Risk Factors for Cryptococcal Meningitis Recurrence in Human Immunodeficiency Virus (HIV)-Infected Patients in a Large Chinese Acquired Immune Deficiency Syndrome (AIDS) Treatment Center

Background: Cryptococcal meningitis (CM) is one of the most common opportunistic neuroinfections in patients with HIV. Most studies have focused on non-HIV CM and there are only a few studies on HIV CM in China. The purpose of the present study was to evaluate the characteristics and risk factors for CM recurrence in patients infected with HIV in the Chongqing Public Health Treatment Center in China.

Material/Methods: From January 2014 to December 2017, all patients with CM aged 18 years or older were enrolled and a case-control study was performed to determine the risk factors associated with recurrence of CM. Antimicrobial susceptibility was determined with a fungal drug sensitivity kit and the sequence types (STs) were analyzed with multilocus sequence typing.

Results: The incidence of CM in the 5185 HIV-infected patients was 3.5% (179). Follow-up data were available for 82 of the patients for whom complete medical records were available and they were included in the present study. There were 7 STs among 82 Cryptococcus neoformans isolates; ST5 and ST31 were the most prevalent genotypes. Testing showed that C. neoformans had high sensitivity to 5 antifungal drugs and no differences in resistance were observed, even when different STs were tested. Risk factors for recurrence were analyzed in 69 patients, excluding those who died. The results of multivariate analysis showed that only hospital stay was associated with recurrence of CM.

Conclusions: Our results indicated that combining education about medication with clinical treatment could help prevent recurrence of CM.

Keywords: Cryptococcus neoformans • Drug Resistance, Fungal • Meningitis, Cryptococcal • Multilocus Sequence Typing • Population Characteristics

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Background

Cryptococcal meningitis (CM) is one of the most common opportunistic infections in patients with immunodeficiency, especially those who are infected with HIV. CM is the second most common opportunistic neuroinfection in patients with HIV [1]. A study estimated that in low-income countries, 1-year mortality after CM was 70% (uncertainty interval 59-81) in those who received care and 100% in those who did not receive care [2-4]. The lethality of cryptococcosis is high, even with routine use of antifungal drugs. The 1-year mortality rate is 20% in those who receive care and 30% in those who do not receive care in the United States. Annual deaths from CM are estimated at 181 100 (95% confidence interval [CI] 119 400 to 234 300) and CM accounts for 10% to 15% of AIDS-related mortality [2,5]. Although global improvements have been made in access to antiretroviral therapy (ART) and most patients consent to medical treatment, the number of HIV-infected people with a CD4 count $<100$ cells/μL is still substantial: 20% to 25% among those presenting for care [6]. The global prevalence of cryptococcal antigenemia in HIV-seropositive individuals with CD4 cell counts $<100$ cells/μL is estimated at 6%, with 223 100 (95% CI, 150 600 to 282 400) incident cases of CM occurring annually [2].

Cryptococcus also can infect HIV-negative patients, leading to severe meningitis, liver cirrhosis, diabetes mellitus, solid organ transplantation, malignancies, rheumatologic diseases, corticosteroid use, and chronic kidney disease [7-9]. Previous studies have reported that immunocompromised Chinese patients are prone to cryptococcal infection [10]. Most studies have focused on non-HIV-associated CM and there are only a few studies on HIV-associated CM in China. According to data from the Chinese Center for Disease Control and Prevention, the number of AIDS cases and deaths in China has risen in recent years. Therefore, in the present study, we analyzed CM in AIDS patients in the largest hospital dedicated to care for AIDS patients in the United States. An estimated 1.1 million people were diagnosed with HIV infection in the United States in 2018 [11]. Annual deaths in the United States from CM are estimated at 181 100 (95% confidence interval [CI] 119 400 to 234 300) and CM accounts for 10% to 15% of AIDS-related mortality [2,5]. Although global improvements have been made in access to antiretroviral therapy (ART) and most patients consent to medical treatment, the number of HIV-infected people with a CD4 count $<100$ cells/μL is still substantial: 20% to 25% among those presenting for care [6]. The global prevalence of cryptococcal antigenemia in HIV-seropositive individuals with CD4 cell counts $<100$ cells/μL is estimated at 6%, with 223 100 (95% CI, 150 600 to 282 400) incident cases of CM occurring annually [2].

