

CLINICAL FEATURES AND PARASITE-SPECIFIC IgM/IgG ANTIBODIES OF PARAGONIMIASIS PATIENTS RECENTLY FOUND IN JAPAN

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Abstract. Clinical features of a total of 30 paragonimiasis *westerni* patients referred to and diagnosed in our laboratory in 1999 were analyzed retrospectively. Most patients were middle-aged (average: 48 years, range: 13-72 years) with the male/female ratio of 19/11. Over 70% of the patients had respiratory symptom and over 80% had peripheral blood eosinophilia and high serum IgE level. All but two cases had radiologic abnormalities on the chest X-ray. Only in 3 cases were *Paragonimus* eggs detected in the sputum smear. We classified the patients into two groups depending on the chest X-ray findings: patients having pleurisy alone and those having nodular/cavitating lesions in the lung parenchyma. We measured parasite specific IgM/IgG antibodies in all patients sera by microplate ELISA. The mean parasite-specific IgM/IgG antibody ratio was significantly higher in the parenchymatous lesion group than in the pleurisy group. While IgM antibody titer had a strong positive correlation with the degree of eosinophilia in peripheral blood, IgG antibody titer had an inverse correlation. Although the degree of eosinophilia in peripheral blood was higher in the pleurisy group than in the parenchymatous lesion group, total IgE level in serum was comparable between the two groups. The present results indicate that pleurisy with eosinophilia and dominant IgM antibody are the characteristic features of the early stage of paragonimiasis, whereas parenchymatous lesions in lungs with low grade eosinophilia and dominant IgG antibody are of the late stage. These results suggest that detection of IgM antibody should always be considered for the immunodiagnosis for paragonimiasis-suspected patients with pleurisy.

INTRODUCTION

Paragonimiasis is a food-borne parasitic disease caused by infection with any of the lung flukes, *Paragonimus* spp. The disease is common in Asia (Yokogawa, 1969), some parts of Latin America (Little, 1968; Ibanes and Fernandez, 1980; Alarcon *et al*, 1985) and Africa (Nwokolo, 1972; Udonsi, 1987). Classically, paragonimiasis was known as a disease occurring in the 10-25-year-old group characterized by chronic cough with rusty-colored sputum, and nodular/cavitating lesions on chest radiographs (Yokogawa, 1969; Gutierrez, 1990). However, the clinical features of paragonimiasis cases in Japan nowadays appears to have changed from those classical type in terms of not only the age and sex distribution but also in their clinical features (Uchiyama *et al*, 1999). Especially, egg detection ratio in the sputum smear or even in BALF samples were only a half. Thus the immunodiagnostic methods have become more important for the diagnosis of paragonimiasis than before (Uchiyama *et al*, 1999). Classical immunodiagnosis for paragonimiasis like the

intradermal test (Yokogawa *et al*, 1955), double diffusion in agarose gel (Katamine *et al*, 1968), and immuno-electrophoresis (Tsuji, 1974) have gradually been replaced by more sensitive and specific methods like the enzyme-linked immunosorbent assay (ELISA) (Waikagul, 1989; Ikeda *et al*, 1992) or immunoblotting (Slemenda *et al*, 1988; Dekumyoy *et al*, 1998). The latter methods are usually focused on the detection of parasite-specific IgG antibody, though some systems have been developed to detect specific IgE antibody (Ikeda *et al*, 1992) or circulating antigen (Zang *et al*, 1993). Recently, we encountered a patient showing seroconversion from IgM to IgG antibody during a follow-up study (Mukae *et al*, 2001). In the present study, we analyzed the clinical features and measured parasite specific IgM and IgG antibodies in the sera of a total 30 paragonimiasis cases referred to and diagnosed in our laboratory in 1999.

MATERIALS AND METHODS

Subjects and samples

A total of 30 cases referred to and diagnosed as having paragonimiasis in our laboratory in 1999 were studied. Clinical data of the patients such as radiologic findings, eosinophil count in peripheral blood, and total IgE level were gathered from the consultation sheets from attending physicians.

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Measurement of IgM/IgG antibody

P. westermani-specific IgM and IgG antibody titers were measured by the microplate ELISA as described previously (Maruyama *et al*, 1996). Briefly, wells of a 96-well microtiter plate were coated with 10 µg/ml of crude somatic extract of *P. westermani* adult worms by incubation at 4°C overnight. Wells were washed with phosphate buffered saline containing 0.05 % Tween 20, blocked with blocking buffer (1% casein in 20 mM Tris HCl, pH 7.6), and then incubated with patients' sera diluted at 1 : 500 at 37°C for 1 hour. After washing, peroxidase-labeled rabbit anti-human IgG (γ-chain-specific; DAKO, Glostrup, Denmark) or IgM (μ-chain specific; DAKO) diluted at 1 : 2000 was added to each well and incubated at 37°C for 1 hour. ABTS peroxidase substrate (one component type; KPL, MD, USA) was added to each well and incubated at 37°C for 15 minutes. The optical density (OD) was read at 405 nm in an ELISA reader (Multiskan Biochromatic: Labsystems Oy, Helsinki, Finland). The cut-off levels were defined as the mean OD value plus three standard deviations (SD) from the sera of 17 patients with pleural effusion definitely caused by non-parasitic diseases.

