ORIGINAL ARTICLE

Oropharyngeal candidosis relative frequency in radiotherapy patient for head and neck cancer

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ABSTRACT

Background: Radiation given during treatment of oral and pharyngeal malignancy frequently causes alteration of the oral environment predisposing to the colonization of the oral mucosa by yeast species most frequently Candida. **Objective:** Thus, this study was undertaken in 107 patients to find out association between radiation therapy and frequency of oropharyngeal candidosis, to quantitate colony forming units (CFUs) to identify Candida at species level and to check the incidence of serotype A and B in *C. albicans*. **Materials and Methods:** The study was done on patients suffering from oropharyngeal cancer who were advised radiotherapy. The oral rinse collection method was used to collect the sample. Sabourauds Dextrose Agar (SDA) was used as primary culture media and subsequently speciation was done using standard techniques. The strains of *C. albicans* were serotyped employing the method described by Hansclever and Mitchell (1961, *J Bacteriol 1961;82:570-3*). **Results:** 26.16% patients were mycologically positive for candida before radiotherapy with CFUs 100. 14 ± 59.11 that increased to 60.74% patients during radiotherapy with an increase in CFUs to 490.15 ± 207.97. Clinically, grading of mucositis was done and also individual signs and symptoms were noted in each patient. The occurrence of erythmatous lesions, ulceration, and xerostomia were found to be statistically significant ($P<0.05$). *C. albicans* was the most frequently encountered species with higher prevalence of serotype A suggesting higher virulent species. **Conclusion:** It is proposed that in such patients taking radiotherapy prophylactic antifungal treatment should be given specially in patients showing development of oral mucosal lesions such as erythmatous lesions, ulcerations, and complaining about dryness of mouth, that is, xerostomia irrespective of presence or absence of clinical oral candidosis.

Key words: *C. albicans*, colony forming units (CFUs) candida species, oral candidosis, radiotherapy, serotyping

INTRODUCTION

Recently fungal infections have greatly increased as a result of the increase in number of patient with immunosuppressive viral infections and malignant tumors.[1] Oral squamous cell carcinoma that constitutes about 90–95% of the head and neck cancers are the most radiosensitive.[2] Candidosis is the most common infection of oropharynx in patients receiving radiation. Radiation frequently causes alteration of the oral environment predisposing to colonization of the oral mucosa by candida causing opportunistic superficial candidosis and occasionally induces deep candidosis in the oesophagus, lungs, kidneys, liver, and intestine. When the immunosurveillance system is strongly suppressed, deep candidosis of these organs becomes lethal.[3] So the morbidity and the potential for death associated with oral candidosis makes an early diagnosis and management of such patients important.

Also recognition can be confusing because of variable white and red presentations and deciding for antifungal treatment can pose a problem.

So the purpose of this study was to support association between radiation therapy and increased Candida colonization, to assess the clinical appearance of oral candidosis in such patients taking radiotherapy, to quantitate CFUs, to identify candida

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at species level and to find out the incidence of serotype A and B in clinical isolates of *C. albicans*.

MATERIALS AND METHODS

The patients of oropharyngeal cancer who were advised radiotherapy were examined and selected to undergo this study. Their consent was taken and they were subjected to thorough extra-oral and intraoral clinical examination. Complete medical and dental history was obtained including accompanying systemic conditions, ongoing medication, and prescribed therapy for their neoplastic lesion. The patients of other risk factors for candidosis such as diabetes, chemotherapy, corticosteroids, or recent use of antibiotics as well as patients using intraoral prostheses and patients who had received antifungal therapy were excluded from the study. All the patients were given oral hygiene instructions and were investigated for complete haemogram and blood sugar level.

There were 95 men and 12 women with an age range of 30 to 76 years. The mean age was 59.12 years.

