SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY OF ESOPHAGEAL CANDIDIASIS IN CANCER PATIENTS IN A TERTIARY CARE HOSPITAL IN CHENNAI.

A R T I C L E   I N F O

Keywords: Antifungal susceptibility pattern cancer patients esophageal candidiasis

Background: Esophageal candidiasis is the most common opportunistic infection in patients with altered immunity such as HIV infection, cancer patients on chemotherapy and radiotherapy. Neutropenia, irradiation and chemotherapy will facilitate deeper mucosal invasion of candida leading onto esophageal candidiasis. Empirical treatment of esophageal candidiasis without antifungal susceptibility testing will lead to the emergence of drug resistant species increasing the morbidity and mortality associated with cancer. Aim: The present study aims to study the frequency of esophageal candida in individuals with cancer, species level identification and Antifungal susceptibility pattern for fluconazole, Itraconazole and Amphotericin B by Microbroth dilution method. Methods: Scrapings of whitish appearing lesions were obtained from a total of thirty five cases of endoscopically identified esophageal candidiasis were obtained from patients with cancer. Identification of the candida isolates were done by cultivation in Sabouraud dextrose agar, Gram staining, germ tube test, colony morphology in Chrom agar and corn meal agar, sugar assimilation and fermentation tests. Antifungal susceptibility was done by Microbroth dilution method for Fluconazole, Itraconazole and Amphotericin B. Results: We found that Candida albicans was the predominant species isolated followed by Candida tropicalis and Candida glabrata. Sensitivity rates were 91.7%, 94% and 100% for fluconazole, Itraconazole and Amphotericin B by Microbroth dilution method. Conclusion: Species level identification of candida isolated from esophageal candidiasis and their Antifungal sensitivity testing should be performed for early identification of Resistant strains and for promptly treating the cases thereby preventing the dissemination of infection in case of immunocompromised individuals. Further the susceptibility pattern will facilitate therapeutic guidance especially in individuals prone to relapse.

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cases and in 7.8% of other malignancies [4]. Alteration in immune status will promote the proliferation of endogenous candida and will facilitate deeper mucosal invasion of candida leading onto esophageal candidiasis. From the esophagus the candida can spread to distant organs via hematogenous route.

Fluconazole is the empirical drug of choice for treating esophageal candidiasis [7]. In individuals with cancer there is mucosal disruption facilitating the colonised candida species to invade deeper tissue leading to esophageal candidiasis or even disseminated candidiasis. Indiscriminately treating all cases of esophageal candidiasis without antifungal susceptibility report can lead to the eradication of drug sensitive species by more inherently resistant species such as candida krusei and candida glabrata. Thus the emergence of Antifungal drug resistance by the isolates has made in vitro antifungal testing methods essential to choose the appropriate antifungal drug and to predict the outcome of therapy.

Hence the present study was undertaken to isolate, speculate and to study the Antifungal susceptibility pattern of esophageal candidiasis in cancer patients.

MATERIALS AND METHODS:

This observational study was conducted over a period of July 2012 – June 2013 at Government Kilpauk Medical College and Hospital, Chennai. The study was approved by the Institutional Ethical Committee.

Cancer patients with endoscopically suspected esophageal candidiasis and not on treatment with any antifungal drugs were included in the study. Esophageal candidiasis was confirmed by the presence of typical coalescent white patches covering the esophageal mucosa. Esophageal scrapings from such lesions were collected in a sterile container and transported to the lab as early as possible. Samples were processed for Microscopy, culture, and characterisation of candida species [8].

From each specimen, smears were made on a grease free clean glass slide air dried and heat fixed and staining was done by Gram staining method. The presence of gram positive budding yeast cells with or without pseudohyphae was observed [8]. The material was then inoculated immediately into two sets of Sabouraud Dextrose Agar culture tubes and incubated at 25°C for 24-48 hours. The medium is also supplemented with chloramphenicol (0.05 g/L) to prevent bacterial overgrowth. Growth usually as creamy, white pasty colonies. Gram staining was performed from the colonies for confirmation of presence of gram positive ovoid 5-7μm sometimes elongated to 4-6×6-10μm in size [9].