Statistical analysis

Data were compared by Student's *t*-test. P value of <0.05 was considered significant.

RESULTS

Clinical data of the patients are summarized in Table 1. The patients consisted of 11 females and 19 males with a mean age of 48 years (range: 13-72 years). Respiratory symptoms were noted in 23/30 (76.7%). *Paragonimus* eggs were detected in the sputum and/or bronchial washings of only 3 patients. All but two cases had radiologic abnormalities. Pleural effusion was seen in 19 patients, whereas parenchymatous lesions such as nodular, cavitating, or infiltrating lesions were seen in 9 patients. Peripheral blood eosinophil count was available from 28 patients, 23 (82.1%) of them showed significant eosinophilia (cut off = 7%). Total IgE level in serum was available in 16 cases, and 14 of them (87.5%) showed elevated serum IgE level (cut off = 250 IU/ml).

Among the 30 patients in this study, 3 patients with pleurisy were diagnosed as having paragonimiasis by detecting IgM, but not IgG, antibody by multiple-dot ELISA. Thus, the patients were divided into two groups; pleurisy group and parenchymatous lesion group and their serum subjected for the measurement of parasite specific IgM/IgG antibody. Among two patients having no remarkable lung lesions, one patient

was classified among the pleurisy group because her mother also suffered from paragonimiasis having pleurisy and they ate freshwater crabs together. The other patient complaining of dry cough was examined only by plain chest X-ray but not by CT. Having had a past history of paragonimiasis he was classified among the parenchymatous lesion group because his symptoms were improved and the parasite-specific antibody titer in his serum drastically decreased after praziquantel treatment. Specific antibodies of IgG and IgM classes to *P. westermani* antigen were, therefore, assayed in all 30 patients' sera by microplate ELISA and the results were analyzed on the basis of clinical stages of the patients. As shown in Fig 1, 27 patients had dominant IgG antibody. The only 3 patients who had dominant IgM but low IgG antibody titer in this assay (marked with * in Fig 1) were diagnosed as having paragonimiasis by detecting specific IgM antibody by multiple-dot ELISA. From this scatter diagram (Fig 1), IgM antibody titers of the pleurisy group patients appeared to be relatively higher than those of the parenchymatous group patients. To confirm this point, IgG/IgM antibody ratio was calculated and compared between the two groups. The IgG/IgM ratio in the parenchymatous group (mean ± SD = 28.7 ± 25.6) was significantly (*p*<0.05) higher than that in the pleurisy group (6.8 ± 5.6) (Nakamura-Uchiyama *et al*, 2001).

The degree of eosinophilia in the pleurisy group (mean ± SD = 24.0 ± 13.7) was significantly (*p*<0.05) higher than that (8.0 ± 5.0) in the parenchymatous lesion group. No significant difference was, however,

Table 1

Summary of paragonimiasis patients in 1999 (n=30).

Age: 48 (range: 13-72 years) M : F = 19:11		
Symptoms		
Respiratory symptoms	23	(76.7%)
Others	2	(6.6%)
No symptoms	5	(16.7%)
Chest X-ray findings		
Pleural effusion	19 ^a	Nodular lesion 5
Pneumothorax	2	Cavitating lesion 2
		Infiltration 3
No lesions	2	
Laboratory data		
Eosinophilia	23	(n=28, 82.1%)
Hyper IgE	14	(n=16, 87.5%)

^a including 2 patients with pneumothorax and 1 patient with nodular lesion.

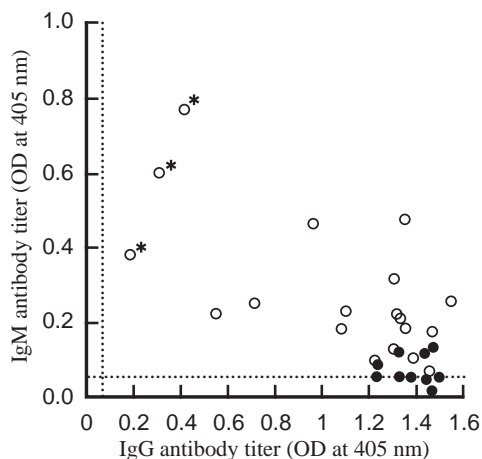


Fig 1- Scatter diagram of parasite-specific IgM and IgG antibody titers in the sera of paragonimiasis patients. ● : patients with parenchymatous lesion, ○ : patients with pleurisy, Dotted line: cut off value. * : patients diagnosed as having paragonimiasis by IgM-multiple dot ELISA.

observed in IgE levels between the pleurisy group (mean ± SD = 1,724 ± 1,484) and the parenchymatous lesion group (2,117 ± 1,344) ($p > 0.1$). Since the degree of eosinophilia was significantly higher in the pleurisy group, we examined the relationship between IgG/IgM antibody titers and the degree of eosinophilia in peripheral blood. As shown in Fig 2, while IgG antibody titer showed inverse relationship ($r^2 = 0.212$) with the degree of eosinophilia, IgM antibody titer correlated positively with the degree of eosinophilia ($r^2 = 0.575$) (Nakamura-Uchiyama *et al*, 2001).