All these selected patients were of squamous cell carcinoma involving oral and/or pharyngeal areas with comparatively normal oral mucosa. They were to undergo radiotherapy. After complete history and thorough clinical examination three visits for these patients were scheduled. First visit was before radiotherapy. Second visit was during radiotherapy and after completion of 20 ± 3 doses, and third visit was after 15 days till when the radiation therapy was also completed. At each visit, the sample was collected by the oral rinse method. Sabouroud's dextrose agar (SDA) was used as primary culture media. With positive culture report of first sample, CFUs/0.1 mL was noted. Then the growth was subcultured on new plate of SDA to obtain pure growth and the growth from a single pure colony was further subjected to Germ Tube test, Carbohydrate Fermentation Test [Figure 1], and Corn Meal Tween 80 agar cut streak culture to identify various species of candida. Thus, at each visit speciation was done using these standard techniques. When the isolates were identified as *candida albicans*, they were serotyped by slide agglutination method as described by Hansclever and Mitchell.[4,5] For this antiserum specific for serotype A was prepared by using the standard strains of *C. albicans* serotype A and B supplied by CDC Atlanta.

Second visit by the patients was made during radiotherapy, after completion of 20 ± 3 doses of radiation. During this visit all the 107 patients were clinically assessed for the presence of oral mucosal lesions and grading of mucositis was done according to the method described by Chen and Webster.[1] Individual signs and symptoms such as erythmatous lesions, ulcerations, white confluents patches, angular chelitis, and xerostomia were also noted for each of 107 patients.

For mycological examination, second oral rinse was collected from all these 107 patients. With positive culture results CFUs/0.1 mL were noted and further mycological examination was done using the standard techniques, as described before to identify different species of candida. When the species were identified as *C. albicans* serotyping was done as described above. Thus, after complete mycological evaluation, 65 (60.74%) patients showed positive culture results during radiotherapy.

These patients were suitably given antifungal treatment for 15 days and were called for third visit after 15 days that also coincided with completion of radiotherapy.

At third visit patients were examined clinically and oral rinse sample was collected. With positive culture results, CFUs/0.1 mL were noted and again complete mycological test were done to identify different species of candida. Also serotyping was done whenever the species was identified as *C. albicans*. The patients were called after 1 month for follow up study. At this time patients were only assessed clinically for the presence or absence of any oral lesions and no mycological tests were done as were not required.

OBSERVATIONS AND RESULTS

Out of 107 patients 28 patients (26.16%) showed positive oral cultures at first visit. The number of CFUs/0.1 mL of each patient was noted. The range and mean value±standard deviation of CFUs/0.1 mL was 100.14 ± 59.11. Serotyping of 22 confirmed strains of *C. albicans* showed that 19 strains reacted quickly with A specific antiserum forming large clumps [Figure 2]. The remaining three strains however failed to agglutinate with A specific antiserum that were of serotype B. Identification of 28 isolates at species level showed that *C. albicans* was seen in 78.57%, C. krusei- 7.15%, and *C. tropicalis*, *C. guilliermondii, C. stellatoidea*, and *C. parapsilosis* each being 3.57% [Figures 3-7].

Since these 28 patients were only mycologically positive without showing any clinical signs and symptums of oral candidosis, they were considered as healthy carriers and were not prescribed any medicine.

