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Prevalence of Candida in Diarrhoeal Stools

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Abstract:
Context*: Candida species inhabit the gut as normal flora and can assume a pathogenic role when host defenses falter.*
Aims: *To determine the percentage isolation of Candida in diarrhoeal stools, to speciate them and to identify the presence of predisposing factors leading to their proliferation in the gut.*
Methods and Material: *Microscopic and cultural evaluation of Candida in faeces of patients with diarrhoea. Statistical analysis used: Pearson Chi-Square: P values*
Results: *Candida species were isolated from 9.7% stools of diarrhoea cases, the predominant species being C. albicans (46.7%). Microscopic correlation showed presence of pseudohyphae in 14 out of 15 (93.3%) culture positive stools. Candida was isolated in all age groups and with no predilection for any gender. A recent history of antibiotic usage was elicited in 60% of patients yielding Candida from stool.*
Conclusions: *It is probable that Candida could have been responsible for diarrhoea in patients, especially those on antibiotics, in the present study, substantiated by the presence of pseudohyphae on microscopy, which is an indirect evidence of invasion.*
Keywords - *Candida, Diarrhoea, antibiotic usage*.

I. Introduction

Fungi, especially Candida form a part of normal flora of various human body sites; with the gut being no exception. This fungus has been isolated from healthy adult stool samples in 65% of cases[1]. However, there is evidence that Candida could be incriminated as a probable cause of diarrhoea. It was frequently associated with diarrhoea in malnourished children[2] and antibiotic associated diarrhoea in infants[3]. Candida is normally kept in check by their bacterial counterparts. However, disruption in bacterial population provides an opportunity to the Candida to cause pathogenic effects, probably leading to diarrhoea. Symptoms ascribed to Candidial diarrhoea include prolonged passing of watery stool which may be frothy and often with a texture of cottage cheese. Passage of blood and mucus is rare.

This study was undertaken to assess the percentage isolation of Candida in stools of patients with diarrhoea, to speciate them and to determine factors predisposing to the clinical condition.

II. Subjects And Methods

The study was undertaken in the Department of Microbiology, Goa Medical College, Bambolim, Goa, over a period of six months, from June to November, 2015. Stool samples were received from all cases of diarrhoea attending the Hospital; either at the Out Patient Department level or those admitted into various wards.

Clinical data was collected and included patient age, duration of diarrhoea, presence of associated conditions, such as Diabetes, Neoplasms or Tuberculosis and History of medication viz. prolonged antibiotic usage and corticosteroid therapy.

All samples were subjected to microscopic wet mount examination with saline, to see for presence of yeast like cells, budding within them and presence of pseudohyphae. In addition, pus cells, RBC's and trophozoites, cysts, ova of parasites were looked for.

The stool samples were then subjected to cultural analysis, using media for primary isolation and enrichment for bacteria. In addition, Sabouraud's Dextrose Agar with Chloramphenicol (SDA) was used for isolation of Candida. All media for bacterial isolation were incubated at 37^0C for 24 hours and SDA at 37^0C for 48 hours.

Growth on SDA was confirmed to be Candida by gram staining. Candida isolates were speciated using germ tube test[4], surface growth on Sabouraud's Broth[4] and growth appearance on HiChrome Candida Differential Agar (Hi- Media).

The HiChrome Candida Differential Agar was prepared as per the instruction manual, with a final pH of 6.3 ± 0.2 at 25^0C. The Candida isolates were inoculated on this medium and cultural characters were observed after incubation at 30^0C for 40-48 hours. C. albicans appeared as light green, smooth colonies; C.

tropicalis as blue to metallic blue raised colonies; C. glabrata as cream to white smooth colonies and C. krusei as purple fuzzy colonies.

The results were tabulated and analysed, using the Chi-Square Test and *P* values (SPSS Software version 21), with respect to gender and correlation of microscopy with culture of stools.

III. Results

A total of 155 diarrhoeal stool samples were assessed for prevalence of Candida by microscopy and culture. Candida species were isolated in 15 cases, the percentage isolation being 9.7% (Table 1). Species of Candida encountered in the study were Candida albicans (46.7%), C. tropicalis (33.3%), C. krusei (13.3 %) and C. glabrata (6.7%) (Table 2).

Correlation of Microscopy with Culture is analyzed in Table 3. 'Microscopy positive' refers to the presence of yeast like cells, budding among them and presence of pseudohyphae. Overall, a total of 14 samples (9%) were positive by microscopy and culture, while a single stool sample (0.7%) was culture positive but did not show evidence of yeast like cells. Microscopy positive but culture negative findings were seen in 29 (18.7%) cases, while 111(71.6%) samples showed no microscopic or culture evidence of Candida. Amongst the culture positive cases (n=15), 14 (93.3%) showed positive findings on microscopy. Using the Chi-Square test, the *P* value was 0.0001 ($x^2 = 35.6$; CI = 6.7), which was significant. This indicated that both microscopy and culture, when used in conjunction, produced conclusive results when compared with microscopy and culture alone.

