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A Study on Oesophageal Candidiasis in HIV Negative Individuals.

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ABSTRACT

This study was done to find the prevalence of Oesophageal candidiasis in HIV negative individuals. The study population included 582 males and 351 females- a total of 933 individuals who had varying complaints related to gastrointestinal tract. All these people were subjected to endoscopy, 40 males and 21 females- a total of 61 cases showed oesophageal candidal lesions out of which 5 showed grade I, 42 grade II, 14 grade III. Only five cases had associated oral thrush. The mean age group was 52 years. Of these 61 cases, 36 cases were without any known predisposing factors. Other 25 cases had predisposing factors such as diabetes mellitus, corticosteroid therapy, alcoholism, anemia, antibiotic therapy and oral contraceptive. Direct smear from these cases were positive for Candida in five cases. Culture on SDA showed growth in 56 cases. Species identification tests showed 49 cases were due to *Candida albicans*, 5 cases were due to *Candida tropicalis* and two undiagnosed.

Keywords: Oesophageal candidiasis, HIV, *Candida albicans,* Endoscopy.

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INTRODUCTION

Candidiasis is a primary or secondary infection involving a member of the genus candida.[1] The important members of this genus which are capable of producing infections are *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida pseudotropicalis, Candida guilliermondii, Candida viswanathii and Candida lusitaniae*.[2] Oesophageal candidiasis though rare it is a serious clinical entity because of its complications. The complications are para oesophageal abscesses, fistulas and perforation. Oesophageal candidiasis also leads to lower intake of food which in due course will lead to various infections and wasting. Since candidal colonization of gastrointestinal tract is an important source of disseminated candidiasis early detection and treatment of Oesophageal candidiasis is necessary. This prospective study was done to find out the prevalence type of complaints predisposing factors and prevalence of Candida species in Oesophageal candidiasis.

MATERIALS AND METHODS

The study population included 933 persons who reported to the Gastroenterologist with the major complaints of odynophagia, dysphagia, nausea and vomiting, hiccups for more than a week where the other causes of hiccup was ruled out, bleching, gastric discomfort and aversion to food.

A detailed clinical evaluation was made in every case. Investigations included routine haematological and biochemical tests. They were also examined for oral thrush. All these people were subjected to upper gastro intestinal endoscopy. Two biopsy samples were taken from the lesions in the oesophagus with the help of endoscopic forceps. One was examined through direct smear. Another one was cut into two and implanted into the tubes containing Sabourauds Dextrose Agar (DDA) with and without antibiotics, aseptically and was transported to the lab immediately. From all these persons two swabs were taken from the oral cavity also. One swab was used to make a direct smear and another swab was inoculated into a SDA tube. The smears which were made from the biopsy tissue and oral cavity were gram stained and examined under oil immersion objective for the presence of yeast cells and hyphal elements.

The inoculated media was incubated at 30ºC and observed for fungal growth after 24 hours. When there was a growth the colonies were studied for the typical morphology. White raised moist colonies with yeast like odour suggestive of *Candida* were seen in the smear was made from the colony, gram stained and looked for gram positive budding yeast cells. If there was no growth at 24 hours the tubes were incubated further for one week after which they were discarded. When gram positive budding yeast cells suggestive of *Candida* were seen in the smear the following tests were done for the species identification.

Germ Tube test

A single colony from the primary culture was lightly touched with a loop and then emulsified in 5ml of pooled human serum in a sterile test tube. The tube was incubated at 37ºC for 3 hours. At the end of this hour a drop of the yeast serum suspension was placed on a sterile slide, overlaid with a cover slip and examined under high power for the presence of germ tube. Controls were put up with a known Candida albicans and germ tube negative strain of Candida tropicalis.

Corn meal agar morphology

An isolated colony of *candida* was obtained from the primary culture media, and was inoculated into plate of Corn Meal Agar by cutting at angles into the medium. A known culture of *Candida albicans* was also inoculated on one part of the plate to serve as a control. A cover slip was placed over an inoculated streak and the plate was incubated at 37ºC. After 48 hours the plate was examined in the areas where the cuts into the agar were made under the microscope at low power and high power for the presence of *chlamydospores*, *blastoconidia* and *pseudohyphae*.

Sugar Fermentation Tests

Sugar fermentation media was inoculated with the test organism by adding a drop of the inoculums suspension prepared from primary culture. Sugars tested were glucose, lactose, maltose, and sucrose. The tubes were incubated in an incubator from 48 to 72 hours and observed for colour change in the medium

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which indicates acid production and gas collection in durhams tube. Control was put up with known strain of *Candida albicans* and uninoculated sets of sugar.

