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Liver function in *Clonorchis sinensis*-infected rabbits

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Abstract: Nine rabbits were fed with *Clonorchis sinensis* metacercariae (MC) and the blood samples chronologically obtained were analyzed biochemically. Rabbits infected by less than 100 flukes were grouped into Group I, and by 100-250 flukes into Group II. The serum level of alanine aminotransferase (ALT) was increased from 3 weeks after the infection of the metacercariae (AIM) and showed a peak at 8 weeks, and decreased from 12 weeks AIM. The serum level of aspartate aminotransferase (AST) was raised to 92.3 ± 65.4 U/L at 3 weeks AIM and stayed high until 8 weeks, then lowered thereafter. The serum level of γ-glutamyl transpeptidase (γ-GT) was increased rapidly to the highest value (18.9 ± 14.6 U/L) at 16 weeks AIM, and decreased to the control level after 20 weeks. The serum level of alkaline phosphatase (ALP) was headed down from the early infection to 52 weeks AIM. The serum cholesterol level was increased from 8 weeks and reached at a peak 16 weeks AIM, and decreased thereafter to the control level. It is suggested that serum ALT, AST, ALP and γ-GT tests be useful to diagnose the early infection of *C. sinensis*.

Key words: *Clonorchis sinensis*, liver function, rabbit, ALT, AST, ALP, γ-GT

INTRODUCTION

Human and mammals become infected by *Clonorchis sinensis* through eating raw frshwater fish. In 1981, it was estimated on the nationwide stool examination result that about 850,000 inhabitants were infected with *C. sinensis* in basins of major rivers in Korea (Seo *et al.*, 1981). *C. sinensis* affects on bile duct epithelium with mechanical stimuli and damages, and produces metabolites toxic to provoke pathologic changes in biliary tree. The pathologic changes are inflammation of bile ducts, hyperplasia and desquamation of endothelial cells, glandular dilatation and thickening of bile ducts, periductal and periportal fibrosis, and increase of mucin-secreting cells and Goblet cells (Komiya, 1966; Gibson and Sun, 1971).

A lot of metabolisms occur in the liver. They are anabolism, catabolism and storage of nutrients; activation and storage of vitamines; inactivation and excretion of hormones; production of bilirubin and bile acids; and metabolism of iron and copper, *etc.* Liver detoxicates many kinds of chemical compounds through oxidation, reduction and hydrolysis, and produces blood coagulation and fibrinogen lysis factors. With kidney, the liver plays an important role in maintaining blood components for homeostasis (Tietz, 1986).

In general, abnormal results are hardly found in the liver function tests of the cases

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having mild liver damage, because the hepatic cells regenerate highly over damages and the remaining normal hepatic tissue compensates well the dysfunctioned portion. To minimize bias between tests and to get better result, it is desirable to do simultaneously several liver function tests which have an independant specificity.

Iwata (1938) reported levels of cholesterol, cholesteol ester and free choletserol were remarkably increased in rabbits infected with *C. sinensis*. Yamagata and Yaegashi (1964) suggested that liver function tests could be adopted as a marker to screen out *C. sinensis* heavy infection cases among light infections, since their tests appeared to be negative in light infections but to be strong positive in heavy infections. Researchers have tried to find any correlation between *C. sinensis* infection and liver function tests in patients, but not chronological changes of liver function in *C. sinensis*-infected experimental animals as far literature concerned.

We analyzed biochemically the liver function of rabbits chronologically after *C. sinensis* metacercaria infection and found the enzyme levels to be fluctuating according to the infection period prolonged. Here we report our results with literature review.

MATERIALS AND METHODS

C. sinensis metacercariae were collected under the stereomicroscope after artificial digestion of *Pseudorasbora parva*. A total of 9 rabbits, New Zealand white, male, 2-4 month-old, 2.0-2.5 kg of body weight, was fed with 200-1,000 metacercariae through gastric tube. For internal control, blood was taken individually from rabbit ear-vein twice with one-week interval before the metacercarial infection. Blood was taken chronologically from 1 to 52 weeks after infection with the metacercariae (AIM), and the sera were transferred to Green Cross Reference Laboratory (Seoul) for biochemical assays. Rabbits were sacrificed at 52 weeks AIM and *C. sinensis* were recovered from their bile ducts. Five rabbits infected by less than 100 flukes were classified into Group I, and four rabbits by 100-250 flukes into Group II.

