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Changes of Anti-*Clonorchis sinensis* IgG Antibody in Serum after Praziquantel Treatment in Human Clonorchiasis

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Abstract: Anti-*Clonorchis* IgG antibody levels in serum were observed by ELISA in 129 egg positive cases and in 25 controls. The antibody levels were 0.063 to 1.216 (0.325±0.202) in clonorchiasis cases and 0.078 to 0.670 (0.255±0.133) in controls. The difference was statistically significant. However, serological diagnosis of clonorchiasis was not satisfactory in lightly infected cases because of low levels of specific IgG antibody. The antibody levels were well correlated with EPG. Changes of the IgG antibody levels were not significant 12~14 days, 4 weeks and 8~9 weeks after praziquantel treatment. Seven and 13 months after treatment, the IgG antibody levels were lowered significantly. The period for serologically negative conversion after praziquantel treatment was between 9 weeks and 7 months in human clonorchiasis.

Key words: *Clonorchis sinensis*, clonorchiasis, IgG, ELISA, EPG, praziquantel

INTRODUCTION

Specific diagnosis of *Clonorchis sinensis* infection is usually made by egg detection in fecal examination. However, it has a certain limitations. The eggs are not produced for the first 3 to 4 weeks during the pre-patent period of the fluke, and the eggs are not detectable inevitably in early phase of infection (Rim, 1986). Also a situation of no egg production is suspected after chemotherapy. Praziquantel is known to have excellent therapeutic effects against clonorchiasis (Rim *et al.*, 1981; Seo *et al.*, 1983). Effect of the drug has been evaluated by egg negative conversion rate and egg reduction rate. However, there is a question whether egg negative conversion in egg positive cases is really a result of complete deworming. Furthermore, praziquantel is known to destroy ovary and testes to inhibit egg production (Lee *et al.*, 1987). Is there any possibility that a live *Clonorchis* remains in the liver without egg production after chemotherapy? Actually an experiment using guinea pigs by Lee *et al.* (1988) revealed that there were a few living worms in liver of the animals which were converted egg negative after treatment.

Recently the practice of chemotherapy with praziquantel in human clonorchiasis in Korea may change the significance of intradermal test. The test becomes a less valuable method for screening of clonorchiasis because of increasing false positive cases.

Serological test is a good supportive diagnostic tool in most infectious diseases. Especially ELISA is popularly used in serodiagnosis of tissue invading helminthiases such as cysticercosis (Cho *et al.*, 1986a), sparganosis (Kim *et al.*, 1984), paragonimiasis (Cho *et al.*, 1981), *etc.* In clonorchiasis, ELISA is known to exhibit sensitivity of about 80% (Lee *et al.*, 1981; Yang *et al.*, 1983; Lee *et al.*, 1983; Han *et*

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al., 1986).

Recently, the sensitivity and specificity of skin test, ELISA and fecal examination in clonorchiasis should have changed. Especially the change of circulating IgG antibody in serum after chemotherapy is one of the interesting aspects to study. Already, Lee *et al.* (1986) and Kim *et al.* (1987) observed lowering of serum levels of specific IgG antibody in 6 to 18 months after treatment. However, most of the cases were treated with 40mg/kg of praziquantel which showed low cure rate (Lee, 1984).

The present study aimed to evaluate the relations between the results of skin test, fecal examination and ELISA, and to observe the change of anti-*Clonorchis* IgG antibody after praziquantel treatment.

MATERIALS AND METHODS

Fecal examination, skin test and serum collection were carried out upon Korean army personnel from 1985 to 1987.

1. Detection of clonorchiasis cases: Thousands of army personnel were examined of their feces by both cellophane thick smear and formalin-ether concentration techniques. Out of them, 135 cases were tested by intradermal injection of VBS antigens of *Clonorchis sinensis* and *Paragonimus westermani* (Green Cross Co., Korea) before fecal examination. Indurations over 60mm^2 were regarded as positive. The *Clonorchis* egg passers were sampled of their blood and treated with praziquantel 25mg/kg× 3 dose. The sera were stored at −20°C until use.

2. Serum collection and follow-up fecal examination after treatment: Treated cases were reexamined of their feces for *Clonorchis* eggs 12 days to 13 months from treatment by the group. The cases who were converted to egg negative were sampled of their sera, and still egg positives were treated again. The sera were stored also under −20°C. ELISA was carried out using all of the sampled sera in Feb., 1988.

