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Cryptococcosis in patients with diabetes mellitus II in mainland China: 1993-2015

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Summary

Diabetes mellitus II (DM II) is a newly defined independent factor contributing to the morbidity and mortality of cryptococcosis. This retrospective case analysis aims to explore the epidemiology, clinical profile and strain characteristics of cryptococcosis in Chinese DM II patients. This study included 30 cases of cryptococcosis with DM II occurring from 1993 to 2015 in mainland China. The hospital-based prevalence of cryptococcosis in DM II was 0.21%. The mean age of the patients was 56.1 years (95% confidence interval: 51.5, 60.6), and 93% of the patients were older than 40 years. Sixty-two per cent of the patients experienced untreated or poorly controlled blood glucose before infection. Multilocus sequence typing analysis categorised all cultured strains as *Cryptococcus neoformans* and sequence type 5. Sixty-nine per cent of pulmonary cryptococcosis patients experienced misdiagnoses and treatment delays. Sixty per cent of cryptococcal meningitis patients received substandard antifungal therapy. The overall death rate was 33%. Considering the large population size of DM II patients in China, improved attention should be paid to the high prevalence of cryptococcosis as revealed by us. We also emphasised the importance of blood glucose control for infection prevention, especially among the elderly.

KEYWORDS
cryptococcosis, diabetes mellitus II, multilocus sequence typing

1 | INTRODUCTION

Infection with yeast of the *Cryptococcus gattii/Cryptococcus neoformans* species complex can lead to a wide array of clinical manifestations, ranging from asymptomatic infections to fatal situations such as meningitis, pneumonia and sepsis.[1,2] Although *Cryptococcus* is a well-known pathogen that was first isolated from the environment and humans >100 years ago,[3,4] most of the current knowledge regarding cryptococcosis has been associated with the human immunodeficiency virus (HIV) pandemic.[5,6] In the post-highly active antiretroviral therapy (HAART) era, the changing epidemiology of cryptococcal diseases among HIV patients has been characterised by decreases in incidence and mortality.[7] However, among non-communicable diseases (NCDs) that affect the human population, such as systemic lupus erythematosus, kidney transplantation, nephrotic syndrome and diabetes mellitus II (DM II), cryptococcosis has received continued attention.[8-11] In contrast with HIV-related cryptococcosis, the clinical features of which have been adequately described in previous studies,[12,13] the epidemiology and manifestations of cryptococcosis in NCDs that affect the human population, including DM II, are unclear.

China has the largest burden of DM II in the world; the morbidity and mortality of DM II has reached epidemic proportions recently.[14] The latest national survey documented that 11.6% or 50.1% of Chinese adults had diabetes or prediabetes, respectively, accounting for nearly 25% of the global diabetes population.[15,16] Life-threatening invasive fungal infections, such as mucormycosis, candidiasis, coccidioidomycosis and cryptococcosis, may be lethal complications in DM II patients.[17-20] The persistent status of hyperglycemia can cause deficiencies in innate and adaptive immunity and can cause growth of microorganisms,[20,21] making patients with DM II more susceptible to opportunistic pathogens. In addition, a high level of blood glucose hinders pathogen eradication.[22] Most of the clinical understanding of cryptococcosis in DM II patients arose from sporadic case reports of the non-Chinese population. Its occurrence among the Chinese DM II population was neglected in scientific journals published in English. This is in contrast to increased attention gained by articles in local Chinese medical journals.

In this study, we retrospectively analysed the pooled data from Shanghai Changzheng Hospital and published studies, aiming to increase the global understanding of the epidemiology, clinical profile and strain characteristics of cryptococcosis in the Chinese DM II population.

2 | PATIENTS AND METHODS

2.1 | Patient selection and data collection

Our study was approved by the Institutional Review Board (IRB) of Shanghai Changzheng Hospital (Approval Number: 2016SL021). Considering the retrospective nature of the study, the IRB agreed to waive the need to obtain informed consent.

