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Clinical and mycological implications of cryptococcal meningitis in Iran

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ABSTRACT

Cryptococcal meningitis (CM) is an uncommon and severe infection that tends to affect both immunocompromised and immunocompetent hosts. To gain insights into the clinical and epidemiological characteristics of CM in Iran, this study evaluated patients with subacute or chronic meningitis referred to 15 Iranian hospitals. Relevant clinical and epidemiological characteristics of the patients were analyzed. Diagnosis of CM cases was performed by microscopic examination, culture, latex agglutination assay, lateral flow assay, and multiplex PCR on cerebrospinal fluid (CSF) samples. The isolates were processed and subjected to molecular identification and *in vitro* susceptibility antifungal profile. Among the 272 evaluated patients, 7 (2.6 %) CM cases were diagnosed. Out of seven CM cases, 6 (86 %) were male with a median age of 36 years. The most common neurological signs were headache (100 %), followed by nausea and vomiting (71.4 %). All CSF samples from CM patients exhibited positive results across all mycological tests conducted. The isolates were identified as *Cryptococcus neoformans* (86 %) and *Cryptococcus gattii* (14 %). All isolates were susceptible to voriconazole and fluconazole, while resistance was observed with itraconazole (MIC value of 0.5 μg/mL) and amphotericin B (MIC values of 4 and 1 μg/mL). The highest mortality (6/7, 86 %) was observed among patients. While a comprehensive study on this subject is currently lacking in Iran, the data acquired through this research play a crucial role in enhancing the clinical and epidemiological understanding of this infection, particularly within low-income countries. Moreover, these findings will serve as a cornerstone for future international comparative studies in this field.

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1. Introduction

Cryptococcal meningitis (CM) is the most typical and perilous manifestation of cryptococcosis, caused by members of *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes, including *C. neoformans, C. deneoformans, C. gattii, C. deuterogattii, C. bacillisporus, C. tetragattii,* and *C. unnamed* [1,2]. These complexes cause subacute and chronic meningitis, which remains a challenging diagnosis even in a referral and experienced center. The HIV/AIDS infection, malignancies, organ transplantation, and the increased use of immunomodulatory therapies are predisposing factors for CM [1]. Cryptococcal meningitis is no longer considered an opportunistic disease only in immunocompromised patients, especially those with HIV/AIDS. There has been an alarming increase in immunocompetent individuals being affected worldwide, presenting a major challenge to healthcare professionals [3,4]. The global burden of HIV-associated CM in adults was reported to be 152,000 cases annually, resulting in 112,000 C M-related deaths in 2020. The rates in Asian and Pacific countries, including Vietnam, India, Indonesia, China, Cambodia, and Thailand, were 44,000 and 26,000 cases in 2020, respectively [5]. According to reports from the World Health Organization (WHO), in 2018, CM was associated with more than 1 in 10 HIV-related deaths, and three-quarters of CM-related mortality occurred in sub-Saharan Africa [6]. Enclosed and silent growth of chronic CM can cause undiagnosed central nervous system (CNS) infections in both immunocompromised and immunocompetent hosts [7]. Due to the importance of this issue, WHO guidelines recommend cryptococcal antigenemia screening for individuals with a $CD4^{+}$ T cell count <100 cells/μL [8,9]. To develop effective public health strategies and reduce the burden of potentially life-threatening CM in Iran, an Asian country, it is important to understand the epidemiological and clinical profiles of patients with CM. This study aimed to evaluate the clinical and mycological characteristics of patients with CM in Iran, where accurate data is lacking.

