European Review for Medical and Pharmacological Sciences 2015; 19: 3801-3810

Clinical study of 23 pediatric patients with cryptococcosis

F.-L. LUO[1,2], Y.-H. TAO[1], Y.-M. WANG[1], H. LI[2]

[1]Department of Pediatrics, West China Second University Hospital, Sichuan University, Sichuan, China
[2]Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Sichuan, China

Abstract. – OBJECTIVE: ***Cryptococcus neoformans*** **is a common opportunistic infection in adults with acquired immunodeficiency syndrome. However, limited data exist for pediatric patients. The aim of this study was to describe the clinical characteristics of cryptococcosis in pediatric patients.**

PATIENTS AND METHODS: A retrospective study was conducted at West China Second University Hospital, Sichuan University. Data on risk factors, clinical manifestations, diagnosis methods, treatment, and prognosis were evaluated. Clinical characteristics associated with cryptococcal meningitis and disseminated cryptococcosis were compared.

RESULTS: Twelve cases of cryptococcal meningitis and 11 cases of disseminated cryptococcosis were identified. Seventeen cases had no underlying disease, but 6 cases had malnutrition. Only 6 cases had a history of exposure to pigeon droppings. No cases had pulmonary cryptococcosis alone. Coughing, pulmonary moist rales, swollen lymph nodes, liver and spleen, and skin rashes were seen only in disseminated cryptococcosis. The prevalence of headache and positive meningeal irritation sign was higher in cryptococcal meningitis than disseminated cryptococcosis. The proportion of increase in eosinophils was higher in disseminated cryptococcosis than cryptococcal meningitis. The primary methods used for diagnosis included cerebrospinal fluid ink staining, cerebrospinal fluid culture, blood culture, and lymph node biopsy. Two patients stopped treatment, and 21 patients received antifungal therapy. The mortality rate was 39.13%.

CONCLUSIONS: Cryptococcosis occurs in non-HIV infected and immunocompetent children. Cryptococcosis lacks specific clinical manifestations. Disseminated cryptococcosis commonly occurs in children. Clinicians should consider a diagnosis of disseminated cryptococcosis for children who present with unexplained long-term fever, mild cough, chest imaging inconsistent with clinical symptoms, lymphadenectasis, hepatosplenomegaly, and eosinophilia.

Key Words:
Cryptococcosis, Cryptococcal meningitis, Disseminated cryptococcosis, Children.

Introduction

Cryptococcosis is a common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS). Cryptococcosis is primarily caused by *Cryptococcus neoformans* infection[1]. *Cryptococcus neoformans* has three variants: the *Neoformans*, *Gert*, and *Shanghai* variants. Most of the clinical isolates in Chinese patients are the *Neoformans* variant[1]. The diversity of primary lesions and difference in invaded organs lead to complex and diverse clinical manifestations of cryptococcosis, difficulties in diagnosis, and high rates of misdiagnosis and fatality.

Cryptococcosis may occur in any age group, but it is more common in people 20 to 50 years old. Many large studies were conducted in adults, particularly in the HIV-positive population, but cryptococcosis is believed to be rare in children[2-4]. Previous case series have only reported on 23 or fewer pediatric patients[5-11]. The clinical characteristics of pediatric patients with cryptococcosis differ across reports, with obvious regional differences. Most of these studies also exclusively investigated children with HIV. Therefore, we performed a retrospective study to investigate the clinical characteristics of cryptococcosis in children.

Patients and Methods

Study Subjects

Pediatric patients who were diagnosed with cryptococcosis between January 1998 and January 2015 in West China Second University Hospital, Sichuan University were enrolled in this

Corresponding Author: Yuhong Tao, MD; e-mail: hxtyh@sina.com

study. Diagnostic criteria were referenced from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group[12]. Isolation of *Cryptococcus* in specimens from cerebrospinal fluid (CSF), blood, and other tissues or body fluids using ink smears, culture, and pathological methodologies was an important basis for diagnosis. All pathological specimens underwent hematoxylin and eosin (HE), mucicarmine, periodic acid Schiff, and methenamine silver staining. A pathological diagnosis of cryptococcosis was made if *Cryptococcus* was detected using any of these staining methods. Disseminated cryptococcosis was diagnosed if two non-adjacent organs were simultaneously affected with cryptococcosis.

Data Collection

"Cryptococcosis" and "*Cryptococcus*" were used as the keywords to collect data from hospital medical records and pathology and microbiology departments. Information on the treatment and prognosis of pediatric patients were collected using telephone follow-up. The following criteria were used to evaluate the therapeutic effect: "recovery" referred to the disappearance of all clinical signs and symptoms and the return of infection-related laboratory parameters to normal levels, and "improvement" referred to improvement in clinical signs and symptoms and infection-related laboratory indicators.

