CASE REPORT

DISSEMINATED CRYPTOCOCCOSIS IN AN HIV-SERONEGATIVE PREGNANT WOMAN WITH TRANSIENT T- LYMPHOCYTOPENIA: A CASE REPORT AND REVIEW OF THE LITERATURE

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Abstract. We report a case of an HIV-seronegative pregnant woman with disseminated cryptococcosis, poorly controlled during gestation. Immunological studies showed T-lymphocytopenia during gestation, but rapid recovery postpartum. T-lymphocytopenia may play a role in increased susceptibility to and severity of cryptococcal infection during pregnancy.

Keywords: T-lymphocytopenia, cryptococcal meningitis, cryptococcosis, *Cryptococcus neoformans*, pregnant woman

INTRODUCTION

Cryptococcus neoformans is an opportunistic pathogen usually affecting immunosuppressed patients, such as those with AIDS, organ transplantation, systemic lupus erythematosus, diabetes mellitus and advanced malignancy (Kiertiburanakul *et al*, 2006). Pregnancy may be considered as a state of relative immunosuppression (Perfect, 2012). During pregnancy, the maternal immunologic status is altered to prevent rejection of the immunologically foreign fetus (Costa *et al*, 2009). Although immune system changes in pregnancy are not completely understood, an alteration in cell-mediated immunity is believed to occur (Costa *et al*, 2009). We report a case of disseminated cryptococcosis in an HIV-seronegative pregnant woman with transient CD4+ and CD8+ T-lymphocytopenia.

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CASE REPORT

A 36-year-old, 28 week estimated gestational age, gravida 2 para 1 Thai woman presented to our hospital with headache, nausea, vomiting and low grade for two weeks. She worked as a clothes vender. She denied a history of exposure to bird droppings or eucalyptus tree. She developed diplopia two days prior to presentation. The past medical history was significant for colonic tuberculosis

treated 15 years previously. Her past obstetrical history revealed no problems. On physical examination she was in moderate distress with a temperature of 39.2°C. Her neurological examination was significant for bilateral sixth nerve palsy without nuchal rigidity. The rest of her physical examination was unremarkable.

She had been receiving prenatal care at our hospital. She was Rh-negative and anti-D immunoglobulin had been given during her 22nd week of pregnancy. Her routine antenatal laboratory results taken during her 6th and 26th weeks of pregnancy were normal, including anti-HIV testing. She was admitted to the hospital and her initial laboratory studies were significant for a white blood cell (WBC) count of 10.8 x 10^9/l, with 86% neutrophils and 5% lymphocytes. Her chest radiography was normal. A non-contrast magnetic resonance image (MRI) of the brain was normal. A lumbar puncture was performed and she had an opening pressure of 26 cmH_2O. Cerebrospinal fluid (CSF) examination showed 1 WBC/mm^3 (mononuclear cell), a reduced glucose level (16 mg/dl), and a normal protein level (37.3 mg/dl). Numerous large encapsulated budding yeasts were found on India ink preparation. Both CSF and serum cryptococcal antigens were positive with titers of >1:1,024 and 1:512, respectively. She was diagnosed with having disseminated cryptococcosis, *C. neoformans* subsequently grew out of both CSF and blood cultures.

The patient was started on amphotericin B deoxycholate at a dose of 0.7 mg/kg/day. Her fever gradually subsided but she continued to have a headache. Despite daily lumbar punctures with large volumes of CSF removed, her opening pressure remained markedly elevated at more than 60 cmH_2O. The results of her HIV serology were repeatedly negative. Two weeks after the onset of treatment, her blood cultures still grew *C. neoformans*. After discussion of the risks and benefits of treatment with the patient and her family, it was decided to deliver the child prematurely. A cesarean-section and lumbo-peritoneal shunt were performed at during her 30th week of pregnancy. A newborn was delivered, weighing 1,550 g, with Apgar scores of 5, 8 and 10 at 1, 5 and 10 minutes, respectively. After delivery, a combination of amphotericin B deoxycholate and intravenous fluconazole (800 mg/day) was given to the mother. However, the maternal management was complicated by renal impairment. Her blood and CSF cultures did not grow any microorganisms after two weeks of combined treatment. After a 6-week course of induction therapy, fluconazole consolidation at a renal dose (400-800 mg/day) was given for 8 weeks followed by maintenance therapy (fluconazole 200 mg/day) for a year.

The placenta was sent for pathological and microbiological studies. Cryptococcosis of the placenta was diagnosed. Pathology of the placenta revealed multiple whitish nodules of the cut surface. Clusters of encapsulated budding yeasts were found in intervillous spaces and in the perivillous areas (maternal compartment), but not in the villous stroma (fetal compartment). A fungal culture of maternal compartment of the placenta grew *C. neoformans*. However, the fetal compartment of the placenta and amniotic fluid did not grow any fungus.

The neonate had no evidence of central nervous system disease. Serum cryptococcal antigen was negative on the first day of life. However, a positive serum cryptococcal antigen with a titer of 1:8 was detected on day 8 of life. CSF and blood cultures from the neonate did not

Table 1
Various laboratory results from reported subject.

