Mycobacterium Avium Complex in HIV-infected Thai Children

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Abstract

Of the 169 human immunodeficiency virus (HIV)-infected children being cared for at Siriraj Hospital from January 1998 to September 2000, 10 had *Mycobacterium avium* complex (MAC) infection; seven had disseminated disease and three had MAC pneumonia. Nine children were in the advanced stage of HIV disease at the time of diagnosis with the median CD4 count of 7 cells/mm^3 and 127 cells/mm^3 and the median age of 65 months and 63 months in disseminated MAC and MAC pneumonia respectively. None of these children had received prior chemoprophylaxis.

Common clinical findings included prolonged fever, weight loss, lymphadenopathy, hepatosplenomegaly, diarrhea, anemia and leukopenia. The outcome of MAC infection was poor, with a mortality rate of 60 per cent. In *in vitro* susceptibility testing, clarithromycin was the least resistant drug.

With the incidence rate of 2.15 per 100 person-years, the high rate of antimicrobial resistance, and the poor outcome, primary chemoprophylaxis for MAC infection in conjunction with effective antiretroviral therapy should be considered for Thai children in the advanced stage of HIV infection.

Key word : *Mycobacterium Avium* Complex, MAC, HIV-Infected Children, Chemoprophylaxis

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Mycobacterium avium complex (MAC) has been known as a cause of serious opportunistic infections (OI) in HIV-infected individuals[1-3]. MAC infection in HIV-infected patients were mostly disseminated. In the US in 1990, MAC was reported in 7.6 per cent adult patients with AIDS[4]. In comparison with adults, infections caused by MAC have been found less frequently in children and exclusively in the severely immune suppressed stage. The Pediatric AIDS Clinical Trials Group (PACTG) conducted a multicenter study among 3,331 HIV-infected children before the era of highly active antiretroviral therapy. There were 126 events of disseminated MAC (DMAC) infection with the event rate of 1.8 per 100 patient-years. The median CD4 count and CD4 per cent at the time of or just before diagnosis of DMAC were 17 cells/mm^3 and 2 per cent respectively[5]. Another study in the US found 7 of 70 HIV-infected children (10%) aged less than 13 years who had prolonged fevers and failure to thrive developed DMAC[2].

In Thailand, DMAC infection was found in 17 per cent of HIV-infected adults with prolonged fever of unknown cause[6]. However, MAC was reported to the Ministry of Public Health in only 1 out of 256 OI episodes in children with AIDS between 1988 and 1995. The lower incidence could be from diagnostic difficulties[7].

The aim of this study was to describe MAC infections in HIV-infected Thai children. This information will be helpful for the consideration of prophylaxis strategy that has not been implemented in Thai children. Another objective was to evaluate the rate of antibiotic resistance of MAC isolates in our institute.

MATERIAL AND METHOD

From January 1998 to September 2000, HIV-infected children in the advanced stage of diseases who were hospitalized with persistent or recurrent fever were investigated for MAC infection. The investigations included blood culture for mycobacteria, and also gastric aspirate (GA) culture in the patients who also had abnormal chest roentgenogram (CXR). The blood culture was carried out by an automate system, BACTEC 9240, using fluorescent detection system.

Identification of MAC was performed by standard biochemical tests[8] and some were confirmed by in-house polymerase chain reaction (PCR). The primers used were based on 16S rRNA gene, NGL-NGR gene, with the following sequences: GGA TAG GAC CTC AAG AC (from bases number 133-149) and TAC CGT CAA TCC GAG AA (from base numbers 438-422).

The MAC isolates were tested for drug sensitivity by proportional method[8,9]. The antimicrobial agents tested were ethambutol, rifampicin, ciprofloxacin, ofloxacin, amikacin, doxycycline, clarithromycin and streptomycin. The antimicrobial agents used were in the form of drug-containing paper discs from which the agents were released to the medium and yeilded the final concentration as shown in Table 1.

The criteria for resistance used was that the number of colonies growing on the drug containing medium was 1 per cent or more than the number on the drug-free medium.

The medical records of the patients with MAC infection were retrospectively reviewed.

RESULTS

During the 33 month period, of the 169 HIV-infected children regularly seen in the clinic, 38 children were investigated for MAC infection. Of these 10 had MAC isolated from blood and/or gastric aspirate (GA); 4 from blood only, 3 from GA only and 3 from both blood and GA. All the children who had MAC isolated from GA had abnormal CXR with respiratory symptoms. The 3 children who had MAC isolated from GA but not from blood were diagnosed with MAC pneumonia. The other 7 children with MAC bacteremia were diagnosed with DMAC infection. The incidence rate of MAC infection in this cohort was 2.15 per 100 person-years. The demographic, clinical, and laboratory findings of these children are shown in Table 2.

