Isolation and speciation of genus candida in patients undergoing chemotherapy and radiotherapy for head and neck tumours

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ABSTRACT

Background: Fungal infections occur as a result of defect in the immune system. The use of wide-spectrum antibiotics, immunosuppressive therapy, HIV and an increasing incidence of diabetes are some of the causes that resulted in raising number of immunocompromised individuals, in the global scenario. Opportunistic fungal infections mainly oral candidiasis is common in patients undergoing chemotherapy (CT) or radiotherapy (RT) for Head & Neck tumors. Objectives: Isolation and speciation of Candida with antifungal susceptibility testing in patients undergoing CT and RT for Head and Neck tumors.

Methods: Study group comprised of 100 saliva samples collected by oral rinse method, (50 chemotherapy and 50 radiotherapy) from inpatients of King George hospital, Visakhapatnam and 50 normal healthy individuals were taken as control group. Standard mycological tests for the Candida isolation, speciation and antifungal susceptibility were done.

Results: In the study group out of the 100 patients, 38% were culture positive for Candida. The most commonly isolated species was C. albicans (60.5%) followed by non albicans species. Most of the candida species showed sensitivity to nystatin, amphotericin B, itraconazole and Ketoconazole. In control group 9 out of 50 samples (18%) were culture positive and all the isolates were Candida albicans.

Conclusion: Increase in frequency of oral candidiasis in patients undergoing chemotherapy and radiotherapy was observed. The increase in positivity may be attributed to inadequate nutritional status and poor oral hygiene during chemotherapy and radiotherapy. Culture positivity is more in RT patients than in CT patients. Though C. albicans is the predominant isolate, non albicans species are also emerging. All the Candida species isolated from study group were sensitive to nystatin, amphotericin B, itraconazole and ketoconazole. In the study group all the Candida species were resistant to clotrimazole and fluconazole and in the control group all the C. albicans were sensitive to fluconazole.

Keywords: Candida albicans, Oral candidiasis, Radiotherapy, Fluconazole, Disk diffusion method

INTRODUCTION

Fungal infections occur as a result of defect in the immune system. The use of wide-spectrum antibiotics, immunosuppressive therapy, HIV and an increasing incidence of diabetes are some of the causes that resulted in raising number of immunocompromised individuals. Opportunistic fungal infections mainly oral candidiasis is common in patients who undergo chemotherapy or radiotherapy. Radiation was given during treatment of
malignancy causes alteration of the oral environment, predisposing to the colonization of the oral mucosa by yeast species most frequently Candida.

Candida species are usually normal oral commensals and their transition to opportunistic pathogens may be associated with the virulence attributes of the organism and also the host resistance. The virulence of Candida strain isolated from patients on chemotherapy and irradiation is largely determined by activities of adherence, multiplication and release of cytoplasmic antigen, enolase and Candida aspartic proteinase.

Although Candida albicans remains the most common causative agent of both superficial and deep fungal infections, an increased incidence of the non albicans species like C. tropicalis, C. krusei and C. parapsilosis were also observed.

Objectives
1) To study the prevalence of Candida in patients undergoing chemotherapy and radiotherapy for head and neck tumors.
2) Isolation and speciation of Candida in those patients.
3) Antifungal susceptibility test for Candida species by Disk diffusion method.

METHODS
The present study was carried out in the dept. of microbiology, Andhra medical college, Visakhapatnam, India. Study group comprised of 50 chemotherapy and 50 radiotherapy inpatients of King George hospital, Visakhapatnam and 50 normal healthy individuals were taken as control group. The patients with other risk factors for candidiasis such as diabetes, recent usage of corticosteroids or antibiotics as well as patients using intraoral prostheses and patients who had received antifungal therapy were excluded from the study.

The samples were collected from control group and study group as follows:

50 patients whose age and sex were matched with study groups and apparently healthy, with no systemic diseases after routine investigations were taken as controls.

50 patients, who were on chemotherapy and 50 patients who were on radiotherapy for head and neck tumours of age 20-80 years were taken as study group.

After an informed consent, saliva samples were collected after oral rinse with phosphate buffer saline for 1 minute in a sterile plastic container. Direct microscopic examination of Gram stained smear (Figure 1) and KOH wet mount (Figure 2) of the sample was done and examined for budding yeast cells and pseudohyphae.

Candida isolates were identified by microscopic examination of Gram stained smear (Figure 4), germ tube
formation, chlamydospore and blastoconidia production which was tested on Cornmeal agar by Dalmau culture plate technique. Rapid method of identifying C. albicans is by its ability to form germ tubes within two hours when incubated in human serum at 37°C (Reynolds-Braude phenomenon) was done (Figure 5). The suspected strains of Candida isolates were grown on cornmeal agar (CMA) by incubating at 25°C for 2-3 days and observed for chlamydospor formation (Figure 6).

For species identification, the isolates from the Sabouraud’s dextrose agar medium were inoculated on CHROM agar medium and incubated at 37°C in dark for 48 hours. Only pigmented colonies were considered for species identification.

- C. albicans - Light green
- C. dubliniensis - Dark green
- C. tropicalis - Blue
- C. glabrata - Pink to purple
- C. krusei - Pink
- C. parapsilosis - Cream to pale pink

Antifungal susceptibility testing was done by disk diffusion method as per CLSI guidelines (M44-A). It recommends the use of Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue dye medium. Inoculum was prepared from the yeast grown on SDA for 24hrs, adjusted to match the turbidity of 0.5 Mc Farlands standard in the spectrophotometer. Sterile applicator swab was moistened in that cell suspension and used to inoculate the surface of Mueller-Hinton agar. Antifungal discs were placed and incubated in BOD for 24hrs and observed for zones of inhibition.