3. Preparation of antigen: Metacercariae of *C. sinensis* were collected from digested *Pseudorasbora parva* which were caught at Nakdong-river. The metacercariae were infected orally to rabbits, 500 to each. Adult worms were recovered from the rabbits after 4 weeks. A total of 1.5g worms were washed in phosphate buffered saline (PBS, pH7.6) 5 times, and ground with 15ml PBS in a tissue homogenizer. Whole worm extract was centrifuged at 4°C, 10,000g for 30 minutes. The supernatant was stored overnight at 4°C, and frozen under −60°C until use. Protein content was 358μg/ml by Lowry method.

4. ELISA: After checkerboard titration, antigen (Ag), sera and conjugate were used after dilution under 1 : 400, 1 : 200 and 1 : 4,000 respectively. The antigen was diluted with carbonate buffer (pH 9.6), and 200μl of diluted Ag was dispensed into 96 wells of micro-plates (Titertek Co.). After overnight storage of the plates at 4°C, the wells were washed 3 times with washing buffer (0.5ml Tween 20 in 1,000ml PBS). The wells were blocked with 3% bovine serum albumin(BSA) solution (30g BSA in 1,000ml washing buffer) at 37°C for an hour. After washing three times, 200μl of diluted sera were dispensed to each well. The sera were diluted 1 : 200 with 0.1% BSA solution (1g BSA in 1,000ml washing buffer). The plates with sera were incubated at 37°C for 2 hours and unbound proteins were washed out 3 times. Peroxidase conjugated anti-human IgG (H & L) goat serum (Cappel lab., USA) was diluted 1 : 4,000 with 0.1% BSA solution and 200μl in a well were incubated at 37°C for 2 hours. After washing, the substrate solution (o-phenylenediamine 4mg and 10μl of 30% H_2O_2 in 10ml phosphate citrate buffer) was dispensed 50μl to each well and stored at room temperature (20°C) for 15 minutes. The reaction was terminated by adding 50μl of 4N H_2SO_4 to each well. Finally the wells of plates were read of their absorbance by an ELISA reader (Dynatek Co.) at 492nm.

RESULTS

1. Skin test: Out of 135 tested cases, 35 (25.9%) were positive for *Clonorchis* antigen and 100 were negative. Among the positive reactors, 12(34.3%) cases were *Clonorchis* egg positive, and one was egg positive out of 100 negative cases.

2. Anti-*Clonorchis* IgG antibody in serum: By absorbance in ELISA, 129 egg positive cases showed 0.063 to 1.216(0.325±0.035). Those of the control cases(parasite egg negative cases) were in absorbance 0.078~0.670(0.255±0.052) (Table 1).

Table 1. Anti-*Clonorchis* IgG antibody levels by ELISA in *C. sinensis* egg positive cases and controls

	No. of cases	Absorbances		
		Mean	S.D.*	95% C.I.**
Clonorchis egg positives	129	0.325	0.202	0.290~0,360
Egg negative controls	25	0.255	0.133	0.203~0.307

* S.D.: Standard deviation

** 95% C.I.: 95% confidence intervals=$\bar{X}\pm 1.96 \times$S.D./$\sqrt{n}$(standard error)

*** The absorbance difference between egg positives and negatives was statistically significant($z=2.18$, $p<0.05$).

Table 2. Anti-*Clonorchis* IgG antibody levels in serum by EPG grades in clonorchiasis cases

EPG grades	No. of cases	Absorbances*		
		Mean	S.D.**	95% C.I.***
0~ 900	68	0.258	0.142	0.224~0.292
1,000~1,900	6	0.463	0.191	0.310~0.616
2,000~3,900	6	0.497	0.103	0.415~0.579
4,000~9,900	7	0.517	0.261	0.024~0.710
Over 10,000	6	0.715	0.342	0.441~0.989
Total	93	0.336	0.217	0.292~0.380

* Correlation analysis between abs. and EPG; $y=0.281+0.00029x$, $y=0.281+0.00029x$, $r=0.575(t=63.85$, $p<0.01)$.

** S.D.: Standard deviation

*** 95% C.I.: 95% confidence intervals=$\bar{X}\pm 1.96 \times$S.D./$\sqrt{n}$

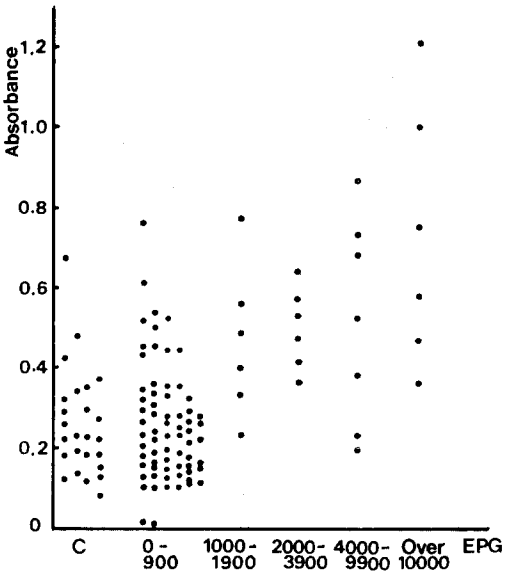

Fig. 1. Anti-*Clonorchis* IgG antibody levels by ELISA plotted by grade of EPG.