Concentration of each component in sera was assayed with blood biochemical analyzer (Hitachi 736-40) by following methods and reagents (Boehringer Mannheim). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by UV method, γ-glutamyl transpeptidase (γ-GT) by calorimetric method, total protein by buret method, albumin by BCG method, total bilirubin by DPD method, alkaline phosphatase (ALP) by calorimetric method using 2-amino-methyl-propanol, and total cholesterol by HOD-PAP method..

The data were analyzed for the statistical difference between the Groups I and II, and between the infection durations with the analysis of variance (ANOVA) in the program Statistical Analysis System (SAS).

RESULTS

Values of biochemical tests according to the worm burden and the period of infection were summarized in Table 1. Serum ALT level decreased to 54.8 ± 9.3 U/L at the first week AIM began to soar from the second week and reached to a peak, 122.6±39.2 U/L, at the 8th week AIM. From 8 weeks AIM, the ALT level decreased slowly to control values by the 20th week and stayed near the control level until the 52nd week. Difference between Group I and II was not significant throughout the infection period ($p < 0.05$).

Serum AST level was increased sharply to 92.3±65.4 U/L at the third week AIM and kept high level until the 8th week. The AST level was decreased near to its control level at the 12th week and ran slightly lower level from the 20th to the 52nd week.

Serum level of γ-GT was increased continuously from the infection to 18.6±8.0 U/L at the 8th week AIM then turned down to 10.7 ± 5.7 U/L at the 12th week. From the 20th week AIM, slightly higher γ-GT level than its control was held until the 52nd week AIM.

Serum ALP level, 133.4±28.8 U/L before *C. sinensis* infection, was fallen headlong down continuously with two small up-rising throughout the infection period. The lowest value, 30.9±12.2 U/L, was observed at the 48th week AIM. Group II showed higher serum

Table 1. (Continued)

Test of liver function	Group[a]	16	20	24	28	32	40	48	52
ALT (U/L)	I	80.8±26.4	64.8±6.5	59.4±7.4	68.2±18.2	62.6±14.0	71.2±24.6	64.8±21.7	50.8±8.0
	II	78.8±18.9	57.3±13.4	61.3±15.8	84.5±12.4	66.0±8.9	52.5±1.7	51.0±1.7	57.5±6.4
	Total	50.3±19.0	70.9±34.9	63.6±22.6	43.8±20.4	42.9±16.7	41.0±15.8	30.9±12.2	40.9±22.4
AST[b](U/L)	I	43.4±37.7	25.0±6.3	27.8±7.6	17.6±4.0	24.0±3.2	34.4±18.3	23.0±4.3	26.0±5.2
	II	29.0±15.0	25.5±15.8	33.5±10.3	27.8±10.2	29.8±13.7	23.8±10.3	22.5±3.3	37.5±9.3
	Total	37.0±30.7	25.2±11.6	30.3±9.3	22.1±8.9	26.6±9.8	39.7±16.2	22.8±3.9	31.1±9.3
γ-GT (U/L)	I	24.2±17.6	10.0±3.7	11.6±3.4	8.4±2.3	11.4±3.1	9.8±4.2	11.0±4.2	9.4±2.3
	II	12.5±3.5	10.3±2.8	13.8±4.8	12.8±7.8	12.8±7.1	12.3±5.8	10.0±4.1	9.0±2.9
	Total	18.9±14.6	10.1±3.3	12.6±4.2	10.3±5.9	12.0±5.1	10.9±4.7	10.6±4.2	9.2±2.6
ALP (U/L)	I	45.6±8.3	63.0±30.3	58.0±23.5	42.8±24.6	43.2±19.7	38.4±14.0	31.0±14.7	42.2±24.7
	II	56.3±25.9	80.8±37.6	70.5±19.1	45.0±13.3	42.5±11.9	44.3±17.1	30.8±8.1	39.3±19.1
	Total	50.3±19.1	70.9±34.9	63.6±22.6	43.8±20.4	42.9±16.7	41.0±16.7	30.9±12.2	40.9±22.4
Cholesterol[b] (mg/dl)	I	186.0±78.4	62.8±31.0	47.4±13.3	53.4±7.2	40.8±19.7	35.6±8.1	40.4±11.8	34.6±7.6
	II	129.5±24.6	82.8±19.5	72.5±45.5	80.3±11.9	72.3±14.5	34.5±5.3	79.5±27.8	86.0±35.5
	Total	160.9±66.9	71.7±28.3	58.6±34.3	65.3±16.4	54.8±23.5	35.1±7.0	57.8±28.3	57.4±35.3
Total Protein (g/dl)	I	6.5±0.5	7.2±0.4	7.1±0.7	6.4±0.7	6.9±0.7	7.0±0.5	6.7±0.8	6.8±0.9
	II	6.2±0.3	6.8±0.4	7.2±0.2	6.5±0.4	7.0±0.1	7.0±0.6	7.4±0.8	7.1±0.3
	Total	6.4±0.5	7.0±0.5	7.1±0.5	6.4±0.6	6.9±0.5	7.0±0.6	7.0±0.9	6.9±0.7
Albumin (g/dl)	I	3.8±0.4	4.0±0.2	4.1±0.2	4.0±0.2	4.5±0.2	4.0±0.1	4.0±0.2	4.0±0.3
	II	3.6±0.3	3.9±0.2	4.1±0.2	4.0±0.1	4.5±0.3	3.9±0.2	4.2±0.2	3.8±0.2
	Total	3.7±0.4	4.0±0.2	4.1±0.2	4.0±0.2	4.5±0.3	4.0±0.1	4.1±0.2	3.9±0.3
A/G ratio	I	1.3±0.4	1.2±0.2	1.4±0.4	1.8±0.7	2.2±0.9	1.6±0.5	1.8±0.8	1.5±0.4
	II	1.4±0.5	0.1±0.3	1.4±0.3	1.6±0.5	1.8±0.3	1.3±0.3	1.4±0.4	1.1±0.1
	Total	1.4±0.4	1.3±0.3	1.4±0.4	1.7±0.6	2.0±0.7	1.4±0.5	1.6±0.7	1.3±0.4

