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Acanthamoebiasis in Korea: Two New Cases with Clinical Cases Review

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The first case was 7-month-old immunodeficiency girl in whom the diagnosis of Acanthamoeba pneumonia was established by culture of a bronchial washing. The patient had been ill for a month when she was admitted due to neonatal thrombocytopenia with respiratory difficulty and treated with gammaglobulin and steroid. Her chest X-ray showed diffuse alveolar consolidation on the left lung with interstitial hazziness and a partial sign of hyperinflation on the right lung. Laboratory tests showed that the Candida antigen was negative and Pneumocystis carinii was not detected. Mycoplasma antigen was negative. All the immunoglobulin levels (IgG, IgA, IgM) were below the normal range. Five days later the patient expired. The second case was an immunosuppressed 7-year-old boy in whom Acanthamoeba trophozoites were found in the skin biopsy, followed by meningitis leading to death. About five days after a laceration on the region of the left eyebrow, a painful bean-sized nodule developed at the suture site and it was treated with antibiotics and corticosteroid. The skin biopsy showed severe inflammatory cell infiltration. Trophozoites were scattered near the blood vessels throughout the inflammatory zone. From one weak prior to admission, the patient had suffered from vomiting, indigestion and mild fever. Skin nodules with tenderness appeared all over his body surface. Examination of cerebrospinal fluid showed clear, Gram stain was negative, bacterial culture negative, India ink preparation negative, and organism on wet smear negaive. On admission day 10, focal seizure of the left extremity occurred. Brain CT revealed calcific density on the left parietal lobe area and hypodensity on the left basal ganglia. He became comatous and died immediately after discharge. Until now in Korea, two cases that are described in this paper, one Acanthamoeba meningoencephalitis case and seven Acanthamoeba keratitis cases including two unreported keratitis cases that are reported in this paper have been presented.

Key Words: Acanthamoebiasis, *Acanthamoeba* sp, *Acanthmoeba* meningoencephalitis, *Acanthamoeba* keratitis, immunosuppression

The pathogenic free-living amoeba, *Acanthamoeba* and *Naegleria,* are well known as the causative protozoa of amoebic meningoencephalitis and keratitis (Willaert, 1973). These amoebae are ubiquitous in the environment, and detected in the nasal cavity (Cerva *et al.*, 1973) and pharynx of healthy humans (Wang and Feldman, 1961).

Since the first case was discovered in Australia in 1965 (Fowler and Carter, 1965), the clinical cases of these amoebae infection have been widely reported throughout the world. By a retrospective study, Dos Santos retrospectively found five fatal primary amoebic meningoencephalitis cases from 16,174 autopsy cases (Dos Santos, 1970).

In many instances pathologists have diagnosed this disease from the results of a review of necropsy material. Unfortunately, in such cases clinical and

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epidemiological descriptions have been rare, and amoeba culture and antiamoebic therapy have not been tried. For these reasons, reviews of the clinical, epidemiological, pathological and protozoological findings with culturally-proven diagnosis, in which all of those cases were investigated antemortem as well as postmortem, have not been satisfactory.

Unitil now in Korea, eight cases of *Acanthamoeba* infection including two unreported keratitis cases that are described in this paper have been presented.

TWO NEW CASES REPORT

Case 1: *Acanthamoeba* pneumonia

This was a case of a congenital immunodeficiency patient in whom the diagnosis of *Acanthamoeba* pneumonia was established by a culture of bronchial washing.