	29 weeks pregnancy	1 week postpartum	Reference range
White blood cell count) $x10^9$/l	7.30	8.60	4.00-10.00
Lymphocytes (%) $x10^9$/l	(6.2) 0.45	(14.0) 1.20	(28-39) 1.60-2.40
Lymphocyte subsets (%) $x10^9$/l			
CD3	(29.5) 0.13	(66.5) 0.80	(67-76) 1.10-1.70
CD4	(18.0) 0.08	(30.9) 0.37	(38-46) 0.70-1.10
CD8	(6.7) 0.03	(18.9) 0.23	(31-40) 0.50-0.90
CD4:CD8 ratio	2.67	1.61	0.65-2.49
CD19	(69.7) 0.31	(20.8) 1.20	(11-18) 0.20-0.40
CD16+56	(20.6) 0.90	(6.9) 0.80	(10-19) 0.20-0.40
Serum immunoglobulin, mg/dl			
IgA	0.636	0.812	0.70-4.00
IgG	8.260	10.100	7.00-16.00
IgM	1.410	1.590	0.40-2.30
Complements			
C3, g/ml	1,350	ND	900-1,800
C4, g/ml	273	ND	100-400
CH50, %	100	ND	100

ND, not done.

grow any organisms. Because of this evidence of possible neonatal cryptococcosis, amphotericin B deoxycholate was given for six weeks followed by fluconazole consolidation and maintenance therapy. The hospital course was complicated by necrotizing enterocolitis. The baby was ultimately discharged home on day 87 of life.

Since disseminated cryptococcosis in pregnancy is rare, maternal immunologic studies were investigated (Table 1). Although her WBC count was in the normal range for pregnancy, she had a marked decrease in her lymphocyte count. A study of lymphocyte subsets revealed low CD4+ and CD8+ T-lymphocytes, but normal B and natural killer (NK) cells. Immunoglobulin and complement levels were within the normal range except for IgA, which was slightly low. One week postpartum, she had a rapid increase in her T-cells, but the numbers were still lower than the normal range. Three months postpartum testing still showed persistently lower than normal CD4+ (34%, 343 cells/mm^3) and CD8+ (14%, 141 cells/mm^3) cell counts.

DISCUSSION

A cryptococcal infection can occur in any trimester of pregnancy. The clinical presentation of cryptococcosis in pregnant women can vary and diagnosis can be challenging. The disease can present as isolated pulmonary cryptococcosis, disseminated cryptococcosis or even as a septic abortion (Ely *et al*, 1998; Costa *et al*, 2009; Mudumbi, 2010). Maternal deaths

Table 2
Summary of published reports of an HIV-seronegative pregnant woman with cryptococcal meningitis.

Cases	Age (years)	Gestational age (trimester)	Country	Blood C/S	CSF C/S	Antifungal used	Maternal outcomes	Fetal outcomes	References
1	34	Third	USA	+	NR	Supportive	Died	Died	Timerman, 1935
2	40	Third	USA	NR	+	NR	Died	Survived	Wager, 1954
3	32	Post-partum	USA	+	NR	NR	Died	Survived	Gantz *et al*, 1958
4	19	Second	USA	NR	+	Amphotericin B	Survived	Survived	Feldman, 1959
5	21	Second	USA	NR	+	Amphotericin B	Survived	NR	Littman, 1959
6	32	Third	Australia	NR	+	Amphotericin B	Survived	Survived	Kuo, 1962
7	26	Second	England	NR	+	Amphotericin B	Survived	Survived	Aitken and Symonds, 1962
8	26	First	Brazil	+	NR	Supportive	Died	NR	Crotty, 1965
9	24	Second	Brazil	NR	+	Amphotericin B	Died	NR	Crotty, 1965
10	29	Third	Brazil	+	NR	Supportive	Died	NR	Crotty, 1965
11	21	Post-partum	Brazil	+	NR	Supportive	Died	NR	Crotty, 1965
12	28	Post-partum	USA	NR	+	Amphotericin B	Survived	NR	Silberfarb *et al*, 1972
13	33	First	Australia	NR	+	Amphotericin B	Died	Survived	Philplot and Lo, 1972
14	16	First	Australia	NR	+	Amphotericin B	Survived	Survived	Philplot and Lo, 1972
15	24	First	USA	NR	+	Amphotericin B	Survived	Survived	Curole, 1981
16	13	Third	USA	NR	+	Amphotericin B	Survived	Survived	Curole, 1981
17	29	Third	USA	NR	+	Amphotericin B plus flucytosine	Survived	Survived	Jones and Graig, 1983
18	30	Second	USA	NR	+	Amphotericin B plus flucytosine	Survived	Died	Stafford *et al*, 1983
19	19	Second	Thailand	NR	+	Amphotericin B	Survived	NR	Chotmongkol and Siricharoensang, 1991
20	25	Second	Thailand	NR	+	Amphotericin B plus flucytosine	Survived	Survived	Chotmongkol and Siricharoensang, 1991
21	29	Second	Brazil	NR	+	Amphotericin B	Survived	Survived	Pereira *et al*, 1993
22	37	First	Brazil	NR	+	Amphotericin B plus flucytosine	Survived	Survived	Pereira *et al*, 1993
23	23	Second	USA	NR	+	Amphotericin B plus flucytosine	Survived	Died	Molnar-Nadasdy *et al*, 1994
24	18	Third	Taiwan	NR	+	Amphotericin B plus flucytosine	Survived	Survived	Chen and Wang, 1996

Table 2 (Continued).