A database was established using Excel software (2007) with double entry of the following clinical data: age, gender, home address, course of disease, risk factors, history of exposure to animals, all signs, main symptoms, laboratory test results, imaging, pathology, misdiagnosis, treatment, and prognosis. Clinical characteristics associated with cryptococcal meningitis and disseminated cryptococcosis were compared.

Statistical Analysis

All data were analyzed using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). Continuous data are presented as the mean ± standard deviation or range. Continuous variables were analyzed using Student's *t*-test, and categorical variables were analyzed using the Chi-square test or Fisher's exact test if any expected value was below five. p value < 0.05 was considered statistically significant.

Results

Affected Organs

Twelve of the total of 23 patients had cryptococcal meningitis (52.17%), and 11 patients had disseminated cryptococcosis (47.83%). None of the patients had pulmonary cryptococcosis alone. The most commonly affected organs were the central nervous system (19 cases; 82.6%) and lungs (11 cases; 47.83%). The affected organs in the 11 children with disseminated cryptococcosis included the lung (11 cases), central nervous system (7 cases) and other organs, such as the lymph nodes (superficial, mediastinal, hilar, abdominal lymph nodes; 10 cases), liver (9 cases), spleen (7 cases), skin (2 cases), heart (1 case), kidney (1 case), and bone (1 case).

Demographic Data

The age of onset ranged from 1 year and 9 months to 13 years and 9 months: < 3 years old (2 cases), 3 to 6 years old (8 cases), and > 6 years old (13 cases). There were 19 boys (82.6%) and 4 girls (17.4%). Fourteen patients were from rural areas, and 9 patients were from urban areas.

Risk Factors

The risk factors included in the analysis were HIV infection, diabetes, use of corticosteroids, lung disease, kidney failure, organ transplant, lymphoma, primary immunodeficiency disease, rheumatic autoimmune disease, and malnutrition. Seventeen cases had no underlying diseases, and 6 cases had malnutrition.

Animal Contact History

A history of exposure to pigeons, horses, cows, dogs, cats, mountain antelope, pigs, and other animals was obtained for all patients. Only 6 cases (26.09%) had a history of exposure to pigeon droppings.

Reasons for First Diagnosis

Disease course before admission ranged from 3 days to 4 months, with an average of 31.5 days. The most common reason for the first diagnosis was fever (19 cases). The remaining reasons included headache (11 cases), vomiting (6 cases), coughing (6 cases), abdominal pain (3 cases), dizziness (2 cases), abdominal distension (2 cases), head rash (1 case), left neck mass (1 case), diplopia (1 case), and neck pain (2 cases).

Primary Symptoms and Signs

Cryptococcosis lacked specific symptoms and signs. The most common symptom was fever. Twenty-one cases had varying degrees of fever, including low fever (1 case), medium fever (8 cases), high fever (10 cases), and very high fever (2 cases). Other symptoms included headache (13 cases), vomiting (11 cases), cough (9 cases), seizures (7 cases), abdominal pain (3 cases), neck pain (2 cases), blurred vision (2 cases), drowsiness (2 cases), abdominal distension (2 cases), paralysis (2 cases), chest tightness (1 case), shortness of breath (1 case), changes in personality (1 case), weight loss (1 case), irritability (1 case), nonresponsiveness (1 case), blurred consciousness (1 case), coma (1 case), meningeal irritation positive (11 cases), hepatomegaly (9 cases), splenomegaly (7 cases), superficial lymph node enlargement (6 cases, including 2 cases of cervical lymph node enlargement and 4 cases of neck lymph node enlargement), positive Babinski sign (5 cases), pimples (5 cases), jaundice (3 cases), moist rales (3 cases), ascites (2 cases), scalp nodules (2 cases), and knee hyperreflexia (1 case).

Laboratory Tests

Total peripheral blood leukocyte counts were high ($> 15 \times 10^9$/L) in 9 patients ($16.8\text{-}44.6 \times 10^9$/L). The proportion of eosinophils was high in 7 cases (6.6-18.1%). C-reactive protein was high in 13 cases (12-180 mg/L). Hemoglobin was low in 14 cases (78-106 g/L), including 6 cases of moderate anemia (78-90 g/L), and 8 cases of mild anemia (90-106 g/L).

Liver function abnormalities were observed in 9 cases, including 5 cases of elevated alanine aminotransferase (83-281 IU/L), 5 cases of elevated aspartate aminotransferase (94-196 IU/L), and 4 cases of decreased albumin (16-29.3 g/L).

Twenty of the 21 pediatric patients who were examined for routine and biochemical tests of cerebrospinal fluid were abnormal. Nineteen of these cases had elevated nucleated cells, primarily lymphocytes, including 10 cases of $22\text{-}100\times10^6$/L, 6 cases of $100\text{-}500\times10^6$/L, and 3 cases of $500\text{-}1080\times10^6$/L. Eleven cases had increased protein levels, including 7 cases of 443-1000 mg/L, 3 cases of 1000-2000 mg/L, and 1 case of 2000-2429 mg/L). Fifteen cases had decreased glucose, including 5 cases of 0.15-1 mmol/L, 3 cases of 1-2 mmol/L, and 7 cases of 2-3.08 mmol/L, and 11 cases had decreased chloride (108.4-119.5 mmol/L).