Patient Choi EJ, a 7-month-old girl, was admitted to the Severance Hospital due to a cardiac problem with cyanosis of about one month on July 12, 1993. She was healthy when she was born by C-section due to CPD. When she was one month old, she was admitted to the Inchon Christian Hospital due to neonatal thrombocytopenia, and treated with gammaglobulin and steroid. Five days before being admitted to the Severance Hospital she was diagnosed with pneumonia. When she was admitted, she was acutely ill looking, and her breathing sound was clear without rales. She had respiratory difficulty with substernal retraction and poor oral intake. Her chest X-ray showed diffuse alveolar consolidation on the left lung with interstitial hazziness and a sign of partial hyperinflation on the right lung, which characterized acute respiratory disease syndrome (Fig. 1). On the next day, oral secretion increased and chest wall retraction was observed, so that oxygen inhalation was done. Until then she had no cardiac problem. Laboratory tests showed that methemoglobin level 0.9 g/dl, *Candida* antigen negative, and LDH 592 IU/L. On the third day after admission, bronchial washing was done. The result of sputum cytology was negative, and *Pneumocystis carinii* was not detected. *Mycoplasma* antigen was negative, but *Acanthamoeba* sp. was cultured on agar plate. All the immunoglobulin levels (IgG, 76 mg/dl; IgA, 4.3 mg/dl; IgM, 34.2 mg/dl) were below the normal range. The next day she was suffering more from respiratory difficulty. Chest AP showed decreased total lung volume and progressive consolidation on both lungs.

Cyanosis became more severe. Antibiotic (Triaxone, Unasyn and Septrin) therapy was done. Physical findings were temperature, 36.4°C; blood pressure, 85/50 mm Hg; pulse 135/min.; pupil, prompt light reflex; motor, uncheckable; no path-

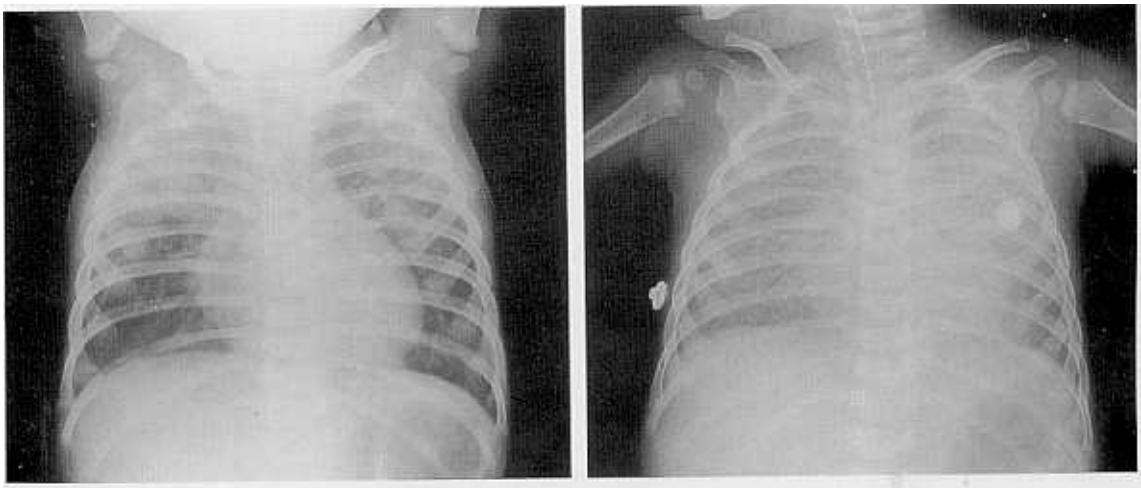

Fig. 1. Chest P-A view showing the ground glass pattern and local consolidation in right upper and left lower lobe (left), and, 10 days later, diffuse consolidation, distinct air bronchogram (right).

Table 1. Laboratory findings of *Acanthamoeba* pneumonia case

		Admission	Day 1	Day 3	Day 5	Day 7	Day 9	Day10
CBC;	Hemoglobin, gm/dl		15.0	15.5	12.8	11.4	11.1	
	Hematocrit, %		48.0	49.9	41.5	36.5	37.6	
	WBC, /mm^3		10900	8420	9300	5490	5460	
	Segmented		78.0	80.5	95.5	79.0	85.0	
	Eosinophil					3.0		
	Lymphocyte		10.0	11.1	9.0	7.3		
	Monocyte		6.0	7.8	5.9	9.0	4.6	
	Platelet count, $\times 10^3$		253	222	182	82	65	
Electrolytes,	Na		137		139	139	137	132
	K		4.9		2.1	3.1	2.8	4.3
	Cl		106		106	107	105	99
	CO_2		19		20	19	25	19
Urinalysis,	pH		6.0					
	specific gravity		1.025					
	protein		–					
	glucose		5					
	ketone		–					
	WBC		–					
	RBC		–					
	Urobilinogen		1.0					
DIC Lab.	PT		25.2 (36%)					
	PTT		more than 20 sec.					
	FOP		1 : 20 (+)					
	Fibrinogen		365					

