



## Study of Prevalence of Cryptococcal Meningitis Among HIV-Infected Patients Receiving Anti-Retroviral Therapy At A Tertiary Care Centre

**Dr Kishor Ingole**

Prof & HOD ,Dept of Microbiology, Dr V M Govt Medical college, Solapur

**Dr Suwarna Pawar**

Assistant Professor, Dept of Microbiology, Dr V M Govt Medical college, Solapur.

**Dr N.K.Shaikh**

Associate Professor, Dept of Microbiology, Dr V M Govt Medical college, Solapur.

**Dr Rebecca Lalngaihzuai**

Student, Dept of Microbiology, Dr V M Govt Medical college, Solapur.

### ABSTRACT

**Aim:** To study the prevalence of Cryptococcal Meningitis among HIV-infected hospitalised patients receiving anti-retroviral therapy (ART) in a Tertiary care centre.

**Methodology:** This study was conducted at tertiary care centre from November 2013 to November 2015. Total 142 clinically suspected cases of Cryptococcal Meningitis amongst HIV positive hospitalised patients on ART were included. Cerebrospinal fluid 0.5-1 ml was collected aseptically. Negative staining with 10% Nigrosin & Gram's stain was performed. Culture was done on Sabouraud's Dextrose Agar (SDA). *Cryptococcus neoformans* was confirmed with the production of brownish colonies on Bird Seed Agar and by Hydrolysis of urea.

**Observation & Result:** Sixteen patients out of 142 (11.27%) were positive for Cryptococcal Meningitis on microscopy and culture. Amongst which 13/16 (81.25 %) were found to have CD4 count < 100 cells/ $\mu$ L. All 16 patients were responsive to intravenous Amphotericin B treatment for 14 days and discharged after duration for 14-21 days.

**Conclusion:** This study shows that Cryptococcal Meningitis is quite high among HIV infected patients receiving ART. So periodic screening is required in a more extensive manner for all HIV-infected patients with low CD4 counts in spite of treatment history.

### KEYWORDS

Cryptococcal Meningitis, HIV-infected patients, Anti-retroviral therapy, *Cryptococcus neoformans*, Bird seed agar.

**Introduction:** *Cryptococcus neoformans* is encapsulated yeast, ubiquitously found worldwide in soil contaminated with bird droppings mainly of pigeons, roosting sites and decaying woods and vegetables<sup>1</sup>. Cryptococcal meningitis, caused by an encapsulated yeast *Cryptococcus spp* is an opportunist mycosis<sup>2</sup>. With the advent of acquired immunodeficiency syndrome (AIDS) pandemic, there has been a dramatic increase in the incidence of cryptococcal infections<sup>3</sup>. 80% - 90% of all patients with cryptococcosis are AIDS-associated<sup>4</sup>. Although effective treatment for HIV disease has led to significant decrease in the incidence of cryptococcal meningitis (CM) in the western countries, it continues to be the common cause of infectious morbidity and mortality especially among the HIV-positive patients living in Sub-Saharan Africa and South-East Asia<sup>1</sup>. The signs and symptoms of *Cryptococcus meningitis* are similar to those of many other causes of meningitis<sup>5</sup> and radiological pictures are not specific in the early stages<sup>6</sup>. Due to the presence of HIV infection, a large number of the population is at risk for this infection. In the developing countries, cryptococcal meningitis remains an important cause of morbidity and mortality in HIV-infected patients<sup>3</sup>. The overall mortality rate from cryptococcal meningitis is approximately 25%-30%<sup>7</sup>. Even if cryptococcosis is an opportunistic infection in AIDS patients, the data on prevalence vary in time and space. It may be because of changing epidemiology of HIV infection and the AIDS management pattern. So this study was conducted to evaluate the prevalence and treatment outcome of Cryptococcal meningitis among HIV-infected patients who were hospitalised and receiving the antiretroviral therapy in a Tertiary care centre.