3. Anti-*Clonorchis* IgG antibody levels by grades of EPG: A total of 93 cases was examined of their EPG. They were grouped into five as in Table 2. Mean absorbance

Table 3. Changes of anti-*Clonorchis* IgG antibody after praziquantel treatment in various groups

Group	Duration after treatment	No. of cases	Absorbances*		
			Mean	S.D.**	95% C.I.***
I	Pre-treatment	31	0.347	0.258	0.254~0.438
	12~14 days	31	0.417	0.272	0.270~0.564
II	Pre-treatment	8	0.320	0.287	0.121~0.519
	4 weeks	8	0.206	0.115	0.126~0.286
III	Pre-treatment	19	0.244	0.117	0.191~0.297
	8~9 weeks	19	0.256	0.134	0.196~0.316
IV	Pre-treatment	12	0.320	0.189	0.213~0.427
	7 months	12	0.201	0.088	0.151~0.251
V	Pre-treatment	12	0.352	0.227	0.224~0.380
	13 months	12	0.216	0.096	0.162~0.270

* O.D. differences were not significant in groups I, II & III and statistically significant ($p<0.05$) in groups IV & V by paired t-test.

** S.D.: Standard deviation

*** 95% C.I.: 95% confidence intervals=$\bar{X}\pm 1.96$ S.D./$\sqrt{n}$

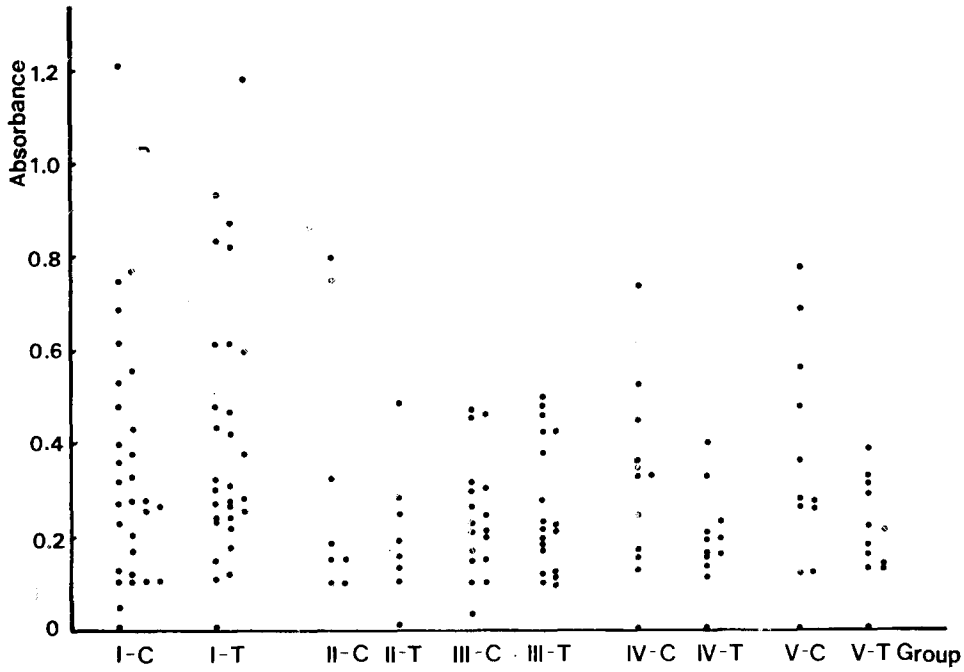

Fig. 2. Changes of Anti-*Clonorchis* IgG antibody levels in serum by ELISA after treatment by Group(Group I-C; pre-treatment of Group I, I-T; 12-14 days after treatment, Group II-C; pre-treatment of Group II, II-T; 4-6 weeks after treatment, Group III-C; pre-treatment of Group III, III-T; 8-9 weeks after treatment, Group IV-C; pre-treatment of Group IV, IV-T; 7 months after treatment, Group V-C; pre-treatment of Group V, V-T; 13 months after treatment).

increased by EPG grades; *i.e.*, from 0.258± 0.034 in 68 cases of EPG 0∼900, to 0.715± 0.274 in 6 cases of EPG over 10,000. The absorbance of each case was plotted by EPG in Fig. 1.