ological reflexes. On the sixth admission day, she was drowsy to stupor and her breathing sound was coarse with rales. No organism was detected in blood culture. Five days later the patient expired.

Isolation, identification, and propagation of amoebae were conducted by the Department of Parasitology, Yonsei University College of Medicine in Seoul, Korea. The organism on agar plate was identified as *Acanthamoeba* cyst, measuring 14.7 μm in mean diameter (12.1 to 17.6 μm in range) (Fig. 2). This isolate was named YM-6.

Case 2: *Acanthamoeba* meningitis

This was a case of an immunosuppressed patient due to long-term treatment with steroid in whom *Acanthamoeba* tropho-zoites were found in the skin biopsy, followed by meningitis leading to death.

Patient KeumSan C.N., a 7-year-old, previously healthy boy was admitted due to meningitis symptoms for one week. In August, 1989, about five days after a laceration on the region of the left eyebrow, a painful bean-sized nodule developed at the suture site of this area, and was treated with antibiotics and corticosteroid. After that, the subcutaneous lesion became larger. Sporotrichosis (R/O Cryptococcosis) was suspected by a dermatologist at C-N University Hospital. He was treated with potassium iodide and

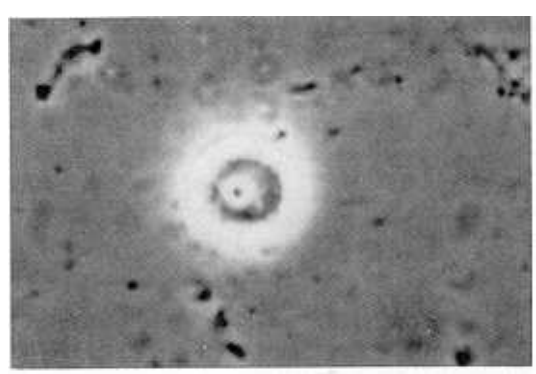

Fig. 2. Acanthamoeba cyst, isolate YM-6, cultured on agar plate from bronchial washing of Acanthamoeba pneumonia patient.