2. Patients and methods

2.1. Study design and setting

We performed a cross-sectional multicenter study across 15 Iranian hospitals in Alborz, East Azerbaijan, Fars, Gilan, Gorgan, Isfahan, Khuzestan, Lorestan, Mazandaran, and Tehran provinces from March 2021 to November 2022. Suspected patients were presented with either chronic (defined as presenting symptoms for a duration of at least 4 weeks) or subacute (defined as presenting symptoms for a duration of at least 5 days) meningitis symptoms, including fever, headache, meningismus, and altered mental status [10,11]. The CM cases were diagnosed by screening clinical manifestations, laboratory tests, and the hospital medical record information systems. This study conducted original research that is approved under the ethical approval code (IR.TUMS.SPH.REC.1400.106) obtained from the Research Ethics Committees of the School of Public Health & Allied Medical Sciences-Tehran University of Medical Sciences.

2.2. Data collection

We collected different patients' data, including demographics, signs and symptoms, laboratory findings, treatment regimens, and clinical outcomes during the study period. The presented symptoms included new headaches, fever, nausea and vomiting, diplopia, vertigo, neuropathies, and impaired level of consciousness. The level of impaired consciousness on admission was assessed using the Glasgow Coma Scale (GCS), which assesses eye opening response, verbal response, and motor response. The GCS score ranges from 3 (unresponsive/coma) to 15 (responsive/alert). Based on the GCS score, the level of consciousness of patients was reported as coma (GCS score of 3–8), stupor/obtundation (GCS score of 9–12), and lethargy/alert (GCS score of 13–15) [12].

2.3. Mycological diagnosis

The diagnosis of CM was confirmed by microscopic examination, culture, latex agglutination assay (LA), lateral flow assay (LFA), and multiplex PCR diagnostic techniques on the cerebrospinal fluid (CSF) specimens of patients. The CSF samples were directly examined using an India ink wet mount. The presence of encapsulated yeast cells was examined using a 40x objective lens. The CSF sediments were cultured on brain heart infusion agar (BHI) (Merck Co., Darmstadt, Germany), Sabouraud dextrose agar (SDA) (Merck Co., Darmstadt, Germany), and Niger seed agar. The culture media were incubated at 37 °C and 28 °C for 15 days and were observed daily. The cryptococcal antigen (CrAg) was detected using the CrAg Latex Agglutination Test System (CryptoLatex®, IMMY, Norman Kew Surrey, OK, USA), and by the Dynamiker CrAg Lateral Flow Assay kit (Dynamiker Biotechnology [Tianjin] Co., Ltd., China), according to the manufacturer's instructions. The positive results of the latex agglutination test, on a four-point visual scale, were graded from weak agglutination (1+) to strong agglutination (4+). The results were interpreted as follows: light flocculent precipitate against a mostly clear background taking 10 min to form (positive 1+), light flocculent precipitate against a mostly clear background taking 5 min to form (positive 2+), heavy flocculent precipitates taking 5 min to form with a clear background (positive 3+), and heavy flocculent precipitates forming within 3 min with a clear background (positive 4+) [13]. DNA extraction from the CSF specimen was conducted using the SinaPure-DNA kit (Sina Clon Bio Science, Iran). Multiplex PCR was performed as described previously [14,15] to detect and identify *Cryptococcus* isolates. Multiplex PCR amplified DNA fragments of 392, 235, and 184 base pairs for *Cryptococcus neoformans*, *Cryptococcus deneoformans*, and *Cryptococcus gattii*, respectively. The internal transcribed spacer (ITS) region was amplified and sequenced using the ITS1/ITS4 primers to confirm the identity of the isolates [14,16]. This experiment was carried out at the Mycology reference Labratory, National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

Table 1
Demographic characteristics, clinical manifestations, and outcome of patients with cryptococcal meningitis.