Seven of the 11 pediatric patients with disseminated cryptococcosis underwent bone marrow examination. Five of these cases were normal, and 2 cases had an increased proportion of eosinophils (10.5% and 27.5%). One case had increased counts of eosinophils in peripheral blood and bone marrow.

Imaging

All pediatric patients underwent chest X-ray or CT examination. All 11 cases of disseminated cryptococcosis were abnormal, but no cases of cryptococcal meningitis were abnormal. Three the disseminated cryptococcosis cases had unilateral lesions, and 8 cases had bilateral lesions. Six cases had patchy lesions, and 5 cases had multiple small nodules. Five cases had interstitial lung disease, and 4 cases had pleural thickening. Four cases had a small amount of pleural effusion, and 9 cases had mediastinal lymph node enlargement. Eight cases had hilar lymph node enlargement, and 3 cases had axillary lymph node enlargement.

Six of the 10 pediatric patients with cryptococcal meningitis who underwent head CT or MRI scans were abnormal. One of the 3 pediatric patients with disseminated cryptococcosis who underwent head CT or MRI was abnormal. Five of these cases had ventricular dilatation, and 1 case had communicating hydrocephalus. One case had low density around the front and rear corners, and 1 case had low density on the right frontal lobe. One case had signal abnormalities in white matter on the anterior and posterior horns of the bilateral ventricles. One case had signal abnormalities in the right basal ganglia, periventricular white matter, and temporoparietal lobe, and 1 case had granuloma.

Seven of the 11 pediatric patients with disseminated cryptococcosis underwent abdominal CT scans (1 case simultaneously underwent abdominal MRI scan). Abnormal results were found in all patients, including 5 cases of hepatomegaly, 2 cases of splenomegaly, 2 cases of parenchymal low density lesions in the liver and spleen, 1 case of diffuse parenchymal nodules in the liver and spleen, 1 case of intrahepatic calcification, 2 cases of retroperitoneal lymphadenopathy, 2 cases of abdominal para-aortic lymphadenopathy, 1 case of portocaval space and inguinal lymph node enlargement, 1 case of lymph node enlargement in the hepatic portal, portocaval space, and mesenteric root, 2 cases of ascites, and 1 case of cystic low density in the left ilium. Five of the 11 pedi-

atric patients with disseminated cryptococcosis underwent abdominal ultrasound scanning. The results demonstrated that 4 cases had hepatomegaly, 2 cases had splenomegaly, 3 cases had retroperitoneal lymph node enlargement, and 2 cases had ascites.

Pathogenic Examination

Cryptococcus laurentii was identified in the blood and cerebrospinal fluid culture of 1 of the 23 pediatric patients. *Cryptococcus neoformans* was identified in the remaining 22 cases. Sixteen of the 20 specimens were positive on cerebrospinal fluid ink staining. Fifteen of the 20 cases had positive cerebrospinal fluid cultures. Five of the 12 patients with blood culture were positive. No statistically significant difference in the positive rates of cerebrospinal fluid ink staining, cerebrospinal fluid culture, or blood culture was found between the two groups. Electronic bronchoscopy of one case with disseminated cryptococcosis revealed no endoscopic abnormalities or negative results in bronchoalveolar lavage fluid smear or culture.

Pathological Examination

Six of the 11 pediatric patients with disseminated cryptococcosis underwent lymph node biopsy because of lymph node enlargement. Biopsies included 3 cases of cervical lymph nodes, 1 case of supraclavicular lymph nodes, 1 case of inguinal lymph nodes, and 1 case of retroperitoneal lymph nodes. Pathological examination revealed granulomatous inflammation, and *Cryptococcus* was detected.

Misdiagnosis

Five of the 13 cases (56.5%) who were misdiagnosed with cryptococcal meningitis were misdiagnosed as purulent meningitis (2 cases), tuberculous meningitis (2 cases), viral encephalitis (1 case), and myopia (1 case). The other 8 cases with disseminated cryptococcosis were misdiagnosed as bronchial pneumonia (4 cases), tuberculosis (3 cases), infectious mononucleosis (2 cases), empyema (1 case), parasitic disease (1 case), and Kawasaki disease (1 case).

Comparison of Clinical Features Between Cryptococcal Meningitis and Disseminated Cryptococcosis

Table I shows that coughing, pulmonary moist rales, lymph node enlargement, hepatosplenomegaly, and skin rashes were only observed in pediatric patients with disseminated cryptococcosis. The rates of headache and positive meningeal irritation were significantly higher in patients with cryptococcal meningitis than disseminated cryptococcosis. Peripheral blood leukocytes and the proportion of increased eosinophils were significantly higher in patients with disseminated cryptococcosis than cryptococcal meningitis. However, the other clinical manifestations were not significantly different between cryptococcal meningitis and disseminated cryptococcosis patients.

