Case Report: The First Direct Evidence of *Gnathostoma spinigerum* Migration through Human Lung

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Abstract. Gnathostomiasis is a helminthic infection caused by the third-stage larvae of nematodes of the genus *Gnathostoma*. The life cycle in humans starts with an enteric phase, with the worm perforating the gastric or intestinal mucosa to reach the peritoneal cavity and migrating through the human body. Subsequent penetration through the diaphragm may produce pleuropulmonary symptoms. We herein present a previously healthy 56-year-old Thai man from Southern Thailand who was an ex-smoker presented with chronic dry cough progressing to hemoptysis after consuming grilled swamp eels and freshwater fish. Chest computed tomography showed consolidation at the lingular segment, and the differential diagnosis was primary lung cancer and pulmonary tuberculosis. The lung tissue biopsied during bronchoscopy displayed segments of organisms with the phenotypic characteristics of *Gnathostoma* spp., and abundant eosinophils were seen in the alveolar tissue. *Gnathostoma spinigerum* infection was confirmed by a Western blot assay for *G. spinigerum*-specific 24-kDa reactive band. The patient received albendazole, and a follow-up chest radiograph revealed improvement in the consolidation in the lung and reduction in hemoptysis. We report the first direct evidence including pathology and immunohistochemistry of *Gnathostoma* invasion via the human lung, with clinical and radiographic presentations mimicking either malignancy or chronic infection.

INTRODUCTION

Gnathostomiasis is prevalent in Southeast Asian countries,^{1,2} East Asia, and Latin America,³ and it is sporadically found in other parts of the world mainly because of a patient visiting an endemic area.⁴ *Gnathostoma* larvae migrate to the skin,^{5,6} gastrointestinal system, genitourinary system, auditory system, central nervous system, and the eyes, and parasitosis of the central nervous system may cause life-threatening complications.^{7–9} Definite diagnosis is made by identification of the parasite taken from the patient or through histopathological section of the suspected lesion. The clinical presentations of gnathostomiasis in the human lung were previously described as transient pulmonary lesions with a marked increase in eosinophils in both blood and sputum (Loeffler's syndrome)⁷ and spontaneous pneumothorax.¹⁰ To the best of our knowledge, no gnathostomiasis study in humans has demonstrated the parasite in the lung tissue, as all previous diagnoses for pulmonary gnathostomiasis depended on evidence of migratory swelling or cutaneous creeping eruption along with transient abnormal chest radiograph.⁷ In the present case, human pulmonary gnathostomiasis was diagnosed by a lung tissue biopsy taken during bronchoscopy supported with confirmatory organism-specific Western blotting, which is the first direct demonstration of *Gnathostoma* larval migration to the lung in humans.

CASE REPORT

A previously healthy 56-year-old Thai man presented with dry cough for 3 months without fever or dyspnea. He was a 20-pack-year ex-smoker, had no current medication, and lived in Nakhon

Si Thammarat Province in Southern Thailand. He had tried antitussive agents without improvement. He had a history of regular consumption of grilled swamp eels and freshwater fish, especially snakehead fish and catfish. He denied either consuming raw or undercooked meats or drinking unclean water. He had a pet cat and a vegetable plot fertilized with organic kitchen waste, where he walked barefooted. He had no known contact with tuberculosis and reported no anorexia or weight loss. He did not notice any cutaneous migratory swelling or creeping eruption. He denied any travel outside his province. Two months after the onset of his cough, he developed hemoptysis. He took a 7-day course of amoxicillin, but his symptoms did not improve.

On physical examination at presentation, he had a body temperature of 36.8°C, a pulse rate of 86 beats/minutes, a respiratory rate of 14 breaths/minutes, a blood pressure of 118/60 mmHg, and an oxygen saturation of 98% while breathing room air. He did not have cyanosis, clubbing, pursed lip expiration, accessory respiratory muscle usage, and nasal flaring. Auscultation of the thorax revealed bronchial breath sound at the left anterior thorax below the nipple and coarse crepitation of the left lung, but no wheezing. The initial chest radiograph showed alveolar opacity silhouette with the left heart border (Figure 1A), whereas the chest computed tomography (CT) showed pan lobular emphysema of the upper lobe and consolidation with central necrosis of the lingular segment (Figure 1C–F). The initial complete blood count showed mild eosinophilia (absolute count 400 cells/ μ L) without other abnormalities. In sputum Gram stain, acid-fast bacilli were negative and sputum cultures for both bacteria and *Mycobacterium* reported later were negative. The differential diagnosis was primary lung cancer and chronic infection with *Mycobacterium tuberculosis* because of his history of being an ex-heavy smoker and CT findings. Bronchoscopy with tissue biopsy was performed, and empirical antibiotic (amoxicillin–clavulanic acid 875 mg/125 mg two tablets per