Case No.	Location Province/City	Gender	Age	NeurologicPresentation	Timeofonset(Daysago)	Impairedlevelofconsciousness(GCS)	Impairedcontentofconsciousness	CranialnervepalsyNO	Seizure	Fever(°C)	Hemodynamicinstability	Extracranialinvolvement	Timetodiagnosis)Days(	Outcome
1	Mazandaran/Amol	Male	31	Headache N/V	7	Stupor/obtundation (11)	No	NO	Yes	36.8	No	No	3	Died
2	Alborz/Hashtgerd	Male	45	Headache Vomiting Decreased level of consciousness	30	Lethargy/alert (15)	No	NO	Yes	37.1	No	No	3	Died
3	Lorestan/Khorramabad	Male	35	Headache N/V Phonophobia delusion	30	Stupor/obtundation (12)	No	NO	No	37.8	No	No	2	Died
4	Mazandaran/Babol	Female	59	Headache	14	Lethargy/alert (15)	No	NO	No	37	No	No	5	Died
5	Gilan/Rasht	Male	33	Headache N/V Decreased level of consciousness	8	Stupor/obtundation (10)	No	NO	No	38.5	Yes	No	3	Died
6	Tehran/Tehran	Male	36	Headache nausea vertigo	14	Stupor/obtundation (9)	Yes	No	No	36.8	No	No	2	Cured
7	Mazandaran/Amol	Male	48	Headache Diplopia	30	Coma (<8)	No	Diplopia, Impaired walking , Global Aphasia	No	37	No	No	2	Died

Abbreviations: GCS: Glasgow Coma Scale, N/V: nausea and vomiting.

Table 2
Laboratory and radiological findings of patients with cryptococcal meningitis.

Case No.	WBC (× 1000/mm3)	Differentiation N-L-M (%)	Creatinine	PT/ INR	Albumin	CSF opening pressure (cmH_2O)	CSF Protein	CSF Glucose	CSF RBC	CSF WBC	CSF WBC diff L/PMN (%)	CT scan findings	MRI findings
1	13.5	76.3-15.5-8.2	0.8	13.7/ 1.12	–	–	59	44	170	70	90/10	Hydrocephalus Ventriculomegaly	Hydrocephalus Ventriculomegaly
2	6.8	84.1-9.7-6.2	0.8	13.5/ 1.11	–	25	129	16	22	60	90/10	Not performed	Normal
3	11.5	78.3- 11.5- 10.2	1.1	13.6/ 1.07	3.7	22	44	57	200	0	–	Increased ICP	Not performed
4	7.8	78-18-4	1.2	13.2/ 1.1	–	–	82	25	5	25	90/10	Normal	Normal
5	9.5	92.5-6.5-1	1.6	15.6/ 1.09	2	70	23	60	7	2	–	Normal	Normal
6	2.9	88-9-3	1	14.0/ 1.2	2.9	15	257	10	80	225	40/60	Normal	Normal
7	7.4	93-5-2	0.96	21.4/ 1.61	2.9	–	157	1	10	1	–	Basal ganglia + Hydrocephalus	Normal

Abbreviations: N: Neutrophils, L: Lymphocytes, M: Mix cells, PMN: Polymorphonuclear leukocytes.

2.4. Antifungal susceptibility testing

Antifungal drug susceptibility profiles of clinical *Cryptococcus* isolates (except for case No. 4) against amphotericin B, fluconazole, voriconazole, and itraconazole (Sigma-Aldrich, St. Louis, MO, USA) were determined using micro-titer broth dilution method according to the Clinical Laboratory Standards Institute (CLSI) M27-A4 and M59 guidelines [17,18]. Yeast suspensions were prepared from recent cultures (48 h) of all isolates on SDA and adjusted to a final inoculum concentration of 5×10^3 colony forming units (CFU)/mL in RPMI 1640 medium (with L-glutamine; without bicarbonate) (Sigma), buffered to pH 7.0 with 0.165 M MOPS (Sigma). Drug powder was dissolved in Dimethyl Sulfoxide (DMSO) at a 10 mg/mL concentration, and different aliquots were prepared and stored at −70 °C. Before use, each working solution was prepared fresh. The concentration ranges of amphotericin B, voriconazole, itraconazole (0.0313–16 μg/mL), and fluconazole (0.062–64 μg/mL) were prepared in RPMI 1640 medium. Equal volumes of drug solutions and yeast suspensions in the test medium were then distributed into sterile 96-well plates. Growth controls in RPMI-1640 medium with 2.0 % of DMSO (v/v) were included. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 strains were included as quality controls. The plates were incubated at 35 °C, and the results were read after 48 h. The minimum inhibitory concentration (MIC) for amphotericin B was recorded as having the lowest drug concentration with no visible growth. While, for the azole drugs, the MICs were read as the lowest concentrations resulting in 50.0 % growth inhibition compared to the growth control. The sensitivity patterns of *Cryptococcus neoformans* isolates were assigned according to the epidemiologic cut-off values (ECV) CLSI M59. In brief, the isolate was considered as wild type or susceptible when MIC value was ≤0.5 mg/mL, ≤8 mg/mL, ≤0.25 mg/mL, and ≤0.25 mg/mL for amphotericin B, fluconazole, voriconazole, and itraconazole, respectively [17].