a) Group I: five rabbits infected by less than 100 flukes; Group II: four rabbits infected by 100-250 flukes

b) Between Group I and II: $p < 0.05$

Table 1. Serum biochemical values from the *Clonorchis sinensis*-infected rabbits according to worm burden and period of infection

Test of liver function	Group[a]	Biochemical values (mean±SD) measured at weeks after the metacercarial infection						
		0	1	2	3	4	8	12
ALT (U/L)	I	66.3±6.0	49.2±4.6	55.4±10.5	99.0±29.6	103.4±38.2	123.6±46.5	80.0±32.4
	II	64.8±13.1	61.8±8.9	68.3±10.8	94.8±27.7	86.3±23.3	121.3±27.2	66.3±13.6
	Total	65.6±9.8	54.8±9.3	61.1±12.4	97.1±28.9	95.8±33.6	122.6±39.2	73.9±26.6
AST (U/L)	I	39.1±14.7	29.0±5.6	34.4±6.5	121.4±75.2	104.0±58.0	88.8±41.3	43.6±24.3
	II	28.1±19.6	24.0±8.9	29.0±8.5	62.3±16.5	62.3±16.5	67.3±22.6	28.8±6.1
	Total	34.2±17.9	26.8±7.7	32.0±7.9	92.3±65.4	85.4±49.2	79.2±35.9	37.0±19.9
γ-GT (U/L)	I	7.4±1.2	7.0±1.7	8.2±1.2	8.4±3.4	10.8±5.7	18.8±6.7	13.0±5.5
	II	7.0±1.9	6.8±1.9	7.8±2.2	9.5±3.8	13.0±4.6	18.3±9.4	7.8±4.6
	Total	7.2±1.5	6.9±1.8	8.0±1.7	8.9±3.6	11.8±5.4	18.6±8.0	10.7±5.7
ALP (U/L)	I	129.9±16.2	114.4±12.7	125.2±16.3	127.4±22.2	122.2±27.5	83.0±27.2	65.8±22.1
	II	137.8±38.7	113.8±37.4	112.5±30.9	126.5±62.9	134.0±60.6	94.5±28.7	60.8±23.8
	Total	133.4±28.8	114.1±26.7	119.6±24.7	127.0±45.1	127.4±45.7	88.1±28.5	63.6±23.0
Cholesterol[b] (mg/dl)	I	88.4±17.6	116.8±44.5	76.6±12.0	66.0±12.1	85.2±14.6	88.8±38.1	106.2±36.5
	II	89.3±25.3	112.3±4.4	58.3±17.8	73.3±32.2	101.8±33.6	109.0±26.4	117.8±50.5
	Total	88.8±21.4	114.8±33.4	68.4±17.5	69.2±23.6	92.6±26.2	97.8±34.9	111.3±43.6
Total Protein (g/dl)	I	6.0±0.2	6.3±0.2	6.1±0.2	5.9±0.1	6.0±0.2	6.2±0.1	6.5±0.2
	II	6.0±0.3	6.1±0.4	5.7±0.4	5.8±0.5	5.8±0.7	5.9±0.3	5.9±0.3
	Total	6.0±0.2	6.2±0.3	5.9±0.3	5.8±0.4	5.9±0.5	6.1±0.3	6.3±0.4
Albumin (g/dl)	I	3.9±0.3	4.0±0.1	3.9±0.1	3.7±0.1	3.9±0.1	3.9±0.1	4.0±0.2
	II	3.8±0.3	3.9±0.3	3.7±0.3	3.7±0.0	3.8±0.2	3.8±0.1	3.8±0.1
	Total	3.9±0.3	3.9±0.2	3.8±0.2	3.7±0.1	3.9±0.2	3.9±0.1	3.9±0.3
A/G ratio	I	1.9±0.5	1.8±0.2	1.8±0.2	1.7±0.2	1.9±0.2	1.7±0.0	1.6±0.3
	II	1.7±0.3	1.7±0.2	1.8±0.3	1.8±0.4	2.0±0.4	1.9±0.3	1.8±0.5
	Total	1.8±0.4	1.8±0.2	1.8±0.3	1.8±0.3	1.9±0.3	1.8±0.2	1.8±0.4