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FIGURE 1. Chest radiograph and computed tomography (CT) of the patient were demonstrated. (A): The initial chest radiograph showed alveolar opacity silhouette with the left heart border (arrows). (B): The 1-month follow-up chest radiograph after bronchoscope and albendazole treatment showed improvement of alveolar opacity (arrow). (C–F): CT chest with contrast shows panlobular emphysema of both upper lobes (arrows) (C) and consolidation with central necrosis of the lingular segment (arrows) (D–F).

day for 14 days) was given. The hematoxylin and eosin (H&E) stain of the biopsied lung tissue revealed segments of parasites with lateral cuticular spines, undulating cuticles, and coelomic muscles, prompting diagnosis of pulmonary gnathostomiasis (Figure 2). Fragments of the alveolar tissue, clusters of inflammatory cells, particularly lymphocytes, and abundant eosinophils were also observed. In-house confirmatory Western blot assay was positive for *Gnathostomiasis spinigerum*, specifically 24-kDa reactive band (Figure 3).² An immunohistochemical study was performed to examine the level of anti-inflammatory and pro-inflammatory cytokines in the peribronchiolar and alveolar tissues from the biopsied lung compared with the normal lung. *H*-score (0–300) was used to determine the expression of those cytokines by the multiplication of intensity score (0–3; 0: absent and 1–3: weak, moderate, and strong reactivities, respectively) and the percentage area of expression/field (0–100%). Digitalized image analysis program (ImageJ, NIH) was used to examine the area of expression.^{11–13} In brief, 10 random fields of color images from each group were acquired and converted to gray scale. Adjusted images were transformed by the threshold mode to locate the area of positive reaction and estimated by the number of black pixels. Thus, the area fraction of positive reaction was determined as the percentage of black pixels/area. The results indicated that there was greater expression of the transforming growth factor (TGF)- β in the peribronchial area than interleukin (IL)-4, IL-10, and tumor necrotic factor (TNF)- α (Figure 4). In addition, the alveolar tissue from patients also exhibited the upregulation of TGF- β when compared with the normal tissue. However, the levels of IL-4, IL-10, and TNF- α

were lower in the patient's lung than those in the normal lung (Figure 5).

The patient was informed the diagnosis of pulmonary gnathostomiasis and ensured the curative treatment because of accidental removal of parasite by tissue biopsy under bronchoscopy. In the follow-up, the chief complains of dry cough and hemoptysis symptoms disappeared, and the physical examination revealed normal finding. None of the bronchial breath sound was detected from chest auscultation together with improvement of alveolar opacity from chest radiograph (Figure 1). Complete blood count showed a decrease in absolute eosinophil count (54 cells/ μ L). A routine stool examination performed to identify any other parasites was negative.

DISCUSSION

Humans are an accidental host of *Gnathostoma* spp. Foodborne zoonosis occurs by ingesting uncooked meats of secondary intermediate hosts in its zoonotic life cycle, mainly freshwater fish or paratenic animals, such as fish-eating reptiles and birds.^{14,15} Our patient had a risk of infection because of habits of consuming grilled swamp eels and some freshwater fish that may have been undercooked. He consumed snakehead fish and catfish, which were proven as important intermediate/paratenic hosts.^{16,17} Also, 48 animal species were reported as reservoir hosts for transmission of infective *Gnathostoma* larvae to humans in Thailand.¹⁸ The larval and immature stages of *Gnathostoma* spp. are hardy and may tolerate the heat if located deep in the flesh or internal organs of improperly grilled fish.

FIGURE 2. Details of the biopsied lung were displayed. **A** and **B**: Biopsy from the lung tissue shows thickening of the alveolar wall (**A**, hematoxylin and eosin [H&E] stain 50 $\times$) and pronounced eosinophilic infiltration (**B**, H&E stain 400 $\times$). (**C** and **D**): Fragments of the cross-sectioned *Gnathostoma* worm were illustrated (**C**, H&E stain 100 $\times$), with higher magnification of the undulating cuticles (inset) from the larger parasite fragment. Another parasite fragment displayed lateral pointed cuticular spines (**D**, H&E stain 400 $\times$). CM = coelomic muscles. This figure appears in color at www.ajtmh.org.

Our patient had *Gnathostoma* larvae in the biopsied lung tissue, which was rare direct evidence of migration to the lung. In most infected cases, especially with pulmonary involvement, the larvae of *G. spinigerum* were not detected.^{7,19,20} Therefore,

a presumptive diagnosis is usually made based on the clinical manifestations of a migratory swelling and a history of consuming of freshwater fish or other intermediate or paratenic hosts in an endemic area. Pulmonary gnathostomiasis is often preceded by episodes of migratory swelling over a period of years^{21–24} together with chest symptoms of cough,^{19,23–25} varying from paroxysmal dry to violent cough,²⁵ pleuritic pain,^{10,23,24} dyspnea,²² hemoptysis,^{20,22} lobar consolidation or collapse,²¹ pleural effusions,^{21–23} pneumothorax,¹⁰ and hydropneumothorax.²³ Thoracentesis was performed in a case with pleural effusion, demonstrating exudative pleural fluid with abundant eosinophils.^{10,23} In all previously reported cases, there was no direct evidence of pulmonary invasion except for indefinite chest symptoms and/or the expectoration of worms. In several cases from Thailand, the larval worm migrated to the upper respiratory tract or even to the pharynx; then, it was ultimately expectorated, and chest symptoms were resolved.^{24,26} In other endemic Asian countries, such as China and Japan, pulmonary gnathostomiasis was reported sporadically and was diagnosed by pleuritic pain and nodular lesion on chest CT and supported by rising antibodies in patient's serum detected with a serological test.^{27,28}

