

Serum Interleukin-12 Levels in Alcoholic Liver Disease

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Background: Interleukin (IL)-12 is a proinflammatory cytokine produced by antigen-presenting cells upon stimulation by diverse stimuli. This study aimed to explore the relationship between IL-12 serum levels and different stages of alcoholic liver disease, alcoholic intake status and abstinence from alcohol.

Methods: A total of 35 healthy controls without alcohol consumption and 94 patients with alcoholic liver disease (17 with alcoholic steatosis, 37 with alcoholic hepatitis, 40 with alcoholic cirrhosis) were included. Their serum IL-12 levels were measured and followed-up at the 3rd, 6th and 9th months. Data were further analyzed according to abstinence from alcohol or not.

Results: Mean serum IL-12 levels were higher in the alcoholic hepatitis group (163.1 ± 57.8 pg/mL) than in the alcoholic liver cirrhosis group (110.5 ± 41.6 pg/mL) and alcoholic steatosis group (74.4 ± 26.2 pg/mL). All of these 3 alcoholic groups had higher serum IL-12 levels than the control group (39.3 ± 8.3 pg/mL; $p < 0.02$). Among the patients who abstained from alcohol, there was no difference in serum IL-12 levels between control and steatosis patients at the 9th month, but the serum IL-12 levels of the hepatitis and cirrhosis groups were still higher than in the control group ($p < 0.001$ and $p = 0.001$, respectively). In addition, the patients who continued to drink alcohol had higher serum IL-12 levels than those who abstained from alcohol in the steatosis, hepatitis and cirrhosis groups. At the cut-off value of 54 pg/mL, IL-12 had good sensitivity and specificity in the diagnosis of alcoholic liver disease.

Conclusion: Serum IL-12 levels reflected the different stages of alcoholic liver disease and can represent the status of continuous alcohol consumption. It has the potential to be a biomarker of alcoholic liver disease. [*J Chin Med Assoc* 2010;73(2):67–71]

Key Words: alcoholic cirrhosis, alcoholic hepatitis, alcoholic liver disease, cytokine, interleukin 12

Introduction

Alcohol has been shown to have multiple effects on the immune system. However, the pathogenesis of alcoholic liver disease is not fully understood. It is postulated that chronic alcohol consumption may be associated with increased proinflammatory cytokines in the serum and deranged balance between proinflammatory cytokine and anti-inflammatory cytokines.^{1–3}

Interleukin (IL)-12 is a proinflammatory cytokine produced by antigen-presenting cells upon stimulation by various pathogens. This cytokine has been shown to have direct effects on natural killer cells and

T cells, and primes T helper 1 response.^{4,5} Laso et al have demonstrated that serum IL-12 levels are increased in chronic alcoholism, with or without alcoholic liver disease.⁶ It has also been shown that serum IL-12 levels return to within normal limits 1 year after cessation of alcohol consumption. The clinical significance of serum IL-12 in the diagnosis of alcoholism and various alcoholic liver diseases has yet to be elucidated. The purpose of this study was to explore the relationship between IL-12 serum levels and the different stages of alcoholic liver disease. The influence of abstinence from alcohol on serum IL-12 level was also evaluated.



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Methods

Subjects

Ninety-four patients with alcoholic liver disease and 35 healthy adults without alcohol consumption were included in this study. All of the alcoholic patients had consumed, on average, at least 50 g of ethanol per day for more than 5 years. Of them, 17 were diagnosed to have alcoholic steatosis based on the findings of liver pathology and abdominal sonography. Their serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) were less than twice the upper limit of normal. Thirty-seven patients had alcoholic hepatitis, diagnosed by the characteristic liver pathological findings of steatohepatitis, with or without fibrosis; however, they did not have any pathological or clinical evidence of liver cirrhosis. Their serum ALT and/or AST were more than twice the upper limit of normal. The remaining 40 alcoholic patients were diagnosed to have alcoholic cirrhosis. In 14 of them, alcoholic cirrhosis was verified by the pathological findings of liver biopsy. In the other 26 patients, liver biopsy was not done due to the presence of coagulopathy, ascites or patient refusal; instead, alcoholic cirrhosis was diagnosed based on the typical clinical stigmata of cirrhosis, including ascites, esophageal varices, hepatic encephalopathy, hypoalbuminemia, and the presence of an uneven liver surface, decreased hepatic size, collateral circulation and splenomegaly on imaging studies. All of the patients and controls were negative for serum hepatitis B surface antigen and anti-hepatitis C antibody, and no autoimmune liver disease was found in any of them.