Treatment and Prognosis

Two pediatric patients with cryptococcal meningitis had serious conditions, and their parents discontinued treatment. Therefore, these patients did not receive antifungal therapy. The remaining 21 pediatric patients received anti-fungal treatment using the inducible/sustaining treatment strategy. Among these patients, 42.86% received amphotericin B combined with 5-fluorocytosine, 28.57% of patients received a single treatment with fluconazole, and 19.05% of patients received amphotericin B combined with fluconazole (Table II). All patients were followed up for more than 6 months. The results demonstrated that 4 cases were cured, 10 cases were improved, and 9 cases were dead. The mortality rate was 39.13%. One case with cryptococcal meningitis suffered paralysis, seizures, mental retardation, aphasia, blindness, and other complications. Fisher's exact test revealed no statistically significant difference in mortality between patients who received fluconazole alone, amphotericin B combined with fluconazole, or amphotericin B combined with 5-fluorocytosine (p = 0.194).

Discussion

Cryptococcus neoformans is widely found in nature, including soil, dry pigeon droppings, vegetables, and fruits[13]. The bacteria are also isolated from animals, such as horses, cows, dogs, cats, mountain antelopes, and pigs. Only 6 of the 23 patients in this study had a history of exposure to pigeon droppings, which suggests that a history of exposure to birds or their droppings is not necessary for disease diagnosis. Cryptococcus is detected in 70% of pigeon droppings, but it cannot be excluded in patients with no history of exposure to pigeon droppings.

Table I. Clinical features of 23 pediatric patients with cryptococcosis[a].

Clinical feature	Cryptococcal meningitis	Disseminated cryptococcosis	Total	*p*-value
Age (year)				0.3426
< 3	0/12 (0)	2/11 (18.18)	2/23 (8.70)	
3-6	4/12 (33.33)	4/11 (34.78)	8/23 (34.78)	
6-14	8/12 (66.67)	5/11 (56.52)	13/23 (56.52)	
Male	9/12 (76.00)	10/11 (90.91)	19/23 (82.61)	0.5901
From rural areas	8/12 (66.67)	6/11 (54.55)	14/23 (60.87)	0.6802
History of exposure to pigeon droppings	3/12 (26.00)	3/11 (27.27)	6/23 (26.09)	1.0000
Duration before admission (days)				0.8201
< 10	1/12 (8.33)	1/11	2/23	
10-20	4/12 (33.33)	2/11	6/23	
20-30	3/12 (26.00)	4/11	7/23	
> 30	5/12 (41.67)	3/11	8/23	
Onset to diagnosis (days)				0.0109
< 30	10	3	13	
30-60	1	6	7	
60-90	1	1	2	
> 90	0	1	1	
Main symptoms and signs				
Fever	10/12 (83.33)	11/11 (100.00)	21/23 (91.30)	0.4783
Headache	11/12 (91.67)	2/11 (18.18)	13/23 (56.52)	0.0000
Vomiting	8/12 (66.67)	3/11 (27.27)	11/23 (47.83)	0.0995
Convulsions	5/12 (41.67)	2/11 (18.18)	7/23 (30.43)	0.3707
Consciousness	4/12 (33.33)	1/11 (9.09)	5/23 (21.74)	0.3168
Meningeal irritation	10/12 (83.33)	1/11 (9.09)	11/23 (47.83)	0.0000
Babinski sign positive	4/12 (33.33)	1/11 (9.09)	5/23 (21.74)	0.3168
Cough	0	7/11 (63.64)	7/23 (30.43)	0.0000
Lung auscultation	0	3/11 (27.27)	3/23 (13.04)	0.0932
Lymphadenopathy	0	6/11 (54.55)	6/23 (26.08)	0.0000
Hepatosplenomegaly	0	9/11 (81.82)	9/23 (39.13)	0.0000
Rash	0	5/11 (36.36)	5/23 (21.74)	0.0000
Blood tests				
Leukocytosis	2/12 (16.67)	7/11 (63.64)	9/23 (39.13)	0.0361
Anemia	6/12 (50.00)	8/11 (72.73)	14/23 (60.87)	0.4003
Eosinophilia	2/12 (16.67)	5/11 (46.45)	7/23 (30.43)	0.0132
C-reactive protein	5/12 (41.67)	8/11 (72.73)	13/23 (56.52)	0.2000
CSF cell count increase	12/12 (100)	2/11 (18.18)	14/22 (8.70)	0.0000
CSF sugar decrease	10/12 (83.33)	5/11 (46.45)	15/23 (66.22)	0.3310
CSF protein increase	8/12 (66.67)	3/11 (27.27)	11/23 (47.83)	0.1748
Abnormal liver function	4/12 (33.33)	5/11 (46.45)	9/23 (39.13)	0.6802
Chest radiographic abnormalities	0/12 (0)	11/11 (100.00)	11/23 (47.83)	0.0000
Neuroimaging abnormalities	6/10 (60.00)	1/3 (33.33)	7/13 (53.85)	0.5594
Abdominal radiographic abnormalities	–	8/8 (100.00)	–	–
Pathogenic examination				
CSF ink staining	11/12 (91.67)	5/8 (62.50)	16/20 (80)	0.2553
CSF culture	8/10 (80.00)	7/10 (70.00)	15/20 (76.00)	1.0000
Blood culture	1/3 (33.33)	4/9 (44.44)	5/12 (41.67)	1.0000
Bone marrow culture	–	2/2 (100.00)	–	–
Ascites culture	–	1/1 (100.00)	–	
Lymph node biopsy	–	6/6 (100.00)	–	–