Further species identification was done by Germ Tube Test. Growth in Chromagar (based on the color of colonies) Growth in Corn Meal Tween 80 Agar (Dalmau Plate Culture Technique), Sugar fermentation test and Carbohydrate assimilation test as per standard microbiological guidelines [10]. Antifungal susceptibility testing was performed by microbroth dilution method as per CLSI guidelines for fluconazole, Itraconazole and Amphotericin B [10, 11].

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by Micro broth dilution method using RPMI 1640 with glucose as per CLSI guidelines [10, 11]. Stock suspension was prepared and diluted with RPMI to obtain an inoculum which contains 1x10^3 to 5x10^3/ml CFU. Antifungal stock solution was prepared by dissolving fluconazole in sterile distilled water. Amphotericin B and Itraconazole were dissolved in Dimethylsulfoxide. The test was performed in a sterile disposable 96 well microtitre plate. The 2X drug concentration in 100μl volume was dispensed into the wells of row 1 to row 10 of the Microtitre plate using a micro pipette. Row 1 contains the highest drug concentration and row 10 contains the lowest drug concentration. Each well was inoculated with the 100 μl of 2X inoculum suspension. The growth control well contains 100 μl of sterile drug free medium and the corresponding diluted 2X inoculums suspensions. Row 11 was used to perform the sterility control containing only the drug free medium only. The microtitreplates were incubated at 35°C for 48 hours. The plates were observed for the presence or absence of visible growth. A numerical score which ranges from 0 to 4 was given to each well [10, 11].

0 - Optically clear
1 - Slightly hazy or approximately 25% of growth control
2 - Prominent decrease in turbidity or approximately 50% of growth control
3 - Slight reduction in turbidity or approximately 80% of growth control
4 - No reduction in turbidity

End point of MIC
Fluconazole and Itraconazole - Score 2 or less
Amphotericin B - Score 0.

RESULTS:

A total of 35 endoscopically confirmed cases of esophageal candidiasis from cancer patients were included in the study. Out of the 35 cases 33 (94.2%) were culture positive. Candida albicans was the predominant species isolated 31 (93.3%) followed by one isolate of candida tropicalis and candida glabrata each. Direct gram staining was done to look for the presence of gram positive pseudo hyphae and budding yeast cells and it showed a sensitivity and specificity of 100% in comparison with culture. The distribution of cases based on the site of malignancy is shown in Table 1. Out of the total 35 cases 15 (42.8%) were from carcinoma esophagus and 14 (40%) were from carcinoma stomach.

All the culture positive isolates were further identified by Gram staining, Germ tube test, Colony morphology on chrom agar, Growth on corn meal agar; sugar fermentation and assimilation tests as per standard microbiological techniques. On chrom agar candida was speciated on the basis of color of the colonies light green color (Calbicans) metallic blue color (C.tropicalis) and pink color colonies (Candida glabrata) [13]. On corn meal agar speciation was done by presence of terminal chlamydosores (Calbicans) oval blastoconidia arranged along the hyphae (C.tropicalis) and only the presence of blastoconidia without hyphae (C.glabrata) [9]. Sugar fermentation and Assimilation tests were interpreted as per standard microbiological techniques.
Antifungal susceptibility testing was done by Microbroth dilution method as per CLSI guidelines for fluconazole, Itraconazole and Amphotericin B and interpreted as per Table 2. Fluconazole and Itraconazole showed 6% and 4% resistance respectively. Both the Non Candida albicans isolated were resistant to fluconazole. All the isolates were sensitive to Amphotericin B as per Figure 1.

Table 1 - Distribution of cases by Risk factors

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td>Carcinoma esophagus</td>
<td>15 (42.8%)</td>
</tr>
<tr>
<td>Carcinoma stomach</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>Carcinoma pancreas</td>
<td>1 (2.85%)</td>
</tr>
<tr>
<td>Others</td>
<td>5 (14.28%)</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 2 - Antifungal susceptibility testing by Microbroth dilution method - Interpretive criteria [11, 17]

<table>
<thead>
<tr>
<th>Antifungal Drug</th>
<th>Susceptible</th>
<th>Susceptible Dose Dependent</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>≤ 8 μg/ml</td>
<td>16-32 μg/ml</td>
<td>≥ 64 μg/ml</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤ 0.125 μg/ml</td>
<td>0.25-0.5 μg/ml</td>
<td>≥ 1 μg/ml</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>≤ 1 μg/ml</td>
<td>-</td>
<td>&gt;1 μg/ml</td>
</tr>
</tbody>
</table>

