

Central nervous system cryptococcosis prevalence in a tertiary care facility

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Abstract

The fungus *Cryptococcus neoformans* is the causative agent of cryptococcosis, which is a systemic opportunistic fungal infection. *Cryptococcus neoformans* is a capsulated opportunistic yeast fungus that mostly infects HIV-infected and other immunocompromised hosts, causing meningitis or meningoencephalitis in the central nervous system (CNS). A significant contributor to death in immunocompromised patients is the presence of cryptococcal disease in the central nervous system. A prospective study was carried out on a total of 160 patients who were suffering from cryptococcal meningitis and other co-morbid conditions, such as diabetes, the use of corticosteroids, chronic debilitating illness, and HIV/AIDS patients. The patients all had symptoms related to the central nervous system (CNS). The purpose of the study was to identify and isolate *Cryptococcus neoformans* from cerebrospinal fluid (CSF) samples taken from immunocompromised patients at the Government General Hospital in Guntur who were suspected of having cryptococcal meningitis. These patients also had other co-morbid conditions, such as diabetes, corticosteroid use, and chronic debilitating illnesses. This work contributes to the assessment of the prevalence of cryptococcosis of the central nervous system (CNS) in immunocompromised persons such as HIV/AIDS patients. This enables the clinician and the community to provide appropriate patient care and to take the appropriate precautions. Various cryptococcal studies, including India ink, gram staining, culture, and CALAS, are performed on two hundred and fifty CSF samples. A prospective study was carried out for a total of six months, beginning in July 2021 and ending in December 2021. Males and girls were equally likely to be engaged in each of the total 160 cases, with the ratio standing at 1:1. The overall prevalence of *Cryptococcus* was found to be 16% in high-risk patients, with HIV-positive patients having a prevalence of 12.6%, diabetic patients having a prevalence of 2.6%, and other patients having a prevalence of 0.8%. A routine mycological evaluation is required for an early and definitive diagnosis as well as appropriate treatment. This will have a beneficial effect on both morbidity and mortality.

Keywords: *Cryptococcus neoformans*, cryptococcal meningitis, HIV/AIDS/CALAS immune compromised patients

Introduction

The fungus *Cryptococcus neoformans* is the causative agent of cryptococcosis, which is a systemic opportunistic fungal infection ^[1]. The infection known as cryptococcosis of the central nervous system is a major contributor to death in immune compromised patients. Inhalation of an aerosolized form of *Cryptococcus* can lead to the development of CM ^[2]. It is most commonly found in soil that has been tainted by the droppings of birds, notably pigeons and chickens ^[3-5]. Reactivation is something that happens most frequently in those who have a weakened immune system, such as HIV/AIDS patients or people with other co-morbid illnesses that involve the central nervous system, such as meningitis or meningoencephalitis ^[6-8]. The capacity of *Cryptococcus* to breach the blood-brain barrier is a characteristic that sets it apart from other bacteria. Yeast cells can migrate in two ways: either directly through the endothelium or by being taken inside the macrophages like a Trojan horse ^[9]. Chronic meningitis caused by cryptococcal infection manifests with symptoms including headache, fever, loss of sensation and memory, paresis of the cranial nerves, and loss of eyesight (due to optic nerve involvement). The cryptococcal disease is found all over the world and is responsible for over one million illnesses and more than six hundred twenty-five thousand fatalities annually. At the Government General Hospital in Guntur, India, a total of one hundred sixty patients who were thought to be suffering with cryptococcal meningitis were included in the current study ^[10-13]. In HIV/AIDS patients, the most prevalent central nervous system (CNS) fungal pathogen is *cryptococcus*. In clinical practice, the signs and symptoms of CM cannot be differentiated from those of any other type of meningitis. If treatment is not provided, death will result from this infection. Because of this, prompt recognition, diagnosis, and treatment are essential in order to lessen the likelihood of morbidity and fatality ^[14-16].

Since the first incidence was recorded in the early 1980s, the human immunodeficiency virus (HIV) infection has risen to become the sixth biggest cause of mortality globally, infecting around 40.3 million people. The encapsulated environmental fungus *Cryptococcus neoformans* is the cause of cryptococcal meningitis, which has become the most prevalent fatal fungal infection in persons with acquired immunodeficiency syndrome (AIDS) around the world ^[17]. After *Candida albicans*, *Cryptococcus neoformans* is the second most frequent fungal opportunist. It is responsible for symptomatic cryptococcosis in up to 8.5% of HIV-infected people. In immune compromised patients, particularly those who have AIDS, *cryptococcus* is the fungus pathogen that affects the central nervous system (CNS) the most frequently ^[18]. In India, the prevalence of HIV infection is extremely high, and cryptococcal meningitis is a typical complication experienced by AIDS patients. According to data that was collected more recently, the prevalence of *C. neoformans* infection is highest in developing nations like India. In our institution, a retrospective research project was carried out with the objective of defining the clinical and laboratory characteristics of CNS cryptococcosis in HIV-positive patients who were hospitalized with meningeal symptoms ^[19-21].