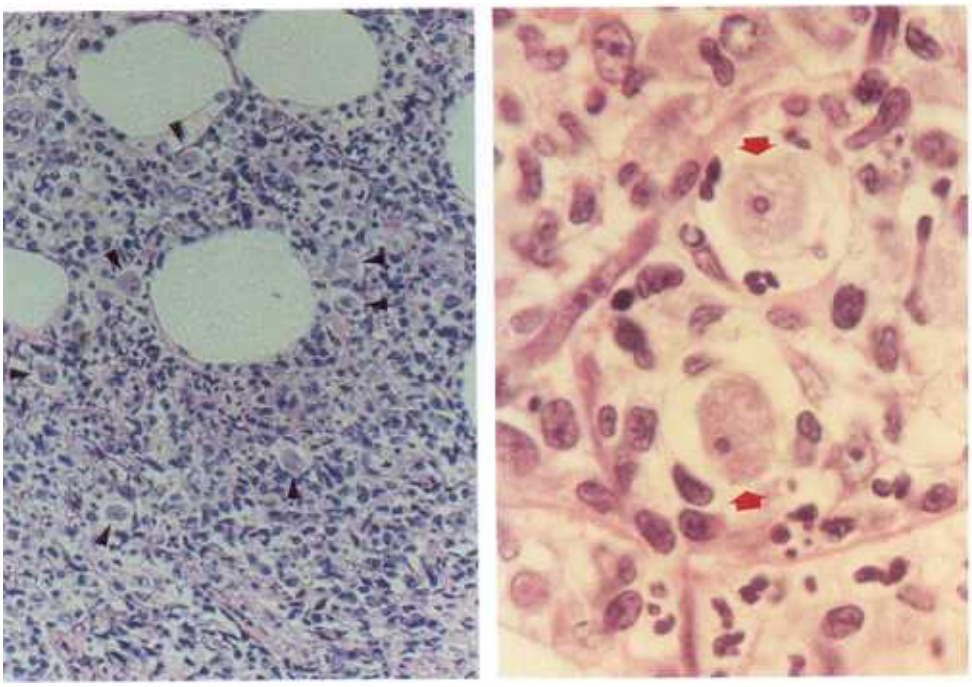

Fig. 3. Biopsy of skin lesion showing the Acanthamoeba trophozoites surrounded by a number of the acute inflammatory cells.

ketoconazole, but the treatment had no effect. A number of nodules appeared on the upper extremity and chest wall. The patient then was transferred to the Department of Dermatology, K-P University Hospital in August, 1991 for better evaluation of his skin lesion. The patient had erythematous annular plaque on the left half of his face below the left eyebrow with severe tenderness and elevated margin. Biopsy of the skin lesion demonstrated negative for fungus culture. The histopathological findings showed severe inflammatory cell infiltration. Trophozoites scattered near the blood vessels throughout the inflammatory zone and granulomatous changes in the dermis through the subcutaneous fatty layer (Fig. 3). Granuloma annulare was suspected, so that diaminodiphenylsulfone and prednisolone were administered orally for 42 days. Skin lesion became flattened a little, transformed continuously, and the inflammation diminished. One and a half months later, the patient looked healthy but his activity had diminished a little.

From one week prior to admission, the patient had suffered from vomiting, indigestion and mild fever, and so was referred to a pediatrician. His meningitis symptoms were not typical. His general condition was poor. Nodules with tenderness, dermal and subcutaneous, appeared all over his body surface. Examination of cerebrospinal fluid showed clear, with a total 40 cell count/ml (40 to 70% of segmented neutrophils), while other results of CSF were all negative. Gram stain was negative, as were acid-fast bacilli stain and culture, bacterial culture, India ink preparation and organism on wet smear. At this time, the symptoms of headache and vomitting became more severe. One week after admission the patient complained of somnia and weakness of the lower extremity. On admission day 10, focal seizure of the left extremity occurred. Brain CT revealed calcific density on the left parietal lobe area and hypodensity on the left basal ganglia. Under the impression of Tuberculour meningitis, antituberculous medication was administered. On admission day 12, the patient became comatous and died immediately after discharge.

The biopsy findings of the skin lesion were not understandable because the history or course of this