**Materials and Methods:** This study was conducted at Department of Microbiology, at tertiary care hospital, Solapur from November 2013 to November 2015 for two years. A Total 142 clinically suspected cases of Cryptococcal Meningitis amongst HIV positive patients who were admitted in the hospital and receiving Antiretroviral Therapy (ART) included in this study. Cerebrospinal fluid (CSF) samples (0.5-1 ml) were collected aseptically. These samples were processed for fungal culture after microscopic examination which includes negative staining with 10% Nigrosin along and Gram's staining. Round budding yeast cells with distinct halo was observed on 10% Nigrosin mount (Figure 1)<sup>8</sup> while gram-positive budding yeast cells were observed on gram staining (Figure 2)<sup>8</sup>. All the patients were started antifungal treatment after the report of Gram staining. Then the samples were inoculated on Sabouraud's Dextrose Agar (SDA) and incubated at 37°C. The fungal cultures were observed for growth for four weeks. The colony morphology was noted. *Cryptococcus neoformans* was identified based on yeast like mucoid colony on SDA (Figure 3) and Hydrolysis of urea (Figure 4). Colony from SDA was subcultured on Bird Seed Agar which incubated at 37°C and brownish colonies were observed suggestive of *Cryptococcus neoformans* (Figure 5).



Fig 1: *Cryptococcus neoformans* in Nigrosin Mount



Fig 2: *Cryptococcus neoformans* in Gram Stain



Fig 3: Growth of *Cryptococcus neoformans* on SDA



Fig 4: Hydrolysis of Urea by *Cryptococcus neoformans*



Fig 5: Growth of *Cryptococcus neoformans* on Bird Seed Agar

All patients were responsive to intravenous Amphotericin B treatment for 14 days and were discharged after duration of hospital stay for 14-21 days.

**Results:**

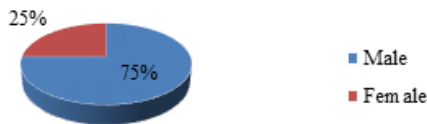
Direct examination in Nigrosin mount of the 142 CSF samples, 16 (11.27%) revealed polysaccharide capsules. These samples were also identified by growth on culture.

**Table 1: Sex distribution among the cases.**

Number of male cases (%)	Number of female cases (%)	Total
12 (75%)	4 (25%)	16

Out of total 142 samples processed 16 (11.27%) were positive for Cryptococcal Meningitis among these, 12 (75%) were male and 4 (25%) were female.

**Sex Distribution Among the Cases**



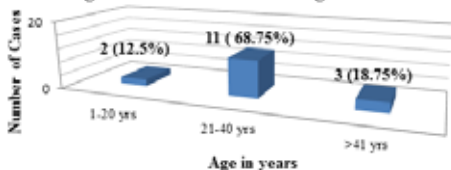
**Fig 6: Sex Distribution Among The Cases**

**Table 2: Age-wise distribution among the cases**

Age in years	Number of cases	Percentage
1-20	2	12.5
21-40	11	68.75
>41	3	18.75

Out of total 16 cases maximum cases 11 (68.75%) were in 21-40 yrs of age group which is followed by 3 (18.75%) cases in >41 yrs age group and lastly 2 (12.75%) cases in 1-20 yrs of age group.

**Age-Wise Distribution Among The Cases**



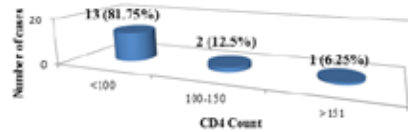
**Fig 7: Age-Wise Distribution Among The Cases**

**Table 3: Correlation of CD4 count with cases**

CD4 Count	Number of cases	Percentage
<100	13	81.25
100-150	2	12.5
>151	1	6.25
Total	16	100

Among the total 16 Cryptococcal Meningitis cases maximum i.e 13/16 (81.25 %) were found to have CD4 count < 100 cells/ $\mu$ L.