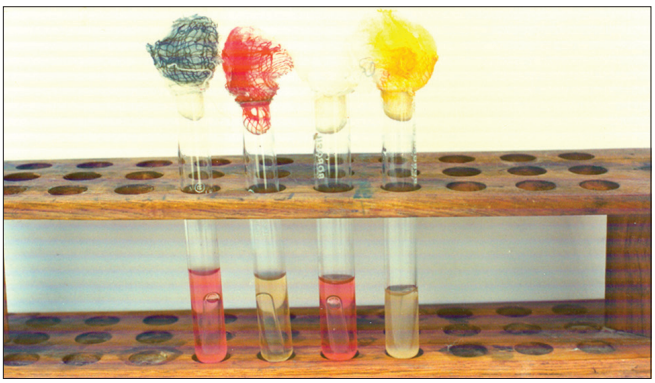

Figure 1: Photograph showing sugar fermentation reaction of *C. albicans*

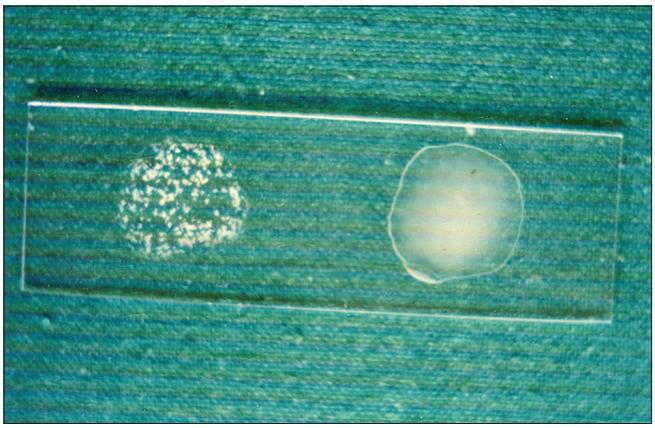

Figure 2: Photograph showing slide agglutination test for serotyping of *C. albicans*. Serotype A showing strong agglutination reaction and serotype B showing weak agglutination reaction with A specific antiserum

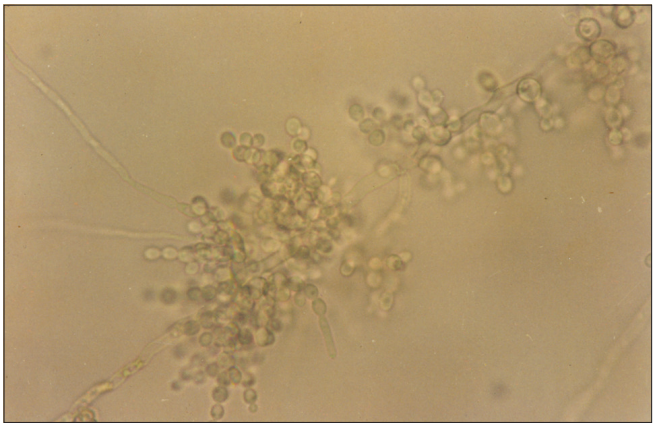

Figure 4: Photomicrograph showing short fine pseudohyphae with clusters of blastospores of *C. guilliermondii* on corn meal Tween 80 agar

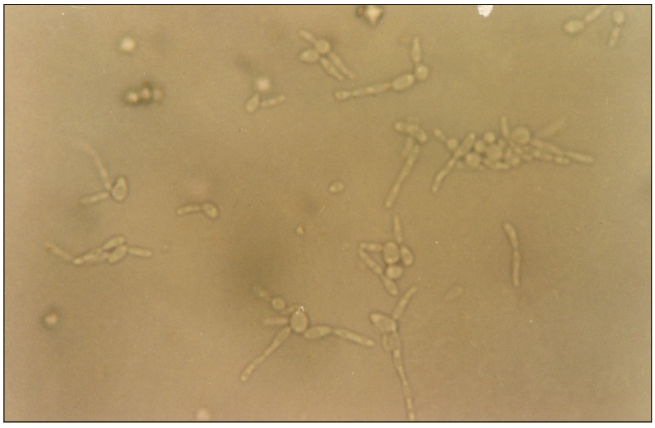

Figure 6: Photomicrograph showing showing giant mycelial cell of *C. parapsilosis* on corn meal Tween 80 agar

At second visit, 46 pts (42.90%) showed mild mucositis, 50 patients (46.72%) showed moderate mucositis, and 11 patients (10.28%) showed severe mucositis. Five patients showed definite occurrence of pseudomembranous candidosis along with severe mucositis.

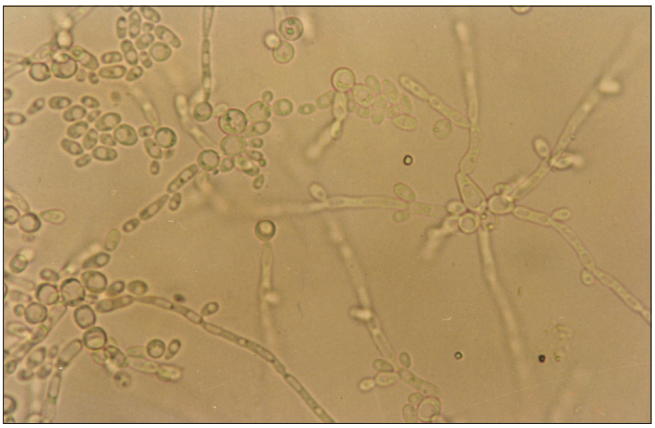

Figure 3: Photomicrograph showing large, terminal, thick-walled chlamydospores of *C. albicans* on corn meal Tween 80 agar