Cases	Age (years)	Gestational age (trimester)	Country	Blood C/S	CSF C/S	Antifungal used	Maternal outcomes	Fetal outcomes	References
25	19	Third	USA	NR	+	Amphotericin B	Survived	Survived	Ely *et al*, 1998
26	21	Second	Brazil	+	NR	Supportive	Died	Died	Nucci *et al*, 1999
27	28	NR	India	+	NR	Supportive	Died	Dieda	Srinivas *et al*, 2008
28	37	First	Brazil	NR	+	Amphotericin B plus fluconazole[b]	Survived	Survived	Costa *et al*, 2009
29	27	Second	Dominican Republic	NR	NR	Fluconazole and Amphotericin B[c]	Survived	Abortion	Mudumbi, 2010
30 (PR)	36	Third	Thailand	+	+	Amphotericin B plus fluconazole[a]	Survived	Survived	

NR, not reported; PR, present case.
[a]Patient presented with sigmoid colonic perforation and peritonitis following termination pregnancy by untrained midwife.
[b]Added fluconazole after delivery.
[c]Changed to amphotericin B after fluconazole failure.

due to cryptococcosis have decreased with better intensive care practices and the use of antifungals, such as amphotericin B deoxycholate (Costa *et al*, 2009). Cryptococcosis in pregnant women has been increasingly reported (Ely *et al*, 1998; Costa *et al*, 2009). Costa *et al* (2009) reviewed 27 cases of cryptococcosis among HIV negative pregnant women, other studies have reported similar cases (Table 2).

Pregnancy is state of relative immunosuppression. Dominance of Th2 cytokines during pregnancy may result in an immunosuppressive state, in which cytotoxic T cells responses are suppressed, leading to decreased cell-mediated immunity. We found T-lymphocytopenia in the cases presented here. CD4+ T- cell counts in HIV-seronegative pregnant women reach a nadir during the third trimester, with a mean (± standard deviation) CD4 count of 800 (± 179) cells/mm^3 (Towers *et al*, 2010). Although pregnancy is associated with a significant reduction in total lymphocytes, it is rare to see a decrease in the absolute CD4+ T cell count more than 30% (Towers *et al*, 2010). In HIV-infected patients, cryptococcal infection is usually seen in patients with a CD4+ T cell count of less than 100 cells/mm^3. In our reported case, the CD4+ T- cell count at diagnosis during the third trimester of pregnancy, was within this range. T-cell responses have been shown to be critically important in establishing protective immunity against cryptococcal infection. In nude mice lacking mature CD4+ and CD8+ T-cells, an avirulent strain of *C. neoformans* can cause an infection and disseminate to the brain (Hill and Harmsen, 1991). Another study found decreased clearance of virulent yeast and dissemination of *Cryptococcosis* from the lungs to other organs among CD4+ and CD8+ T-cell depleted mice (Huffnagle *et al*, 1991). Rapid recov-

ery of CD4+ and CD8+ T-lymphocytes after delivery, along with the improving clinical course, suggest a critical role for CD4+ and CD8+ T-lymphocytes in controlling cryptococcal infection.

The cause of transient T-lymphocytopenia in this patient is unknown. A possible cause could be an alteration in the immune system due to the provision of anti-D immunoglobulin. The patient received anti-D immunoglobulin two weeks before the onset of symptoms. Evidence of anti-D immunoglobulin induced immune suppression has never been reported. However, we cannot exclude the possibility the anti-D immunoglobulin induced immune suppression. Further investigations are needed to evaluate this in more depth.

Although cryptococcosis has been reported in pregnancy, there are no reports of proven transplacental transmission of cryptococcosis (Sirinavin *et al*, 2004). Without evidence of the fungus in the fetal compartment of the placenta or amniotic fluid, we believe the neonate acquired the cryptococcal infection intrapartum rather than transplacentally. Evidence to support this hypothesis is the delayed detection of cryptococcal antigen after birth.

The optimal duration of antifungal therapy for cryptococcosis during pregnancy has not been determined. We adopted the guidelines for the management of cryptococcal disease for non-HIV-infected and nontransplant patients as provided by the Infectious Diseases Society of America (Perfect *et al*, 2010). The maternal and fetal outcomes were favorable in this case.

In conclusion, we present a rare case of cryptococcosis during pregnancy and a possible neonatal case of cryptococcosis. We found transient CD4+ and CD8+ lymphocytopenia during pregnancy in this patient. This may have contributed to increased susceptibility and severity of cryptococcal infection.

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