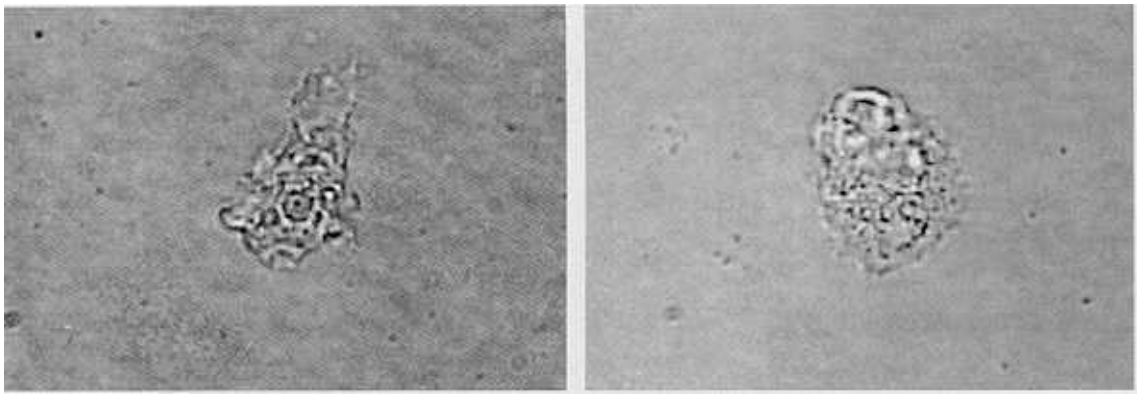

Fig. 4. Acanthamoeba sp. YM-7 trophozoite isolated from the corneal scraping of a patient who was suffering from corneal keratitis reported by Kim et al. (1995).

skin disease was chronic. However, the skin lesion showed an acute inflammatory reaction. In this case, the problem was determined that the patient had been treated with steroid for a long time.

PUBLISHED CASES REVIEW

Acanthamoebic meningoencephalitis case

Ringsted *et al.* first reported a case of *Acanthamoeba* meningoencephalitis in Korea (Ringsted *et al.*, 1976). This case was a 5-year-old boy who developed multiple subcutaneous, nontuberculous granulomas and died with meningoencephalitis. Autopsy disclosed amebic granulomas in subcutaneous tissue, the left adrenal gland, and the pancreas, with more acute inflammatory lesions in the liver, kidney, and brain. The causative organism was believed to be an *Acanthamoeba* sp.

Acanthamoeba keratitis cases

Two cases of *Acanthamoeba* keratitis/conjunctivitis were reported by Cho *et al.* (1992). This is the first report of *Acanthamoebic* ketatitis in Korea. These two patients were suffering from a chronic relapsing central corneal ulcer and chronic conjunctivitis with mucoid discharge. These two cases had no history of contact lens wearing or eye injury. Both patients were transferred to hospital from a local clinic because of no improvement with long-term treatment of broad-spectrum antibiotics topically and systemically. Specimens from corneal scraping and conjunctival mucoid discharge were examined with calcofluor white staining. In dark-field fluorescent microscopy, *Acanthamoeba* cysts of light-green colored wall were observed with orange-red colored cytoplasm in both cases.

Kim *et al.* presented a case of a contact lens wearing patient who was suffering from corneal ulcer with conjunctival injection (Kim *et al.* 1995). In spite of broad-spectrum antibiotic therapy, corneal lesion was not improved and bacterial smear and culture were negative. Examination of corneal scrapings and specimens of ulcered cornea was done by Giemsa-Wright stain and hematoxylin-eosin stain. Double walled *Acanthamoeba* cysts measuring 15 μm in mean diameter (14.0 to 16.9 μm in range), were detected (Fig. 4). The specimens were cultured on 1.5% non-nutrient agar plate with *E. coli* suspension, and *Acanthamoeba* cysts and trophozoites were found with H & E stain. Identification and propagation of amoebae were conducted by the Institute of Tropical Medicine, Yonsei University College of Medicine in Seoul, Korea. This isolate was named YM-7 and cultured well axenically in CGV medium which was identified probably as *Acanthamoeba castellanii.*

Two cases of Acanthamoeba keratitis by Chung *et al.* were presented. A 16-year-old female who wore contact lenses, had a severe corneal ulcer of the right eye and nodular scleritis (Chung *et al.* 1996). She had been complaining of eye pain and

lacrimation for previous 3 months, and was treated for bacterial keratitis. But *Acanthamoeba* cysts were detected from the corneal scraping and confirmed by acridine orange staining. Treatment was done with 0.02% PHMB, 0.1% hexamidine, itraconazole and ofloxacin. Three weeks after treatment, central corneal perforation occurred. Immediately, a corneal graft was performed and antiamoebic therapy was done. Two months later, visual power was restored up to 20/70. Isolate KA/E3 isolated from this patient was observed to be very close to *Acanthamoeba castellanii* by riboprints. The other case was a 32-year-old male, who had been complaining of eye pain, congestion and diminished visual power for two months. This patient was not wearing contact lenses. Under the diagnosis of fungal keratitis in the local clinic, therapy was done without improvement. So, he visited the out-patient clinic of K-P University hospital, Taegu, Korea. Physical examination revealed large corneal trauma and parenchymal infiltration of an oval shape, and his vision was in the perception of hand movement. Treatment with PHMB, neomycin, prednisolone acetate and homatropine was done. Isolate KA/E4 from corneal scraping was confirmed to be *Acanthamoeba* cysts by acridine orange stain. Riboprints showed this isolate was similar to, but not same as, *Acanthamoeba castellani, A. quina, A. polyphaga* or *A. triangularis.*

