Serological Diagnosis of Human Sparganosis 
by means of micro-ELISA

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INTRODUCTION

Human sparganosis, at present, is a surgical disease because its diagnosis depends almost entirely on the recovery of the larva(e) from lesion or finding the worm section in surgical pathology specimens. In general, human sparganosis can hardly be anticipated before surgery; the surgeon himself who met sparganum was surprised when he found the moving creature in the lesion. In endemic areas of sparganosis the presumptive diagnosis may be preoperatively made by experienced surgeons if the patient's history of eating raw snakes or frogs, size, migration of mass and crepitation of the subcutaneous mass etc. are considered. However, those relevant informations were not always helpful for correct diagnosis, because human sparganosis showed great variations in manifesting the disease (Cho et al., 1975). Furthermore, because the incidence of the disease is relatively low even in the endemic areas, it is hardly expectable for a surgeon to place the possibility in the diagnostic priority list.

One more fact concerning the sparganosis is that up to present there is no known anthelminic effective in sparganosis (Torres et al., 1981; Maki et al., 1981). Still the only proper way of treatment is removing the larva by surgery.

In such context, the serological diagnosis has only a limited value in preoperative diagnosis of the disease. Probably because of these facts, only a few reports are available so far on the serological approach to its diagnosis. Okabe and Murase (1957) observed the precipitin reaction in sparganosis patients in which the reaction was observed in antigen dilution of $1:800\sim1:3,200$ whereas the control showed the reaction up to $1:100\sim1:480$ dilution of antigen. Ishii (1973) applied indirect fluorescent antibody test in five sparganosis cases. The patients' sera exhibited high antibody titers of $1:32\sim1:256$ dilution whereas the control revealed lower than $1:16$. Those two papers indicated the high sensitivity and specificity in serological reaction of sparganosis.

Even though the usefulness in preoperative diagnosis is limited, serology may have its value in other respects. First of all, there exist highly susceptible groups to sparganosis in our country who used to eat raw wild animals (Cho et al., 1971). For the epidemiologic investigation in these susceptible population, serological survey appears to be the only reasonable approach. Secondly, serological test may be necessary to confirm whether more spargana are still remaining in the patient or not, after surgery. Though the majority of infected cases had a single worm, a report showed that up to 12 worms were removed by repeated surgeries in a patient (Lee et al., 1967). Thirdly, some of helminth sections in surgical pathology specimens disclosed it very difficult for morphologic identification. In such cases serology may provide the specific differentiation of tissue invading helminths.

With above facts in mind, we started to collect sera from the confirmed sparganosis cases, and conducted serologic test by the method of enzyme-
linked immunosorbent assay (micro-ELISA). As a first step of the studies, the sensitivity, the specificity and cross reactivity with other helminth infections were investigated.

MATERIALS AND METHODS

1. Antigens

(1) Sparganum antigen:
Saline extract of spargana from Korean snakes, Natrix tigrina lateralis (Cho et al., 1982) was used as antigen. Washed spargana were ground with teflon coated tissue homogenizer in ice bath. After adding saline to tissue emulsion, then shaking it for 2 hours at room temperature, the emulsion was kept at 4°C for 21 hours. It was centrifuged at 4°C by 10,000xg for 30 minutes. The supernatant was used as antigen. The protein content of the supernatant was 5 mg/ml as measured by Lowry method.

(2) Other helminth antigens:
Saline extract of Paragonimus westermani was prepared in same way as described above. Cystic fluid of Cysticercus cellulosae was used as antigen after measuring the protein content.

2. Micro-ELISA Procedure

The general procedure of micro-ELISA techniques of McLaren et al. (1978) was followed.
Antigens of respective parasites were coated to flat bottom polystyrene micro-titer plate in protein content of 5μg/ml; sera were diluted to 1:100, and conjugate (peroxidase-labelled anti-human IgG (H&L) goat serum, Cappel Lab., USA) was diluted to 1:1,000. In tests of cerebrospinal fluid, undiluted ones were used. Absorbance was read at 492nm.

3. Sera
Sera from seven patients who were surgically diagnosed as human sparganosis were subjected in this study. The clinical details of the cases will be presented below. For the comparative study and controls, sera from 10 cases of biopsy confirmed cysticercosis, 20 cases of pulmonary paragonimiasis and 4 cases of confirmed ectopic paragonimiasis, 20 cases of egg positive clonorchiasis, 7 cases of Taenia saginata infections and normal control cases were included in the study. Normal sera were obtained from the medical students of Chung-Ang University in Seoul, who had no history of exposure to the infection sources of tissue helminths.