Current non-culture methods of diagnosing CM can produce false-positive and false-negative results. To ensure the accuracy of our study, we chose the culture method as the criterion standard. HIV-infected patients were diagnosed with CM disease if their CSF culture was positive for Cryptococcus. Recurrence was defined as a positive CSF culture after at least 2 negative cultures, with a 30-day asymptomatic period [13,14]. Patients with CM were screened for cryptococcal antigen, and to prevent development of cryptococcal disease, those who were positive for cryptococcal antigen were preemptively treated with antifungal therapy. According to the Chinese expert consensus on CM, treatment with amphotericin B (AMB) combined with fluocytosine (5-FC) is recommended during the induction period and fluconazole (FLU) during the consolidation period.

Material and Methods

Patients and Data Collection

All patients with CM aged 18 years or older admitted to the Chongqing Public Health Treatment Center from January 1, 2014 to December 31, 2017 were enrolled. Located in Chongqing, it is the largest AIDS treatment center in China, with 160 beds. Patients were included in the study if they were HIV-positive; had culture-proven Cryptococcus species-positive cerebrospinal fluid (CSF); had clinical features of meningitis and HIV positivity; had a first onset of CM during the study period; and agreed to be followed up once a month for 1 year and every 3 months for an additional year if they had a recurrence of CM. Data collected from electronic medical records included age, sex, history of underlying disease, presenting clinical symptoms, and laboratory results.

The present study was approved by the Ethics Committee of Chongqing Public Health Center and was registered with the hospital registration system for clinical trials (2018-006KY).

Diagnostics and Treatment Regimens

HIV screening and confirmation were performed using an enzyme-linked immunosorbent assay (ELISA) and western blot. Patients were diagnosed as HIV-positive if their blood samples were positive on both tests. The tests were performed following standard laboratory procedures from the Chinese Center for Disease Control and Prevention. Clinical staging of HIV infection was defined based on the diagnosis and treatment of AIDS in China (2018). In accordance with Chinese policy, all HIV-positive patients who met national treatment guidelines of a CD4 cell count $<350$ cells/mm$^3$ or had World Health Organization stage III or IV disease were eligible to receive ART [11,12].

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Strains and Drug Resistance Assays

All clinical isolates were obtained from patients suspected of having CM. Identification of Cryptococcus was carried out with a bacterial detection system. Then, the isolates were stored as glycerol stocks and cultured on yeast peptone dextrose medium before they were used [15]. In strict accordance with the manufacturer’s instructions, a Sensititre Yeastone Colorimetric Antifungal Susceptibility Panel (Trek Diagnostic Systems, Thermo Fisher Scientific, West Sussex, UK) was used to test for AMB, 5-FC, FLU, voriconazole (VOR), and itraconazole (ITR) susceptibility. Candida parapsilosis ATCC 22019 was used as the control strain for the susceptibility assays. Based on
domestic and foreign research reports, we set the epidemiological cut-off values (ECVs) at 8 mg/L for 5-FC and FLU, 0.25 mg/L for ITR and VOR, and 1 mg/L for AMB for *Cryptococcus neoformans* [16-18]. In the present study, isolates with minimum inhibitory concentration values above the ECVs were considered resistant.

**Multilocus Sequence Typing**

Genomic DNA was extracted following the manufacturer's instructions for the Fungal Genomic DNA Extraction kit (Solarbio, Beijing, China). Multilocus sequence typing (MLST) analysis consists of 7 unlinked loci, including 6 housekeeping genes (*CAP59, SOD1, GPD1, LAC1, PLB1* and *URA5*) and the non-coding region *IGS1*. Both forward and reverse amplicons of each locus were amplified according to the International Society for Human and Animal Mycology consensus MLST scheme for *Cryptococcus* and then subjected to bidirectional sequencing for all isolates [19]. All sequences were submitted to the *C. neoformans/C. gattii* species complex database (http://mlst.mycologylab.org) and an allele number was assigned to each of them. Seven allele type numbers and a sequence type (ST) number were given to each specimen after being compared to the MLST database website. The MLST data were analyzed with the hierarchical clustering algorithm in PHYLOVIZ 2.0 software (http://www.phyloviz.net/) to construct a rooted tree (dendrogram) that reflected the structure present in a pairwise dissimilarity matrix. At each step, the nearest 2 clusters were combined into a higher-level cluster. The Hamming distance was used as a measure of genetic distance.