4. Changes of anti-*Clonorchis* IgG antibody levels after praziquantel treatment: The treated cases of clonorchiasis were grouped into five by duration of serum sampling after treatment; 12∼14 days, 4 weeks, 8 weeks, 7 months and 13 months(Table 3). Mean absorbance of the Group I increased slightly 12∼14 days after treatment but the change was not statistically significant. Thereafter, 7 months or more after treatment, their absorbance decreased significantly(Table 3 & Fig. 2).

DISCUSSION

High sensitivities of ELISA have been reported in serodiagnosis of clonorchiasis; 87% by Lee *et al.* (1981), 83.3% by Yang *et al.* (1983), 88.3% by Lee *et al.* (1983) and 78.2% by Ham *et al.* (1984). Those authors recommended ELISA as a good serodiagnostic technique of clonorchiasis. However, the present results showed low sensitivity. Especially the IgG antibody levels of light worm burden cases of EPG below 900 were almost identical with those of controls. However, the absorbances were increased by increase of EPG as in Table 2 and Fig. 1. The increasing pattern of absorbance by EPG was also described by Yang *et al.* (1983). Therefore, ELISA is regarded as a sensitive method only in cases of moderately or heavily infected cases. Inevitably detection of anti-*Clonorchis* IgG antibody using ELISA may be greatly influenced by the epidemiological characteristics. The more the lightly infected cases, the lower the sensitivity. Actually most

of the clonorchiasis cases in Korea are lightly infected, therefore, serodiagnosis of clonorchiasis is not so practical although the difference of absorbance between egg positive cases and control was statistically significant.

Han *et al.* (1986) recorded that as the duration of serum storage became longer even in frozen state, the antibody levels decreased. However, such lowering effect was observed not so remarkable, and the effect was also negligible in this study. Low sensitivity of the present results may be mainly due to the low immunogenicity of *C. sinensis*. High sensitivity of serological tests in other tissue parasitic trematodiases such as schistosomiasis, fascioliasis and paragonimiasis were well known (Tsuji, 1984). In clonorchiasis, other serological methods, such as complement fixation test, indirect hemagglutination test, immunofluorescence test or immunoelectrophoresis showed also low sensitivity(Tsuji, 1984). Therefore it is suggested that anti-*Clonorchis* IgG antibody levels increased above that of controls only as the worms are infected numerously.

Negative conversion of serological test should take a certain period of time after treatment. Mean absorbance of the Group I was slightly increased 12~14 days after treatment, but not significant statistically. Thereafter in 4 or 8 weeks after treatment, mean absorbance decreased but not statistically significant. Only in Groups IV and V, the decrease of absorbance in 7 or 13 months after treatment was found significant. Therefore, anti-*Clonorchis* IgG antibody levels began to decrease significantly between 9 weeks and 7 months after the treatment.

In human cases of clonorchiasis, Soh *et al.* (1985) observed significant reduction of absorbance 1 year after treatment. Lee *et al.* (1986) reported that mean absorbance decreased after 6 months although most of them remained still in positive ranges. They treated the cases with 40mg/kg of praziquantel single dose, which showed the cure rate 87.1%(Lee, 1984). Also Kim *et al.*(1987) observed 22 cured cases and 24 egg reduced cases 9 and 18 months after treatment. The results showed negative conversion of serum IgG antibody levels 18 months after treatment by ELISA not only in cured cases but also in the egg reduced cases. The decrease of the IgG antibody in egg reduced cases after treatment suggested that significant reduction of worms by praziquantel treatment should convert the IgG antibody level negative. Such a result of negative serological test in cases of low worm burden was well compatible with the present results.