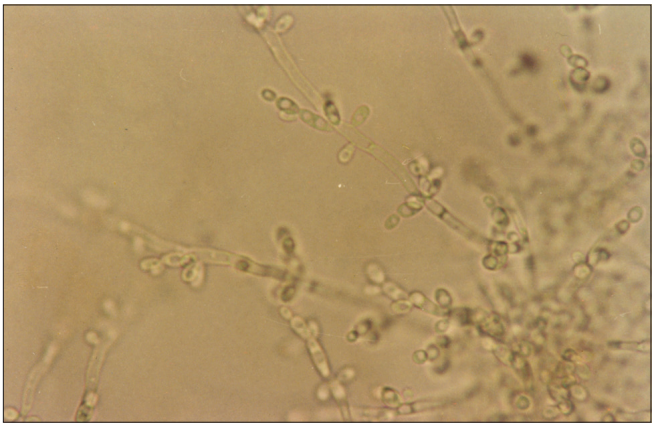

Figure 5: Photomicrograph showing pseudohyphae with scanty irregularly arranged blastopores of *C. tropicalis* on corn meal Tween 80 agar

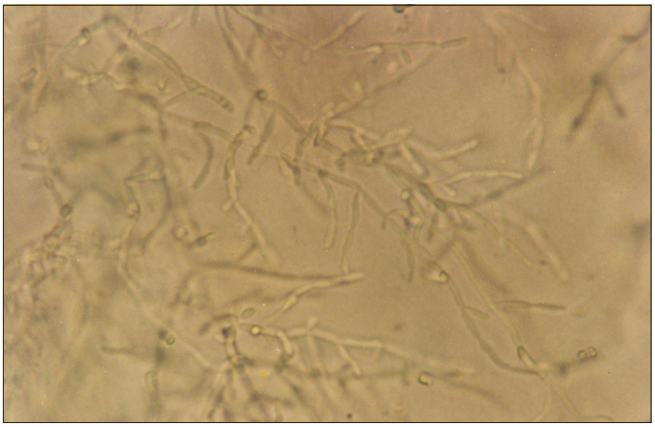

Figure 7: Photomicrograph showing pseudo hyphae with branching of *C. krusei* on corn meal Tween 80 agar

Individual signs and symptoms such as erythmatous lesions, ulcerations, white confluent patches, angular chelitis, and xerostomia were also noted for each of 107 patients. Distribution of which is shown in Table 1.

After radiation, it was found that 37 more patients who did not have positive cultures before radiotherapy and 28 patients who

Table 1: Distribution of clinical signs and symptoms of mycologically positive 65 patients and mycologically negative 42 patients

Lesions	Total patients showing lesions	Mycologically positive patients	Percentage	Mycologically negative patients	Percentage
Erythmatous lesions	57	45	69.23	12	28.57
Ulcerations	55	40	61.53	15	35.71
White confluent patches	5	5	7.69	0	–
Xerostomia	52	42	61.61	10	23.81
Angular chelitis	11	10	15.38	1	2.38

were mycologically positive at first visit were all positive also at the second visit with more candidal colonization. Thus, total number of positive patients were 65 at the time of second visit.

The observations of mycological positivity and CFUs in positive cases at each visit are summarized in Table 2. From the table, it is evident that the number of CFUs/0.1 mL of 28 patients were increased markedly during radiotherapy indicating more candidal colonization and thus suggestive of candidal infection during radiotherapy.