**Statistical Analysis**

All analyses were performed using the Statistical Package for the Social Sciences software, version 17.0 (SPSS Inc., Chicago, Illinois, United States). Data were expressed as numbers and percentages for categorical variables and continuous variables with normal distribution were presented as mean±standard deviation; non-normal variables were reported as medians with interquartile ranges. In a first analysis, an independent-sample t test or Mann-Whitney U-test was used to compare the continuous variables between groups and a chi-square test or Fisher’s exact test was used to compare categorical variables between groups. In a second assessment, we used logistic regression in univariate and multivariate analyses to evaluate risk factors affecting the recurrence of CM patients. Variables with *P*≤0.05 in the univariate analysis were included in the multivariate analysis. The results were evaluated with odds ratios and 95% CIs, and *P*≤0.05 was considered statistically significant.

**Results**

During the present study, records from 5185 HIV-infected patients at the center were examined. In total, 179 of them (3.5%) who had a CSF culture positive for *Cryptococcus* species were diagnosed with CM. A total of 174 cases involved *C. neoformans*, 2 involved *Cryptococcus laurentii*, and 3 involved both *C. neoformans* and *C. laurentii*. Among the patients with complete medical records, 101 cases were considered first onset; for 19 cases, follow-up information was incomplete. Among the 82 cases analyzed in the present study, 5 patients died in the hospital, 8 patients died within 1 year without recurrence, and 32 patients experienced recurrence (*Figure 1*). The recurrence rate was 39.0%. Twenty-seven patients had 1 recurrence, 4 had 2, and 1 had 3 recurrences. Seven patients with recurrence died within 1 year after it. In the present study, the mortality rate within 1 year after first onset of CM was 24.4% (20/82). All 82 patients with CM were *C. neoformans*-positive, 2 of whom were coinfected with *C. neoformans* and *C. laurentii*. We included all of the *C. neoformans* isolates collected in the drug sensitivity and MLST experiments. In the analysis of risk factors for recurrence, we excluded fatal cases and included 69 cases.

**Figure 1.** Flowchart showing the flow of patients through the study.

![Flowchart showing the flow of patients through the study.](image-url)
Demographic and clinical characteristics of patients with CM who had first onset or recurrence are shown in Table 1. Regardless of recurrence or non-recurrence, the majority were men. At first admission, the mean age of patients with recurrence was greater than that of patients without recurrence. Except for cough, hospital stay, and length of medication continuation after discharge, there were no significant differences in other characteristics between the first-onset and recurrence groups. Univariate analysis was performed to determine whether demographic and abnormal clinical characteristics were risk factors for recurrence of CM (Table 2). Except for cough and liver disease, there was no correlation between clinical symptoms and recurrence. The statistical analysis showed that fungal resistance was not associated with recurrence. In the present study, time-related indicators (hospital stay, duration of medication use after discharge) were associated with recurrence of CM. A multivariate analysis of risk factors was undertaken after adjustment of the logistic regression model for liver disease, cough, hospital stay, and duration of medication use. The results showed that only hospital stay was an independent risk factor for recurrence of CM (Table 3).

The results of the univariate and multivariate analyses indicated that time of administration was an important risk factor for recurrence of CM. Therefore, we divided the total medication time for patients with CM recurrence into 2 groups: >75 days and <75 days. Then, time to recurrence after treatment termination was analyzed, as shown in Figure 2. There were 21 patients with recurrence whose total medication time was <75 days. Of them, 4 (19.05%), 7 (33.33%), 2 (9.52%), and 2 (9.52%) patients presented with recurrence in the 1st, 2nd, 3rd, and 4th months after discharge, respectively. There were 11 patients with recurrence whose total medication time was >75 days. Of them, 2 (18.18%), 2 (18.18%), and 3 (27.27%) patients experience recurrence in the 7th, 8th, and 9th months after discharge, respectively.