Sugar Assimilation Tests:

Yeasts and yeast like fungi utilize specific carbohydrate substrates. Organisms are inoculated into a carbohydrate free medium after exhausting their endogenous metabolites. Carbohydrate containing filter paper discs are added and utilization is determined by presence of growth around the disc.

A portion of the colony from primary culture was emulsified in four ml sterile saline to produce a density equivalent to McFarland No.4 standard and was kept at room temperature overnight. A tube containing basal medium II was melted and allowed to cool. Into this medium a few grains of yeast extract was added and mixed well. The yeast saline suspension was added mixed well and poured into a petridish and allowed to harden at room temperature. Sugar discs were distributed on the plate 30mm apart. Sugars tested were glucose, sucrose, lactose, maltose, galactose, raffinose and trehalose. The plates were incubated at 30ºC for 18 to 24 hours at which time it was examined for presence or absence of growth of yeast around each disc.

RESULTS

Endoscopy showed *Oesophageal candidasis* in 61 cases (Table 1), 5 cases showed typeI lesion, 42 cases showed type II lesion, 14 cases type III lesion. (Table: 2)

Table 1: Prevalence of Oesophageal candidiasis

Particular	Incidence	Percentage
Positive with Grade I lesions	5	8.19%
Positive with Grade II lesions	42	68.85%
Positive with Grade III lesions	14	22.95%
Total	61	99.99%

Table 2: Nature of lesion in endoscopic view

Sex	Total number subjected to endoscopy	Positive for oesophageal candidiasis	Percentage
Male	582	40	6.8
Female	351	21	6

Among the positive cases considering their general health condition based on clinical and lab investigations 36 cases were apparently normal, 9 cases were diabetics, 6 on cortisteroid therapy, 3 alcoholics, 3 cases of anemia, 3 cases on antibiotic therapy for more than 3 weeks, 1 female patient on contraceptive. (Table 3)

Table 3: Occurrence of predisposing factors among the *Oesophageal candidiasis* positive

S.no	Factors	Occurrence
1	Diabetes mellitus	9
2	Corticosteroid therapy	6
3	Alcoholism	3
4	Anemia	3
5	Antibiotic therapy	3
6	Oral contraceptive	1
7	No predisposing factors	36
	Total	61

For all these 61 cases direct smear from oral cavity was made and gram staining of these smear showed yeast cells in 5 cases. Direct smear from biopsy tissue from these 61 cases showed yeast cells and hyphal elements in 48 cases. (Table: 4)

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Table 4: Direct smear examination

Specimen	Total No. Tested	Direct smear		Percentage
		Positive	Negative	
Biopsy tissue	61	48	13	78.7%
Oral swab	61	5	56	8%

Culture on SDA with and without antibiotics (Chloramphenicol) there was no growth in 5 cases in both tubes. All the other cases 56 there was growth within 48 hours. Culture done from oral cavity produced growth in 5 cases in SDA. (Table:5)

Table 5: Culture Results on SDA

Specimen	No. Tested	Growth on SDA with antibiotics	Growth on SDA without antibiotics	Percentage
Biopsy tissue	61	56	56	92
Oral swab	61	5	Not tested	8

Germ tube test was positive in 48 cases out of the 56 cases that showed growth. Chlamydospore formation on cornmealagar was seen in 49 cases out of 56 cases. **(Table 6)**

Table 6: Characterization of fungal pathogens isolated from biopsy specimens

S.No	Test	Total No. Tested	Positive
1	Germ tube test	56	48
2	Chalmydospore formation	56	49
3	**Fermentation**		
	Glucose	56	56
	Sucrose		7
	Lactose	56	
	Maltose		-
		56	
			56
		56	

The results of sugar assimilation tests is shown in table 7

From the various tests the species were identified as 49 cases of *C. albicans*, 5 cases of *C. tropicalis* and 2 undiagnosed. Of these 49 cases due to *C. albicans*, 48 cases were positive for germ tube one was negative, but it was positive for chlamydospores formation sugar fermentation and assimilation were identical with that of *C.albicans*. So taking into consideration the fact 5% of *C. albicans* can be germ tube negative this was included in *C. albicans*. 2 cases were undiagnosed because their assimilation pattern did not confer with the standard patterns given in the text.