DISCUSSION

The present results together with our previous observation (Uchiyama *et al*, 1999) clearly show that paragonimiasis in Japan today tends to be more prevalent in middle-aged men, who are considered to be much more conservative in their traditional eating habits. Typical paragonimiasis symptoms are chronic cough, bloody sputum, and nodular/cavitating lesions on chest X-ray and other imaging techniques. In the present study, however, about 2/3 of the patients showed pleurisy without parenchymatous lesions in lungs. Considering the migration route of *Paragonimus* worm in the human body, pleurisy develops prior to the development of parenchymatous lesions. In fact, parasite-specific IgM antibody titer was higher in the pleurisy group than in the parenchymatous lesion group. High detection rate of the patients having

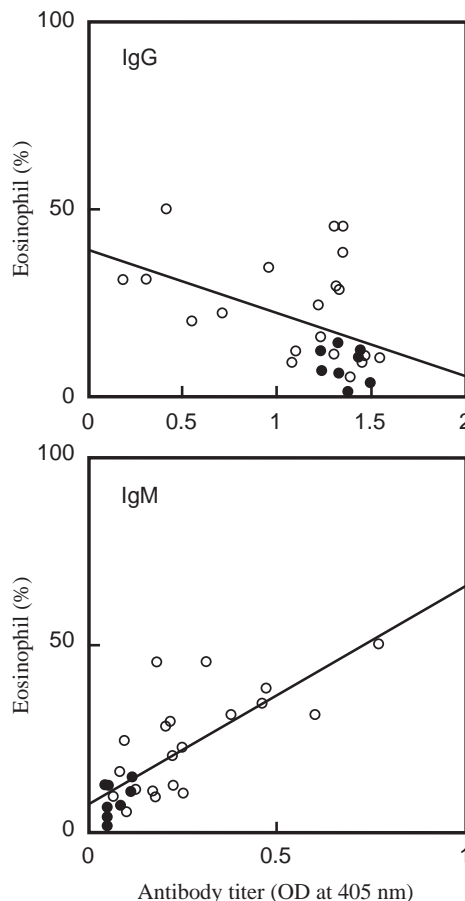


Fig 2- Relationship between IgM and IgG antibody titers and the degree of eosinophilia in paragonimiasis patients. ● : patients with parenchymatous lesion, ○ : patients with pleurisy.

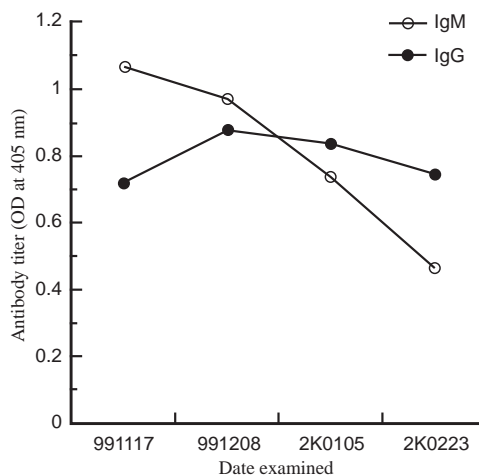


Fig 3- Typical antibody change pattern of one case. IgM and IgG antibody titers were reversed along with the time course.

pleurisy with relatively high IgM antibody titer indicate that the diagnosis of the disease has been made much earlier than before, probably due to frequent access to chest X-ray examinations in regular health check-ups. In some cases, we could follow up the antibody titer after treatment. Typical antibody change pattern of one case is shown in Fig 3. As expected, IgM and IgG antibody titers were reversed along with the time course.

Although 27 out of 30 patients in the present study were diagnosed as having paragonimiasis by IgG antibody detection, 3 patients were not clearly diagnosed by IgG-multiple-dot ELISA, but were diagnosed by IgM antibody in multiple-dot ELISA. Surprisingly, the degree of eosinophilia, but not IgE level was correlated well with the IgM antibody titers. Therefore, the present results clearly show that pleurisy with eosinophilia and relatively high IgM antibody are the characteristic features of the early stage of paragonimiasis, whereas parenchymatous lesions in lungs with relatively low eosinophilia and dominant IgG antibody are of the late stage. Thus, in addition to the detection of parasite-specific IgG antibody, detection of IgM antibody should always be considered for the immunodiagnosis of paragonimiasis-suspected patients with pleurisy.

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