In our present study single isolate of candida tropicalis and candida glabrata were identified (3.03%). Reports from various parts of the world have indicated the emergence of Non Candida albicans species as a cause of infections [15], [16]. Candida albicans and Non candida albicans differ in their antifungal susceptibility pattern. Few candida species show innate resistance to drugs like azoles which are usually prescribed as the first line of drug for treating esophageal candidiasis. Empirical treatment with drugs like fluconazole might eliminate the susceptible species like candida albicans and promote more discerning growth of species that are naturally resistant like candida krusei. Prophylactic treatment with antifungal drugs like fluconazole without analysing the antifungal susceptibility pattern may escalate the chances of colonisation of esophagus by drug resistant Non candida albicans species. Such colonisation may lead to invasion of the epithelial layer and development of esophageal candidiasis with drug resistant strains when mucosal disruption occurs such as in the case of malignancy, cancer chemotherapy and irradiation [16].

Antifungal susceptibility testing was done by broth microdilution method. Resistance rates were 6% and 4% for fluconazole, Itraconazole, and Amphotericin B respectively.

DISCUSSION

Esophageal candidiasis is the most common opportunistic infections in patient with altered immunity such as HIV, individual on corticosteroid therapy chemotherapy and radiotherapy. Development of Esophageal candidiasis is a two step process consisting of colonisation and subsequent invasion of epithelial layer. Once colonisation has been established impaired cellular immunity permits invasion of epithelial layer. Neutropenia, irradiation, chemotherapy will lead to mucosal disruption facilitating deeper invasion of esophagus by candida. Naito et al and underwood et al had reported the prevalence of esophageal candidiasis to be 0.71% and 1.17% respectively [14,15]. Most cases of esophageal candidiasis remain silent and invasion of esophageal wall is usually limited to the superficial epithelium leading on to extensive tissue necrosis and ulceration resulting in esophageal perforation. Candidiasis can develop secondary to malignancy possibly due to impaired antifungal host defence due to mucosal damage [5,16]. Candida esophagitis is an important problem in cancer patients. The diagnosis can be missed in some cases leading on the more invasive form of infection which can be recalcitrant to treat. In the present study cancer patients with endoscopically diagnosed esophageal candidiasis were included and candida species causing infection were isolated, identified, characterised and antifungal susceptibility testing was done by Microbroth dilution method.

In this study all the patients have underwent chemotherapy, steroid therapy and antibiotic treatment. The age and sex distribution were analysed. Male patients 21 (60%) comprised of the predominant population. Most of the cases were in the age group of 41-50 years 14(40%). In this study we observed that candida albicans was the predominant species isolated which is consistent with Wilhelm et al [5], Sajith et al [17] (97.4%) and Badarinayanan et al [18] 87.5%.
flucanazole and Itraconazole respectively which was consistent with Goldman et al [19] and Pfaffer et al[20]. Wilhelmit et al has reported flucanazole resistance as 4%[19] and Pfaffer et al[20]. Wilhelmit et al has reported flucanazole resistance as 4%[19] and Pfaffer et al[20]. In our study the two Non candida albicans isolated were resistant to flucanazole. In individuals with cancer and those who are on therapy like radiation and chemotherapy mucosal disruption may lead to development of esophageal candidiasis with drug resistant isolates. For treating such isolates antifungal susceptibility pattern will provide us with an idea to choose the appropriate antifungal drug to prevent further dissemination of the infection which might have serious consequences in immunosuppressed individuals such as in cancer patients.

The emergence of antifungal resistance within Candida spp., particularly in cancer patients, necessitates routine investigations into antifungal resistance pattern. Such type of studies will facilitate us to get a competent awareness about their drug resistance pattern and may help the physician in selecting the appropriate antifungal agent for empirical therapy.

CONCLUSION:

Non Candida albicans is emerging as an important pathogen causing esophageal candidiasis. The finding that significant proportion of candida albicans showed reduced susceptibility to azoles may have implications for changes in the antifungal drug regimens for treatment. Species level identification and antifungal susceptibility testing is of importance in cancer patients with esophageal candidiasis to prevent invasiveness of the infection. Antifungal susceptibility testing must be done for all the isolates to prevent the emergence of drug resistant isolates.

References


