IMMUNODIAGNOSIS OF HUMAN GNATHOSTOMIASIS IN ECUADOR BY SKIN TEST AND ELISA USING GNATHOSTOMA DOLORESI ANTIGEN

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Abstract: Present study evaluated the sensitivity of skin test and enzyme linked immunosorbent assay (ELISA) using antigen of Gnathostoma doloresi adult obtained from wild boars for immunodiagnosis of the human gnathostomiasis in Ecuador. Examinations were performed on 17 subjects clinically diagnosed as gnathostomiasis, 18 Ecuadorian controls and 10 Japanese controls. Based on the criteria of the positivity, 9 mm or more in diameter of the wheal size of the skin test, the positives were 14 (82.4%) out of 17 patients by the injection with 1 μg antigen. Using 10 μg antigen, the positive rate in patients attained to 100%, but 3 (16.7%) out of 18 controls also showed positive reaction. There was a statistically significant correlation (r=0.68, P<0.01) between the wheal size in the skin test and the period from the appearance of the initial symptom. These results suggest that the skin test was highly sensitive in gnathostomiasis. When ELISA value of 0.4 or more was considered positive, 15 (93.8%) out of 16 were positive in patients, 5 (27.7%) out of 18 were positive in Ecuadorian controls and none in 10 Japanese controls. The study revealed that ELISA showed high sensitivity.

INTRODUCTION

Human gnathostomiasis caused by Gnathostoma spinigerum is widely distributing in the coastal regions of Ecuador (Ollague, 1985). The diagnosis of gnathostomiasis is rather difficult since the parasite is usually immature and rarely recovered by surgical operation. Therefore, the diagnosis is usually presumptive on the basis of clinical manifestations and laboratory findings particularly eosinophilia in the peripheral blood. There are few reports of immunodiagnosis of gnathostomiasis except for skin test using G. nipponicum adult antigen (Tada et al., 1966) and enzyme linked immunosorbent assay using G. spinigerum third stage larvae antigen (Suntharasamai et al., 1985) and G. doloresi adult antigen (Tada et al., 1987). For the immunodiagnosis, it will be desirable to use homologous species of the parasite as the source of antigen. However, it is difficult to obtain a large amount of G. spinigerum for antigen. Therefore, in this study, we

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evaluated adult *G. doloresi* antigen, which is easily obtainable from wild boars, instead of *G. spinigerum* antigen in skin test and enzyme linked immunosorbent assay for immunodiagnosis.

**MATERIALS AND METHODS**

*Patients*

Seventeen subjects living in Guayaquil in the coastal region, were consulted in Instituto Ecuatoriano de Seguridad Social and were clinically diagnosed as gnathostomiasis by dermatologists. The period from the appearance of the initial symptom to the diagnosis ranged from 5 days to 5 years. The age of the subjects, 11 males and 6 females, ranged 25–61 years old. As controls, 18 apparently uninfected Ecuadorian outpatients and 10 Japanese were examined. In the blood smear of patients and controls, the eosinophil ratio (%) in the 200 white blood cells was calculated.

*Skin test*

Antigen for skin test was prepared due to the method of filarial skin test antigen (Tada and Kawashima, 1964). Adult worms of *G. doloresi* were homogenized and defatted with cold acetone. After drying, the material was extracted with 0.1 N HCl solution. The suspension was centrifuged and the supernatant was brought to pH 7.0 by 1.0 N NaOH solution. After centrifugation, the supernatant was added to an equal volume of saturated picric acid. The precipitate obtained was extracted with 3% HCl in 99.9% ethanol. After centrifugation, the supernatant was concentrated to 1/10 volume under reduced pressure. The concentrate was added by cold acetone, and the precipitate appeared was collected by centrifugation. The precipitate was washed with cold acetone and finally dried. This powder (GPT) was used as skin test antigen for gnathostomiasis. Injections of 0.1, 1 and 10 µg (dry weight) of antigen in 0.05 ml saline were intracutaneously injected to the forearm of the examined. After 15 min., the average size of the wheal was measured.

*Enzyme linked immunosorbent assay (ELISA)*

The procedure of ELISA was of slightly modified one described by us (Korenaga *et al.*, 1983). The flat bottom wells in EIA microtitration plate (Flow Laboratories, Inc., U.S.A.) were sensitized for 1 hour at 37°C with 100 µl of the crude antigen (20 µg/ml) of *G. doloresi* in 0.05 M carbonate-bicarbonate buffer, pH 9.6. The plates were washed three times with 0.05% Tween 20 in saline. 100 µl of test sera diluted at 1:200 in 0.02 M phosphate buffered saline containing 0.05% Tween 20 and 1% bovine serum albumin (PBS-T-BS) were placed in sensitized wells and incubated at 37°C for 45 min., and washed. Then, the conjugate (peroxidase-labelled anti-human IgG goat serum; MBL, No. 206) diluted at 1:500 in 0.05 M PBS-T-BS was added and incubated at 37°C for 1 hour. After washing, 0.1 mg of orthophenylendiamine per ml and 0.03% of H₂O₂ in 0.05 M acetate acetic acid buffer, pH 4.5, was applied in the amount of 150 µl per well and incubated at 25°C in the dark. The enzyme reaction was stopped by the addition of 50 µl 4 N H₂SO₄. The absorbance was read by a spectrophotometer (Corona MTP-12, Nissey Sangyo) at 500 nm. The concentrations of antigen, sera and conjugate mentioned above were determined by box titrations in serial dilutions.
RESULTS