2.5. Statistical analysis

Descriptive statistics of qualitative variables (e.g., sex) were shown as frequency (percentage). Minimum, maximum, mean, median, and standard deviation were used to describe quantitative variables (e.g., age) using Excel and SPSS software.

3. Results

3.1. Clinical findings

In total, among the 272 referred patients, seven CM cases (2.6 %) were definitively diagnosed. Demographic characteristics and clinical manifestations of the patients are shown in Table 1. The confirmation of diagnosis typically occurs within two to five days from the initial clinical suspicion. Notably, a majority of patients were male (86 %), with a median age of 36 (range: 31–59). The disease exhibited a diverse range of manifestations, varying from chronic (43 %) to subacute (57 %) meningitis presentations. Remarkably, all patients had been experiencing symptoms for at least one week before seeking medical attention. Foremost among the neurological symptoms was headache (100 %), reported by all patients, followed by symptoms of nausea and vomiting in 71.42 % of cases. Upon admission and subsequent diagnosis, it was observed that two CM patients presented with impaired levels of consciousness, and one patient displayed impaired content of consciousness. This indicates the notable frequency of alterations in consciousness within CM cases. Interestingly, only one patient demonstrated a cranial nerve disorder (diplopia) at the point of diagnosis. In the context of brain parenchymal involvement, either directly or as a consequence of meningitis, seizures were identified in two patients, underscoring its significance as a potential sign in CM cases. Regarding extracranial involvement, no patients exhibited clinical extracranial

Table 3
Underlying conditions and treatment information of patients with cryptococcal meningitis.

Case No.	Underlying disease	Primary immune deficiency	Treatment			Treatment adverse effects	CSF shunting
			Induction	Consolidation	Maintenance		
1	Pulmonary & neurosarcoidosis	No	Amphotericin B 50 mg (IV) + fluconazole 150 mg (P·O.)	Amphotericin B 50 mg (IV) + fluconazole 150 mg (P·O.)	_	No	Yes
2	HIV/AIDS	No	Ambisome 350 mg (IV) + fluconazole 400 mg (IV/BD)	Ambisome 350 mg (IV) + fluconazole 400 mg (IV/BD)	_	No	No
3	No	No	Ambisome 350 mg (IV) + fluconazole 800 mg (IV)	Ambisome 350 mg (IV) + fluconazole 800 mg (IV)	_	No	No
4	Colon cancer	No	Fluconazole 400 mg (IV)	Fluconazole 400 mg (IV)	_	No	No
5	HIV/AIDS	No	Ambisome 350 mg (IV)	Ambisome 350 mg (IV)	_	No	No
6	Autoimmune hepatitis, on steroid treatment	No	Ambisome 350 mg (IV) + Ambisome 20 mg (IT)	Fluconazole 400 mg (IV/BD)	Fluconazole 400 mg (IV/BD)	No	No
7	Tuberculosis, on anti-TB and steroid treatment	No	Amphotericin B 50 mg (IV) + fluconazole 800 mg (IV)	Amphotericin B 50 mg (IV) + fluconazole 800 mg (IV)	_	No	No

manifestations at the time of diagnosis.