Antifungal discs used were:

- Amphotericin B - 20 µg
- Itraconazole - 10 µg
- Fluconazole - 10 µg
- Ketoconazole - 10 µg
- Clotrimazole - 10 µg
- Nystatin - 100 units/disc

For azoles, sensitivity zones were:

- Susceptible - when ≥17 mm diameter
- Intermediate - in between 14 mm -16 mm diameter
- Resistant - when ≤13 mm diameter.
For Amphotericin B, sensitivity zones were:
- Susceptible - when ≥15 mm diameter
- Intermediate – in between 13 mm -14 mm diameter
- Resistant - when ≤12 mm diameter.

**RESULTS**

Majority of patients were between 51-60 years (22%) of age followed by 41-50 years of age (9%) and 61-70 years (5%) (Table 1).

**Table 1: Age wise distribution of cases.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Chemotherapy Culture positive No.</th>
<th>Radiotherapy Culture positive No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>2 Nil</td>
<td>-</td>
</tr>
<tr>
<td>31-40</td>
<td>6 1</td>
<td>8 1</td>
</tr>
<tr>
<td>41-50</td>
<td>10 3</td>
<td>16 6</td>
</tr>
<tr>
<td>51-60</td>
<td>22 10</td>
<td>20 12</td>
</tr>
<tr>
<td>61-70</td>
<td>10 2</td>
<td>6 3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50 16</strong></td>
<td><strong>50 22</strong></td>
</tr>
</tbody>
</table>

In study group of the 100 samples tested, 38% were culture positive for Candida. Majority of culture positives were from male patients, 24 out of 38 culture positives were from both chemotherapy and radiotherapy cases. Out of 38 culture positive samples, 22 (57.9%) were from RT patients and 16 (42.1%) were from the CT patients. Majority of patients were of carcinoma larynx and oral cavity (Table 2).

**Table 2: Gender wise distribution.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Chemotherapy Culture positive</th>
<th>Culture negative</th>
<th>Radiotherapy Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>10</td>
<td>18</td>
<td>14</td>
<td>16</td>
<td>58</td>
</tr>
<tr>
<td>Females</td>
<td>6</td>
<td>16</td>
<td>8</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>34</strong></td>
<td><strong>22</strong></td>
<td><strong>28</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

The most commonly isolated species in both chemotherapy and radiotherapy patients is *C. albicans* 60.5% (23), followed by non albicans species, *C. tropicalis* 18.4% (7), *C. glabrata* 10.5% (4), *C. krusei* 7.9% (3), *C. parapsilosis* 2.6% (1) (Table 3).

**DISCUSSION**

Opportunistic fungal infections are increasing as there is increase in the number of immunocompromised individuals due to CT and RT for malignancies, increased number of patients undergoing transplantations, prolonged hospital stay in intensive care units, metabolic disorders like diabetes, HIV.6,7 Radiation induced fragility of oral mucosa which leads to erythematous lesions, ulcerations and compromised salivary function is thought to be a major contributing factor for oral candidiasis. Suppression of antifungal activity of leukocytes and increase in candidal cell virulence are the predisposing factors for candidiasis in CT & RT undergoing patients.8
The increased number of non albicans species may be due to changing ecology of the pathogens and evolution of non albicans species as pathogens in immunocompromised patients.

In the present study Candida albicans was the most common isolate 23 (60.5%) out of 38 isolates, which correlates with the studies of Amador et al. who reported 68%, Shaheen & Taha who reported 56% and Hema Suryawanshi et al. reported 84.62% of C. albicans.

In the present study the non albicans species isolated were 15 (39.5%) out of 38 isolates which correlates with Shaheen & Taha who reported 44% and Amador et al. who reported 32%.

In the present study the non albicans species isolated were C. tropicalis, C. glabrata, C. krusei, C. parapsilosis which correlates with Redding et al. and Dahia et al.

Among non albicans species C. tropicalis was the common isolate 7 (46.7%) out of 15 non albicans species which correlates with Shoba Rani Bakki et al.

In the control group C. albicans was isolated in 9 (18%) out of 50 which correlates with Shoba Rani Bakki et al. who reported 22% of controls and Cohen et al. who reported 20-60% of controls.

In the present study the prevalence of candida is more in males (63.1%) when compared to females which correlate with Mohd Suhail Lone et al. who reported 54% in males and Shoba Rani Bakki et al. and Gooris et al.

All candida species isolated were sensitive to nystatin, amphotericin B, itraconazole and ketoconazole in the present study. All the Candida isolates were resistant to clotrimazole and fluconazole. In the control group all the C. albicans were sensitive to Fluconazole which correlates with studies of Mohd Suhail Lone et al.

CONCLUSIONS

Increase in frequency of oral candidiasis in patients undergoing chemotherapy and radiotherapy was observed. This increase in positivity may be attributed to inadequate nutritional status and poor oral hygiene during chemotherapy and radiotherapy. Culture positivity is more in RT patients than in CT patients and more in males than in females. Though C. albicans is the predominant isolate, non albicans species are also emerging. All the Candida species isolated from study group were sensitive to nystatin, amphotericin B, itraconazole and ketoconazole. In the study group all the Candida species were resistant to clotrimazole and fluconazole and in the control group, all the C. albicans were sensitive to fluconazole.

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