

Cryptococcal meningitis: An under-reported disease from the hills of Uttarakhand: A hospital-based cross-sectional study

Aroop Mohanty¹, Mohit Bhatia¹, Ankita Kabi², Kuhu Chatterjee¹,
Neelam Kaistha¹, Balram Ji Omar¹, Puneet K. Gupta¹, Pratima Gupta¹

¹Department of Microbiology, All India Institute of Medical Sciences, ²Department of Emergency Medicine, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India

ABSTRACT

Background: Cryptococcal meningitis is a fatal opportunistic neuroinfection and an AIDS defining illness. It can also occur in non-HIV patients who are immunodeficient due to chronic glucocorticoid use, organ transplantation, malignancy and sarcoidosis. **Materials and Methods:** A cross-sectional study was conducted in a tertiary care hospital from July to December 2018. CSF samples of 364 patients were received by Microbiology laboratory during this period for the purpose of aerobic bacterial, fungal and TB culture, respectively. All samples were subjected to examination by direct wet mount, Gram stain and India ink preparation. Ziehl Neelsen staining, solid culture for *Mycobacterium tuberculosis* on Lowenstein Jensen medium and Gene Xpert was also performed on all CSF samples. These samples were further subjected to fungal culture on Sabouraud's dextrose agar. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) was used for identifying all bacterial (except *M. tuberculosis*) and fungal isolates. **Results:** Out of 364 CSF samples received, 288 were sterile after 48 hours of aerobic incubation. Bacterial isolates, *M. tuberculosis* and *Cryptococcus spp.* were obtained in culture from 51, 21 and 4 samples, respectively. The prevalence of cryptococcal meningitis in our study was 1.09% (4/364). *Cryptococcus neoformans var grubii* was the most common isolate (2/4; 50%) followed by *Cryptococcus neoformans var neoformans* (1/4; 25%) and *Cryptococcus neoformans var gattii* (1/4; 25%), respectively. **Conclusion:** Cryptococcal meningitis is a rapidly fatal condition which requires a high index of suspicion and calls for a collective effort from family physicians and diagnosticians alike. This disease is under-reported from Uttarakhand and therefore calls for further research from this region.

Keywords: Amphotericin B, cryptococcal meningitis, MALDI-TOF

Introduction

Cryptococcosis is an acute, subacute or chronic fungal infection caused by encapsulated heterobasidiomycetous yeast like fungus *Cryptococcus neoformans*. Till date five serotypes have been described. Serotypes A, D and AD hybrids are globally responsible for 98% of all cryptococcal infections in AIDS patients, whereas serotypes B and C predominantly affect immunocompetent individuals, but also have been recently reported in AIDS patients.^[1,2]

Cryptococcal meningitis is a fatal opportunistic neuroinfection and an AIDS defining illness seen in up to 69% of HIV-positive patients.^[3] It can also occur in non-HIV patients who are immunodeficient due to chronic glucocorticoid use, organ transplantation, malignancy and sarcoidosis.^[4] It is a rapidly fatal infection which requires early diagnosis and prompt treatment.

With the advent of antiretroviral therapy, the incidence of cryptococcosis has shown a steady decline in developed countries^[5] when compared with developing where access to

Address for correspondence: Dr. Mohit Bhatia,

Department of Microbiology, All India Institute of Medical Sciences, Virbhadr Marg, Rishikesh, Uttarakhand - 249 203, India.

E-mail: docmb1984@gmail.com

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anti-retroviral therapy and other necessary healthcare resources are a major limitation.

The incidence of cryptococcal meningitis in India has risen dramatically over the past 20 years.^[3] However, there is paucity of literature on cryptococcosis from the state of Uttarakhand. This study was undertaken to evaluate the prevalence and clinical presentation of cryptococcal meningitis in patients who presented to a tertiary care teaching hospital.