MLST has been established as the criterion standard for identifying the major molecular types or populations of C. neoformans and the standardized typing technique is convenient for comparison of the prevalence of Cryptococcus worldwide. The phylogenetic tree of 82 C. neoformans isolates is shown in Figure 3. The results showed that all of the STs belonged to molecular type VNI. ST5 (44) and ST31 (24) were the most common genotypes, followed by ST191 (8), ST7 (3), ST359 (1), ST63 (1) and ST38 (1). Antifungal drug susceptibility results are presented in Table 4. C. neoformans was highly susceptible to the antifungal drugs tested. The MLSTs of the 5 C. neoformans isolates from the patients with recurrence were different from those from the first episode. Among the strains with the same ST, 3 strains in the patients with recurrence were found to be resistant to AMB.

Discussion

Previous studies from other regions have shown that most patients with CM are HIV-positive [2,5]. In China, most study results and viewpoints suggest that immunocompetent individuals are the most likely to develop CM, and researchers believe that the Chinese population may be more susceptible to cryptococcal infections than other ethnic populations [20-22]. The null hypothesis was that HIV patients have a low incidence of CM, but related studies are limited. During the present study, 3.5% of HIV-positive inpatients with a CSF culture positive for Cryptococcus species were diagnosed with CM. Similar to previous studies [23,24], C. neoformans was the dominant species. No C. gattii infections was found in our study because that species is more common in tropical and subtropical regions [25,26]. To our surprise, C. laurentii and C. neoformans coinfection, which previously has been reported, was observed [27]. The cause of coinfection was unclear, and further research may be helpful for development of clinical treatment strategies. In our study, 1 of the 2 coinfected patients died, and the other patient survived to 1-year follow-up without recurrence. All 79 medical records included in the risk factor analysis documented C. neoformans infection. The mortality rate within 1 year after first onset was 24.4%, which is similar to that in developed countries [2]. These results suggest that the infections were treated appropriately in our hospital and that timely and effective intervention significantly reduced the mortality of patients with CM.

AMB in combination with FC during the induction period followed by FLU during the consolidation period is the recommended antifungal regimen for management of acute CM [28]. In our center, only 39.1% of patients adhered to the recommended therapeutic regimen. Although the recurrence rate in patients after receiving the recommended treatment regimen was lower than that for those who did not follow the recommended treatment regimen, there was no significant difference in the recurrence rate between the patients who followed the recommended treatment regimen and those who did not. The reason may be an insufficient number of samples; additional samples will help us obtain more accurate results. Moreover, cryptococcal resistance was found to be associated with recurrence in other reports [29,30], in contrast with the results of our study. This may be because there were few resistant cryptococci. In the present study, the rate of Cryptococcus resistance was lower than that in previous reports [31-33]. Antifungal susceptibility tests have become essential tools for guiding treatment of fungal diseases, determining local and global epidemiology of diseases, and identifying resistance to antifungals. Therefore, we must pay attention to resistance of cryptococci when treating CM.
Table 1. Demographic and clinical characteristics of 69 cryptococcal meningitis patients with first onset or recurrence.