Quantitative assessment of candida species colonization at second visit

1. The number of CFUs recovered from those 28 patients who were mycologically positive at first visit was compared to their CFUs recovered after 20 ± 3 doses of radiation therapy by means of the paired *t*-test Degree of freedom=27, calculated T=30.50. Therefore, P<0.01. It was found that the difference between mean number of CFUs before radiotherapy and mean number of CFUs during radiotherapy of these 28 patients was significantly greater.
2. The number of CFUs at second visit recovered from the 28 patients were compare with the number of CFUs of the 37 patients who were mycologically negative at first visit and became positive during radiotherapy by mean of the unpaired *t*-test. It was found that the difference between mean±standard deviation of CFUs of these two groups was highly significant statistically. Degree of freedom=65, calculated t=36.76. Therefore, P<0.001. The number of CFUs recovered from 28 patients before starting radiotherapy were compared with the number of CFUs recovered from all 65 patients during radiotherapy by mean of unpaired *t*-test. It was found that the difference between mean±standard deviation of CFUs was highly significant statistically. Degree of freedom=91 calculated T=13.87. Therefore, P<0.0001.

Species identification of candida strains of these 65 patients showed that 55 (84.62%) were *Candida albicans*, 3 (4.62%) *Candida tropicalis*, *Candida krusei*, *Candida guilliermondii were* each 2 (3.07%) and *Candida pseudotropicalis*, *Candida stellatoidea*, and *Candida parapsilosis* were each 1 (1.54%).

Table 2: Distribution of CFUs of mycologically positive patients at each visit

	Number of patients mycologically positive	Range of CFUs/0.1 mL	Mean±standard
Before radiotherapy	28	40 to 224	100.14 ± 59.11
During radiotherapy			
a	28	564 to 792	274 ± 53.69
b	37	248 to 392	312.78 ± 28.82
Combined a+b	65	248 to 792	490.15 ± 207.9776
After antifungal treatment	24	100 to 228	125 ± 50.182267

Serotyping of 55 confirmed strains of *C. albicans* showed that 43 reacted quickly with A specific antiserum and the remaining 12 failed to agglutinate which were of serotype B.

To check relationship between the increased candidal colonization and the severity of acute radiation reaction (mucositis) during radiotherapy the clinical assessment of 65 mycologically positive patients was compared with the clinical assessment of mycologically negative 42 patients at their second visit and it was observed that there is no correlation between the grades of mucositis and occurrence of candida species during radiotherapy in head and neck cancer patients and the result were found to be statistically insignificant (x^2=3.6880). From this result it was concluded that the severity of mucositis is not influenced by the positive and negative culture for Candida organisms.

When the signs and symptoms of mycologically positive 65 patients were correlated with the signs and symptoms of mycologically negative 42 patients, the occurrence of erythmatous lesions, ulcerations, and xerostomia were found to be statistically significant (P<0.05) whereas the occurrence of white confluent patches and angular chelitis were statistically insignificant (P>0.05). The number of CFUs of 24 patients whose cultures were positive at third visit, i.e., after antifungal treatment were compared with the number of CFUs before antifungal treatment and were found to be markedly reduced and was highly significant statistically by paired *t*-test. Degree of freedom=23, Calculated t=13.259. Thus, P<0.0001.

Upon further mycological examination of these 24 isolates, 20 were *C. albicans* (83.33%) and *C. krusei*, *C. pseudotropicalis*, *C stellatoidea* and *C. parapsilosis* were each 1 (4.16%). Out of 20 *C. albicans* 18 showed strong reaction to antiserum and two failed to agglutinate. Thus, 18 were of serotype A and 2 were of serotype B.

DISCUSSION

26.16% patients were mycologically positive for candida before radiotherapy with CFUs 100.24 ± 59.11. The CFUs of these 28 patients increased from 100.24 ± 59.11 before radiotherapy to 724 ± 53.69 during radiotherapy showing high statistical significance. So it can be considered that they were healthy carriers at the first visit and the marked increase at second visit is probably suggestive of candidal infection during radiotherapy. Thirty seven more patients became positive for candidosis with CFUs 312.78 ± 28.82 during radiotherapy who were negative for candida before radiotherapy. A marked difference between CFUs of 28 patients and 37 patients during radiotherapy was found with high statistical significance. These findings are consistent with the findings of Rossie *et al*.[6] who has done quantitative assessment of increase in the incidence of the Candida species in a similar study by measuring CFUs of Candida. Possibly the reason being the healthy carriers for candida shows more colonization due to radiation than noncarriers.

