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Original Article

Oropharyngeal candidiasis and resistance to antifungal drugs in patients receiving radiation for head and neck cancer

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Abstract

BACKGROUND: Oropharyngeal candidiasis is a common infection in patient receiving radiotherapy for head and neck cancer. Accurate and rapid identification of candida species is very important in clinical laboratory, because the incidence of candidiasis continues to rise after radiotherapy. The genus Candida has about 154 species that show different level of resistance to antifungal drugs and have high degree of phenotypic similarity. The aim of this study was to investigate oral yeast colonization and infection and resistance to antifungal drugs in these patients.

METHODS: Thirty patients receiving a 6-week course of radiation therapy for treatment of head and neck cancer at the Oncology Unit in Shafa Hospital, in 2008, were enrolled in the study. Specimens from patients were cultured weekly for Candida. All isolates were plated on CHROM agar and RPMI-based medium. They were subcultured and submitted for antifungal susceptibility testing (nystatin, fluconazole, clotrimazole and ketoconazole) and molecular typing.

RESULTS: Infection (clinical and microbiological evidence) occurred in 50% of the patients and Candida colonization (only microbiological evidence) occurred in 70% of subjects in the first week. Candida albicans alone was isolated in 94.9% of patient visits with positive cultures. Candida tropicalis was isolated from 5.1% of patient visits with positive cultures. All isolates were susceptible to nystatin, but did not respond to the other antifungal drugs

CONCLUSIONS: The irradiation-induced changes of the intraoral environment such as xerostomia lead to increased intraoral colonization by Candida species. All yeast isolates were susceptible to nystatin. Thus prophylactic therapy with nystatin should be considered for these patients.

KEY WORDS: Oropharyngeal candidiasis, radiation, colonization, antifungal drugs, cancer.

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Patients receiving therapy for head and neck cancer are particularly susceptible to oropharyngeal candidasis.[1-3] In these patients, radiotherapy involves oral and facial structure, including the major salivary glands that lead to prolonged xerostomia.[4-6] The symptoms of this infection are pain and/or burning and can lead to significant patient morbidity.[2] Results of past studies have shown that Candida albicans has been by far the most predominant organism isolated in patients receiving cancer therapies. Recently, an increase in non-Candida albicans has been reported. It seems that non-Candida albicans strains are responsible to development of resistance to antifungal drugs.[3] However, taking a single culture and performing specific techniques may not be sensitive in distinguishing all can-

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dida strains.[7-9] Therefore, use of multiple cultures, chromogenic medium, and molecular typing technique will allow the identification of multiple Candida species.[10]

Redding and colleagues showed that Candida infections occurred in 27% of 30 patients receiving radiotherapy that were predominantly due to Candida albicans (78%). Yeasts other than Candida albicans had been isolated from 59% of patients. In this study all infections responded to fluconazole therapy.[1] Leung and colleagues showed that irradiation-induced xerostomia can lead to intraoral colonization of Candida species, particularly Candida albicans.[6]

Antifungal drugs are used for treating fungal infections such as candidiasis and serious systemic infections. These drugs belong to 3 principal classes: polyenes, pyrimidines and azoles. Immunocompromised patients are increasingly susceptible to drug resistance and create potentially life-threatening fungal infections.

Resistant strains exhibit a modification in the quality or quantity of target enzyme, reduced access to the target or some combination of these mechanisms.[9] Drug resistance depends on several factors including the use of inappropriate drugs, inadequate or excessive doses of medication, poor patient cooperation, quality of available drugs and geographical differences of Candida isolates. Hence, it obviously varies in different geographical areas.[9,11]

Early diagnosis and management of oral candidiasis in patients receiving therapy for head and neck cancer is very important. Furthermore, identification of Candida species has remained problematic because high degree of phenotypic similarity between candida strains and deciding for antifungal treatment can pose a problem.[12,13] The aims of this study were evaluation of oropharyngeal Candida colonization and infection as well as resistance to antifungal drugs in patients receiving radiation for head and neck cancer.

Methods

We enrolled thirty patients that received a 6-week course of radiation therapy for treatment of head and neck cancer at Shafa Hospital. We examined these patients for signs of oropharyngeal candidiasis at baseline and weekly thereafter during their radiation treatment, and obtained oral cultures at each visit and from any clinical infection. Positive clinical signs including white intraoral plaques, erythematous lesions, ulcerations, and angular cheilitis, confirmed by use of 10% potassium hydroxid (KOH) preparation and/or positive culture were defined as infection.