[a]Results are n/N (%).

Cryptococcus neoformans is an opportunistic pathogen. The infection rate in the immunocompetent population is approximately 1/100,000[14]. The infection rate in immunosuppressed patients can reach 5-10%, and it can reach 30% in patients with AIDS. The incidence of AIDS-related cryptococcosis has decreased significantly with the increased use of highly active antiretroviral therapy, but the incidence of AIDS-unrelated cryptococcosis increases annually. Notably, epi-

Table II. Treatment and prognosis of 23 pediatric patients with cryptococcosis.

Drug for induced remission	Cryptococcal meningitis				Disseminated cryptococcosis				Total			
	Number of cases	Cured	Improved	Dead	Number of cases	Cured	Improved	Dead	Number of cases	Cured	Improved	Dead
Fluconazole	1	0	0	1	5	1	4	0	6	1	4	1
Amphotericin B	0	0	0	0	1	0	0	1	1	0	0	1
Amphotericin B + Fluconazole	3	0	1	2	1	0	0	1	4	0	1	3
Amphotericin B + 5-fluorocytosine	6	2	2	2	3	1	2	0	9	3	4	2
Fluconazole + 5-fluorocytosine	0	0	0	0	1	0	1	0	1	0	1	0
Total	10	2	3	5	11	2	7	2	21	4	10	7

demiological data from Europe, the United States, Australia, and South Africa demonstrated that only 7-32% of pediatric patients with AIDS-unrelated cryptococcal meningitis had normal immune function[3,15,16]. Joshi et al[3] investigated patients with cryptococcosis from 42 children's hospitals in the United States between 2003 and 2008, and the results demonstrated that 16% of patients had HIV infection, 63% of patients had other potential immunocompromising factors, and 21% of patients were immunocompetent. However, normal immune function in patients with cryptococcosis is not uncommon, and the age of onset may be as young as infant[2,17]. In Chinese patients from mainland China, Hong Kong, Taiwan, and Singapore, 50-77% of patients with cryptococcal meningitis exhibited normal immune function[2,11,18,19] . Ninety-one of the 129 clinical strains isolated from patients from mainland China were isolated from patients who had no apparent immune deficiency[20]. Among the patients in the present study, 73.91% had normal immune function, and only 6 patients experienced malnutrition. Our patients had no underlying diseases and normal immune function, which is similar to most patients with cryptococcosis in China. Assessments of whether children with normal immune function will develop cryptococcosis should consider the definition of normal immune function. Some patients with cryptococcosis who exhibit so-called "normal" immune function may also have genetic defects in mannose-binding lectin and Fc receptors (Fc R) 2B[21,22].

Previous studies suggested that cryptococcal meningitis is common and that disseminated cryptococcosis is rare[4,8-11,23,24]. However, disseminated cryptococcosis accounted for 47.83% of patients with cryptococcosis in the current study, which suggests that childhood cryptococcosis is easily spread, possibly due to the weaker immune system in children. *Cryptococcus* can invade any tissue or organ of the human body, and clinical manifestations are not specific to each infected area. Therefore, misdiagnosis and missed diagnosis of this disease are often observed.

The clinical manifestations of disseminated cryptococcosis are not specific, but all patients presented pulmonary cryptococcosis. The correct diagnosis of pulmonary cryptococcosis can prevent or reduce the occurrence of disseminated cryptococcosis. Acute pulmonary cryptococcosis presents acute pneumonia, which is easily misdiagnosed as bacterial or mycoplasma pneumonia.