| Characteristics                        | First onset (n=37) | Recurrence (n=32) | P     |
|----------------------------------------|-------------------|-------------------|-------|
| Age (years)                            | 41.4±14.2         | 46.3±16.7         | 0.196 |
| Sex (Male/Female)                      | 26/11             | 26/6              | 0.291 |
| Underlying diseases                    |                   |                   |       |
| Diabetes                               | 0                  | 3 (9.4)           |       |
| Malignant tumor                        | 11 (29.7)         | 10 (31.3)         | 0.891 |
| Autoimmune diseases                    | 4 (10.8)          | 1 (3.1)           | 0.446 |
| Cryptococcal pneumonia                 | 9 (24.3)          | 14 (43.8)         | 0.088 |
| Infection in other areas               | 33 (89.2)         | 26 (81.3)         | 0.554 |
| Kidney disease                         | 1 (2.7)           | 2 (6.3)           | 0.898 |
| Liver disease                          | 4 (10.8)          | 10 (31.3)         | 0.071 |
| Presenting clinical symptoms           |                   |                   |       |
| Headache                               | 34 (91.9)         | 28 (87.5)         | 0.839 |
| Dizziness/vertigo                      | 6 (16.2)          | 4 (12.5)          | 0.925 |
| Fever                                  | 30 (81.1)         | 26 (81.3)         | 0.986 |
| Vomiting                               | 13 (35.1)         | 9 (28.1)          | 0.533 |
| Cough                                  | 16 (43.2)         | 22 (68.8)         | 0.034 |
| Abnormal vision                        | 2 (5.4)           | 3 (9.4)           | 0.866 |
| Abnormal hearing                       | 0 (0)             | 1 (3.1)           | 0.464 |
| Disturbance of consciousness           | 2 (5.4)           | 0 (0)             | 0.495 |
| Altered mental status                  | 4 (10.8)          | 3 (9.4)           | 1.000 |
| Weakness                               | 11 (29.7)         | 13 (40.6)         | 0.343 |
| Laboratory data                        |                   |                   |       |
| CD4/CD8                                | 0.12 (0.01-0.39)  | 0.1 (0.01-0.4)    | 0.343 |
| CD4 >100 count/mm³                     | 5 (13.5)          | 3 (9.4)           | 0.874 |
| HIV viral load (log_{10} copy/mL)      | 5.18±0.89         | 5.20±0.85         | 0.906 |
| Resistance rate                        |                   |                   |       |
| Amphotericin B                         | 2 (5.4)           | 6 (18.8)          | 0.177 |
| Fluconazole                            | 3 (8.1)           | 4 (12.5)          | 0.839 |
| 5-fluorocytosine                       | 2 (5.4)           | 4 (12.5)          | 0.539 |
| Itraconazole                           | 3 (8.1)           | 6 (18.8)          | 0.342 |
| Voriconazole                           | 6 (16.2)          | 7 (21.9)          | 0.549 |
| Treatment plan during hospitalization  |                   |                   |       |
| Drug combination                       | 25 (67.6)         | 22 (68.8)         | 0.916 |
| Use of recommended chemotherapy regimens| 17 (45.9)        | 11 (34.4)         | 0.329 |
| Hospital stay (d)                      | 41.5±16.1         | 28.1±10.8         | <0.001|
| Fluconazole treatment after discharge  | 33 (89.2)         | 29 (90.6)         | 1.000 |
| Length of medication continuation after discharge | | | |
| <30 d                                  | 2 (5.4)           | 6 (18.8)          |       |
| 30-60 d                                | 19 (51.4)         | 21 (65.6)         |       |
| >60 d                                  | 16 (43.2)         | 5 (15.6)          | 0.023 |
| ART treatment                          | 32 (86.5)         | 28 (87.5)         | 1.000 |

ART – antiretroviral therapy.
| Risk factor                        | OR   | 95% CI         | P   |
|----------------------------------|------|----------------|-----|
| **Age**                          | 1.021| 0.989-1.054    | 0.194|
| **Sex**                          | 1.605| 0.510-5.051    | 0.419|
| **Diabetes**                     | –    | –              | 0.999|
| **Malignant tumor**              | 1.074| 0.385-3.002    | 0.891|
| **Autoimmune diseases**          | 0.266| 0.028-2.514    | 0.248|
| **Cryptococcal pneumonia**       | 2.420| 0.868-6.748    | 0.091|
| **Infection in other areas**     | 0.525| 0.134-2.058    | 0.355|
| **Kidney disease**               | 2.400| 0.207-27.781   | 0.483|
| **Liver disease**                | 3.750| 1.044-13.472   | 0.043|
| **Headache**                     | 0.618| 0.127-2.994    | 0.550|
| **Dizziness/vertigo**            | 0.738| 0.189-2.899    | 0.663|
| **Fever**                        | 1.011| 0.301-3.392    | 0.986|
| **Vomiting**                     | 0.722| 0.259-2.012    | 0.534|
| **Cough**                        | 2.887| 1.072-7.778    | 0.036|
| **Abnormal vision**              | 1.810| 0.283-11.579   | 0.866|
| **Abnormal hearing**             | –    | –              | 1.000|
| **Disturbance of consciousness**| –    | –              | 0.999|
| **Altered mental status**        | 0.853| 0.176-4.135    | 0.844|
| **Weakness**                     | 1.617| 0.597-4.384    | 0.345|
| **CD4/CD8**                      | 0.112| 0.001-9.942    | 0.338|
| **CD4 >100 count/mm³**           | 0.662| 0.145-3.018    | 0.594|
| **HIV viral load**               | 1.035| 0.594-1.802    | 0.904|
| **Amphotericin B**               | 4.038| 0.754-21.643   | 0.103|
| **Fluconazole**                  | 1.619| 0.334-7.847    | 0.550|
| **5-fluorocytosine**             | 2.500| 0.426-14.657   | 0.310|
| **Itraconazole**                 | 2.615| 0.597-11.455   | 0.202|
| **Voriconazole**                 | 1.447| 0.431-4.856    | 0.550|
| **Drug combination**             | 1.056| 0.382-2.917    | 0.916|
| **Use of recommended chemotherapy regimens** | 0.616 | 0.232-1.633 | 0.330 |
| **Hospital stay**                | 0.928| 0.888-0.970    | 0.001|
| **Fluconazole treatment after discharge** | 1.172 | 0.242-5.677 | 0.844 |
| **Length of medication continuation after discharge** | 0.970 | 0.949-0.992 | 0.009 |
| **ART treatment**                | 1.094| 0.267-4.476    | 0.901|