Materials and Methods

A cross-sectional study was conducted in a tertiary care hospital located in Rishikesh, Uttarakhand with a study period of 6 months starting from July to December 2018. CSF samples of 364 patients were received by Microbiology laboratory during this period for the purpose of aerobic bacterial, fungal and TB culture, respectively. All samples were subjected to examination by direct wet mount, Gram stain and India ink preparation. Ziehl Neelsen staining, solid culture for *Mycobacterium tuberculosis* on Lowenstein Jensen medium and Gene Xpert was also performed on all CSF samples. These samples were further subjected to fungal culture on Sabouraud's dextrose agar. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) (Bruker Biotyper Microflex, MA, USA) was used for identifying all bacterial (except *M. tuberculosis*) and fungal isolates.

Results

Out of 364 CSF samples received, 288 were sterile after 48 hours of aerobic incubation. Bacterial isolates, *M. tuberculosis* and *Cryptococcus spp.* were obtained in culture from 51, 21 and 4 samples, respectively. The bacterial profile of 51 CSF samples has been depicted in Table 1.

The prevalence of cryptococcal meningitis in our study was 1.09% (4/364). *Cryptococcus neoformans var grubii* was the most common isolate (2/4; 50%) followed by *Cryptococcus neoformans var neoformans* (1/4; 25%) and *Cryptococcus neoformans var gattii* (1/4; 25%), respectively. Out of four laboratory-confirmed cryptococcal meningitis patients, 3 (75%) were HIV positive. Clinical and laboratory profile of these patients has been shown in Table 2. Gram stain, India ink and culture findings of these patients have been depicted in Figures 1-3, respectively.

Table 1: Microbiological profile of CSF samples

Organisms identified	Total (%)
<i>Acinetobacter spp.</i>	21 (41.1)
CONS	13 (25.5)
<i>Pseudomonas aeruginosa</i>	07 (13.7)
<i>Staphylococcus aureus</i>	07 (13.7)
<i>Klebsiella pneumoniae</i>	05 (9.8)
Total	51 (100)

Discussion

The genus *Cryptococcus* contains at least 39 species, but only few are able to cause disease in human beings. Most human infections

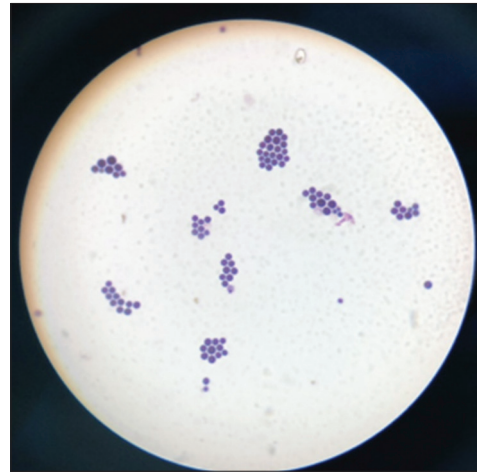


Figure 1: Gram stain showing budding yeast cells



Figure 2: India ink showing capsulated round yeast cells

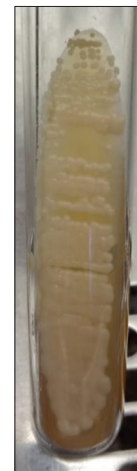


Figure 3: SDA tube showing mucoid, creamy pasty colonies of *Cryptococcus spp.*

Table 2: Clinical and laboratory profile of cryptococcal meningitis patients

Patient/ age, Sex	Risk factor	Clinical presentation	Sample	Microcopy-Wet mount and India ink	Organism-isolated confidence interval (MALDI-TOF)	Other OIs	Treatment	Outcome
66 year, M	HIV	Fever×7 days Headache×3 days Breathing difficulty×3 days Altered sensorium×3 days	CSF	Budding yeast cell with capsule	<i>Cryptococcus neoformans var grubii</i> (2.01)	PTB	Liposomal Amphotericin B	Expired
60 years, M	HIV	Headache×1 month Altered sensorium×1 week Seizure 1 episode	CSF	Budding yeast cell with capsule	<i>Cryptococcus neoformans var grubii</i> (2.11)	–	Liposomal Amphotericin B + Fluconazole + Flucytosine	Survived
44 year, F	HIV	Headache×2 months Fever×10 days Cough×10 days Decreased vision×10 days	CSF	Budding yeast cell with capsule	<i>Cryptococcus neoformans var neoformans</i> (2.24)	–	Liposomal Amphotericin B + Fluconazole + Flucytosine	Expired
32 years, M	-	Headache×4 months Seizure episodes since 2008	CSF	Budding yeast cell with capsule	<i>Cryptococcus neoformans var gattii</i> (2.35)	–	Liposomal Amphotericin B + Flucytosine	Survived

are caused by *C. neoformans*. The portal of entry is via inhalation of airborne particles, with bird droppings and associated soil being major environmental source following which it frequently pitches itself in the meninges, lungs, bones, adrenals, kidneys, liver and spleen.

