Abstract

The candidial infection of cerebrospinal fluid is an uncommon manifestation but may have an usual occurrence in an immunocompromised patients treated with broad spectrum antibiotics in association with intravenous hyper alimentation following the surgical manipulation of mucosal surface colonized with *Candida* species. In the present study, the CSF samples of the patient were collected and the organism isolated was identified as *C. albicans*. So far, there is no report of *C. albicans* in CSF from Vidarbha region, hence an attempt has been made in the present study.

Keywords: *Candida albicans*, Cerebrospinal fluid, Immunocompromised.

Introduction

Cerebrospinal fluid is an isotonic solution and acts as a “cushion” or buffer for the cortex, providing a basic mechanical and immunological protection to the brain inside the skull. From the last decade or so, the incidence of fungal infections have been increased out of which, *Candida* species are becoming an important cause of nosocomial infection, primarily affecting immunocompromised patients (1, 2). In practice, the majority of cases of CNS candidiasis are associated with disseminated or invasive candidiasis (IC). Another form of candidal CNS infection is the one occurring as a postoperative complication of neurosurgical procedures, especially ventriculo-peritoneal (VP) shunt placement. *Candida* meningitis is the most frequent clinical manifestation of IC-related CNS Candidiasis (3).

*Candida* species are now the fourth most common isolated organism. The crude mortality rates ranges from 70 to 100 % in CNS candidiasis, and are increasingly isolated from surgical site and urinary tract infections (4). *Candida* remained a relatively uncommon CNS pathogen until the 1960s when use of chemotherapeutic agents, glucocorticoids, and intravenous drugs rendered increasing numbers of patients susceptible to opportunistic infections (5). Meningitis is the most common form of CNS infection caused by *Candida*. The clinical symptoms are highly variable and range from acute to chronic (6). Typically, the onset of meningitis evolves subacutely over several days to weeks with fever, headache, meningismus and diminished consciousness. More acute manifestations are often indistinguishable from bacterial meningitis. Meningitis is among the ten most common infectious causes of death and is responsible for approximately 135,000 deaths throughout the world each year (7). Oral thrush is also one of the common clinical manifestations of candidiasis, seen in both HIV seropositive as well as seronegative patients (8).

In the current study, the patient was a 23 years old female admitted to the hospital. She was suffering from fever with chills and constant
vomiting. CSF samples were collected by a procedure called lumbar puncture, processed and *C. albicans* was isolated repeatedly. So far there is no report of infection of *C. albicans* in CSF from the Vidarbha region, it is being reported for the first time here.

**Materials and Methods**

The pathogen was isolated on Sabouraud Dextrose Agar (40g glucose, 10g peptone, 15g agar-agar dissolved in 1 liter of distilled water and incubated at 37°C). For the identification, samples were processed for Gram staining, culture, germ tube test, production of chlamydospores, sugar fermentation and assimilation tests.

**Culture**

The samples were cultured on Sabouraud Dextrose Agar (SDA), incubated at 37°C and examined twice a week for growth showing cream coloured pasty colonies suggestive of *Candida* species (Figure 1 [a] and [b]).

**Gram stain**

Smears were prepared from the CSF samples. Gram stained smears were used for detection of gram-positive budding yeast cells with pseudohyphae (Figure 2).

**Germ tube Test**

*C. albicans* was inoculated in human serum and incubated at 37°C. After 2-4 hours, wet mount was prepared and examined under the microscope for the presence of germ tube (Figure 3).

**Formation of Chlamydospores**

All Candida isolates were tested for the production of chlamydospores in corn meal agar. The Candida strains were inoculated in corn meal agar (CMA) and then incubated at 25°C. After 72 hours, the plates were examined under the microscope for the presence of chlamydospores.

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Figure 1: Growth of *Candida albicans* on the Sabouraud Dextrose Agar [a] after 3 days and [b] after 14 days. budding yeast cells of *Candida albicans*

Figure 2: Micrograph showing gram positive budding yeast cells of *Candida albicans*
Sugar fermentation test

All Candida isolates were subjected to carbohydrate fermentation test. Carbohydrate solutions used were 6% solution of dextrose, maltose, lactose and sucrose with basal media.

Sugar assimilation test

The assessment of the ability of yeast to utilize carbohydrates is based on the use of carbohydrate-free yeast nitrogen base agar and observing for the presence of growth around carbohydrate impregnated filter paper disks after an appropriate period of incubation. Carbohydrates used were glucose, lactose, maltose, sucrose and galactose.

Results and Discussion

During the past decade, there has been increasing incidence of fungal pathogens (9). The prevalence of systemic fungal infections has increased significantly and has been reported from other parts of India. Rao et al. (10), reported fungal infections in neonates. Disseminated Candidiasis is a significant source of mortality and morbidity in neonatal intensive care nurseries. Nabi et al. (11) found a very low birth weight infant with disseminated invasive candidiasis including meningitis. In the present investigation, Candida albicans was repeatedly isolated from cerebrospinal fluid. Recently, a case of Candida parapsilosis has been reported to cause meningitis in 50 year old HIV seropositive male presented with chronic headache, altered sensorium and neck rigidity (12). Wabale et al (8) reported oral thrush in both HIV seropositive as well as seronegative patients. The isolation rate of Candida species is found to be 90% in HIV-seropositive group, as compared to HIV-seronegative group (60 %). Thus, Candida species has emerged as an opportunistic pathogen with severe manifestations since the last two decades. The other Candida species isolated by Wabale et al (8) were, C. dubliniensis, C. parapsilosis, C. glabrata, C. krusei and C. lusitaniae.

In the present study, the smear prepared, was stained by Gram staining method and the micrograph reveals the gram-positive budded yeast cells, characteristic to the Candida species. Formation of germ tube was observed which is a rapid and a presumptive test for Candida species. A conventional (Sabouraud dextrose agar) and a chromogenic media (HiCrome® agar) were used for the preliminary identification. HiCrome® agar is a novel, differential culture medium that is claimed to facilitate the isolation and presumptive identification of some clinically important yeast species (Figure 4). Besides, HiCrome® agar,
there are other chromogenic media utilized such as, CHROMagar Candida (13), Albicans ID2® media (14), BBL™ CHROMagar™ Candida (15) for the identification of Candida species. Candida glabrata can be identified more rapidly on the basis of trehalose assimilation of fermentation (16). This new differential culture medium allows selective isolation of yeasts and simultaneously identifies colony of C. albicans, C. tropicalis, C. krusei and C. glabrata. Also, other biochemical tests were performed which shows the characteristic features of Candida albicans.

**Conclusion**

Incidences of candidal infection have been increased drastically since the past two decades. Thus, with the advent of new technologies new diagnostic tools are now being used for the characterization of different pathogenic organisms. In general medical laboratories, characterization of Candida species is based on the traditional diagnostic methods. However, with the introduction of different diagnostic tools like DNA-microarrays, PCR and immunodiagnostic tools the laboratory diagnosis of Candida species up to the strain level can be achieved.

In the present study, characterization of Candida species was carried out with the traditional methods, which reveal that the organism isolated from the CSF samples was Candida albicans. As in past, there has been no such report of candidal infection in CSF from Vidharba region, so it is reported here.

**References**