Characterization of the Humoral Immune Response against *Gnathostoma binucleatum* in Patients Clinically Diagnosed with Gnathostomiasis

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**Abstract.** Gnathostomiasis is an emerging systemic parasitic disease acquired by consuming raw or uncooked freshwater fish infected with the advanced third-stage larvae of *Gnathostoma* spp. This disease is endemic to the Pacific region of Mexico, and one of its etiologic agents has been identified as *Gnathostoma binucleatum*. We characterized the humoral immune response of patients clinically diagnosed with gnathostomiasis by detecting total IgM, IgE, and IgG class and subclasses against a crude extract of the parasite by Western blotting. Our results do not show differences in the antigens recognized by IgM and IgE. However, we found that the specific humoral immune response is caused mainly by IgG, specifically IgG4. We found that 43.6%, 65.2%, 54.1%, and 26.3% of the patients recognize the 37-kD, 33-kD, 31-kD, and 24-kDa antigens, suggesting that the 33-kD antigen is the immunodominant antigen of *G. binucleatum*.

**INTRODUCTION**

Gnathostomiasis is a parasitic infection caused by advanced third-stage larvae (ADV3) of the helminths *Gnathostoma* spp., which are seen mostly in tropical and subtropical regions. It is a food-borne zoonosis, and is endemic to areas where humans eat raw freshwater fish or shellfish, especially Thailand and other parts of Southeast Asia, Japan, and increasingly Latin America, particularly Mexico. Adv3 is acquired by consuming raw or uncooked fresh-water fish infected with ADV3, in particular, swamp eels, catfish, sleeper perch, bream, Nile tilapia, butterfly fish, loaches, or snake-headed fish. Epidemiologic studies have shown that more than 2,000 cases of this disease have been reported in Mexico since 1999, of which more than 500 were reported in the state of Nayarit. Although 18 species of *Gnathostoma* have been recognized worldwide, *G. binucleatum* is the only species found to infect humans in Mexico to date. However, other species may also infect humans.

In humans, this disease is characterized by a combination of signs and symptoms caused by the parasite that include mechanical damage caused by migration of larvae, release of toxic substances, and an inflammatory reaction in the host. Although neurologic and ocular symptoms have also been described, the most prevalent symptoms in Mexico are cutaneous, and no cases of invasion of the central nervous system have been reported.

The definitive diagnosis of gnathostomiasis can be made by recovering the migrating larvae from skin lesions, but this procedure can be difficult because of the migratory behavior of this particular parasite. However, it can be clinically diagnosed by obtaining a history of eating raw or partially cooked fish, intermittent subcutaneous or cutaneous migratory swelling, and eosinophilia. Immunologic approaches have been developed to diagnose gnathostomiasis, including a cutaneous test, agglutination, immunofluorescence, enzyme-linked immunosorbent assay, and Western blotting. Some of these tests use excretion-secretion products of nematodes as invasion, migration through host tissues, facilitation of feeding, and evasion of host immune responses. However, for the development of these tests, previous characterization of the humoral immune response against the *Gnathostoma* spp. was necessary.

The IgG subclasses have been shown to provide improved specificity over the total IgG antibody array for the diagnosis of many parasitic infections, such as ascaridiasis, echinococcosis, leishmaniasis, filariasis, and gnathostomiasis caused by species of *Gnathostoma* other than *G. binucleatum*. Therefore, the purpose of this study was to characterize the humoral immune response to a crude extract of *G. binucleatum* in patients with clinical diagnoses of gnathostomiasis to detect a possible antibody class or subclass that could be used in the diagnosis of gnathostomiasis.

**MATERIALS AND METHODS**

**Patients and Controls.** Serum samples from 73 patients with clinical diagnoses of gnathostomiasis who came to the Hospital General in Tepic, Nayarit, Mexico, were included in this study. Diagnoses were attained by following the following criteria: 1) subcutaneous or cutaneous migratory swelling, itching, and pain; and 2) a history of eating raw freshwater fish. In addition, serum samples from 20 healthy persons with no history of cutaneous or cutaneous migratory swelling or previous symptoms compatible with migratory swelling, and no history of eating raw or uncooked fish; 14 samples from persons positive for intestinal parasites, and nine samples from persons negative for intestinal parasites at the time of the study were analyzed by using the formalin–ether concentration technique. Serum samples from two infants born at the same hospital and given a diagnosis of infection with *Toxoplasma gondii* (toxoplasmosis) were also included.

**Isolation of ADV3 of *G. binucleatum*.** Advanced third-stage larvae of *G. binucleatum*, were isolated from fish (*Cathorops furthii*) obtained from local fishermen. To visualize areas of infection, thin fillets of fish musculature were compressed in polyethylene bags by using a metallic press and examined under a stereomicroscope. The ADV3 were found free or encysted. Larvae were suspended in phosphate-buffered saline. Cysts were treated with artificial gastric fluid (0.259% HCl, 0.1% pepsin A) and isolated ADV3 were then stored at −20°C.

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