We systematically mined the medical record system of Shanghai Changzheng Hospital, two international medical databases (PubMed and Embase) and three Chinese medical databases (WanFang databases, China National Knowledge Infrastructure (CNKI) and Chinese Biomedical Literature Service System (SinoMed)), for cases of cryptococcosis in DM II patients with detailed information. "Cryptococcosis," "cryptococcal meningitis," "cryptococcal pneumonia," "diabetes mellitus" and "hyperglycemia" were the main search terms both for literature and medical record retrievals. The confirmation of cryptococcosis was based on positive India ink staining, histopathological examination, culture, Latex Agglutination test (LA) and/or Lateral Flow Assays Cryptococcal Antigen Lateral Flow Assay (CrAg LFA).[23] DM II was diagnosed in accordance with the ICD-10-CM (International Classification of Diseases, Tenth revision, Clinical Modification). The diagnosis of DM II should be made prior to cryptococcosis. Patients with DM I or hyperglycemia as a physiological reaction to severe infections were not considered.[24] The following data were collected: admission or publication data, gender, age, duration of DM II course, blood glucose control, other underlying diseases, affected sites, clinical symptoms and signs, laboratory findings, genotype, *in vitro* antifungal susceptibility test, therapeutic regimens and outcomes.

2.2 | Mycological study of the clinical strains

All clinical *Cryptococcus* strains at our hospital were preserved in the Chinese Type Culture Collection Center of Medical Mycology, Shanghai Institute of Medical Mycology. The clinical strains related to our study were searched and subcultured for molecular type and antifungal susceptibility analyses.

Multilocus sequence typing (MLST) analysis was performed (Promega, Madison, American) using seven unlinked housekeeping loci: capsule polysaccharide (*CAP59*), glycerol 3-phosphate dehydrogenase (*GPD1*), intergenic spacer (IGS1), laccase (*LAC1*), phospholipase B1 (*PLB1*), superoxide dismutase (*SOD1*) and orotidine monophosphate pyrophosphorylase (*URA5*). Genomic DNA extraction was performed as described previously.[9] Polymerase chain reaction primers (Sangon Biotech, Shanghai, China) and conditions for each housekeeping gene were strictly based on the International Society for Human and Animal Mycology (ISHAM) consensus MLST scheme for *C. neoformans* and *C. gattii*.[25] Each locus was bidirectionally sequenced and uploaded to the International Fungal MLST Database (http://mlst.mycologylab.org). After the alignment with the MLST database, a *Cryptococcus* sequence type was given to each sample.

The antifungal susceptibility test was performed as previously described by the Clinical and Laboratory Standards Institute (CLSI).[26] Five antifungal agents (amphotericin B, fluconazole, itraconazole, voriconazole and fluorocytosine) were obtained commercially (Sigma-Aldrich, Saint Louis, USA) and were stored at −20°C until use. *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 2159) were used as quality control strains. After incubating for 72 h, the fungal yields between wells were visually compared to determine the minimal inhibitory concentration (MIC) of each antifungal for each strain. All tests were conducted with triple experimental replicates on different days.

2.3 | Statistical analysis of the pooled data

SPSS (version 21, International Business Machines Corporation, New York, NY, USA) and GraphPad Prism (version 5, GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical analysis, and results were presented as mean ± standard deviation (SD) for normal data. A probability (P) value <.05 implies statistically significant difference.

3 | RESULTS

3.1 | Original and published cases identified

From 1997 to 2015, 7,714 DM II patients were admitted to our hospital, and among these were 16 cryptococcosis cases. Hence, the hospital-based prevalence of cryptococcosis in the DM II population was 0.21% at our centre. Additionally, 14 cases were collected via systematically searching the international and Chinese local literature databases. Notably, all of the published cases were written in Chinese and were published in local Chinese journals.

3.2 | Demographical and epidemiological characteristics

The general information of each case is shown in Table S1 and is summarised in Table 1. No statistically significant differences were observed between the hospital cases and published cases (P>.05). Regarding the case distribution, most of the hospital cases were from East China, whereas the published cases were mainly from North China (Figure 1). The sex ratio of the included cases was close to one (M/F=1.13). The mean age was 56.1 years (95% confidence interval (CI): 51.5, 60.6), and 37% to 89. 93% of the patients were middle-aged to elderly (>40 years).