4. Brief description of sparganosis cases
Case 1:
HJN, 26 year-old female, residing in Seoul, visited a local clinic on June 1982 because of a bulging painful mass at right upper abdominal wall for past 8 months. Surgical operation revealed a sparganum of 9 cm long and 3 mm in width. No further details on the case were available. This case was referred to us by Dr. K.W. Song of St. Peter’s Hospital in Seoul.
Case 2:
XXLee, 33 year-old male, visited St. Paul’s Hospital, Catholic Medical College in Seoul on August 1981 firstly, because of 5×10 cm, indurated mass on left upper thigh, fixed at the place for 7 years. The mass suddenly became painful for 7 days before the hospital visit. Surgery on the mass recovered two live spargana each of which was 12 cm long, and 3 degenerated worm masses together with pus discharge of fair amount. Case description of the present case was already made by Byun et al. (1982).
One year later that initial surgery, the patient complained again of a mass in the scrotal area. And at the second surgery about 100 calcified fragments of each 1 cm long with some viable pieces of spargana were removed from the lesion. The serum from this patient was collected about one month after the second surgery. The patient admitted that he had eaten undercooked frogs in the past. This case was referred to us by Dr. B.K. Cho in Department of Dermatology, St. Paul’s Hospital, Catholic Medical College.
Case 3:
JHB, 50 year-old male, living in Kosung Gun, Kyungsangnam Do, developed a single, left mid-thigh mass of 1 cm diameter on its medial side. The mass was tender and firm. Excisional biopsy on March 1983 revealed one sparganum. The patient agreed that he had eaten raw snakes
in the past. This case was referred to us from Sejong Hospital in Buchun City, Kyunggi Do through Dr. K.Y. Song, Department of Pathology, Chung-Ang University.

Case 4:

JEK, 71 year-old male leprosy patient (since his age of 21-year) living in a Leprosarium in Yangpyung Gun, Kyunggi Do, revealed a hard, bean-sized mass on right thigh since one month before the surgery. The patient said he ate 5 raw snakes last summer in the Leprosarium. Surgery undertaken on April 1983 recovered 4 spargana from the lesion. This case was referred to us by Dr. Byung Eun Kwak in the Leprosarium.

Case 5:

AJY, 40 year-old male laborer living in Cheju Do, developed pain on thoracolumbar region, radiating to both lower extremities, gait disturbance and urinary and fecal incontinence during past 1 months. After X-ray and CT scan, the lesion was found at T10-L2 level, shutting off the spinal canal lower than T10 with loss of epidural fat density. Surgery revealed a fragment of a sparganum and necrotic tissue debris. The detail of the case history was already described by Park et al. (1983). Both serum and cerebrospinal fluid were delivered to us for serological confirmation on August 1983.

Case 6:

KSL, 28 year-old male developed seizure attacks and left hemiparesis 15 days prior to the admission to National Medical Center in Seoul. He had been lived in Pyungtaek Gun, Kyunggi Do and had the history of eating snakes. CT revealed highly enhanced contrast with surrounding edema at right frontal lobe of brain. Surgery revealed some fragments of a sparganum in the lesion. Serum, cerebrospinal fluid and worm fragments were sent to us from Department of Neurosurgery, National Medical Center on August 1984. The details of the present case history will be described in a separate paper.

Case 7:

SWL, 22 year-old male soldier with the complaint of a scrotal mass was surgically treated at Army Hospital in Taegu City, Korea. Pathologic examination of the mass disclosed granulomatous lesion with highly degenerated worm which made multiple tunnels, with dense infiltration of eosinophils, and with many calciospherules. These findings were similar with descriptions of histology in human sparganosis (Chi et al., 1980). The history of the patient revealed that he had eaten raw snakes during survival training in Army. This case was referred to us to confirm the diagnosis by Dr. S.T. Hong on September 1984.