Chronic pulmonary cryptococcosis is similar to tuberculosis, but it rarely exhibits pulmonary symptoms. Therefore, it is easily misdiagnosed as tuberculosis and lung cancer. A diagnosis of pulmonary cryptococcosis should be considered in pediatric patients who present respiratory symptoms, imaging findings that are similar to pneumonia, tuberculosis, or lung cancer with a lack of common bacteria, mycoplasma or mycobacterial infection, and treatment response. The characteristics of lung damage in pediatric patients with cryptococcosis in this study included a light cough, clinical manifestations that were inconsistent with imaging changes, diffuse or scattered small nodular shadows in the lung, distribution under the pleura, and accompanied with a small piece of lung consolidation, hilar and mediastinal lymphadenopathy, or all three characteristics. The current results are consistent with the literature[25].

Cryptococcosis often affects the central nervous system, lung, skin, bones, and eyes. Recent cryptococcosis cases with abdominal cavity, hilar, and mediastinal lymph node involvement as the first manifestation were also reported[26-30]. The rate of lymph node, liver, and spleen enlargement in patients with disseminated cryptococcosis was significantly higher than cryptococcal meningitis in this study. Ten of the 11 patients with disseminated cryptococcosis had superficial or deep lymph node involvement, which suggests that lymph nodes are the most affected sites in cryptococcosis. This result may occur because cryptococcal infection activates the monocyte-macrophage system, which results in a strong reaction in lymph nodes, liver and spleen. *Cryptococcus* can spread along blood and lymphatic vessels, and it directly invades lymph nodes. Lymph node enlargement is often observed in multiple sites, including the neck, mediastinum, abdominal cavity, armpits, and groin. Therefore, cryptococcosis is very easily misdiagnosed as lymphoma and tuberculosis.

Abnormal increases in peripheral blood eosinophil levels were reported in recent case reports of cryptococcosis[31,32]. In this study, 46.45% of patients with disseminated cryptococcosis exhibited increased peripheral blood eosinophil counts, and 1 case, who had been misdiagnosed as parasitic infection and eosinophilic pneumonia, exhibited high numbers of eosinophils in peripheral blood, bone marrow, and lung lavage fluid. Therefore, a diagnosis of possible disseminated cryptococcosis should be considered in pediatric patients with cryptococcosis who exhibit increased eosinophils in peripheral blood. The current results also demonstrated that peripheral blood eosinophil levels fell as the patient's condition improved with clinical treatment, which indicates that an increase in the proportion of eosinophils may be one hematological characteristic of the acute phase of cryptococcosis, and the decreased proportion of eosinophils may contribute to the evaluation of clinical treatment. The mechanism underlying the increase in eosinophil counts in cryptococcosis is not clear. One possibility is that some specific components of the capsular of *Cryptococcus neoformans*, such as capsular polysaccharides and glucose-mannopyranose, serve as antigens to stimulate the production of IgE, the induction of type I hypersensitivity, the binding of IgE to local mast cells to cause degranulation, the release of histamine and eosinophil chemotactic factors, and lymphocyte production of eosinophil colony-forming factor and interleukin-5, which increase the differentiation of eosinophils in bone marrow and leads to an increased level of peripheral blood eosinophils. Chen et al. demonstrated that the Th2 cell-mediated immune response is related to the manifestations of cryptococcal infection in a mouse model, such as an increased number of eosinophils, severe lung damage, increased serum IgE levels, and the spread of the infection to the central nervous system[33].

Accurate pathological examination is key to reducing the rates of missed diagnosis and misdiagnosis of cryptococcosis. The cryptococcal cell wall contains little 1,3-β-D-glucan, and it is wrapped in a layer of capsule. Therefore, it is difficult to release 1,3-β-D-glucan into the blood[34], and the value of the G test in the diagnosis of cryptococcosis is limited. The sensitivity and specificity of cryptococcal capsular polysaccharide antigen in the diagnosis of cryptococcosis can reach 96%[35]. Serum cryptococcal capsular polysaccharide antigen levels should be determined in patients with fever and swollen lymph nodes, livers, and spleens to avoid a misdiagnosis of cryptococcosis. Five of the 6 pediatric patients with superficial lymph node enlargement in this study who underwent lymph node biopsy exhibited granulomatous lesions and *Cryptococcus*. The rate of lymph node involvement is high in childhood cryptococcosis. Lymph node biopsy is a simple and reliable technique that involves little

trauma, and it may be used to confirm the diagnosis of this disease. Notably, cryptococcosis is not as common as tuberculous lymphadenitis in the cases of lymph node biopsy, and patients with granulomatous nodules in lymph nodes and cheese-like necrosis are easily misdiagnosed with tuberculosis. Cryptococcal cells were lightly stained or colorless in HE staining specimens, which made these cells difficult to observe. The microscopic light must be turned down to clearly visualize the capsule. Cryptococcal cell walls are red in PAS staining and silver and black in methenamine silver staining. The cryptococcal capsule is crimson in mucicarmine staining. Therefore, a combination of these special staining methods should be used to confirm the diagnosis of cryptococcosis when the number of *Cryptococcus* is small[36].