ALP level than Group I.

Serum cholesterol level rising above the control at the first week AIM was dropped abruptly to 68.4 ± 17.5 mg/dl, below the control, at the second week. The cholesterol level was risen again from the 4th week and gave a peak, 160.9±66.9 mg/dl, at the 16th week. The level was fell down from its peak below control level at the 20th week, and remained lowered thereafter. Serum cholesterol level of Groul II was higher than that of Group I ($p < 0.05$).

After 20 weeks AIM, serum total protein level was around 7.0 g/dl, higher than its control level. Change of serum albumin level was not significant. A/G ratio was lowered below its control level after the 16th week except at the 32nd week AIM. Serum levels of total and direct bilirubins were 0.1 mg/dl, but their change was not recognizable (data not shown).

Values in all item appeared to be different statistically along the entire infection period ($p < 0.01$).

DISCUSSION

A large increase of serum AST has been found at early stage of diseases associated with extensive tissue necrosis. Specific assay of serum AST level was suggested as a useful tests to estimate the degree of necrosis in liver disease (Zakim and Boyer, 1990). Changing pattern of serum levels of AST and ALT, in this study, elevated between 3 and 8 weeks AIM and lowered to control level after 12 weeks, can be explained well with the pathologic progress in the liver after *C. sinensis* infection. In liver parenchyma infected with *C. sinensis*, the pathologic change was insignificant until the first week. The portal veins were dilated and their limiting plates were focally necrotized at the 4th week. At the 8th week, hepatic lobules were mildly damaged due to the focal invasion of fibrotic tissue increasing in periepithelial and periductal layers of portal veins. Fibrosis of the veins, however, was not prominent at the 12th week (Lee *et al.*, 1978). Serum levels of AST and ALT from *C. sinensis*-infected patients were resting in normal range, even though they were belonging to heavy worm burden groups (Kim *et al.*, 1982). It is, therefore, considered that they are on cholestasis and/or chronic infection stage rather than on acute infection (Zakim and Boyer, 1990).

ALP has been identified in liver, intestine, kidney, bone, placenta and leukocytes. In the liver, ALP is associated with both sinusoidal and canalicular membranes, and is present in the cytosol (Hagerstrand, 1975). Elevations of serum ALP are associated with a wide variety of pathologic lesions. The highest elevations of ALP occur in patients with cholestasis. Certain bile acids are responsible for the induction of ALP and its release in plasma (Hatoff and Hardison, 1981). In contrast to these characters of ALP, in the course of *C. sinensis* infections of rabbits, the serum level of ALP was lower than the control level from the metacercarial infection in this study or after 100 days in the experiment by Kuwamura (1966). In the patients infected with *C. sinensis* the proportion showing lower serum level of ALP was 63% of the light infections, 72% of moderate infections, 47% of heavy infections, and 24% of very heavy infections. Higher than normal value was only observed in very heavy infections by 16% (Kim *et al.*, 1982). Causes recently reported for the low serum ALP level include Wilson's disease, pernicious anemia, congenital hypophosphatase, hypothyroidism, and zinc deficiency (Zakim and Boyer, 1990; Shaver *et al.*, 1986). It is suggested that a lower serum level of ALP might be an indication of *C. sinensis* early infection.