ART – antiretroviral therapy; CI – confidence interval; OR – odds ratio.
Our results showed that only hospital stay was an independent risk factor for recurrence of CM. Considering the follow-up data, we believe that a short hospitalization time and limited medication use outside the hospital resulted in the low compliance rates in patients. The reasons for poor medication compliance were as follows: patients mistakenly believed that they had fully recovered because their symptoms had improved and they were concerned about the cost of the treatment. There may be additional reasons for noncompliance; however, poor adherence to antifungal drug prophylaxis leading to recurrence has been reported in previous research [34]. The costs of antifungal drug treatment result in a heavy economic burden for patients, regardless of hospitalization or discharge. In contrast, patients generally have good compliance with free ART drugs. These results suggest that economic support was an important factor in treatment.

In our study, ST5 and ST31 were the most common epidemic genotypes, similar to results from domestic research reports [16,35]. Notably, in the adjacent province of Sichuan, the ST5 prevalence is 82.9% (34/41) [36]. In contrast to international reports [37,38], our results suggest that there were regional differences in genotypes. MLST genotypes are helpful in identifying the source of Cryptococcus infection. For the same incident and recurrent CM MLST genotypes, the recurrent cryptococcal strain may not be eradicated because of treatment failure during the initial infection. There has been no research on the genetic relationships between incident and recurrent strains in China. In our study, 84.4% of patients with recurrence (27/32) were positive for the same MLST genotype as in the first onset. Because most are epidemic genotypes, we cannot exclude secondary infection by the same genotype. However, at the same time, we analyzed the time of recurrence and inferred that patients with total medication times <75 d had shorter recurrence intervals than those with total medication.

### Table 3. Multivariate analysis of risk factors for recurrence in patients with cryptococcal meningitis.

| Risk factors                             | B    | OR   | 95% CI          | P   |
|------------------------------------------|------|------|-----------------|-----|
| Liver disease                            | 1.272| 3.570| 0.802-15.878    | 0.095|
| Cough                                    | 0.834| 2.303| 0.696-7.617     | 0.172|
| Hospital stay                            | -0.066| 0.936| 0.890-0.984     | 0.010|
| Length of medication use after discharge | -0.024| 0.977| 0.953-1.001     | 0.065|

B – coefficient of logistic regression analysis; CI – confidence interval; OR – odds ratio.

### Figure 2. Distribution of patients with recurrence by month after discharge. The patients with recurrence were divided into 2 groups according to total medication time. Of the patients, 21 had total medication times >75 d and 11 had total medication times <75 d.