Material and Methods

The study population includes 160 individuals who may be suffering from cryptococcal meningitis. Following standard operating procedures and screening several cryptococcal investigations on 160 samples a sample of 1 milliliter of the cerebrospinal fluid is obtained and then centrifuged before being separated into three portions. The first portion is for gram staining and the India ink method, the second part is for fungal culture by inoculating on SDA, and the third part is for antigen detection by latex agglutination test by using the Meridian CALAS kit in accordance with its instructions. Examine the sample using direct microscopy by preparing a clean glass slide, adding one drop of India ink to the sample, and covering it with a cover slip. For more information, refer to the sections on low power and high power. This will demonstrate the capsule, which against the dark background looks like

a retractile, clearly demarcated region encircling the developing yeast cell. It is possible for the capsule to be twice or even three times the diameter of the yeast cell. The India ink stain is less sensitive, anywhere between 60 and 70 percent. The gram stain reveals that the cells are budding yeast that is gramme positive. In the culture centrifuge sediment of CSF is inoculated onto SDA without antibiotic, blood agar, and chocolate agar, and then it is incubated at 37 degrees Celsius for twenty-four hours. Colonies manifested themselves as a mucoid, creamy white colour. The determination of the species of Cryptococcus through inoculation into Niger seed agar and subsequent testing with urease. The meridian cryptococcal antigen latex agglutination test was utilized in order to identify cryptococcal antigen in the centrifuge sediment of two hundred different CSF samples. If the titre is eight or higher, then it is determined that Cryptococcus is present. The clinical features of chronic meningitis, along with laboratory findings such as a positive India ink preparation, Grams stain, positive culture of Cryptococcus, and demonstration of Cryptococcal antigen in the CSF by latex agglutination test, are used to make a definitive diagnosis of CNS Cryptococcal infection. This diagnosis is based on the clinical features of chronic meningitis. CALAS is a test that can diagnose cryptococcal meningitis in a short amount of time, is very sensitive (with a sensitivity level of 95%), and is specific.

Table 1: Age and sex wise distribution of cryptococcal meningitis

Sr. No	Age group	Study group	
		Male	Female
1	15-45	20	10
2	26-35	23	10
3	36-45	45	30
4	46-55	12	10
	Total	100 (50%)	60 (50%)

Table 2: Analysis of clinical symptoms in chronic meningitis

Sr. No	Age group	Study group	Percentage
1	Head ache	120	95%
2	Fever	130	85%
3	Neck stiffness	22	11%
4	Altered sensorium	50	25%

Table 3: Prevalence of Cryptococcus in India ink/gram staining / culture /CALAS

Sr. No	Age group	India ink 12%	Gram staining 11.3%	Culture 12.6%	CALAS 16%
1	15 -25	2	2	2	2
2	26 – 35	3	3	3	3
3	36 -45	12	12	13	17
4	46 – 55	1	0	1	2
Total		18	17	19	24

Table 4: Prevalence of Cryptococcus in high risk patients

HIV	DM	Others
19 (12.6%)	4 (2.6%)	1 (0.8%)

Results

During the period of time spanning July 2021 through December 2021, a total of 160 patients who had been clinically diagnosed with meningitis were subjected to testing for Cryptococcus neoformans using a variety of techniques. The results of an investigation of the age and

gender distribution of chronic meningitis are presented in Table 1. Patients with ages ranging from 15 to 55 years old were seen here. The number of males to females is equal to one another. The males were more vulnerable than the females in this situation. In patients aged 36 to 45, the prevalence of chronic meningitis is at its highest. The clinical signs of patients diagnosed with chronic meningitis are broken forth in Table 2. According to this data, the most common symptoms were headache (95%), fever (85%), neck stiffness (11%) and altered sensorium (25%). The prevalence of Cryptococcus was determined using India ink, gram staining, culture, and CALAS, as shown in table no. 3. According to this table, the percentage of positive in India ink is 12%, whereas the percentage of gram staining is 11.3%, culture is 12.6%, and CALAS is 16%. The prevalence of Cryptococcus in people at high risk is outlined in table no. 4. It is 12.6% for HIV/AIDS, 2.6% for diabetes mellitus, and 0.8% for other conditions.