3.3 | Underlying diseases

We analysed the time interval from the diagnosis of DM II to that of cryptococcosis (Figure 2), and the mean duration was 5.0 years (95% CI: 3.3, 9.7). The details of blood glucose control were available in 21 cases. Five DM II patients were untreated before infection. The remaining 16 cases received insulin or oral hypoglycemic agents (OHAs); however, eight patients were reported with poor control of blood glucose. Among the 10 casualties, DM II was untreated or poorly controlled in six cases.

In addition to DM II, 43% of the patients were diagnosed with other underlying diseases (n=13), including solid organ transplant (n=6, five kidney transplant and one liver transplant), autoimmune haemolytic anaemia (n=2), hypertension (n=2), chronic renal failure (n=1), hepatitis B (n=1) and vasculitis (n=1). Only three patients had a history of exposure to pigeon droppings.

3.4 | Clinical characteristics

Fifteen patients were diagnosed with cryptococcal meningitis. Among them, severe headache and fever were presented as the

TABLE 1 Epidemiology and clinical manifestations of the included cases

		Total (n=30)	Hospital cases (n=16)	Published cases (n=14)
General information	Mean age	56.1 (95% CI 51.5, 60.6)	54.3 (95% CI 48.2, 60.3)	58.1 (95% CI 50.5, 65.8)
	Male	16 (53%)	7	9
	Disseminated cryptococcosis	17 (57%)	11	6
	Duration of DM II course (years)	5.0 (95%CI 3.3, 9.7)	4.2 (95% CI 2.0, 9.3)	6.1 (95% CI 3.1, 9.1)
	Underlying diseases except DM II	13 (43%)	8	5
	Pigeon contact	3 (10%)	2	1
	Death	10 (33%)	5	5
Constitutional signs	Fever	21 (70%)	9	12
	Nausea	11 (37%)	6	5
	Vomiting	12 (40%)	7	5
	Weakness	1 (3%)	1	0
	Weight loss	1 (3%)	1	0
Respiratory	Cough	13 (43%)	5	8
	Sputum	12 (40%)	5	7
	Chest pain	1 (3%)	1	0
Neurology	Headache	14 (47%)	9	5
	Signs of meningeal irritation	9 (30%)	4	5
	Conscious disturbance	8 (27%)	5	3
	Hearing damage	4 (13%)	3	1
	Visual damage	2 (6%)	2	0

95% CI: 95% confidence interval; DM II: diabetes mellitus II.
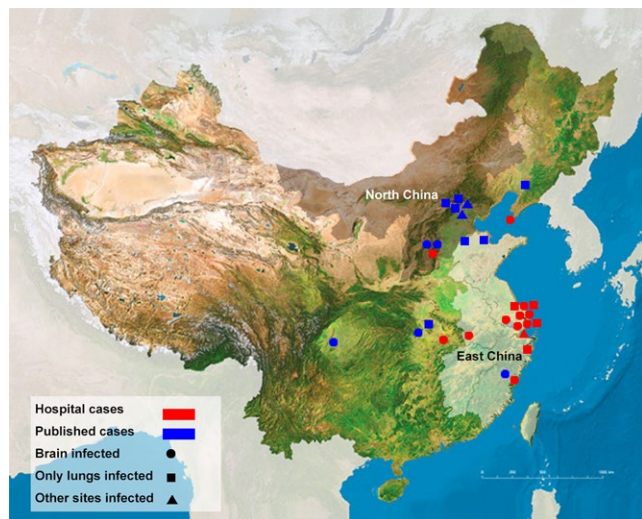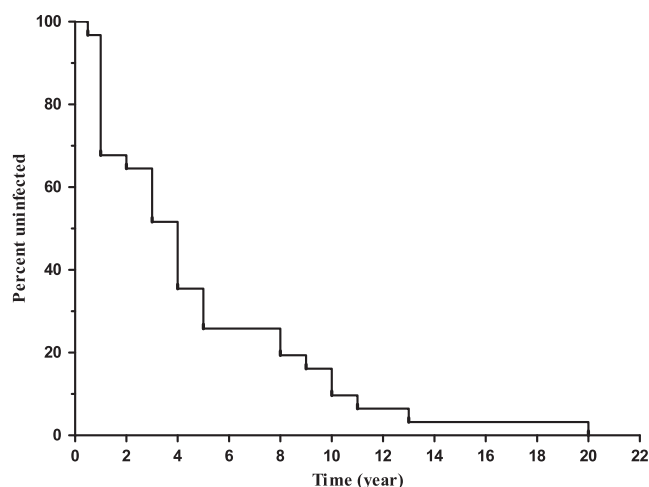
FIGURE 1 Distribution of the included cases

