Isolates of Cryptococcus Neoformans from Non-HIV and Non-Transplant Hospitalized Patients

Yun Xi[1]* Donglin Zhu[1] Jieming Dong[1] Fanhua Huang[1] Changzhi Xu[1] Gang Xiao[2]*

1. Department of Laboratory Medicine, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, 510630, China

2. Department of Laboratory Medicine, The Third Affiliated Hospital of South China Medical University, Guangzhou, Guangdong, 510630, China

Abstract: A retrospective cross-sectional study for patients with confirmed Cryptococcus neoformans meningitis (CM) in non-HIV-infected and non-transplant hosts in two class-A tertiary hospitals in Guangzhou, China is reported. 181 CM patients were enrolled during the study period, 48% (87/181) of which died. Underlying diseases were risk factor associated with higher mortality, among which diabetes mellitus ranked first for the incidence of CM. The mortality was not related to antifungal drug susceptibility. All strains were considered susceptible to amphotericin B, although interpretative breakpoints for amphotericin B have not yet been established. According to the CLSI guidelines, most of the strains in our study were susceptible to voriconazole, fluconazole, fluorocytosine and dose-dependently susceptible to itraconazle.

Keywords: Cryptococcus neoformans; Drug resistance; Nosocomial infection

***Corresponding Author:** *Gang Xiao, Department of Laboratory Medicine, The Third Affiliated Hospital of South China Medical University, 183 Zhongshan dadao west, Guangzhou, Guangdong, China Email: *xiaogang*2993*@yeah.net*; *Yun Xi, Department of Laboratory Medicine, The Third Affiliated Hospital of Sun Yat-sen University, 600 Tianhe road, Guangzhou, Guangdong, China Email: *xiyun*1993@163.*com*

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1. Introduction

Cryptococcus neoformans is a ubiquitous encapsulated human yeast pathogen causing infections ranging from asymptomatic pulmonary colonization to the life threatening meningoencephalitis, mainly in patients with cellular immune defects, such as those with acquired immunodeficiency syndrome (AIDS).[1] This pathogen is estimated to cause one million annual cases globally and nearly 625,000 deaths/year.[2] It has been well reported that Cryptococcosis frequently occurs in two risk groups: 1) human immunodeficiency virus (HIV)–infected individuals, 2) organ transplant recipients. However, we report a retrospective cross-sectional study for patients with confirmed Cryptococcus neoformans meningitis (CM) in non–HIV-infected and non-transplant hosts in two class-A tertiary hospitals in Guangzhou, Guangdong, China.

2. Methods

2.1 Patients

Patients were from the Third affiliated hospital of Zhongshan university and the Third affiliated hospital of Southern medical university, both of which are tertiary medical center located in Guangzhou, the largest city in south China. Cryptococcal antigen was measured in serum and CSF samples using IMMY CrAg® LFA Cryptococcal Antigen Lateral Flow Assay (Immuno-Mycologics, USA). During the period from January 2011 to May 2015, patients diagnosed with CM were recruited in this study. CM diagnosis was established on the basis of the presence of symptoms and/or signs of meningitis, and a CSF positive for India ink. Brain computed tomography and/or magnetic resonance imagings were performed dependent on the physician's decision. Medical records for all CM cases were manually reviewed. Only the first CM episode of each

patient during the study period was included for statistical analysis. We excluded the patients presented any of the following features: HIV-positive, incomplete data.

2.2 Microbiological Testing of Cryptococcus Isolates

Cerebrospinal fluid (CSF) samples were collected for white blood cell counts, glucose and protein concentrations, India ink stain and culture. The CSF and blood specimens were cultured using the BACTEC 9120 system (Becton Dickinon, USA). When the system notified the presence of presumptive positive vials, a gram staining was performed and a yeast-like organism was observed. Identification was performed by culture on Sabouraud agar plates and analyzed using MicroScan walkAway-96 (Siemens AG, German). The drug susceptibility tests were accomplished using Biomerieux (France) yeast susceptibility cards. Briefly, after incubation on Sabouraud agar plates at 35°C for 72 h, the standardized 2.0 McFarland inoculum suspension was prepared and placed into a yeast susceptibility test card for each organism. The yeast suspensions were diluted appropriately, after which the cards were filled, incubated, and read. The time of incubation varied from 12 to 24 h, based on the rate of growth in the drug-free control well. The drug susceptibility was expressed as minimal inhibitory concentration (MIC) in micrograms per milliliter. Quality control was conducted by testing the strains C.parapsilosis ATCC 2209 or C. krusei 6258 as recommended by CLSI. These isolates were tested between 21 and 26 times in the two laboratories, and all MICs were in the reference ranges respectively.

3. Statistics

The X2 or Fisher's exact test was employed for comparisons of attribute data between groups using the software SPSS (version 17).