Brain imaging, particularly Magnetic Resonance Imaging (MRI), did not show any remarkable results in the cases conducted. Two patients had hydrocephalus, which was detected in a Computerized Tomography (CT) scan of the brain. The CSF opening pressure (OP) in the patients who underwent the procedure was higher than the normal range (5–20 cmH2O), indicating increased intracranial pressure (ICP). However, one patient had an OP of 15 cmH2O, which falls within the normal range. Most patients (6/7) had hypoglycemia, and showed lymphocytic pleocytosis, except for one patient with a higher percentage of neutrophilia (60 %). The laboratory and radiological findings of the patients on the first day of admission are presented in Table 2. None of the patients were diagnosed with primary immunodeficiency. One patient was immunocompetent, and six patients exhibited a range of underlying conditions, including HIV infection, cancer, autoimmune hepatitis, pulmonary and neurosarcoidosis, and pulmonary tuberculosis. Most of CM cases underwent treatment with amphotericin B, either alone or in combination with fluconazole during the induction phase based on clinical suspicion and diagnosis (Table 3). Despite receiving antifungal regimens, most CM patients died (6/7, 86 %) and did not reach the maintenance therapy after undergoing induction and consolidation therapies.

3.2. Mycological finding

Mycological diagnostic results of CM patients are shown in Table 4. Notably, microscopic examination, fungal culture, and CrAg LA, CrAg LFA, and molecular test results were positive for all seven CM patients. Detection of encapsulated yeast cells within the CSF using India ink staining identified the presence of these cells in all seven patients, regardless of their HIV infection status. Furthermore, the culture of CSF samples resulted in the growth of mucoid yeast colonies in all seven cases, indicating the viability and potential for the proliferation of the fungal organisms.

Six *Cryptococcus neoformans* and one *Cryptococcus gattii* isolate were initially identified based on the size of the fragments observed in the multiplex PCR and confirmed via ITS rDNA sequencing. The sequences obtained from the studied isolates have been deposited in the GenBank database and their accession numbers are provided in Table 4.

3.3. Antifungal susceptibility testing

Table 5 summarizes the *in vitro* susceptibilities of the six *Cryptococcus* isolates to amphotericin B, fluconazole, voriconazole, and itraconazole, as measured using the broth microdilution method. The MICs of tested antifungals ranged from 0.062 to 4 μg/mL for amphotericin B, 0.25–8 μg/mL for fluconazole, 0.0313–0.125 μg/mL for voriconazole, and 0.125–0.5 μg/mL for itraconazole. All isolates were susceptible to voriconazole and fluconazole, while resistant isolates were indicated for other antifungals. One isolate (16.7 %) was resistant to itraconazole (MIC = 0.5 μg/mL), and two isolates (33.3 %) were resistant to amphotericin B (MICs = 4 and 1 μg/mL).

4. Discussion

Cryptococcal meningitis poses a considerable burden on the healthcare system and is associated with high morbidity and mortality rates. Prompt diagnosis and treatment are crucial for improving patient outcomes. The existing body of research on CM in Iran primarily consists of case reports, indicating a notable gap in comprehensive studies addressing this subject. The current study was conducted to fill the significant gap through describing various aspects of CM, including its frequency, risk factors, treatments, and outcomes of patients. Previous studies conducted in Iran have indicated a spectrum of CM frequencies up to 4.4 % during the period spanning from 2004 to 2019 [19]. In the current study, a frequency rate of 2.6 % was reported. CM primarily manifests in immunocompromised individuals, including those diagnosed with conditions like HIV/AIDS, malignancies, autoimmune diseases, a history

Table 4
Mycological diagnostic methods and results in patients with cryptococcal meningitis.