FIGURE 2 Time interval from DM II diagnosis to cryptococcosis diagnosis

initial presentation among 80% (n=12) and 87% (n=13) of patients respectively. Nausea and vomiting occurred in nearly half (53%) of the patients. Seven patients had signs of meningeal irritation, and seven showed conscious disturbance. Twelve patients were diagnosed with non-disseminated pulmonary cryptococcosis, and fever (n=7), cough (n=9) and sputum (n=8) were the most common symptoms and signs. One case was diagnosed as thoracic vertebrae cryptococcosis, and thoracic dull pain was the only symptom. Two cases of cryptococcal sepsis were both presented as conscious disturbance in the whole process of the disease, and both patients finally died. Fever was the only sign in the patient whose adrenal glands were involved.

3.5 | Mycological study

The details of the mycological tests are shown in Tables 2 and S2.

Among the 26 patients who underwent India ink staining analysis, 18 cases showed positive results (69%). Strain isolation was successful in 18 cases and failed in 10 cases. Cryptococcal antigen

tests of CSF were used in 10 patients with cryptococcal meningitis, and all showed positive results (titres ranged from 1:32 to 1:5120). Cryptococcal antigen tests of serum were performed in six patients, and five showed positive results (titres ranged from 1:128 to 1:160). India ink staining, culture and cryptococcal antigen tests were all positive in five cases.

All isolated *Cryptococcus* strains from hospital cases (n=7) were collected from the Chinese Type Culture Collection Center of Medical Mycology in Shanghai. MLST analysis revealed that all isolates belonged to the *Cryptococcus neoformans* and sequence type 5 (ST5). The mating types of all isolates were all *MATα*. The *in vitro* antifungal susceptibility test results demonstrated the following MIC (minimal inhibitory concentration) ranges, which failed to identify resistant strains: amphotericin B (AmB), 0.25-0.5 μg/mL (MIC$_{50}$: 0.5 μg/mL); fluconazole (FCZ), 0.5-1 μg/mL (MIC$_{50}$: 1 μg/mL); itraconazole (ITR), 0.03-0.12 μg/mL (MIC$_{50}$: 0.06 μg/mL); voriconazole (VRI), 0.03-0.5 μg/mL (MIC$_{50}$: 0.25 μg/mL); and flucytosine (5-FC), 0.5-2 μg/mL (MIC$_{50}$: 1 μg/mL).

3.6 | Treatments and outcomes

Twelve patients who first presented pulmonary symptoms experienced misdiagnoses and treatment delays: seven were diagnosed with bacterial pneumonia, and five patients were mistaken for having tuberculosis. Therefore, all of them received antibacterial agents as initial treatments in error. Seven of the misdiagnosed pulmonary cryptococcosis cases finally progressed to cryptococcal meningitis.

The therapeutic details were recorded in 26 cases (Table S3). Fifteen cases were diagnosed as cryptococcal meningitis, and nine of them did not follow the recommended combined sequential therapeutic strategy using AmB (or Lipid formulations of AmB), 5-FC, FCZ or ITR.[27] The death rate among patients with cryptococcal meningitis was 33%. There were no cases of substandard therapy in cases of focal pulmonary cryptococcosis, except for one patient who died before antifungal treatment. The death rate among cases of focal pulmonary cryptococcosis was also 33%.