Clinical findings and blood figure

The swelling or eruption of the skin was observed in all of patients and the location recorded was as follows: 2 eruptions on the face, 2 on the neck, 2 on the chest, 4 on the abdomen, 3 on the arms and 4 on the legs. Most of these eruptions accompanied with erythema, pain and itching. In 11 cases of patients, the eruptions showed migration. The average eosinophil ratio, $5.4\pm2.2\%$, seen in the patients was significantly higher than that, $3.7\pm2.3\%$, of controls ($t=2.14, P<0.05$). In the patients, the eosinophil ratio, $6.7\pm2.3\%$, of the group whose onset of the symptom less than 1 year was higher than that, $4.3\pm1.4\%$, of the group with the history longer than 1 year ($t=2.56, P<0.05$).

Skin test

The wheal size of skin test using different concentration of antigens of *G. doloresi* was shown in Figure 1. The average wheal size increased with the rise of antigen concentrations. Based on the criteria of the positivity, 9 mm or more in diameter (Tada et al., 1966), the positives were 14 (82.4%) out of 17 patients with $1\,\mu$g antigen (Table 1). Using $10\,\mu$g antigen, the positive rate attained to 100%, but 3 (16.7%) out of 18 controls also showed positive reaction (Table 1). Relation between the wheal size in the skin test and the period between the onset of the symptom and the test was shown in Table 2. Further, the wheal size was smaller among patients whose eruption appeared within 3 weeks. On the contrary, the reaction was strong among those whose infection continued more than 3 years. There was a statistically significant
correlation between two parameters ($r=0.68$, $P<0.01$).

**DISCUSSION**

The present study evaluated the sensitivity of skin test and ELISA using *G. doloresi* adult antigen for immunodiagnosis of the human gnathostomiasis in Ecuador.

In the skin test, the wheal size became larger with the rise in antigen concentration in patients. However, with the criteria of positivity, 9 mm or more in diameter, 3 (16.7%) out of 18 controls showed positive reactions at 10 $\mu$g antigen injection. Therefore, it was considered that optimum concentration of antigen would be 1 $\mu$g. Tada et al. (1966) described that 48 (82.7%) cases out of 58 gnathostomiasis were proved to be positive in the skin test using *G. nipponicum* antigen. In the skin test, antigen of *G. doloresi* was useful material as well as that of *G. nipponicum*. There was a significant correlation ($r=0.68$, $P<0.01$) between the reaction in
the skin test and the period since the onset of the symptom. This result suggests that the skin test was highly sensitive in gnathostomiasis.

In the ELISA, the positive rate was 93.8% in gnathostomiasis. However, 27.7% of Ecuadorian controls were also showed positive. We do not know if these people showed cross reactions to other helminths, or were ever infected with gnathostomes. Suntharasamai et al. (1985) evaluated ELISA for gnathostomiasis using crude extract of the third stage larvae of *G. spinigerum* as antigen. The test yielded cross positives in 33% of 24 angiostrongylosis cases and 23% of 92 cases with other parasitic diseases. Tada et al. (1987) also showed cross reaction to paragonimiasis in the ELISA employing *G. doloresi* antigen. ELISA revealed high sensitivity in gnathostomiasis but the specificity is apparently not so high.

REFERENCES
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エクアドル国の颚口虫症における *Gnathostoma doloresi* 抗原を
用いた皮内反応および酵素抗体法による免疫診断

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エクアドル国の颚口虫症における免疫診断法として、イノシシから得た *Gnathostoma doloresi* 成虫抗
原を用いた皮内反応と酵素抗体法を試みた。これらの反応は、臨床的に颚口虫症と診断された17名
と、非感染者と考えられるエクアドル人18名、および日本人10名に対して行った。皮内反応の丘疹径
9 mm 以上を陽性と判定した場合、1 µg 抗原量接種で17名の患者のなかで14名（82.4％）が陽性を示
した。10 µg 抗原量では患者全てが陽性であったが、18名のエクアドル人対照群で3名が陽性を示し
た。また、皮内反応の丘疹径と臨床症状が発症してからの期間に相関が認められた (r=0.68, \( P< \\
0.01 \))。これらの結果は皮内反応が颚口虫症に対して感度が高いことを示唆している。一方、酵素抗体
法において OD 値0.4以上を陽性とすると、16名の患者のうち15名が陽性であったが、18名のエクア
ドル人対照群で5名（27.7％）が陽性を示した。酵素抗体法は、颚口虫症に対して感度は高いが、特
異性はやや低いと考えられる。