Table 1. Amount of antimicrobial agents on the paper discs and final concentration in the medium for drug susceptibility testing.

Drugs	μg of drug/disc	Final concentration in medium (μg/ml)
Amikacin	30	6
Ciprofloxacin	5	1
Clarithromycin	15	3
Doxycycline	30	6
Ethambutol	25	5
Ofloxacin	5	1
Rifampicin	5	1

Table 2. Demographic, clinical and laboratory findings in HIV-infected children with MAC infection.

Characteristic	DMAC (N=7)		Probable MAC pneumonia (N=3)	
Demographic				
Median (range) age (months)	65 (20-101)		63 (24-74)	
Sex				
Male	4		2	
Female	3		1	
Clinical stage before the MAC infection episode				
B	-		1	
C	7		2	
Immunological status				
Median (range) CD4 count (cells/mm^3)	7 (4-48) (N=4)		127 (N=1)	
Median (range) CD4 (%)	1.67 (0.22-4.31)(N=4)		3.77 (N=1)	
On ART	1 (d4T+ddI+HU)*		2 (ddI, d4T+ddI)	
Presenting symptoms		%		%
Prolonged fever	5	71	3	100
Weight loss	7	100	2	67
Lymphadenopathy	6	86	3	100
Hepatomegaly	7	100	3	100
Splenomegaly	1	14	2	67
Diarrhea	4	57	3	100
Respiratory symptoms				
Cough	5	71	2	67
Dyspnea	2	29	2	67
Tachypnea	1	14	2	67
Laboratory (mean and range)				
Hematocrit (%)	25 (13-31.5)		31.4 (10.5-36.5)	
WBC (cells/mm^3)	7,020 (1,600-28,940)		2,660 (1,920-5,800)	
Liver function tests				
Alkaline phosphatase (U/L)	283 (125-342)		222 (93-351)	
GGT (U/L)	192 (45-300)		70 (31-109)	
LDH (U/L)	1,074 (871-1,596)		936 (750-1,122)	
CXR (N=8)				
Focal infiltration	2		-	
Diffuse infiltration	2		3	
Hilar lymphadenopathy	2		1	
Death	4	(57%)	2	(67%)

* d4T: stavudine, ddI: didanosine, HU: hydroxyurea

The median age of the children with DMAC infection and MAC pneumonia was 65 and 63 months old respectively. Nine (90%) children were in the advanced stage of HIV before MAC infection was diagnosed. The CD4 level available in 5 patients revealed severe immunosuppressive stage with the median of 7 cells/mm^3 (1.67%) in DMAC and 127 cells/mm^3 (3.77%) in MAC pneumonia. Only 3 patients were on antiretroviral therapy (ART) with single or dual nucleoside reverse transcriptase inhibitors. Prolonged fever (80%), weight loss (90%), lymphadenopathy (90%) and hepatomegaly (100%) were the common presenting signs and symptoms. Respiratory symptoms were also common and more pronounced in cases of MAC pneumonia than these of DMAC infection.

Nine (90%) children were anemic (Hematocrit <33%) and four (40%) were leukopenic (WBC <4,000 cells/mm^3). Of the eight patients who had CXR done, all were abnormal. The distribution of the infiltration was mostly diffused, although localized infiltration was also found in 2 patients. Hilar lymphadenopathy was infrequently found.

Five of the seven children with DMAC infection and one of the three with MAC pneumonia received antibiotics treatment including clarithro-

Table 3. Detailed clinical findings in each patient.

No	Sex	Age (Months)	Clinical staging**	Immuno staging**	CD4 count	CD4 %	Symptoms	Specimen***	ART****	Treatment *****	Outcome and timing
1	M*	48	C	3	4	0.22	fever, weight loss, diarrhea, hepatosplenomegaly, lymphadenopathy	B	D4T+ddI+HU	CER	Survive at 24 months of F/U (cleared bacteremia)
2	F	65	C	3	8	0.4	weight loss, anorexia, hepatosplenomegaly, lymphadenopathy	B		CER	Survive at 17 months of F/U (cleared bacteremia)
3	F	67	C	-	-	-	fever, weight loss, diarrhea, anorexia, hepatomegaly, lymphadenopathy	B & GA		CER	Survive at 12 months of F/U
4	F	91	C	-	-	-	fever, weight loss, diarrhea, anorexia, hepatomegaly, lymphadenopathy	B & GA		2IRZE	Died from septic shock at 7 months after diagnosis
5	M	101	C	3	48	2.93	fever, weight loss, anorexia, diarrhea, hepatomegaly, lymphadenopathy	B & GA		-	Died from unknown cause at 10 days after diagnosis
6	M	20	C	-	-	-	fever, weight loss, diarrhea, hepatosplenomegaly, lymphadenopathy	B	ddI	CER	Died at 3 weeks of treatment (liver, spleen and lung necropsy found AFB)
7	M	60	C	-	6	4.31	fever, weight loss, diarrhea, hepatomegaly, lymphadenopathy	B		CER	Died at 7 months of F/U (liver necropsy found AFB)
8	M	24	C	3	-	-	weight loss, anorexia, hepatomegaly, lymphadenopathy	GA		IRZEC	Died at home of unknown cause at 3 months of F/U
9	F	74	B	3	127	3.77	fever, diarrhea, hepatosplenomegaly, lymphadenopathy	GA	D4T+ ddI	IRZEC	Refer at 2 months of F/U
10	M	63	C	-	-	-	fever, weight loss, diarrhea, anorexia, hepatomegaly	GA		-	Died at home of unknown cause at 3 months of F/U