Discussions

In immunocompromised and weakened individuals, *Cryptococcus neoformans* is one of the most prevalent opportunistic fungal infections that can occur. There are at least 39 different species in the genus *Cryptococcus*, however only a small fraction of them are capable of making people sick. *Cryptococcus neoformans* is the causative agent in the majority of human infections. Encapsulated *cryptococcus neoformans* was discovered to be a human pathogen for the first time in 1894, when it was isolated from the tibia of a patient in Germany by Buese and Buschke. That same year, it was also isolated from peach juice in San Felice. Both of these discoveries took place in the same year. Although a case of chronic meningitis that was documented in 1861 by Zenker before the pathogen was isolated was possibly the earliest case history, the cryptococcal meningitis was not described for the first time until 1905 when it was published by Van Han Semann ^[22]. There are both asexual and sexual variants of the fungus *Cryptococcus neoformans*. The asexual form is distinguished by budding yeast cells that range in shape from oval to spherical and are surrounded by a polysaccharide capsule. The sexual form has not been described in combination with clinical specimens, and it is only detected in when the organisms are mating. In clinical specimens, the asexual form that has a capsule is observed more commonly. When a negative stain with 10% nigrosin in India ink is used, it is possible to establish that the virulence of the organism is attributable to oxidase, protease enzymes, and a carbohydrate capsule ^[23]. Pathogenicity is absent in non-capsulated mutant versions of the organism. Infection occurs when yeast cells act as a respiratory pathogen and are inhaled, after which the yeast either dies or becomes dominant depending on the host immune system. The significant flaw in the host immune system is the cause of the disease's spread. The advanced HIV stage, the use of corticosteroids, organ transplantation in the terminal stage of cancer, lymphomas, sarcoidosis, lymphoproliferation disorders, hypogammaglobulinemia, and systemic lupus erythematosus are all factors that increase the likelihood of contracting systemic lupus erythematosus. Even though cryptococcal disease has been around for a long time, before the AIDS epidemic its frequency was relatively low all across the world. The pandemic of HIV had an effect on the rate at which cryptococcal diseases became prevalent. According to the findings of a number of Indian studies, cryptococcal meningitis is a newly emerging opportunistic infection that is particularly prevalent among HIV-positive patients. Cryptococcal meningitis is also a major cause of mortality among patients. Both previous research that has been published and these most recent data point to the high frequency of the virus in India. In our nation, HIV-positive patients are not the only patients at risk; leukaemia patients and patients with diabetes mellitus have also emerged as significant risk categories. In the course of our research, we came to the conclusion that males are more frequently implicated than females at a ratio of 1:1. This disparity may be attributable to differences in exposure rather than variations in host

susceptibility. Male female ratio (1:1). The prevalence of *Cryptococcus neoformans* was found to be 16% in the present investigation (32 out of 160). This figure is consistent with the findings that were reported in the studies conducted by KV. Prasad *et al.* (16.6%) 2016 and Mukti Keth *et al.* (14.3%) (2012). The prevalence of cryptococcal meningitis in immune compromised patients has been shown to range significantly, from 19.8% all the way up to 45.8%, according to the findings of numerous studies that have been carried out in various regions of the world, including India. According to the findings of our research, the prevalence of Cryptococcal meningitis in HIV-positive patients is 12.5 percent, or 25 out of a total of 160 cases [24-26].

Comparing the results of our research with those of KN Prasad *et al.* (3%), we found that the prevalence of cryptococcal meningitis in DM is 3%, with 6 cases out of 160 examined.

In this particular investigation, the clinical symptoms seen included headaches 150/160 (95%) of the time, fever 130/160 (85%) of the time, stiffness (22/160) 11% of the time, and altered sensorium 50/160) 25% of the time. Muktikasu Das and colleagues found that all patients had 100% head pain, 100% fever, 90% neck stiffness, and 100% altered sensorium. Lakshmi & al found headache in 92% of patients, fever in 49.79% of patients, and altered sensorium in 79% of patients. Prasad *et al.* 1 noted headache in 89.5% of patients, fever in 78.9% of patients, and neck stiffness in 13.2% of patients. Our findings are consistent with those of previous research conducted in India. In parallel with the rise in the number of HIV cases, the prevalence of HIV coinfection with cryptococcal disease increased from 20% in the years 1992–1996 to 30% in the years 1996-2000 and 49% in the years 2000-2004, according to the findings of three studies that were conducted in AIMS, New Delhi over a period of 12 years (1992-2004). ART, or antiretroviral therapy, has been shown in this recent study to be effective in HIV (AIDS) patients across India, resulting in a decline in the disease's prevalence over the course of a period of six months. Culture is considered to be the "gold standard" method of diagnosis for *C. neoformans*; nevertheless, the growth of the culture might take anywhere from three days to a maximum of one month. The second method involves demonstrating encapsulated yeast in India ink prepared from CSF [27]. This method utilizes specialized stains that have poor sensitivity (between 50 and 80 percent). Enhancing diagnostic procedures for *Cryptococcus* can be done in a number of ways, the most immediate and expedient of which is antigen detection. It is a test that is both very sensitive and very quick, and it can detect antigen for a period of time that extends well beyond illness.

Conclusions

Meningitis caused by *Cryptococcus* continues to be a major contributor to morbidity and mortality, particularly among patients whose immune systems are impaired, most notably those with HIV/AIDS. The current study reveals that immune compromised patients hospitalized in tertiary care facilities have a significant prevalence of cryptococcal meningitis. Mycological testing should be performed on a routine basis in order to arrive at an accurate diagnosis as soon as possible and, following that, to choose the most effective course of treatment for the majority of patients. Antigen detection by latex agglutination was shown to be an effective method for the diagnosis of cryptococcal meningitis. This method was both sensitive and specific. The presence of HIV infection is the single most important risk factor for developing cryptococcosis of the central nervous system (CNS). HIV infection is also a significant contributor to morbidity and mortality in HIV-positive patients. Because the majority of patients are amenable to treatment, an elevated index of clinical suspicion as well as routine mycological surveillance are essential to assist in the process of early diagnosis and appropriate treatment.

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