4 | DISCUSSION

China has the world's largest DM II population. It is estimated that in China, the case number of adult patients with DM II will rise from 109.6 million (95% CI: 99.6, 133.4) at present to 150.7 million (95% CI: 138.0, 179.4) in 2040. This figure does not include the undiagnosed, whose numbers are approximately equal to those of the diagnosed.[28] DM II patients are highly susceptible to pathogens, including common and rare microorganisms. Although not supported by previous risk factor investigations, several studies have focused on the possible relationship between cryptococcosis and DM II.[29,30] In 2016, Lin et al. conducted the largest epidemiological study, which recruited nearly 23,000 cryptococcosis cases and other-disease controls, and first confirmed DM II as a definite independent contributing factor for cryptococcosis-related morbidity and mortality in the Chinese population.[11] However, there has not

TABLE 2 Mycological tests of cryptococcosis with DM II

No.	India ink stain	Culture	Cr Ag	MIC AmB	MIC FCZ	MIC ITR	MIC VRI	MIC 5-FC	Genotype	Sequence type (ST)	Mating type
P1	Neg	Neg	1:128 (Blood)	ND	ND	ND	ND	ND	ND	ND	ND
P2	Pos pathological examination			ND	ND	ND	ND	ND	ND	ND	ND
P3	Pos	Pos	1:5120 (CSF)	0.25	1	0.06	0.25	1	VN I	ST5	MATα
P4	Pos	Pos	1:1280 (CSF)	0.5	1	0.06	0.03	0.5	VN I	ST5	MATα
P5	Neg	Pos	1:160 (CSF)	0.5	1	0.06	0.25	1	VN I	ST5	MATα
P6	Neg	Pos	ND	0.5	1	0.12	0.25	1	VN I	ST5	MATα
P7	Neg	Neg	1:128 (blood)	ND	ND	ND	ND	ND	ND	ND	ND
P8	Pos	Neg	1:640 (CSF)	ND	ND	ND	ND	ND	ND	ND	ND
P9	Pos	Neg	1:2560 (CSF)	ND	ND	ND	ND	ND	ND	ND	ND
P10	Pos	Neg	Neg (Blood)	ND	ND	ND	ND	ND	ND	ND	ND
P11	Neg	Neg	1:160 (Blood)	ND	ND	ND	ND	ND	ND	ND	ND
P12	Pos	Pos	ND	0.5	0.5	0.06	0.12	1	VN I	ST5	MATα
P13	Pos	Neg	1:160 (CSF)	ND	ND	ND	ND	ND	ND	ND	ND
P14	Pos	Neg	1:320 (CSF)	ND	ND	ND	ND	ND	ND	ND	ND
P15	Pos	Pos	1:160 (CSF)	0.5	1	0.03	0.5	2	VN I	ST5	MATα
P16	Pos	Pos	1:640 (CSF)	0.5	1	0.06	0.06	2	VN I	ST5	MATα
S1	Neg	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S2	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S3	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S5	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S6	Pos	Neg	ND	ND	ND	ND	ND	ND	ND	ND	ND
S7	ND	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S8	Neg	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S9	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S10	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S11	Pos	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND
S12	ND	ND	Pos (Blood)	ND	ND	ND	ND	ND	ND	ND	ND
S13	Pos	Pos	1:32 (CSF)	ND	ND	ND	ND	ND	ND	ND	ND
S14	Neg	Pos	ND	ND	ND	ND	ND	ND	ND	ND	ND

Cr Ag, cryptococcal capsule polysaccharide antigen tests; MIC, minimal inhibitory concentration (µg/mL); AmB, amphotericin B; FCZ, fluconazole; ITR, itraconazole; VRI, voriconazole; 5-FC, 5-flucytosine; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; ND, no data.

yet been a molecular epidemiological, demographical and clinical summarisation of cryptococcosis in DM II patients until now, especially among the Chinese population.

Our study identified 16 hospital cases from 7,714 DM II patients, which demonstrated that the prevalence of cryptococcosis among the DM II population is approximately 0.21% (during 1997 to 2015). Considering the large population base of DM II patients in China, much attention should be paid to the high prevalence of cryptococcosis. Although not population-based, these data represent the only prevalence data available until now. Additionally, our comprehensive searches identified 14 cases, all of which were published in local journals in Chinese. This lack of international publication records reflected the global underestimation of cryptococcosis among the Chinese DM II population and emphasised the necessity of revealing the disease profile of people living in mainland China. We observed that the sex ratio of included cases was close to one (M/F=1.13). Most of the published and hospital cases occurred in North and East China, where the DM epidemic is the most serious (Fig. S1).