* M : male, F : female,
** CDC classification for HIV-infected children,
*** B : blood, GA : gastric aspirate,
**** d4T : stavudine, ddI : didanosine, HU : hydroxyurea,
***** C : clarithromycin, E : ethambutol, R : rifampicin, O : ofloxacin, I : isoniazid, Z : pyrazinamide F/U : follow up

Table 4. Results of drug susceptibility testing.

Patient number	Specimens	Drug susceptibilities***							
		etham	rifam	cipro	oflox	amik	doxy	clari	strep
1	Blood	-	-	-	-	-	-	-	-
2	Blood	S*	R**	-	-	S	S	S	R
3	Blood	R	R	-	R	R	R	S	R
	Gastric wash	R	R	-	-	R	R	R	R
4	Blood	R	R	-	-	R	R	S	R
	Gastric wash	R	R	-	-	R	R	R	R
5	Blood	-	-	-	-	-	-	-	-
	Gastric wash	-	-	-	-	-	-	-	-
6	Blood	R	R	-	-	R	R	S	R
7	Blood	R	R	-	R	-	-	-	R
8	Gastric wash	R	R	-	R	R	R	R	R
9	Gastric wash	-	-	-	-	-	-	-	-
10	Gastric wash	-	-	-	-	-	-	-	-
Total sensitivity rate (%)		1/8 (12.5)	0/8 (0)	-	0/3 (0)	1/7 (14.2)	1/7 (14.2)	4/7 (57.1)	0/8 (0)

* S : sensitive
** R : resistant
*** etham = ethambutol, rifam = rifampicin, cipro = ciprofloxacin, amik = amikacin, doxy = doxycycline, clari = clarithromycin, strep = streptomycin

mycin, ethambutol and rifampicin. Only 2 patients were able to get rid of MAC bacteremia after treatment. The treatment, however, did not resolve the symptoms in any patients. Four of the patients with DMAC infection died at 10 days, 3 weeks, 7 months, and 7 months after diagnosis. Two of these had acid fast bacilli in various organs postmortem. Another 3 are still alive after 13, 17, 23 months of follow-up. In 2 of these, bacteremia was cleared. The other patient continue to have positive blood culture taken 3 months after treatment. Of the patients with MAC pneumonia, two died 3 months after diagnosis and the other patient was referred to another hospital with unknown outcome.

Eight MAC isolates were available for drug sensitivity testing, 5 from blood and 3 from GA. All the isolates from blood were sensitive to clarithromycin, however, all the isolates from GA were resistant. All isolates from both blood and GA were resistant to rifampicin. All but one isolate from blood were resistant to ethambutol. All the isolates from GA resisted all the antibiotics tested. The overall mortality rate was 60 per cent. The median survival time after diagnosis was 3.3 months.

DISCUSSION

This report confirmed that MAC infection is not a rare OI in HIV-infected children in Thailand. The incidence rate of 2.15 per 100 person-years found in this study was higher than that previously reported in the US[5]. Patients with MAC infection were all older than 2 years old and in the advanced stage of HIV. These findings were similar to that found in adults[10-12]. The median age found in the present study was 63-65 months, close to that found in previous reports[2,3].

In the present study, three children in whom MAC were isolated from GA without mycobacteremia were diagnosed as MAC pneumonia. However, it was possible that the isolates were from respiratory or gastrointestinal colonization. With the compatible signs and symptoms, the authors suspected that they were cases of DMAC infection with false negative blood culture. A study in adults reported that respiratory colonization associated with 65 per cent positive predictive value for DMAC infection [13]. For these reasons, these patients were treated as if they had DMAC. According to the current recommendation for prophylaxis[14], all but one child in this study fulfilled the criteria for MAC prophylaxis. However, none had received chemoprophylaxis before the episodes.

The clinical signs and symptoms found in this study were similar to those previously reported [1,15]. Anemia and leukopenia were common findings in DMAC infection, although some medications

such as zidovudine and cotrimoxazole, as well as HIV infection itself, may also contribute to bone marrow dysfunction[1,4].