In rabbits infected with *Schistosoma japonicum*, specific IgG antibodies to adult worm antigen began to lower 4 to 14 weeks after treatment (Matsuda *et al.*, 1984), and the changes of anti-egg antibody levels were significant 8 weeks after treatment in baboons infected with *S. mansoni*(Sturrock *et al.*, 1987). However, Roberts *et al.*(1987) observed that the level of anti-tegumental membrane Ag IgG antibody in human schistosomiasis mansoni increased twofold shortly after chemotherapy and declined to pre-treatment level. The level was maintained up to 18 months. In paragonimiasis the duration for serologically negative conversion was 2 to 6 months(Tsuji, 1984; Choi *et al.*, 1986) and longer than two years in neurocysticercosis (Cho *et al.*, 1986). The decrease of specific IgG antibodies after treatment is an outcome of no more antigenic stimuli by death or by complete deworming. In clonorchiasis, discharge of damaged worms is easier than that of other tissue inhabiting parasite infections. *Clonorchis* is expelled within a few days after treatment, and the liver was observed under healing process after one week in rabbit clonorchiasis (Lee *et al.*, 1987). Unlike the pathological improvement, the period for antibody decrease after treatment in clonorchiasis seems similar with that in schistosomiasis or paragonimiasis.

Serological test should be a valuable tool in follow-up examination of heavily infected cases when IgG antibody levels before and after treatment were compared. However, low IgG antibody levels do not necessarily mean the cure

after treatment. It needs at least 2 weeks for anti-*Clonorchis* IgG antibody to become positive in early phase of moderate or heavy infection of *Clonorchis*. Many lightly infected cases were falsely negative for the IgG antibody, and negative conversion by ELISA needs rather longer duration after treatment than egg negative conversion. Furthermore, anti-*Clonorchis* IgG antibodies are lowered to negative range by only worm reduction. These characteristics of serological diagnosis made detection of anti-*Clonorchis* IgG antibody by ELISA less sensitive and less valuable than egg detection by fecal examination in human clonorchiasis.

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=국문요약=

간흡충 감염자의 프라지콴텔 치료후 혈청내 IgG 항체가의 변화

서울대학교 의과대학 기생충학교실 및 풍토병연구소
홍 성 태

프라지콴텔이 사용되어 많은 간흡충 감염자가 치료되고 있는 현재의 상황에 비추어, 간흡충증의 진단에서 피내반응검사와 혈청검사의 효용성과 치료 후의 혈청 항체가의 변동 양상을 관찰하고자 이 연구를 실시하였다. 그 결과를 요약하면 다음과 같다.

1. 피내반응검사를 실시한 135명 중에서 간흡충의 VBS항원에 양성 반응자가 35명(25.9%)이었고 이 중에서 간흡충란 양성자는 12명(34.3%)이었다.

2. 전체 간흡충란 양성자 129명에서 간흡충 성충의 조항원을 이용한 효소면역법(ELISA)의 흡광도가 0.063~1.216(0.325±0.202)이고 25명의 충란음성자에서는 0.078~0.670(0.255±0.133)이었다.

3. 총 93례에서 실시한 EPG와 흡광도의 관계를 보면 68명의 EPG 900이하 감염례에서 0.063~0.761(0.258±0.142)이고, EPG 1,000~1,900의 6례에서 0.231~0.773(0.463±0.191), EPG 2,0000~3,900의 6례에서 0.361~0.640(0.497±0.103), EPG 4,000~9,900의 7례에서 0.196~0.874(0.517±0.261), 6명의 중감염자(EPG 10,000 이상)에서 0.359~1.216(0.715±0.342)이었다.

4. 치료 전후에 관찰한 혈청내 특이 IgG항체가는 각 군에 따라서 다음과 같다. 제Ⅰ군의 치료 전 흡광도는 0.075~1.216(0.347±0.258)이고 치료 12~14일 후에는 0.065~1.181(0.417±0.272)이었고, 제Ⅱ군의 흡광도는 0.102~0.796(0.320±0.287)이고 치료 4주 후에 0.107~0.544(0.206±0.115)이었다. 제Ⅲ군에서는 흡광도가 치료 전에 0.102~0.470(0.244±0.117)이며 치료 8~9주 후에 0.101~0.500(0.256±0.134)이었다. 제Ⅳ군의 경우 흡광도가 0.063~0.735(0.320±0.189)에서 치료 후 7개월에 0.090~0.404(0.201±0.088)로 감소하고, 제Ⅴ군에서 흡광도 0.063~0.773(0.352±0.227)이 치료 13개월 후에 0.076~0.386(0.216±0.096)으로 감소하였다. 치료 전후의 흡광도는 7개월 및 13개월 후의 변화만이 통계적으로 유의하였다.

이상의 결과를 미루어보면 간흡충증의 진단에서 효소면역법을 이용한 혈청 내 특이 IgG항체 검사는 EPG 1,000 이상의 중등도 이상 감염자에서만 우수한 진단적 가치를 가진다고 판단된다. 또한 프라지콴텔로 치료하여 충란음전이 된 상태에서 혈청내 IgG항체는 투약 후 9주와 7개월 사이에 유의하게 감소하였다.