Case No.	Diagnostic methods					Molecular identification	NCBI accession No.
	India ink microscopy	Culture	Latex agglutination assay	Lateral flow assay	Multiplex PCR		
1	Encapsulated budding yeast cells	Positive	Positive (4+)	Positive	Positive	*Cryptococcus neoformans*	OR502798
2	Encapsulated budding yeast cells	Positive	Positive (3+)	Positive	Positive	*Cryptococcus neoformans*	OR498213
3	Encapsulated budding yeast cells	Positive	Positive (4+)	Positive	Positive	*Cryptococcus gattii*	OR498641
4	Encapsulated budding yeast cells	Positive	Positive (2+)	Positive	Positive	*Cryptococcus neoformans*	OR505937
5	Encapsulated budding yeast cells	Positive	Positive (4+)	Positive	Positive	*Cryptococcus neoformans*	OR505935
6	Encapsulated budding yeast cells	Positive	Positive (4+)	Positive	Positive	*Cryptococcus neoformans*	OR498836
7	Encapsulated budding yeast cells	Positive	Positive (4+)	Positive	Positive	*Cryptococcus neoformans*	OR505936

Table 5
In vitro activities of four antifungal agents against *Cryptococcus* isolates from six patients with cryptococcal meningitis.

Antifungal drug	MIC Range (μg/mL)	MIC (μg/mL)/Sensitivity pattern					
		Case 1	Case 2	Case 3	Case 5	Case 6	Case 7
Amphotericin B	0.062–4	4/R	0.25/S	1/R	0.062/S	0.125/S	0.125/S
Fluconazole	0.25–8	0.25/S	4/S	4/S	2/S	4/S	8/S
Itraconazole	0.125–0.5	0.125/S	0.25/S	0.25/S	0.125/S	0.125/S	0.5/R
Voriconazole	0.0313–0.125	0.0313/S	0.125/S	0.125/S	0.0313/S	0.062/S	0.125/S

Abbreviations: MIC: minimum inhibitory concentration, R: resistant, S: susceptible.

of organ transplantation, recipients of immunosuppressants, and sarcoidosis [20,21]. Notably, HIV/AIDS stands as the most significant risk factor for CM, particularly in regions with a high prevalence of HIV/AIDS, especially in Southeast Asian countries [22]. However, non-HIV/AIDS patients are experiencing an increase in CM due to advances in medical technology, such as increased organ/stem cell transplantation rates or increased immunosuppressive therapies [23]. In our study, we observed notable heterogeneity in immune profiles, encompassing one patient who was immunocompetent and others who were immunocompromised, including both HIV/AIDS patients and those without HIV/AIDS. This diversity presents an opportunity for conducting comparative analyses that can yield invaluable insights. By scrutinizing outcomes, disease progression, or responses to interventions among these distinct immune states, researchers can attain a more nuanced perspective regarding the influence of immunodeficiency on health outcomes.

The demographics of the CM patients revealed a predominantly male with ages ranging from 31 to 59 years. This gender and age distribution might indicate potential risk factors associated with CM, warranting further investigation [24]. It seems that increased susceptibility to this infection in men may be related to underlying factors such as work-related exposure, certain behaviors like drinking and smoking, and HIV infection. Moreover, testosterone can increase capsule production and decrease macrophage efficiency [25]. CM presents with insidious, progressive, subacute-to-chronic meningitis, a pattern that is also noted in the current investigation [11]. All the patients had mild symptoms, such as headaches, which were either ignored or not properly managed as neurological symptoms.