The clinical details of two cases were previously unreported (Kong and Chung, 1998). These Korean isolates from patients were named KA/E1 and KA/E2.

DISCUSSION

A case of amoebic meningoencephalitis was first reported in Korea by Ringsted *et al.* in 1976. Im demonstrated the six clinical cases of *Acanthamoeba* infection up until 1996 (Im, 1996). *Acanthamoeba* meningoencephalitis may present as an acute disease. However, it has frequently occurred in chronically-ill patients. Generally, this disease is usually of gradual onset and has a prolonged chronic course.

Acanthamoeba generally causes a slowly progressive illness characterized by hematogenous dissemination of amoebae from an unknown portal of entry to the brain in all known cases and to other organs in some cases (Jager and Stamm, 1972; Robert and Rorke, 1973). *Acanthamoeba* sp. may enter through ulcerated or broken skin or the eye, and possibly through the respiratory tract. The case of meningoencephalitis presented here was nearly the same as the case presented by Ringsted *et al.* (1976) in its clinical course. These two cases gave an instance of hematogenous dissemination of *Acanthamoeba* from a skin injury to the brain. And these two patients probably were in the state of immunosuppression leading to the amoebic meningoencephalitis, which may have developed as a result of long-term steroid therapy, especially in the presented case. Because fatal *Acanthamoeba* meningoencephalitis has occurred in debilitated patients or in those whose immunity has been impaired, careful immunosuppressive therapy may be necessary.

Tissue invasion tends relatively towards granuloma formation. The tissue pathological findings are necrotizing, granulomatous inflammation with focal hemorrhage and multinucleated giant cells. Trophozoites are scattered throughout the inflammatory zone and were observed abundantly near blood vessels. Chronic granulomatous lesions caused by *Acanthamoeba* sp. have been reported in the skin, kidneys, liver, spleen, uterus and prostate (Ringsted *et al.* 1976). In the cases described here where the primary focus of invasion was the skin, the period from exposure to onset in central nervous system was one year or longer.

Eye lesions caused by *Acanthamoeba* are primarily keratoconjunctivitis, corneal ulcer and uveitis. *Acanthamoeba* sp. was detected in all the present cases, and confirmed to be probable *Acanthamoeba castellani, A. polyphaga* and other species. Genus *Acanthamoeba* has been detected in a number of contact lens containers (Lee *et al.* 1997). For soft contact lens wearers, it is necessary to consider the effectiveness of the disinfectant used to clean the lenses.

The diagnosis depends firmly on the detection of the amoebae in cerebrospinal fluid, in scrapings from the eye or skin lesion or in cultures of materials from those sources. Cultivation, on 1.5%

non-nutrient agar plate overlayed with dead *Escherichia coli,* is highly recommended. Immunological tests are useful. Indirect fluorescent test or immunoperoxidase staining may be useful in identifying amoeba from tissue sections. Tisssue stains are also available with Giemsa stain, Gomori's silver stain and calcofluor white.

A number of *Acanthamoeba* sp. have been isolated from sewage, water puddles, storage reservoirs, and even the gills of freshwater fish in Korea. These isolates proved to be pathogenic to mice infected intranasally, which led to experimental meningoencephalitis (Im *et al.* 1998). The *Acanthamoeba* cysts can tolerate drying, which enables them to be waterborne or airborne. General preventive controls include increasing public awareness of the possible dangers of contaminated water.

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