All fungal cultures were performed by an oral swab that been applied in different parts of the mouth and a swish sample of 10 ml of normal saline instilled in the mouth for 10 S and then collected in a sterile container. All swabs were tested through following techniques:

1- Direct test with KOH 10 %, to confirm the yeast
2- Plate on SC medium (Sabouraud's dextrose agar + chloramphenicol), for Initial growth of yeast
3- Plate on CMA medium (corn meal agar), to confirm Candida albicans
4- Culture in human or cheap serum for three hours for germ tube, to supplement confirm of candida albicans
5-Use of standard techniques (diagnostic kit and PCR-RFLP) for diagnosis of other candida spices

The PCR assay was done with 1 µL of sample (around 1ng) in a total reaction volume of 50 µL, including of 10 mM Tris-HCL, 1.5 mM $MgCL_2$, 50 mM KCL, 0.2 mM of each primers and 5U of Taq DNA polymerase. Thirty-five cycles of amplification were performed in a thermal cycler model Techne- progene.[13]

For evaluation of antifungal drugs resistance, serial dilutions of all drugs (nystatin 100000 U/L, clotrimazole 100 mg, Ketoconazole 100 mg, fluconazole 100 mg), were prepared and minimum inhibitory concentration (MIC) were measured. First, different fungi were mixed with Sabouraud's dextrose agar and were added to plate to be solid. Then, wells were created in the plates, and various dilutions of the drugs were poured into the wells and diameter of the area around them was measured.

All tests were repeated three times (triplicate) and Candida albicans was used as standard strain. We conducted data analysis using SPSS version17. We assessed the effect of time on Candida growth by generalized estimating equations. The associations were considered statistically significant at $P < 0.05$.

Results

The demographic data (including age, sex and type of cancer) of the patients are shown in table 1. In the first week, Candida infections occurred in 15 of 30 patients (50%) and Candida colonization occurred in 21 of 30 subjects (70%). The total cultures (6-week course of radiation therapy) of 3 patients were negative. The cultures of 21 of 27 patients were positive in first week, and cultures of 6 of 27 subjects became positive during the next weeks (Table 2).

Table 1. Demographic characteristics of patients

Characteristics	Number (percent)
Sex	
Male	18 (77.8 %)
femalea	12 (22.2%)
Age (mean)	45.6 ± 15.6
Site of lesion	
Larynx	15 (50%)
Oropharynx	9 (30%)
Oral cavity	5 (17%)
Salivary gland	1 (3%)

Table 2. Growth of Candida in consecutive visits

Number of patients / Week	1	2	3	4	5	6
9	+	+	+	+	+	+
2	-	-	-	-	+	+
3	-	-	-	-	-	-
6	+	+	+	+	+	_
4	-	-	-	+	+	+
3	+	+	-	-	-	-
3	+	+	-	-	+	+

- No growth of Candida
+ Growth of Candida

The results showed that time has had a significant effect on Candida growth ($P = 0.001$). Although Candida growth in the fifth week was significantly higher than other weeks, there was not a significant relationship between Candida growth in other weeks (Figure 1).

The results of PCR showed that Candida albicans was detected in all of patients with positive cultures (27 subjects), and in 6 of these patients (22%) Candida albicans and Candida tropicalis were isolated. Overall, 118 of 180 (65.6%) patient visits (30 visits in 6-week radiation therapy) were positive for Candida carriage (infection and colonization). Candida albicans was isolated from 112 of 180 patient visits (94.9%), and Candida tropicalis with Candida albicans were detected in 5.1% of patient visits. All infections in cultivated environments responded to nystatin therapy at a dosage of 100000 U/L, but candida species did not respond to other drugs. Candida species did not grow to 1/40 or 1/80 dilutions of nystatin in total cultures. There was no growth of candida spices to 1/160 dilution of nystatin in 3 cultures after fifth and sixth weeks.