Previous studies demonstrated that immunocompetent patients may also exhibit a high mortality rate that is similar to immunocompromised patients with cryptococcosis[37]. Amphotericin B combined with 5-fluorocytosine is recommended for initial induction therapy in patients with HIV-unrelated symptoms in the central nervous system or disseminated cryptococcosis[38]. Only 1 of the 6 patients who were treated with fluconazole alone died in this study. The number of cases in this study was small, but fluconazole exhibited good antibacterial activity, and it is safe and easy to administer. We assert that fluconazole may be used in patients who cannot tolerate the side effects of amphotericin B or prolonged treatment. Further clinical observations are required to confirm this assertion.

Conclusions

Cryptococcosis may occur in non-HIV-infected and immunocompetent children. Patients with cryptococcosis may have no history of risk factors or exposure to pigeon droppings. Cryptococcosis lacks specific clinical manifestations. Disseminated cryptococcosis commonly occurs in children. There are similarities and differences between cryptococcal meningitis and disseminated cryptococcosis. Disseminated cryptococcosis should be suspected in pediatric patients with unexplained long-term fever, mild cough, chest imaging that is inconsistent with clinical symptoms, lymphadenectasis, hepatosplenomegaly, and eosinophilia. The rate of lymph node involvement is high in children with cryptococcosis. Pathological examinations should be performed regardless of obvious enlargement. Lymph node biopsy may be used to confirm the diagnosis. Effective antifungal agents should be commenced promptly after a diagnosis is rendered.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Fang W, Fa Z, Liao W. Epidemiology of Cryptococcus and cryptococcosis in China. Fungal Genet Biol 2015; 78: 7-15.
2) Yuanjie Z, Jianghan C, Nan X, Xiaojun W, Hai W, Wanqing L, Julin G. Cryptococcal meningitis in immunocompetent children. Mycoses 2012; 55: 168-171.
3) Joshi NS, Fisher BT, Prasad PA, Zaoutis TE. Epidemiology of cryptococcal infection in hospitalized children. Pediatr Infect Dis J 2010; 29: e91-95.
4) Lizarazo J, Escandon P, Agudelo CI, Castaneda E. Cryptococcosis in Colombian children and literature review. Mem Inst Oswaldo Cruz 2014; 109: 797-804.
5) Leggiadro RJ, Barrett FF, Hughes WT. Extrapulmonary cryptococcosis in immunocompromised infants and children. Pediatr Infect Dis J 1992; 11: 43-47.
6) Leggiadro RJ, Kline MW, Hughes WT. Extrapulmonary cryptococcosis in children with acquired immunodeficiency syndrome. Pediatr Infect Dis J 1991; 10: 658-662.
7) Gonzalez CE, Shetty D, Lewis LL, Mueller BU, Pizzo PA, Walsh TJ. Cryptococcosis in human immunodeficiency virus-infected children. Pediatr Infect Dis J 1996; 15: 796-800.
8) Abadi J, Nachman S, Kressel AB, Pirofski L. Cryptococcosis in children with AIDS. Clin Infect Dis 1999; 28: 309-313.
9) Likasitwattanakul S, Poneprasert B, Sirisanthana V. Cryptococcosis in HIV-infected children. Southeast Asian J Trop Med Public Health 2004; 35: 935-939.
10) Severo CB, Xavier MO, Gazzoni AF, Severo LC. Cryptococcosis in children. Paediatr Respir Rev 2009; 10: 166-171.
11) Huang KY, Huang YC, Hung IJ, Lin TY. Cryptococcosis in nonhuman immunodeficiency virus-infected children. Pediatr Neurol 2010; 42: 267-270.
12) De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J,

Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46: 1813-1821.

13) Baddley JW, Perfect JR, Oster RA, Larsen RA, Pankey GA, Henderson H, Haas DW, Kauffman CA, Patel R, Zaas AK, Pappas PG. Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. Eur J Clin Microbiol Infect Dis 2008; 27: 937-943.

14) Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS--100 years after the discovery of Cryptococcus neoformans. Clin Microbiol Rev 1995; 8: 515-548.

15) Miglia KJ, Govender NP, Rossouw J, Meiring S, Mitchell TG. Analyses of pediatric isolates of Cryptococcus neoformans from South Africa. J Clin Microbiol 2011; 49: 307-314.

16) Chen S, Sorrell T, Nimmo G, Speed B, Currie B, Ellis D, Marriott D, Pfeiffer T, Parr D, Byth K. Epidemiology and host- and variety-dependent characteristics of infection due to Cryptococcus neoformans in Australia and New Zealand. Australasian Cryptococcal Study Group. Clin Infect Dis 2000; 31: 499-508.

17) Laman M, Hwaihwanje I, Davis TM, Manning L. Cryptococcal meningitis in immunocompetent Papua New Guinean children. Trop Doct 2010; 40: 61-63.

18) Lui G, Lee N, Ip M, Choi KW, Tso YK, Lam E, Chau S, Lai R, Cockram CS. Cryptococcosis in apparently immunocompetent patients. QJM 2006; 99: 143-151.