Current recommendations for treatment of DMAC including clarithromycin or azithromycin plus ethambutol, and a third drug such as rifampicin, rifabutin, clofazimine, ciprofloxacin or amikacin may be added[16-18]. However, the outcome even with the recommended treatment was very disappointing [3], as found in the present study. The high rate of drug resistance may explain this unfavorable outcome. Moreover, the patients in this study did not receive adequate antiretroviral therapy and therefore were in a poor condition before the episodes.

The high resistant rate in this study is alarming. Clarithromycin is the only effective drug in *in vitro* test. However, monotherapy with clarithromycin induced resistance quite rapidly and also induced cross-resistance to azithromycin[19,20]. A larger antibiotic-resistance surveillance study in our institution testing 127 clinical isolates from July 1997 to July 1999 revealed resistant rate as follows: amikacin 91 per cent, rifampicin 88 per cent, ethambutol 69 per cent, and clarithromycin 44 per cent. Of the 103 isolates from HIV-infected individuals, 38 per cent resisted all drugs tested[21].

With the high resistant rate and poor outcome, prevention is the important strategy. Effective antiretroviral therapy is probably the best measure, however, is not feasible in places with limited resources. Primary chemoprophylaxis has not been used in Thailand due to the belief that MAC is rare and antibiotics for prophylaxis (clarithromycin/azithromycin) are expensive. Diagnostic difficulties probably resulted in missing the diagnosis in many cases. This study has shown that the incidence of MAC infection in Thai children is even higher than in the US, and therefore chemoprophylaxis should be considered in children in the advanced stage of HIV.

In conclusion, the authors have shown that MAC infection in HIV-infected Thai children is not rare. The host factors, clinical, and laboratory findings were all similar to other reports. The outcome is generally unfavorable. Primary chemoprophylaxis in conjunction with effective antiretroviral therapy, the important measures to prevent MAC infection, should be implemented whenever possible particularly in places with a high rate of drug resistance.

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การติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์ในผู้ป่วยเด็กไทยที่มีการติดเชื้อเอ็ชไอวี

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การติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์เป็นปัญหาโรคติดเชื้อฉวยโอกาสที่สำคัญในผู้ป่วยเด็กที่มีการติดเชื้อเอ็ชไอวี จากจำนวนผู้ป่วยเด็กติดเชื้อเอ็ชไอวี 169 รายในโรงพยาบาลศิริราชระหว่างเดือนมกราคม 2541 ถึงเดือนกันยายน 2543 พบผู้ป่วยมีการติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์ 10 ราย โดย 7 รายมีการติดเชื้อแบบแพร่กระจายและ 3 รายมีการติดเชื้อในปอด ผู้ป่วย 9 รายอยู่ในระยะท้ายของการติดเชื้อเอ็ชไอวีโดยมีค่ามัธยฐานของจำนวน CD4 เท่ากับ 7 เซลล์/ลูกบาศก์มิลลิเมตรและ 127 เซลล์/ลูกบาศก์มิลลิเมตรและค่ามัธยฐานของอายุเท่ากับ 65 เดือนและ 63 เดือนในผู้ป่วยที่มีการติดเชื้อแบบแพร่กระจายและผู้ป่วยที่มีการติดเชื้อในปอดตามลำดับ ไม่มีผู้ป่วยรายใดได้รับยาป้องกันการเกิดโรคนี้มาก่อน

อาการและอาการแสดงที่สำคัญ ได้แก่ ไข้เรื้อรัง น้ำหนักลด ต่อมน้ำเหลืองโต ตับม้ามโต ท้องเสีย ซีดและเม็ดเลือดขาวต่ำ ผู้ป่วยที่มีการติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์มีอัตราการเสียชีวิตสูงถึง 60% จากการทดสอบความไวของเชื้อต่อยาพบการดื้อยา clarithromycin น้อยที่สุด

จากการที่อัตราการติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์ในผู้ป่วยเด็ก ที่มีการติดเชื้อเอ็ชไอวีสูงถึง 2.15 รายต่อ 100 person-years รวมทั้งมีอัตราการดื้อยาและอัตราการเสียชีวิตที่สูง จึงควรจะมีการพิจารณาให้ยาป้องกันการติดเชื้อแบบปฐมภูมิร่วมกับการให้ยาต้านไวรัสที่ เหมาะสมในเด็กไทยที่มีการติดเชื้อเอ็ชไอวีในระยะท้าย

คำสำคัญ : การติดเชื้อ*มัยโคแบคทีเรียม เอเวียม* คอมเพล็กซ์, เด็กที่ติดเชื้อเอ็ชไอวี, การใช้ยาป้องกันการเกิดโรค

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