3.1 Results

181 CM patients were enrolled during the study period, of which 87 were male and 57 were female. The age of patients ranges from 13 to 74 years old (Table 1). Before admission, of the CM patients, 84/181 (46.4%) had fever and 31/181 (17.1%) had cough. Forty-eight (26.5%) patients had diabetes mellitus, 36 (19.9) were diagnosed hepatitis and 23 (12.7%) were receiving anti-tuberculosis therapy (Table 2). Among thirty-seven (20.4) patients, no underlying disease was found. Although CM was diagnosed in 7 HIV-positive patients, these patients were excluded in this study. Because when HIV positive was confirmed, the patients were routinely transferred to specialized infectious diseases hospital, so the data associated with HIV-positive patients in these two hospitals were not typical.

Table 1. Age of Patients

Age (years)	n=181 (%)
<19	22 (12.2)
19 - 45	48 (26.5)
45 - 65	93 (51.4)
>65	18 (9.9)

Table 2. Distribution of Cryptococcal Meningitis in Terms of the Underlying Diseases

Underlying disease	n = 181 (%)
Diabetes mellitus	48 (26.5)
Hepatitis	36 (19.9)
Tuberculosis	23 (12.7)
Renal diseases	16 (8.8)
Autoimmune diseases	13 (7.1)
Malignancies	8 (4.4)
non-basic disease	37 (20.4)

Table 3. A total of 234 Isolates Were Recovered and Tested for Drug Susceptibility from 181 Patients

	N=234(%)		
	R	I	S
5-flucytosine	10 (4.3)	12 (5.1)	212 (90.7)
Lipid-amphotericin B	0	0	234 (100)
Fluconazole	14 (6.0)	32 (13.7)	188 (80.4)
Itraconazole	32 (13.8)	79 (33.8)	123 (52.6)
Voriconazole	4 (1.7)	3 (1.2)	227 (96.9)

Notes: *R, Resistant; I, Intermediate; S, Sensitive.*

In this study, 48% (87/181) of CM patients died. Underlying diseases were risk factor associated with higher mortality. More patients in the underlying diseases group had fever than those in the non-underlying diseases group. The mortality was not related to antifungal drug susceptibility. And there was no difference in chest imaging. Table 4 summarized the major clinical findings in both groups of patients with and without underlying diseases.

Table 4. Comparison of Clinical Characteristics between CM Patients with and without Underling Diseases (%)

Varialbes	with underlying diseases (n=144)	non-underlying diseases (n=37)	P
CSF CrAg positive	53/68(77.9)	14/25(56.0)	0.251
Fever	73/144(50.7)	11/37(29.7)	<0.001
Cough	23/144(16.0)	8/37(21.6)	1
Chest CT positive	113/144(78.4)	28/37(75.7)	1
Survival	71/144(49.3)	23/37(62.2)	0.037

3.2 Discussion

We used clinical data and isolates collected in two class-A tertiary hospitals in Guangzhou city of Southern China to analyze Cryptococcus distribution in the medical settings and the clinical presentations. Left untreated, CM is a uni-

formly fatal disease, even with antifungal treatment. The prognosis is influenced by factors associated with fungal species, underlying diseases, or the host status.[3] Diabetes mellitus (DM) is a group of metabolic diseases with high blood sugar levels over a prolonged period. DM is very common globally and known to suppress the cell mediated immunity and increases the frequency of infections.[4, 5] Infectious diseases in diabetic patients are always more severe than in non-diabetic ones, as observed in this study. However, there are some limitations to this study. Hepatitis patients also constitute a large portion of infections in the study. Because the Third affiliated hospital of Zhongshan university is famous for its liver diseases department, we have much more hepatitis patients than other hospitals of the same rank, which resulted in a bias in the statistics associated with underlying diseases.

In this study, all strains were considered susceptible to amphotericin B, although interpretative breakpoints for amphotericin B have not yet been established, due to a lack of correlation between in vitro and in vivo results.[6] Current data suggest that the CLSI M27-A methodology does not permit reliable detection of amphotericin B resistance.[7] According to the CLSI guidelines, most of the strains in our study were susceptible to voriconazole, fluconazole , fluorocytosine and dose-dependently susceptible to itraconazle.

4. Conclusion

In contrast to Candida albicans, there is only limited reported experience of resistance testing for Cryptococcus neoformans. Resistance in C.neoformans clinical isolates remains uncommon. But we observed that MICs increased in serial isolates, supporting the paradigm that resistance may evolve during antifungal therapy. However, we also observed that some fluconazole-resistant isolates with increased MIC values remained susceptible to another triazole agent, such as itraconazole.

We provide evidence for the understanding of the fungal pathogen and parameters potentially useful for the management of the diseases it causes.

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