RESULTS

1. Results of micro-ELISA for sparganum antigen

As shown in Fig. 1, all of seven, surgically confirmed human sparganosis cases showed high antibody levels being the absorbance value of 0.13 1.14 by micro-ELISA. The Case 2, who was old sparganosis, showed the lowest antibody level (the absorbance, 0.13) out of all cases. Except for this case, the antibody levels were higher than the absorbance value, 0.30.

All sera of 10 normal control, 10 biopsy confirmed cysticercosis, 21 ectopic and pulmonary paragonimiasis, 20 egg positive clonorchiasis showed the antibody levels of absorbance value lower than 0.09. However, out of 7 Taenia saginata infected cases, 3 showed high antibody level, the absorbance value of 0.12-0.52, and the remaining 4 cases revealed lower antibody levels the absorbance value being 0.01-0.04.

2. Cross reactivity of sparganosis sera to Paragonimus and Cysticercus antigens

Sera of seven cases of sparganosis showed lower levels of cross reactivity to Paragonimus antigen when tested by micro-ELISA. One case revealed the absorbance of 0.01, 3 cases 0.02 and the remaining 3 cases 0.04, 0.06 and 0.08 respectively.

Compared with Paragonimus antigen, some of sparganosis sera showed a little higher antibody levels to Cysticercus antigen. One case showed the absorbance 0.16, two cases 0.12 and the
reactivity to *Paragonimus* antigen whereas the CSF from Case 6 showed no such cross reactivity.

**Table 1.** Antibody levels in cerebrospinal fluid of two CNS sparganosis cases against 3 different helminthic antigens

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Absorbance in Case 5</th>
<th>Absorbance in Case 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sparganum</td>
<td>0.69</td>
<td>0.34</td>
</tr>
<tr>
<td>Cysticercus</td>
<td>0.75</td>
<td>0.01</td>
</tr>
<tr>
<td>Paragonimus</td>
<td>0.18</td>
<td>0.00</td>
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</tbody>
</table>

**DISCUSSION**

Sera from seven human sparganosis did contain higher levels of specific IgG antibody demonstrated by micro-ELISA than in other helminthic infections and normal controls. This implies that serology can be a method of diagnosis of human sparganosis. The applicability of serology was already explored by Okabe and Murase (1957) and by Ishii (1973) in this infection. However, unlike the report of Ishii (1973) who could not observe any cross reactivity with sparganum antigen, there were some cases of other helminthic infections in the present study that showed a rather high levels of specific antibody to sparganum antigen. For example, three out of seven *Taenia saginata* cases showed high antibody levels. So far, sera from cysticercosis did not show cross reactivity to sparganum antigen; but the possibility of such cross reaction is always present if we consider the close taxonomic relation between *Taenia solium* and *T. saginata*. Actually, undiluted CSF from a spinal sparganosis (Case 5 in this study) showed high antibody levels both to sparganum and to Cysticercus antigens.

Reverse cross reactivity, *i.e.*, high antibody level of sparganosis sera to *Paragonimus* or *Cysticercus* antigens was not observed in the present study yet.

Reviewing our results on serologic reactions of sera from sparganosis patient and other helminthic...
infections, a certain cross reaction between cestode infections seems to be inevitable unless the antigens are specifically purified. Crude saline extracts of sparganum is considered to share considerable common antigens that react with either adult or larval cestodes.

If we neglect the applicability in the preoperative diagnosis, the serology using micro-ELISA itself was found to be a very sensitive method anyway. Six out of seven cases in this study showed sufficiently high levels of specific IgG antibody above the absorbance of 0.25. However, serum of Case 2 who showed a rather lower level of specific IgG antibody was collected about one month after the second surgery when only calcified lesions were found. Therefore Case 2 could be considered as a cured patient. Then the sensitivity of micro-ELISA of this study could be regarded as 100% if the positive criterion of the test is set at the absorbance value of 0.25 as in paragonimiasis (Cho et al., 1981). If we include the result of Case 2 in estimation of the sensitivity, it became 85.7%. Anyway, serological diagnosis is quite different from pathologic one; only active infection can be properly diagnosed by serology.

By differential point of the absorbance value of 0.25, the specificity of micro-ELISA is calculated to be 95.7% because only 3 out of 71 cases of other infections and normal controls were in positive range.

As an epidemiological tool, serology seems very useful if the positive reactions are carefully interpreted. For example, the positive reactors should be examined whether the cases were infected with other cestode infections especially with adult *Taenia* or *Diphyllobothrium latum*. When the results of serologic test are carefully interpreted, it would provide valuable information which has been otherwise impossible hitherto, on the infection status of sparganosis in susceptible population.