Table 7: Auxanogram profile of the fungal isolates isolated from the Biopsy specimens.

Sugar tested	**Total No. Tested**	**Positive**
Glucose	56	56
Sucrose	56	56
Lactose	56	2
Maltose	56	56
Galactose	56	56
Raffinose	56	-
Trehalose	56	56

Of the 25 cases of Oesophageal Candidiasis with pre disposing factors, 21 cases were due to *C.albicans*, 2 due to *C. tropicalis*, 2 there was no growth. Of the 36 cases of Oesophageal candidiasis without any predisposing factors 28 cases were due to *C. albicans* 3 cases were due to *C. tropicalis* 2 undiagnosed. 3 was culture negative.

Of the five cases which produced growth from the specimens taken from the oral cavity all were found to be *Candida albicans*.

DISCUSSION

Prevalence of oesophageal candidiasis

Among 933 cases studied which included 582 males and 351 females for varying complaints related to gastrointestinal tract were subjected to endoscopy 61 cases (6%) were positive for Oesophageal candidiasis (40 in male and 21 in female). This is consistent with the study of odds 1983 which showed a prevalence of 1-7% of Oesophageal candidiasis in 3 large endoscopic studies.[3] In the study conducted by kodsi et al 27 cases of Oesophageal candidiasis was detected in 370 (7.3%) endoscopic studies.[4] In our study the prevalence was higher among males than the females.

Predisposing factors

In our study 25 cases showed predisposing factors and 36 cases were without any predisposing causes. In the study conducted by Aleman et al all the 7 cases studied were without any predisposing factor.[5] In the study, of Ortuno Cortes TA et al 1997, 9 cases out of 31 cases of Oesophageal candidiasis were without any predisposing factors.[6] In our study we found that the prevalence of Oesophageal candidiasis in apparently normal persons was more than those with underlying causes. This is consistent with the statement of Gazzard that prevalence can be more among non-immunosuppressed than the immuno suppressed. In our study the predisposing factors were diabetes 15%, corticosteroid therapy 5%, antibiotic therapy 3%, alcoholism 3%, anemia 3% and oral contraceptive 1%.

Diagnosis of Oesophageal candidiasis

In the study on Oesophageal candidiasis conducted by Kodsi et al 1976 the diagnosis of Oesophageal candidiasis was established by endoscopy, combined with microscopic techniques directed on biopsy tissue.[4] According to the study of Walsh T.J et al and Aleman et al endoscopic findings are highly suggestive of Oesophageal candidiasis.[7], [5] According to Mathieson et al the endoscopic findings and demonstration of tissue invasion by fungal mycelia is confirmatory of Oesophageal candidiasis.[8] In our study also endoscopy, direct smear from biopsy lesion and culture studies were found to confirm the diagnosis of Oesophageal candidiasis. Out of 61cases showing Oesophageal candidiasis on endoscopy, 48 were positive on direct smear, 56 cases showed candidial growth on culture, 5 cases with no growth the lesions in the oesophagus were of grade I.

In the study conducted by zillessen et al only 3 cases out of 43 cases of Oesophageal candidiasis had simultaneous oral thrush. In our study, 5 cases out of 61 cases had associated oral thrush.[9]

Prevalence of Candida species

In our study five cases did not produce any growth in culture out of the 61 specimens. out of 56 cases that produced growth 49 cases (87%) was due to Candida albicans, five cases (8.9%) was due to Candida tropicalis and two cases (3.4%) was undiagnosed. According to Mackie Candida albicans is responsible for 90% of candidal infections.[10] According to Bailey Candida albicans is isolated in 75% of cases, Candida tropicalis comes next to Candida albicans in its pathogenic capacity.[11] A number of studies conducted on Candidiasis have shown that Candida albicans is the most common aetiological agent. [12], [13] Candida tropicalis stands next to Candida albicans as shown by various study.

CONCLUSION

From this study we incur that the endoscopy, direct smear examination of biopsy tissue and lab techniques are confirmatory in the diagnosis of oesophageal candidiasis. Oesophageal candidiasis can occur as a separate clinical entity. Oesophageal candidiasis can occur without any known predisposing factors given in the various texts and the incidence can be more in apparently healthy individuals. *Candida albicans* is the most common aetiological agent in oesophageal candidiasis, *Candida tropicalis* is the second most common

aetiological agent in oesophageal candidiasis. Since *candida albicans* is a commensal in the gastrointestinal tract, the source of infection is probably endogenous.

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