γ-GT distributes widely in membranes of the liver, pancreas, spleen, kideney, heart, seminal vesicle, and brain. γ-GT is localized to the whole hepatobiliary tree in the liver and to pancreatic acini and ductules by histochemical techniques. The greatest concentration of this enzyme is associated with the luminal border of the epithelial cells lining fine biliary ductules (Naftalin *et al.*, 1969). Serum γ-GT is elevated in association with hepatobiliary disease as well as in pancreatic disease, chronic alcoholism, renal failure, myocardial infarction, and diabetes (Goldberg and Martin, 1975). The increase of serum γ-GT in early infection stage correlates well with the expansion of damaged area on biliary epithelium in accordance to the growth of *C. sinensis*. Decrease of serum γ-GT in later

infection stage, atfer 20 weeks AIM in this study, can be speculated that the increased fibrotic tissues in periepithelial and periductal layers barrier the absorption of released γ-GT in plasma. Serum γ-GT is a very sensitive test showing up to 90% accuracy for detecting biliary tract disease and correlates with serum ALP (Whitfield *et al.*, 1972). However, the elevation of γ-GT has limited usefulness, because the enzyme is distributed ubiquitously, and its elevation is associated with variety of diseases as well as hepatobiliary disease. In consideration of the high sensitivity but poor specificity of this enzyme, it can paradoxically be said a normal test result predicts the absence of hepatobiliary disease.

Iwata (1938) reported the serum level of cholesterol was increased in rabbits infected with *C. sinensis* from one week and augmented by two times of the normal value. Elevation of serum cholesterol imlpies the release of lipoprotein X in plasma and accelerated synthesis of cholesterol in the liver due to obstructive liver disease (Brocklehurst *et al.*, 1978). In this experiment the cholestasis was unlike, because the serum levels of total and direct bilirubins were not changed. However, serum cholesterol level was eleveted during the infection period. Cholestatic effect in the liver by *C. sinensis* infection is a subject to be elucidated in the future.

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= 국문초록 =

간흡충을 감염시킨 토끼의 간기능 검사

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간흡충 피낭유충을 9 마리의 토끼에 200-1,000개씩 감염시킨 후 1주, 2주, 3주, 4주, 8주, 12주, 16주, 20주, 24주, 28주, 32주, 40주, 48주 및 52주에 각각 채혈하여 혈청의 생화학적 검사를 실시하였다. 감염 52주후에 토끼를 도살하여 간흡충을 회수하고 감염 충체수가 100마리 미만인 토끼는 제1군으로, 100마리 이상 250마리 이하인 토끼는 제2군으로 분류하여 검사 결과를 분석하였다. 혈청 ALT 수준은 모든 감염군에서 감염 후 3주부터 증가하여 8주에 최고치를 나타내었으며 12주 이후에는 현저히 감소하였으나 52주까지 대조군보다 높은 수준으로 유지되었다. AST는 감염후 3주에 급격히 증가하여 높은 수준으로 8주까지 유지되었으며 12주 이후에는 대조군보다 약간 높은 수준으로 유지되었다. 혈청 γ-GT는 감염후 2주부터 8주까지 급속히 증가하여 16주에 최고치를 나타내었으며 20주에는 현저히 감소되었으나 대조군보다 높은 수준으로 52주까지 유지되었되었다. 혈청 ALP는 감염 초기부터 52주까지 계속 감소하였다. 혈청 cholesterol은 감염후 8주부터 점차 증가하여 16주에 높은 검사치를 기록하고 다시 감소하여 24주 이후에는 대조군 수준을 유지하였다. Total bilirubin과 direct bilirubin은 모든 감염군에서 변화가 없었다. 이상의 결과로 볼 때 토끼에서 혈청 AST, ALT 및 γ-GT는 간흡충 감염 초기에 증가하고 혈청 ALP는 저하되는 양상을 보이므로 이러한 간기능 검사는 인체 간흡충증을 초기에 진단하는 자료로 활용될 수 있다고 생각된다.

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