Following the mycological confirmation of CM, induction therapy is initiated in accordance with the guidelines provided by WHO [26]. Although amphotericin B plus flucytosine are the first-choice drugs for induction therapy, especially in HIV patients, other drugs are also considered. However, no large trial has been conducted to compare alternative and standard treatments. To overcome treatment challenges, combining azoles with amphotericin B, which exerts fungicidal action, is recommended [27]. The pharmaceutical arsenal for managing these conditions in Iran is relatively restricted, encompassing amphotericin B in both its conventional and liposomal formulations, in addition to fluconazole. Within the scope of the present study, it is worth noting that, apart from a single patient who exclusively received fluconazole, the remaining individuals underwent treatment with amphotericin B either alone or in combination with fluconazole during the induction phase.

The *in vitro* activity of this antifungal agent revealed that two isolates exhibited resistance to amphotericin B. Numerous studies have shown an obvious contrast between clinical and *in vitro* resistance results [17,28,29]. Factors such as control of the underlying disease, early diagnosis, appropriate antifungal and dose selection, patient follow-up and monitoring, and the concentration of drugs in the blood, CSF, and brain parenchyma should be considered in these studies [30].

The findings of this study reveal a distressing outcome, indicating a high mortality rate of 86 % within the studied scope. The high mortality rate of patients is a discussion of the potential reasons and implications of missed and delayed diagnosis of CM [24]. Diagnosing CM can be challenging due to the lack of information or awareness about distinguishing it from acute meningitis, as well as the similar epidemiological, clinical, and laboratory characteristics of other subacute meningitis cases reported in immunocompromised patients such as tuberculosis. *Haemophilus influenza* type B meningitis due to a lack of routine vaccination and tuberculous meningitis is more common than CM in Iran, which can lead to misdiagnosis and incorrect treatment, as seen in one patient in our study [31,32]. The use of steroids to manage underlying conditions might mask the clinical symptoms of CM, leading to delayed recognition and treatment initiation. Additionally, the unavailability of flucytosine, a valuable medicine in treating and managing the disease, may be a contributing factor to the high mortality rate. Moreover, there is a discrepancy in the treatment regimen, particularly in the induction phase, which is another important reason for the high mortality in this case series. The findings of this study should be interpreted while considering certain limitations. These limitations encompass the relatively limited number of positive cases available for analysis within the study's designated timeframe. Additionally, there is constrained access to advanced molecular and rapid diagnostic techniques across various mycological diagnostic centers in Iran. Despite the limitation of sample size, the current study provides a holistic understanding of CM practices in the Iranian context. It also provides valuable insights that can inform healthcare policies, guide clinical practices, and pave the way for further in-depth studies on CM in Iran.

5. Conclusions

CM can be associated with high mortality and present with a variety of unspecific symptoms. Strengthening laboratory capacities for accurate identification and antifungal susceptibility testing and improving treatment options are essential for guiding appropriate treatment decisions. Addressing the clinical and mycological implications of CM in low-income countries such as Iran requires a comprehensive approach involving healthcare providers, policymakers, researchers, and international collaborations. These findings need to be confirmed by further investigations.

Ethics statement

This study was approved by the Research Ethics Committees of the School of Public Health & Allied Medical Sciences-Tehran University of Medical Sciences, under the ethical approval Code of IR. TUMS.SPH.REC.1400.106.

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Informed consent statement

Informed consent was obtained from all individual participants included in the study.

Data availability statement

The data associated with this study have not been deposited into a publicly available repository; however, they will be made available upon request.

CRediT authorship contribution statement

Bahareh Bashardoust: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ilad Alavi Darazam:** Writing – original draft, Supervision, Conceptualization. **Roshanak Daie Ghazvini:** Supervision, Resources. **Seyed Jamal Hashemi:** Resources. **Mohammadreza Salehi:** Resources, Project administration, Conceptualization. **Ladan Abbasian:** Supervision, Resources, Conceptualization. **Seyed Ali Dehghan Manshadi:** Resources, Conceptualization. **Mahsa Abdorahimi:** Methodology. **Afsaneh Mohamadi:** Methodology. **Fariba Zamani:** Writing – review & editing. **Pegah Ardi:** Methodology. **Sadegh Khodavaisy:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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