There was no difference in the isolation of yeasts with respect to age or gender (Table 4). Cases occurred in all ages. Males affected were 46.7% and females being 53.3%. The *P* value was 0.347 for gender, indicating that there was no significant relationship between gender and type of cases.

Nine patients (60%), yielding Candida on culture, gave a history of recent antimicrobial therapy, while 2 each (13.3%) were on steroids and had diabetes. HIV Infection was present in one case (6.7%).

IV. Discussion

Fungi, previously considered as commensals, are now emerging as opportunistic pathogens. Candida resides as a normal resident flora of the gut along with enteric bacteria. However, when this balance is upset, Candida can dramatically increase in number and result in tissue penetration and inflammation of the mucosa[5].

In the present study, Candida was isolated from 9.7% of diarrhoeal stools. Forbes et al[6] encountered Candida in 39% of stools from diarrhoeal cases.

A number of reports suggest the probable causal role of Candida in diarrhoea. Danna et al reported antibiotic associated diarrhoea in elderly patients with heavy growth of Candida in stools. These patients improved with treatment with Nystatin[7]. Gupta et al encountered heavy faecal growth of Candida as a sole pathogen in critically ill and malnourished patients with diarrhea[8]. Bishop et al suggested that Candida albicans can cause prolonged diarrhoea with mucosal injury by another pathogen[5].

Transition of Candida from commensal to pathogenic state needs to be evaluated, to assign the causal role of diarrhoea. This can be done by assessing colony phenotype and genetic similarity, using hybridization and probes[9]. In the absence of these facilities in the present study, signs of invasion such as budding in the yeast like cells and presence of pseudohyphae were looked for. Amongst 15 stool samples yielding Candida on culture, microscopic evidence of invasion was seen in 14 cases (93.3%). Kozinn et al, similarly reported the presence of mycelia in the stools, as a marker of invasive enteric Candida diarrhoea[10]. The invasiveness of hyphal forms is probably related to the concentration of phospholipases at the tip, promoting invasion[11].

Apart from the established Candida albicans, the non albicans Candida are also gaining clinical importance. They comprised 53.3% of the total isolates in the present study. The emergence of non albicans Candida is probably related to selection of less susceptible species by the pressure of antifungal agents[12].

In the present study, age and gender had no bearing on the prevalence of Candida in diarrhoeal cases. However prevalence of Candida, in general, is common in extremes of age, attributable to a compromised general immune status[12].

A total of 60 % patients with Candida isolated from the stools, gave a history of recent antibiotic usage. Similar findings were encountered by Forbes et al[6]. Antibiotics suppress bacteria that compete with Candida for nutrition. This destruction creates an ecological vacuum, permitting Candida to assume invasive powers and induce bowel irritability.[13]

V. Figures And Tables

Table 1

Percentage isolation of Candida from diarrhoeal stools

Number of Samples studied	Number of Candida isolated	Percentage
155	15	9.7

Table 2
Species of Candida encountered in the study

Species of Candida	Number	Percentage
C.albicans	7	46.7
C.tropicalis	5	33.3
C.krusei	2	13.3
C.glabrata	1	6.7
Total	15	100

Table 3
Correlation of microscopy with culture of stools (number=155)

Microscopy positive, Culture positive	Microscopy negative, Culture positive	Microscopy positive, Culture negative	Microscopy negative, Culture negative
14 (9%)	1 (0.7%)	29 (18.7%)	111 (71.6%)

Table 4
Age and sex distribution of patients yielding Candida

Age in years	Total number	Male	female
<1	1 (6.7%)	-	1 (100%)
1-10	2 (13.3%)	-	2 (100%)
11-20	2 (13.3%)	-	2 (100%)
21-30	2 (13.3%)	1 (50%)	1 (50%)
31-40	3 (20%)	2 (66.6%)	1 (33.3%)
41-50	2 (13.3%)	2 (100%)	-
>50	3 (20%)	2 (66.6%)	1 (33.3%)
Total	15 (100%)	7 (46.7%)	8 (53.3%)

Table 5
Predisposing conditions in patients yielding Candida in stools (number=15)

Predisposing condition	Number	Percentage
Prolonged antimicrobial treatment	9	60
Corticosteroids	2	13.3
Malnutrition	1	6.7
Presence of diabetes	2	13.3
Presence of HIV infection	1	6.7

VI. Conclusion

Although Candida has been frequently isolated from stools of diarrhoeal cases, it has not assumed the status of a proven enteropathogen. In the present study, however, indirect evidence of invasion on microscopy along with culture positivity could hypothesize a causal role in diarrhoea.

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