**Co-relation of CD4 Count with cases**



**Fig 8: Co-relation of CD4 Count with cases**

**Discussion:**

*Cryptococcus neoformans* infection is often fatal in immunocompromised patients<sup>7</sup>. *Cryptococcus neoformans* has become one of the most prevalent cause of fungal disease affecting more than one million people and causing at least 5,00,000 deaths annually worldwide due to HIV-associated cryptococcosis<sup>1</sup>. The genus *Cryptococcus* contains at least 39 species of yeasts, but few are able to cause disease in human beings. Even those that cause infection are not primarily pathogens; they have so called 'readymade virulence' as a side effect of their adaptation to their environments. Most human infections are due to *Cryptococcus neoformans*. *Cryptococcus neoformans* is encapsulated yeast first identified as human pathogen in 1894, when it was isolated from tibia of a patient in Germany by Buese and Buschke<sup>8</sup>. In the same year, it was also isolated from peach juice by Sanfelice. The first description of *Cryptococcal meningitis* was published in 1905 by Van Han Semann, although a case of chronic meningitis described in 1861 by Zenker, prior to pathogen isolation, was probably the first case history<sup>9</sup>.

Although Cryptococcosis is an established disease, its prevalence was low worldwide before AIDS era<sup>8-10</sup>. The HIV pandemic has a profound impact on the prevalence of cryptococcal disease. Its prevalence varies place to place. The prevalence of infection in this study is 11.27%. Similar percentage of prevalence (11%) was found in a study done in South-west area of France<sup>11</sup>. Paris and its suburbs noted the prevalence as 54% while in the Mediterranean area it is 15%. The prevalence in Manipur is 23.88%<sup>1</sup>. Various studies have been conducted in different parts of the world, including India, to find the prevalence of cryptococcosis in HIV-reactive patients and it has been found to range from 2.09% to 68.6%<sup>12-15</sup>.

In our study it was observed that among the 16 positive cases, 12 (75%) were male and 4 (25%) were female which indicates that prevalence of infection is slightly more in male as compared to female. Similar findings were also noticed in some of the studies which include Lungran et al<sup>1</sup>, Thakur et al<sup>3</sup>, Baradkar et al<sup>6</sup>, Cameroon et al<sup>7</sup>, Dromer et al<sup>11</sup>. There is hypothesis that differential interaction between Cryptococcus and macrophages within different gender environments and hormone contribute to the increased prevalence of cryptococcosis in males<sup>1</sup>. According to some authors, the reason for male predominance is not known although increased environmental exposure, hormonal influences and D or genetic predisposition of the patients have been postulated to be the contributing factors<sup>16,17</sup>.

In our study the maximum number of patients 11 (68.75%) were observed in the age group of 21-40 yrs. The similar findings were also noticed in the study performed by Lungran et al<sup>1</sup>, Baradkar et al<sup>6</sup>, Laxmi V. et al<sup>12</sup>. The predominance of cryptococcosis in the third and fourth decades of life may be because of the presence of HIV infection. As HIV infection is

most prevalent during this age range<sup>1</sup>.

The CD4 count is the best indicator of immediate state of immunologic competence and also the strongest predictor of HIV-related complications in patients. Among the total 16 positive for Cryptococcal Meningitis maximum i.e. 13/16 (81.25 %) were found to have CD4 count < 100 cells/ $\mu$ L. In a study done by Baradkar et al<sup>5</sup> the percentage of CD4 count <100 cells/ $\mu$ L was observed in all 19 cases which were yielded growth on culture.

It has been shown that HIV-infected patients who are given combined antiretroviral therapy have a lower incidence of cryptococcosis, and the risk of cryptococcal disease is substantially reduced among persons receiving these treatments<sup>17</sup>. Antinori et al also observed that, with the introduction of HAART for HIV treatment in HIV patients, the incidence of cryptococcosis, along with other opportunistic infections, has decreased<sup>18</sup>. All the patients which included in this study were already started on ART. Non compliance of the patient may be the proposed reason for the high prevalence of infection in spite of the treatment.

**Conclusion:** Cryptococcosis is an important cause of mortality and morbidity for HIV-infected patients. This study shows that Cryptococcal Meningitis is quite high among HIV infected patients who are on ART so periodic screening of such patients is required in a more extensive manner. Screening should be carried out for all HIV-infected patients with low CD4 counts in spite of treatment history.

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