19) Chen YY, Lai CH. Nationwide population-based epidemiologic study of cryptococcal meningitis in Taiwan. Neuroepidemiology 2011; 36: 79-84.

20) Chen J, Varma A, Diaz MR, Litvintseva AP, Wollenberg KK, Kwon-Chung KJ. Cryptococcus neoformans strains and infection in apparently immunocompetent patients, China. Emerg Infect Dis 2008; 14: 755-762.

21) Hu XP, Wu JQ, Zhu LP, Wang X, Xu B, Wang RY, Ou XT, Weng XH. Association of Fcgamma receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. PLoS One 2012; 7: e42439.

22) Ou XT, Wu JQ, Zhu LP, Guan M, Xu B, Hu XP, Wang X, Weng XH. Genotypes coding for mannose-binding lectin deficiency correlated with cryptococcal meningitis in HIV-uninfected Chinese patients. J Infect Dis 2011; 203: 1686-1691.

23) Guo J, Zhou J, Zhang S, Zhang X, Li J, Sun Y, Qi S. A case-control study of risk factors for HIV-negative children with cryptococcal meningitis in Shi Jiazhuang, China. BMC Infect Dis 2012; 12: 376.

24) Meiring ST, Quan VC, Cohen C, Dawood H, Karstaedt AS, McCarthy KM, Whitelaw AC, Govender NP. A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. AIDS 2012; 26: 2307-2314.

25) Rozenbaum R, Goncalves AJ. Clinical epidemiological study of 171 cases of cryptococcosis. Clin Infect Dis 1994; 18: 369-380.

26) Haramati LB, Choi Y, Widrow CA, Austin JH. Isolated lymphadenopathy on chest radiographs of HIV-infected patients. Clin Radiol 1996; 51: 345-349.

27) Lee MY, Chung JH, Shin JH, Hwang TJ, Kim KS, Lee JH, Nam JH, Lee MC, Park CS, Juhng SW, Choi C. Lymphonodular cryptococcosis diagnosed by fine needle aspiration cytology in hyper-IgM syndrome. A case report. Acta Cytol 2001; 45: 241-244.

28) Zou CC, Yu ZS, Tang LF, Liang L, Zhao ZY. Primary abdominal lymphonodular cryptococcosis in children: 2 case reports and a literature review. J Pediatr Surg 2006; 41: e11-15.

29) Gazzoni AF, Severo CB, Barra MB, Severo LC. Atypical micromorphology and uncommon location of cryptococcosis: a histopathologic study using special histochemical techniques (one case report). Mycopathologia 2009; 167: 197-202.

30) Kim SH, Kim SD, Kim HR, Yoon CH, Lee SH, Kim HY, Park SH. Intraabdominal cryptococcal lymphadenitis in a patient with systemic lupus erythematosus. J Korean Med Sci 2005; 20: 1059-1061.

31) Bassetti M, Mikulska M, Nicco E, Viscoli C. Pulmonary cryptococcosis with severe eosinophilia in an immunocompetent patient. J Chemother 2010; 22: 366-367.

32) Yamaguchi H, Komase Y, Ikehara M, Yamamoto T, Shinagawa T. Disseminated cryptococcal infection with eosinophilia in a healthy person. J Infect Chemother 2008; 14: 319-324.

33) Chen GH, McNamara DA, Hernandez Y, Huffnagle GB, Toews GB, Olszewski MA. Inheritance of immune polarization patterns is linked to resistance versus susceptibility to Cryptococcus neoformans in a mouse model. Infect Immun 2008; 76: 2379-2391.

34) Al SM, Bahu RR, Chaftari AM, Al WI, Shomali W, Jiang Y, Debiane L, Raad S, Jabbour J, Al AF, Hachem RY, Raad I. Pro-adrenomedullin as a novel biomarker for predicting infections and response to antimicrobials in febrile patients with hematologic malignancies. Clin Infect Dis 2013; 56: 943-950.

35) Antinori S, Radice A, Galimberti L, Magni C, Fasan M, Parravicini C. The role of cryptococcal antigen assay in diagnosis and monitoring of cryptococcal meningitis. J Clin Microbiol 2005; 43: 5828-5829.

36) Gazzoni AF, Severo CB, Salles EF, Severo LC. Histopathology, serology and cultures in the diagnosis of cryptococcosis. Rev Inst Med Trop Sao Paulo 2009; 51: 255-259.

37) Ecevit IZ, Clancy CJ, Schmalfuss IM, Nguyen MH. The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. Clin Infect Dis 2006; 42: 1443-1447.

38) Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, Harrison TS, Larsen RA, Lortholary O, Nguyen MH, Pappas PG, Powderly WG, Singh N, Sobel JD, Sorrell TC. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. Clin Infect Dis 2010; 50: 291-322.