In the present study, we found the *G. spinigerum* larval stage in the human lung by the tissue biopsy technique, and the Western blot assay confirmed the diagnosis by organism-specific 24-kDa reactive band with the antibody against *G. spinigerum*.² As clinical manifestations and radiographic findings of pulmonary gnathostomiasis are not specific and scanty, morphological identification of *Gnathostoma* larval detection including histopathology, immunohistochemistry,

FIGURE 3. Specifically reactive band by Western blot analysis was diagnosed as gnathostomiasis. (**A**): Patient's serum and (**B**): positive control (patient's serum with *Gnathostoma* larva detected); the diagnostic reactive band of gnathostomiasis was indicated by arrow. This figure appears in color at www.ajtmh.org.

FIGURE 4. Expressions of anti-inflammatory and pro-inflammatory cytokines were demonstrated. (A–E): interleukin (IL)-4, IL-10, transforming growth factor- β , and tumor necrotic factor- α (A–D) scoring with *H*-score (E) in the peribronchial tissue from lung biopsy by immunohistochemical staining. This figure appears in color at www.ajtmh.org.

and confirmatory Western blotting is useful for definite diagnosis. However, obtaining the larval worm from a patient via the biopsied lung tissue is difficult because of its very small size and migrations. Moreover, the biopsy technique may reduce the specificity of morphological diagnosis because of distortions in the organism or only a small part of the larval worm being extracted from the lung tissue. However, some characteristics of the worm can be observed, allowing for identification. These included several characteristic features of the body shape, the number of rows of hooks at the cephalic end, the number of hooks in each row, the character of the spines which cover the body, and the extent to which the body was covered by spines, which vary according to species.^{29,30} In the present case, histopathological findings of the fragmented parasite with features of lateral cuticular spines, undulating cuticles, coelomic muscles, and eosinophilic response were highly suggestive of *Gnathostoma* spp.

Host inflammatory responses to the parasitic diseases have been discussed for many decades. Acute inflammation with eosinophil and neutrophil infiltrations or chronic granulomatous inflammation was mainly observed depending on disease progression. In gnathostomiasis, host cellular immune responses with the production of cytokines, for example, IL-4, IL-5, IL-10, and IL-13, were described in promoting granulomatous formation.³¹ However, encyst formation has never been reported in the human gnathostomiasis. Interleukin-5 plays a crucial role in growth, differentiation, and activation of eosinophils³² in relation to helminth-induced eosinophilia and eosinophilic inflammation. In the present study, high levels of circulating and tissue eosinophilia were observed which

would be a result of upregulation of IL-5. Further studies on IL-5 levels are required in gnathostomiasis. Moreover, helminthic secretions induce T-cell Foxp3 expression through the TGF- β pathway, and this regulates the immune response to create an environment for the parasite to persist.³³ Recently, the level of Fc gamma receptor I, a high-affinity receptor for initial phagocytic signaling, was inhibited by *G. spinigerum* excretory–secretory products,³⁴ which was similar to the effect from TGF- β production.³⁵ The immunohistochemical study of our patient's biopsied lung tissue showed upregulation of TGF- β compared with IL-4, IL-10, and TNF- α . This evidence in the infected human lung tissue supports those of the previous in vitro and animal model studies for the role of TGF- β in retarding the host's innate immune response.

In conclusion, we report the first direct evidence of *Gnathostoma* migration to the human lung with clinical presentation mimicking either malignancy or chronic infection. Confirmatory Western blot assay, histopathology, and additional immunohistochemistry are useful for definite diagnosis.

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FIGURE 5. Expressions of anti-inflammatory and pro-inflammatory cytokines in the alveolar tissue from the biopsied lung tissue compared with the normal tissue were demonstrated. **A–L:** Immunohistochemical staining of interleukin (IL)-4, IL-10, transforming growth factor-β, and tumor necrotic factor-α (**A–C**, **D–F**, **G–I**, and **J–L**, respectively) scoring with *H*-score (**C**, **F**, **I**, and **L**) between patient lung biopsy (**B**, **E**, **H**, and **K**) and normal lung tissue (**A**, **D**, **G**, and **J**). This figure appears in color at www.ajtmh.org.

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