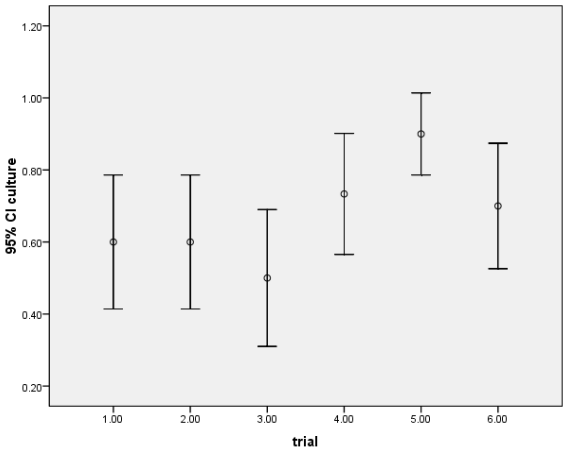

Figure 1. The effect of time on Candida growth

Discussion

The epidemiology of oral candidiasis shows a wide variation (from 17 to 52.5%) in different studies.[14-16] The main reason for this wide variation is the difficulty in diagnosing oral candidiasis (especially oral pseudomembranous candidiasis), from the radiation-induced mucosal reaction, and the fact that oral candidiasis may be superimposed on radiation-induced mucosal reactions, causing additional problems in the differential diagnosis.[14]

In the present study, positive clinical signs of white intraoral plaques confirmed by use of

10% KOH preparation and/or positive culture were defined as infection. Infection was reported in 50% of patients after first week. Candida infections were predominantly due to Candida albicans (94.9%), and Candida tropicalis was isolated in 5.1% of patient visits. The results of Suryawanshi and colleagues study,[13] showed that Candida albicans was seen in 78.57%, Candida krusei in 7.15%, and Candida tropicalis, Candida guilliermondii, Candida stellatoidea and Candida parapsilosis each being 3.57%. This finding is similar to our study that showed Candida albicans was the predominant organism.

In other study Redding and colleagues[1] showed that Candida infections occurred in 27% of the patients receiving radiation for head and neck cancer. This finding is less than our study, but similarly, in their study, Candida albicans was the predominant organism (78%). The other yeasts were Candida dubliniensis and Candida rugosa.[1] Identification of 28 isolates at species level showed that in six other patients who initially had negative growth of Candida, the culture was positive in the weeks after it. This finding is similar to the Redding and colleagues study that showed Candida colonization was common and was detected in 73% of patients.[1]

In our study PCR showed that Candida albicans was detected in all of patients with positive cultures (94.8%) and in 6 of these patients (in 3 patients after the first and in 3 subjects after the fifth week) Candida tropicalis were also isolated. Overall, Candida tropicalis was isolated in 5.1% of patient visits with positive cultures. Redding and colleagues study showed that Candida albicans was the predominant organism (78%), and yeasts other than Candida albicans (ten different species) were detected in 13 of 22 patients (59%) with positive cultures and at 49 of 95 patient visits with positive cultures (52%).[1] Moreover, Leung and colleagues study showed that candidiasis were reported in 9 of 12 patients and Candida albicans and Candida tropicalis were the predominant organisms.[6] As mentioned, the main reason for this variation in various studies is the difficulty in diagnosing oral candidiasis from the radiation-induced mucosal reaction.

In this study, response to nystatin (100000 U/L) therapy was significant. Our findings showed that resistance to nystatin was not developed during consecutive weeks, even there was no growth of candida spices to 1/160 dilution of nystatin in 3 cultures in fifth week. In these patients in fifth week, Candida tropicalis in addition to Candida albicans were grown, and in the sixth week following growth of Candida tropicalis, response to nystatin therapy was created to 1/80 dilution. It seems that 1/40 dilution of nystatin is appropriate for treatment of Candida spices.

In this study, Candida spices did not respond to other drugs. Perhaps one reason for the lack of response is that the selected dose of other drugs was not sufficient. However, Redding and colleagues study showed that fluconazole 100 mg was effective in the treatment of all candida species except candida rugsa.[1] Overall, as Candida albicans was the predominant organism in our study, and response to nystatin therapy was significant, and resistance to nystatin was not developed during consecutive weeks, so designing therapy programs including meeting with the dentist before radiation therapy, establishing proper oral hygiene status, and prescription of nystatin during the weeks of radiation therapy to reduce infections and improve quality of life for these patients is very effective.

Conclusion: The irradiation-induced changes of the intraoral environment such as xerostomia, lead to increased intraoral colonization by Candida species. All isolated yeast were susceptible to nystatin 100000 U/L, thus prophylactic therapy with nystatin should be evaluated for these patients.

Conflict of Interest

Authors have no Conflict of Interest.

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