showed a significant difference from the control group \( (P < 0.05) \); however, the difference between moderate and severe grades was not statistically significant \( (P = 0.352) \).

In Table 9, it was seen that severe leukoplakia showed a positive germ tube test giving approximately 66.7% positive growth while no growth was seen in cases of moderate dysplasia. Germ tube positivity was higher in severe grade as compared to moderate grade; yet, this difference was not significant statistically.

Tables 10 and 11 show a quantitative analysis of chlamydospore formation in milk serum and cornmeal broth; this shows a significant difference in both. The reasons for such results could be attributed to well establish the fact that cornmeal broth is a better media for growth of \( \text{C. albicans} \) while Table 12 shows that \( \text{C. albicans} \) is only associated with severe dysplasia.

Thus, from the above result, we can conclude that severe dysplasia is mostly affected by \( \text{C. albicans} \). Severe dysplasia shows maximum quantitative growth when differentiated using cornmeal broth as compared to milk serum. While observing a qualitative aspect, both media are equivalent efficient for the growth of chlamydospore.

Hence, it can be said that cornmeal broth +5% milk and milk serum, both the media, are capable of producing chlamydospores; however, quantitatively and qualitatively, cornmeal broth +5% milk is a better media in comparison to milk serum in the identification of \( \text{C. albicans} \).

**CONCLUSION**

Our study has proved beneficial in bringing forth many estimations and association related to the commonly encountered fungal species Candida Albicans in oral cavity of Leukoplakia and Oral squamous Cell Carcinoma patients.

In our study we have tried to compare the efficacy of Corn Meal Broth +5% milk and Milk serum for identification of Candida Albicans. Both the culture media were able to promote growth of chlamydospore in Candida Albicans.
Our study also indicated that among different grades of Leukoplakia the growth of Candida Albicans was seen in severe dysplastic patient only. While mild and moderate dysplasia showed on candida growth.

In the cases of Oral squamous Cell Carcinoma occurrence of Candida Albicans was seen in 36.7% of cases that shows Candida Albicans is usually associated with Oral squamous Cell Carcinoma.

Here it was also proved that quantitively and qualitatively the Media Corn Meal Broth +5% milk was a better medium in the identification of chlamydospore producing Candida Albicans.

This concluded that evaluation of salivary parameters can be a cost effective and non-invasive alternative for screening, diagnosis and monitoring of severe dysplasia and for presence of Oral squamous Cell Carcinoma in the patient.

This would surely help us in conclusively establishing the presence of Candida in Oral squamous Cell Carcinoma and severe dysplasia patients and hence a larger sample size and further research could be done for the association of Candida Albicans with the two debilitating disease.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Saigal S, Bhargava A, Mehra SK, Dakwala F. Identification of Candida albicans by using different culture medias and its association in potentially malignant and malignant lesions. Contemporary Clinical Dentistry 2011;2:188-93.
2. Alcia Z-S, Blanca O-S, Mariana G-H, Magdalena C-C, Alexandre B. rapid production of Candida albicans chlamydospores in liquid media under various incubation conditions. Nihon Ishinkin Gakkai Zasshi 2006;47:231-4.
3. Ariyawardana A, Panagoda GJ, Fernando HN, Ellepola AN, Tilakaratne WM, Samararayake L, et al. Oral submucous fibrosis and oral yeast carriage – A case control study in Sri Lankan patients. Mycoses 2007;50:116-20.
4. Byadarahally Raju S, Rajappa S. Isolation and identification of Candida from the oral cavity. ISRN Dent 2011;2011:487921.
5. Mahmoudabadi AZ. Isolation and Identification of Candida species from the oral cavity using CHROMagar Candida. Iran Biomed J 2000;4:57-61.
6. Vuckovic N, Bokor-Bratic M, Vuckovic D, Picuric I. Presence of Candida Albicans in potentially malignant oral mucosal lesions. Arch Oncol 2004;12:51-4.
7. Canković M, Bokor-Bratić M. Candida albicans infection in patients with oral squamous cell carcinoma. Vojnosanit Pregl 2010;67:766-70.
8. Arendorf TM, Walker DM. The prevalence and intra-oral distribution of Candida albicans in man 1980;25:1-10.
9. Mosca CO, Moragues MD, Llovo J, Al Mosaid A, Coleman DG, Pontón J, et al. Casein agar: A useful medium for differentiating Candida dubliniensis from Candida albicans. J Clin Microbiol 2003;41:1259-62.
10. Casal M, Linares MJ. The comparison of six media for chlamydospore production by Candida albicans. Mycopathologia 1981;76:125-8.
11. Nakamoto S. Promotion of chlamydoconidium formation in Candida albicans by corn meal broth incubation. Med Mycol 1998;36:123-5.
12. Dany A, Kurian K, Shanmugam S. association of Candida in different stages of oral leukoplakia. J Indian Acad Oral Med Radiol 2011;23:14-6.
13. Barrett AW, Kingsmill VJ, Speight PM. The frequency of fungal infection in biopsies of oral mucosal lesions. Oral Dis 1998;4:26-31.
14. McCullough M, Jaber M, Barrett AW, Bain L, Speight PM. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncol 2002;38:391-3.