And the serology was definitely helpful in correct identification of worm sections in surgical pathologic specimens; Case 5 and Case 7 in the present study were such examples.

**SUMMARY**

Seven cases of surgically proven sparganosis were serologically tested by means of micro-ELISA for their specific IgG antibody levels. For that purpose, crude saline extract of spargana from snake, *Natrix tigrina lateralis* was prepared and used as antigen. The sparganosis sera were also tested with *Paragonimus* and *Cysticercus* antigens to observe the cross reactivity. A total of 71 sera from normal control, ectopic and pulmonary paragonimiasis, clonorchiasis, cysticercosis and *Taenia saginata* cases were also included.

Except for one case of old calcified infection, all of 6 human sparganosis showed higher serum levels of specific IgG antibody when the differential point of positive reaction was set at the absorbance value of 0.25 (the sensitivity being 85.7%). In control and other helminthic infections, all except 3 cases of *T. saginata* infection showed negative reaction to sparganum antigen (the specificity being 95.7%).

None of sparganosis cases showed cross reactivity to *Paragonimus* and *Cysticercus* antigens. Undiluted cerebrospinal fluid also showed high levels of antibody when central nervous system was invaded.

The serologic diagnosis by means of micro-ELISA could be a useful tool in epidemiological study of human sparganosis in susceptible population, as well as in individual diagnosis.

**ACKNOWLEDGMENT**

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REFERENCES


효소면역측정법을 이용한 스파르가눔증의 혈청학적 진단

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김 혁·김 성 일·조 승 엽

우리말 요약

과거적으로 총력을 기울여 진단한 스파르가눔증 환자 7명으로부터 혈청을 수집하고 혈청체 스파르가눔 특이 IgG 항체가 효소면역측정법으로 측정하였다. 스파르가눔 환자는 유약독이에 저항 감염된 총체로 간아 생리시험수로 추출한 것으로 단백질 함량은 5mg/ml 이었다.

효소면역측정법은 McLaren 등(1978)의 방법에 따라 실시하였다. 스파르가눔줌 환자 혈청이 제음출 혈원 또는 유구란미중 혈원에 교차반응이 있는지 여부로 측정하였다. 또한 스파르가눔 환자에 대해서 검정인, 백혈증환자, 간유증 환자, 유구난미중 환자와 부구조증 환자 등 모두 71명에 대해서도 교차반응을 나타내는지를 관찰하기로 효소면역측정법을 실시하였다. 그 결과를 요약하면 다음과 같다.

1. 스파르가눔증 환자 7명중 6명이 같은 스파르가눔에 감염되어 있었고 수온후 1개월에 원질을 수집한 1배를 제외하면 모두 수온도 0.3 이상을 나타내었다. 양성관절가중은 수온도 0.25로 하였을 때 효소면역측정법의 민감도 (sensitivity)는 85.7%이었다.
2. 기타 기병중 감염자와 정상인 71명중에서 스파르가눔 환자에 대한 특이항체 양성자는 무구조증검진 결과 3명으로 특이도 (specificity)는 97.5%이었다.
3. 스파르가눔증 환자 혈청이 제음출 혈원 및 유구난미중 혈원에 대해서 항체가 남는 값을 보이고 있어 교차 반응은 밝혀진 수 있었다.
4. 중추신경계를 감염한 스파르가눔증 환자 2례에서 비특수역을 피하하지 않은 상태로 효소면역측정법을 실시하였다. 2례는 제음출 및 유구난미중 혈원에 대해 교차반응 없이 스파르가눔혈청에 대해 높은 항체가 보이며 비해 다른 1례에서는 스파르가눔 혈원 및 유구난미중 혈원에 대해 비슷한 항체를 나타내고 있었다.

이상의 결과에서 효소면역측정법을 이용하여 스파르가눔증을 진단적으로 진단하는 경우 매우 특이하고 민감한 결과를 얻을 수 있을 것으로 생각하였다. 스파르가눔증의 수술적 진단이 석회로 어려울 것이 현실이므로 진단적 진단을 억지하는 경우가 흔히 발생하면, 이런 발병을 변모로 진단법으로 이용할 수 있을 것으로 생각한다.