In their 15-year retrospective survey, Zheng et al. revealed that cryptococcosis more likely occurs in younger than in older people.[31] However, in our study, 93% and 30% cases were >40 and 60 years old, respectively, indicating the different distribution characteristics of cryptococcosis between the overall patients and the DM II population. Serious infections among elderly DM patients remain a cause of concern. It is estimated that 18%-40% of the elderly population have been diagnosed as DM II with an equal number of potentially undiagnosed DM II cases.[32,33] In addition to DM II, ageing-associated low functional immune status also acts as a risk factor for infections.[34] Considering the increasing rates of DM II and ageing individuals among the global human population in the coming years, further attention should be paid to cryptococcosis among those populations.

On average, the included cases had 5-year histories of DM II. However, 62% of DM patients (n=13) were reported to be untreated or had poor control of blood glucose despite treatment. We also found that more than half of the cases who died had a history of uncontrolled or poorly controlled blood glucose level, indicating that blood glucose management may be a probable cause of the poor prognosis of cryptococcosis.

It is very common to find the co-existence of multiple comorbid diseases in one patient among the non-HIV cryptococcosis population,[11] including but not limited to those with DM II. The cryptococcosis cases in this study were characterised by the relatively frequent existence of underlying diseases besides DM II (43%). Similarities were also found in other researches on cryptococcosis with certain underlying diseases, 50% (n=7/14) of the cryptococcosis in kidney transplant recipients were with secondary underlying diseases; 34.8% (n=8/23) of the cryptococcosis/tuberculosis co-infected cases were diagnosed as another comorbid condition; 16.7% of the cryptococcosis in SLE patients were noticed to be with other underlying diseases.[8,9,35] Among other underlying diseases besides DM II, posttransplant diabetes was the most frequent (46%) in our study. Solid organ transplantation

patients, especially host with kidney transplantation, showed a high incidence of fungal infection (1%-14%).[36] Because antimycotic prophylaxis via drugs is not recommended among transplant patients based on clinical evidence, early recognition and timely antifungal treatment is of great urgency.[36]

Cryptococcal meningitis remains the most frequently observed type of cryptococcosis in DM II patients. Headache, fever and meningeal irritation signs were identified as the earliest signs and symptoms of infection and may serve as meaningful clinical clues to determine the presence of infection dissemination and the need for subsequent diagnostic lumbar puncture. Most cases of pulmonary cryptococcosis with DM II revealed misdiagnoses and treatment delays. Pulmonary cryptococcosis represents a relatively earlier stage of cryptococcosis than cryptococcal meningitis, which tends to lack clinical specificity for diagnosis.

Espinel-Ingroff et al. revealed that the molecular type of the *Cryptococcus neoformans-Cryptococcus gattii* species complex was associated with epidemiological cut-off values for AmB, 5-FC, FCZ, ITR, posaconazole and VRI.[37,38] In this study, we studied the MLST types and MICs of strains isolated from cryptococcosis cases among DM II patients. MLST revealed that all seven clinical isolates in our study belonged to ST 5, which is in agreement with previous reports indicating that ST 5 is the predominant sequence type in China.[39-42] Antifungal susceptibility tests did not identify any resistant isolates.

According to the clinical practice guidelines for cryptococcal meningitis,[27] the antifungal treatment for non-HIV-infected, non-transplant hosts is AmB plus 5-FC for at least 4 weeks as the first choice of induction therapy, and FCZ for 8 weeks as consolidation therapy. Among the included 15 cryptococcal meningitis cases, nine received substandard antifungal therapy. Hence, we recommended that more effort is made to highlight the importance of adequate dosage and course of appropriate antifungals for treatment of cryptococcosis in DM II patients.

In conclusion, this study is the first that evaluated the epidemiological and clinical profiles of cryptococcosis in the Chinese DM II population. Considering the large population size of DM II patients in China, much attention should be paid to the high prevalence of cryptococcosis as revealed by us. We also emphasised the importance of blood glucose control for infection prevention, especially among the elderly. Considering the retrospective nature of our research, we recommend further prospective multicenter studies on cryptococcosis in DM II patients.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

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