Irradiation does have significant detrimental effects that provides a more favorable environment for the growth of the fungi. As the fungi flourish, they are more easily detected resulting in higher positive culture rate.[3] This is possibly the reason why 28 patients showed such a significant difference in CFUs before and after radiotherapy which was even statistically significant. The occurrence of oral candidosis in patients taking radiotherapy is mainly related to subsequent quantitative and qualitative changes in salivary gland after radiotherapy.

The growth of oral Candida is locally regulated not only by phagocytes flowing in saliva but also by saliva itself.[6] Saliva exhibits mechanical cleaning activity in healthy individuals with normal salivary gland function.[1] A dry environment and/or lack of salivary enzymes and antibacterial proteins fosters a desirable environment for fungal proliferation.[7] Xerostomia and alteration in saliva result in alteration of normal host defenses and microbial equilibrium.[6]

The suppression of salivary polymorphonuclear leukocyte function such as chemotaxis and reactive oxygen generation along with already suppressed cellular immunity allows the overgrowth of weak pathogens such as Candida.[8]

However, pathogenesis of candidal infection is complex encompassing both fungus and host factors. The adherence of Candida to the epithelial cells of oral cavity is recognized as an essential prerequisite for the development of a Candida pathosis which is probably dependent on and reflective of proteins expressed by Candida, host cell and extracellular matrix proteins, hostcation influence on adhesion, possible co-aggregation between fungi and bacteria as well as patient's immune competence.[9]

The first step of Candida multiplication is adhesion of Candida to the oral mucosa.[1] After adhesion Candida begins to multiply if the abilities of Candida growth inhibitory agents are suppressed.[6] Multiple proteins in saliva have inhibitory activities against both adhesion and multiplication of Candida.[1,6] Secretary immunoglobulin A which consists of IgA binds mannons of Candida cell walls and inhibits adhesion of Candida to the mucous membrane.[10] Secretary component (SC) has mannose residues and it also inhibits adhesion of Candida to the mucous membrane.[10] Lactoferrin (Lf), an iron binding protein which is released from phagocytes by inflammatory stimulation, suppresses fungal growth and protects the tissue from microbial invasion.[11] Histatins are proteins generated from epithelial cells including the salivary glands and exhibit potent antifungal activities. Defensins-α defensins secreted by neutrophils and β defensins secreted by the epithelial cell, strongly suppresses the growth of bacteria as well as fungi.[1]

Thus, saliva contains multiple potent antimicrobial proteins and peptides and if salivary secretion is decreased or if salivary levels of the antimicrobial agents are decreased, oral microbial overgrowth is allowed and oral mucosal infection such as Candidosis is induced.[1] Thus radiotherapy induced xerostomia does have relationship with oral Candida overgrowth.

In our study also during radiotherapy 61.61% (52 pts) patients complaining about dryness of mouth showed presence of Candida mycologically.

The role of Candida species in oral mucositis is unknown. But it is observed that areas of mucositis due to radiation may become infected by Candida secondarily and even if candidosis does not play a role in the cause of oral mucositis, it may contribute to the duration and discomfort of mucositis.[1] When the mycologically positive and negative patients were compared according to grades of mucositis, the results were statistically insignificant (x=3.6880) which shows that the severity of the acute radiation reaction was not influence by the positive and negative culture for Candida organism. This is similar to the findings of Chen and Webster.[3]

The total number of 65 mycologically positive patients during radiotherapy showed mean CFUs 490.15 ± 207.97/0.1 mL.

After completion of radiotherapy and treatment of oral candidosis when these 65 patients were mycologically

evaluated, 41 patients showed complete eradication of culture and the remaining 24 patients who still showed positive cultures had very low CFUs than before treatment and thus are also benefited to some extent.