The clinical presentation of this disease is variable and therefore difficult to differentiate from other multisystem ailments such as tuberculosis, other tropical infections and malignancies. The most common presenting complaint in our study was headache (100%) which is in concordance with other studies from India.^{16,71} Fever (50%), altered sensorium (25%) and seizure history (25%) were next in line, similar to a study by Abhilash *et al.* (75%, 40% and 18%, respectively).⁶¹

Current identification methods for yeasts rely heavily on physical characteristics and biochemical properties of the isolate. Rapid tests such as India ink are often used to quickly and presumptively identify the *Cryptococcus spp.* But even experienced observers can confuse the halo around cells (suggestive of capsule) with artifacts produced by reactions between leukocytes and carbon particle in the India ink stain. For these limitations, rapid tests must be confirmed by additional methods. We used MALDI-TOF-MS to identify all our clinical isolates which is known to have very high sensitivity and specificity. It also allows rapid identification of microbes, which in turn guides the clinicians in early initiation of appropriate therapy, thereby reducing overall time and cost of care. All the four isolates generated confidence scores of more than 2.0 which is considered to be secure species level identification.¹⁸¹

There has been a substantial increase in reporting of cryptococcosis in both immunosuppressed and immunocompetent individuals in recent years, which reflects an enhanced clinical awareness and improved diagnostic capability.

To the best of our knowledge, there is only one case report on cryptococcal meningitis from the state of Uttarakhand by Patil

Table 3: Cumulative records of cryptococcal meningitis from different parts of India

Part of India	No of confirmed cases	No of immunosuppressed cases (%)	Name of Author
East India	16	16 (100)	Dash <i>et al.</i> ¹¹⁰¹
	16	14 (87)	Lungram <i>et al.</i> ¹¹¹¹
North India	40	17 (42)	Kumar <i>et al.</i> ¹¹²²
	06	06 (100)	Thakur <i>et al.</i> ¹¹³³
	117	102 (87)	Abhilash <i>et al.</i> ¹¹⁷⁷
South India	39	39 (100)	Laxmi <i>et al.</i> ¹¹⁴⁴
	97	88 (91)	Naik <i>et al.</i> ¹¹⁵⁵
	27	27 (100)	Patel <i>et al.</i> ¹¹⁶⁶
	19	19 (100)	Baradkar <i>et al.</i> ¹¹⁷⁷
West India	19	19 (100)	Baradkar <i>et al.</i> ¹¹⁷⁷
	16	16 (100)	Kadam <i>et al.</i> ¹¹⁸⁸

Table 4: Cumulative records of cryptococcal meningitis from different parts of world

Part of world	Total no of confirmed cases	Total no of immunosuppressed cases (%)	Name of Author
Taiwan	219	54 (25)	Tseng <i>et al.</i> ¹¹⁹⁹
Vietnam	34	00 (0)	Chau <i>et al.</i> ¹²⁰⁰
Brazil	129	111 (86)	Nunes <i>et al.</i> ¹²²¹
Argentina	06	06 (100)	Frola <i>et al.</i> ¹²²²
Nepal	15	09 (60)	Kharel <i>et al.</i> ¹²³³

*et al.*⁹¹ Ours is the first cross-sectional study on prevalence and clinical profile of cryptococcal meningitis from the state of Uttarakhand. Cumulative records of cryptococcal meningitis from India and rest of the world have been depicted in Tables 3 and 4, respectively.

Conclusion

Cryptococcal meningitis is a rapidly fatal condition which requires a high index of suspicion and calls for a collective effort from family physicians and diagnosticians alike. This disease is

under-reported from Uttarakhand and therefore calls for further research from this region.

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Conflicts of interest

There are no conflicts of interest.

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