### Figure 3. Maximum likelihood phylogenetic analysis of Cryptococcus neoformans.

### Table 4. Antifungal drug susceptibility results (mg/L).

| Antifungal agent | MIC<sub>90</sub> | MIC<sub>50</sub> |
|------------------|------------------|------------------|
| Amphotericin B   | 2                | 0.25             |
| Itraconazole     | 0.5              | ≤0.125           |
| Voriconazole     | 1                | ≤0.125           |
| Fluconazole      | 8                | 2                |
| 5-fluorocytosine | 4                | 0.5              |

MIC – minimum inhibitory concentration.
times >75 days. These results suggest that most patients with recurrence were not completely cured. C. neoformans also can form a biofilm on medical devices, which protects the pathogens from attack by the host immune system and promotes fungal drug resistance, potentially leading to infection recurrence. In addition, patients with AIDS have a low immune clearance ability. Therefore, sustained antifungal therapy is necessary for CM in patients with AIDS. When treating these individuals, patient education should be emphasized throughout the process, including the induction and consolidation periods. In our study, only 5 patients with recurrence had different cryptococcal MLST genotypes from those in the first onset, suggesting that their recurrent CM may have been due to a secondary infection.

Our study had the following limitations. First, the clinical information and isolates were collected from a single center and the sample size was small. Second, only a 4-year period was considered, which was short.

Conclusions

In summary, the present study showed that C. neoformans clinical isolates from patients with AIDS had high drug sensitivities and a good therapeutic effect was achieved with standardized treatment. Recurrence was mainly related to the duration of standardized treatment. Combining education about medication with clinical treatment will promote enhanced therapeutic effects.

References:

1. Currie BP, Casadevall A. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. Clin Infect Dis. 1994;19(6):1029-33
2. Rajasingham R, Smith RM, Park BI, et al. Global burden of disease of HIV-associated cryptococcal meningitis: An updated analysis. Lancet Infect Dis. 2017;17(8):873-81
3. Milanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adequate support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: An open-label, randomised controlled trial. Lancet. 2015;385(9983):2173-82
4. Govender NP, Roy M, Mendes JF, et al. Evaluation of screening and treatment of cryptococcal antigenemia among HIV-infected persons in Soweto, South Africa. HIV Med. 2015;16(8):468-76
5. Pyngos V, Seltz AE, Steiner CA, et al. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PLoS One. 2013;8(2):e56269
6. Lahueruta M, Wu Y, Hoffman S, et al. Advanced HIV disease at entry into HIV care and initiation of antiretroviral therapy during 2006-2011: Findings from four sub-saharan African countries. Clin Infect Dis. 2014;58(3):432-41
7. Shen L, Zheng J, Wang Y, et al. Increased activity of the complement system in cerebrospinal fluid of the patients with Non-HIV Cryptococcal meningitis. BMC Infect Dis. 2017;17(1):7
8. Qu J, Zhuo T, Zhong C, et al. Comparison of clinical features and prognostic factors in HIV-negative adults with cryptococcal meningitis and tuberculous meningitis: A retrospective study. BMC Infect Dis. 2017;17(1):51
9. Liao CH, Chi CY, Wang YJ, et al. Different presentations and outcomes between HIV-infected and HIV-uninfected patients with Cryptococcal meningitis. J Microbiol Immunol Infect. 2012;45(4):296-304
10. Yuchong C, Fubin C, Jianghan C, et al. Cryptococcus in China (1985-2010): Review of cases from Chinese database. Mycopathologia. 2012;173(5-6):329-35
11. Hao Y, Sun H, Xe Y, et al. Progress of the National Pediatric Free Antiretroviral Therapy program in China. AIDS Care. 2010;22(10):1182-88
12. Gils C, Crowley S, Ekpini R, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet. 2006;368(9534):505-10
13. Powderly WG, Saag MS, Cloud GA, et al. A controlled trial of fluconazole or amphotericin B to prevent relapse of cryptococcal meningitis in patients with the acquired immunodeficiency syndrome. The NIAID AIDS Clinical Trials Group and Mycoses Study Group. N Engl J Med. 1992;326(12):793-98
14. Bicanic T, Harrison T, Niepieklo A, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis: An international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. Antimicrob Agents Chemother. 2012;56(6):3107-13
15. Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, et al. Cryptococcus neoformans-Cryptococcus gattii species complex: An international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. Antimicrob Agents Chemother. 2012;56(6):3107-13
16. Hong N, Chen M, Xu N, et al. Genotypic diversity and antifungal susceptibility of Cryptococcus neoformans isolates from paediatric patients in China. Mycoses. 2019;62(2):171-80
17. Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, et al. Cryptococcus neoformans-Cryptococcus gattii species complex: An international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for Cryptococcus neoformans-Cryptococcus gattii species complex using the CLSI M27-A3 broth microdilution method. Antimicrob Agents Chemother. 2015;59(1):666-68
18. Prakash A, Sundar G, Sharma B, et al. Genotypic diversity in clinical and environmental isolates of Cryptococcus neoformans from India using multilocus microsatellite and multilocus sequence typing. Mycoses. 2020;63(3):284-93
19. Liu Y, Kang M, Wu SY, et al. Different characteristics of cryptococcal meningitis between HIV-infected and HIV-uninfected patients in the Southwest of China. Med Mycol. 2017;55(3):255-61
20. Chen J, Varma A, Diaz MR, et al. Cryptococcus neoformans strains and infection in apparently immunocompetent patients, China. Emerg Infect Dis. 2006;12(5):755-62
21. Sloan DJ, Parris V. Cryptococcal meningitis: Epidemiology and therapeutic options. Clin Epidemiol. 2014;6:169-82
22. Fang W, Fa Z, Liao W. Epidemiology of Cryptococcus and cryptococcosis in China. Fungal Genet Biol. 2015;78:7-15
23. Lawrence DS, Boyer-Chammard T, Jarvis JN. Emerging concepts in HIV-associated cryptococcal meningitis. Curr Opin Infect Dis. 2019;32(1):16-23
24. Chen SC, Meyer W, Sorrell TC. Cryptococcus gattii infections. Clin Microbiol Rev. 2014;27(4):980-1024
25. Lizarazo J, Escandon P, Agudelo CI, et al. Retrospective study of the epidemiology and clinical manifestations of Cryptococcus gattii infections in Colombia from 1997-2011. PLoS Negl Trop Dis. 2014;8(11):e3272
26. Krajden S, Summerbell RC, Kane J, et al. Normally saprobic cryptococcus isolated from Cryptococcus neoformans infections. J Clin Microbiol. 1991;29(9):1883-87
27. Stone NR, Rhodes J, Fisher MC, et al. Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. J Clin Invest. 2012;129(2):699-709
28. Musubire AK, Boulware DR, Meya DB, et al. Diagnosis and management of cryptococcal infection in apparently immunocompetent patients, China. Emerg Infect Dis. 2007;13(5):873-81
29. Jhamb R, Kashyap B, Das S, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis: Recurrent cryptococcal meningitis or Cryptococcus-related immune reconstitution inflammatory syndrome? Int J STD AIDS. 2014;25(5):369-72
31. Bongomin F, Oladele RO, Gago S, et al. A systematic review of fluconazole resistance in clinical isolates of Cryptococcus species. Mycoses. 2018;61(5):290-97
32. Worasilchai N, Tangwattanachuleeporn M, Meesilpavikkai K, et al. Diversity and antifungal drug susceptibility of Cryptococcus isolates in Thailand. Med Mycol. 2017;55(6):680-85
33. Chen YH, Yu F, Bian ZY, et al. Multilocus sequence typing reveals both shared and unique genotypes of Cryptococcus neoformans in Jiangxi Province, China. Sci Rep. 2018;8(1):1495
34. Jarvis JN, Meintjes G, Williams Z, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis in South Africa: The role of inadequate secondary prophylaxis. S Afr Med J. 2010;100(6):378-82
35. Dou H, Wang H, Xie S, et al. Molecular characterization of Cryptococcus neoformans isolated from the environment in Beijing, China. Med Mycol. 2017;55(7):737-47
36. Wu SY, Lei Y, Kang M, et al. Molecular characterisation of clinical Cryptococcus neoformans and Cryptococcus gattii isolates from Sichuan province, China. Mycoses. 2015;58(5):280-87
37. Rocha DFS, Cruz KS, Santos C, et al. MLST reveals a clonal population structure for Cryptococcus neoformans molecular type VNI isolates from clinical sources in Amazonas, Northern-Brazil. PLoS One. 2018;13(6):e0197841
38. Cogliati M, Zani A, Rickerts V, et al. Multilocus sequence typing analysis reveals that Cryptococcus neoformans var. neoformans is a recombinant population. Fungal Genet Biol. 2016;87:22-29