Oral rinse culture technique: The oral rinse culture technique used in this study appears to be relatively simple and sensitive. This technique was used by various investigators effectively for the quantitative assessment of oral *C. albicans* colonization and infection.[6,12-14] However, despite the sensitivity of the oral rinse culture it is uncertain whether patients whose culture were first negative and then positive after radiotherapy were newly colonized by Candida or whether the organisms were initially present but at undetectable level. Though it was stated by various investigators that no definite colony counts have been established that allows for the differentiation between commensalism and disease,[3] this study showed statistically highly significant difference in CFUs before and after radiotherapy.

Species-wise evaluation: In this study, speciation of Candida organisms showed that *C. albicans* was the most commonly encountered species. Other species more or less equally isolated were *C. tropicalis*, *C. krusei*, *C. guilliermondii*, *C. stellatoidea*, *C. parapsilosis*, and *C. pseudotropicalis*, *C. glabrata*.

In normal population also, *C. albicans* is the most prevalent yeast isolated from the human body as a commensal or as an opportunistic pathogen and it is likely that patients of oral cancer taking radiotherapy shows *C. albicans* more frequently.

In this study, there was no significant difference in the percentage of isolation of different species other than *C. albicans* and thus the difference in the other candidal species did not appear to be related to increased pathogenicity.

Even after antifungal treatment, *C. albicans* was isolated more frequently than other species as seen before and during radiotherapy. There was not a particular species which could be related to the resistance to antifungal treatment.

Serotyping of *Candida albicans*

In 1961 Hansclever and Mitchell[4] demonstrated two distinct serotypes -serotype A and B of *C. albicans* by using whole cell agglutination to rabbit antisera. Initial investigation by these clinical isolates revealed that 68% were serotype A and 32% were serotype B. The preponderance of serotype A from clinical isolates has been confirmed in the studies of Auger *et al*.,[15] Martin *et al*.,[16] and Brawner *et al*.[17]

These investigators also suggested that since serotype A is isolated more frequently from pathologic conditions, some relationship exist between serotype and virulence. However, in the study by Hansclever *et al*.,[5] there was no relationship between virulence and serotype in *in vitro* pathogenicity studies using mice. So to see the relationship if any between serotype and pathogenic potential, serotyping of *C. albicans* isolates was done.

In this study, *C. albicans* of Serotype A was isolated more frequently than serotype B both before and after radiotherapy from patients infected with *C. albicans*, thus suggesting its pathogenic potential and virulent nature. Other proposed virulence factors for *C. albicans* are presence of pseudohyphae in the tissue, proteinase enzyme production by infecting strains, adherence to epithelial cells and virulence to laboratory animals.[18] However, the tests required to determine these virulent factors are technically demanding and require sophisticated equipments and other facilities and are not routinely possible in a laboratory with moderate feasibility. Serotyping is technically simple, inexpensive and easy to perform. This typing system can be used routinely to detect virulent strains of *C. albicans* in the isolate.

With this method of typing of the strains of *C. albicans*, discrimination between avirulent and virulent strains can be undertaken routinely in the laboratory. At third visit after antifungal treatment when *C. albicans* was present in still mycologically positive patients, 90.00% showed presence of serotype A and 10% strain of *C. albicans* showed serotype B. Thus even after antifungal therapy, higher prevalence of serotype A was found.

Serotype A is known to be more virulent. However no correlation was found between serotype and mycological failure of antifungal drug to eliminate Candida.

CONCLUSION

These results conclusively suggest that radiation definitely increases frequency of occurrence of oral candidosis. Radiation induced fragility of oral mucosa with occurrence of specific lesions like erythmatous lesions, ulceration, and dryness of mouth shows statistically significant role in making oral mucosa more susceptible to oral candidosis with occurrence of high number of colony forming units. *C. albicans* is still the most frequently isolated species more frequently being of serotype A. Thus, *C. albicans* of serotype A definitely has some pathogenic potential and virulent nature which can be studied further. We propose that in such patients taking radiotherapy prophylactic antifungal treatment should be given specially in patients showing development of oral mucosal lesions like erythmatous lesions, ulcerations and complaining about dryness of mouth that is xerostomia, irrespective of presence or absence of clinical oral Candidosis.

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