After enrolment, the patients were followed-up every 3 months for a total of 9 months; serum IL-12 concentration was measured at each follow-up. Education and abstinence from alcohol was advised to all the patients. Careful interviews with patients and their families, in addition to regular follow-up liver enzyme tests, were done to determine if the patient had quit alcohol drinking or not.

This study was approved by the institutional review board of our hospital. All of the study subjects signed informed consent forms.

Serum IL-12 levels were measured using a commercial enzyme-linked immunosorbent assay (PeproTech Inc., Rocky Hill, NJ, USA). The IL-12 detection range of this kit is from 32 to 3,000 pg/mL. For the convenience of statistical analysis, all undetectable IL-12 levels (i.e. < 32 pg/mL) were recorded as being 32 pg/mL.

Statistical analysis

The data are expressed as mean \pm standard deviation unless otherwise noted. Comparisons of variables among

the different alcoholic liver disease groups and healthy controls were carried out using ANOVA and Tukey's test. Multiple linear regression was applied to evaluate the correlation between IL-12 and age, amount and duration of alcohol drinking. Student's *t* test was used to compare serum IL-12 levels between the alcohol abstinence group and the continuous alcohol intake group at different time periods. For further confirmation, repeated measures general linear model was used to evaluate the sequential changes in IL-12 in the abstinence and continuous alcohol drinking groups. Receiver operating characteristic curve was applied to select the best cut-off value of IL-12 for the discrimination of alcoholic liver disease. Statistical tests were based on 2-tailed probability. A *p* value < 0.05 was considered significant. All analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

The patients with alcoholic liver cirrhosis were older, had a higher Child-Pugh score, higher volume of daily alcohol intake, and longer duration of alcohol consumption than the patients in the other groups (Table 1). The patients with alcoholic hepatitis had higher serum ALT, AST and γ -glutamyltransferase levels than those in the other groups. Serum IL-12 levels were highest in the alcoholic hepatitis group (163.1 ± 57.8 pg/mL), followed by the alcoholic liver cirrhosis group (110.5 ± 41.6 pg/mL) and the alcoholic steatosis group (74.4 ± 26.2 pg/mL; $p < 0.02$ between groups) (Table 1). All of these 3 groups had higher serum IL-12 levels than the control group (39.3 ± 8.3 pg/mL; $p < 0.02$). Furthermore, there was no association between IL-12 levels and age, average daily amount of alcohol consumption, and duration of alcohol consumption ($p = 0.962, 0.155$ and 0.084 , respectively).

Ten of the steatosis patients, 17 of the hepatitis patients, and 30 of the cirrhosis patients were noted to have abstained from alcohol during the follow-up period. The serum IL-12 levels between the abstinence and non-abstinence groups at the 9th month are shown in Figure 1. Among the patients who abstained from alcohol, there was no difference in serum IL-12 levels between the control and steatosis patients. However, the serum IL-12 levels of the hepatitis and cirrhosis groups were still higher than in the control group ($p < 0.001$ and $p = 0.001$, respectively). The patients who continued to consume alcohol had higher serum IL-12 levels than those who had abstained from alcohol in the steatosis, hepatitis and cirrhosis groups (Figure 1).

Table 1. Clinical characteristics and serum interleukin-12 levels in controls and patients with alcoholic steatosis, alcoholic hepatitis and alcoholic cirrhosis*

	Controls (n=35)	Steatosis (n=17)	Hepatitis (n=37)	Cirrhosis (n=40)
Age (yr)	44.4±14.5	45.6±15.9	46.4±10.3	54.9±9.6 [†]
Sex (M/F)	21/14	11/6	26/11	31/9
ALT (U/L)	25.4±7.2	64.2±10.2	203.8±112.2 [†]	91.7±76.2 [†]
AST (U/L)	27.7±7.3	75.9±12.8	272.2±121.9 [†]	125.7±95.7 [†]
GGT (U/L)	35.6±12.4	100.1±76.9	134.2±97.6 [†]	92.3±54.9 [†]
Alk-P (U/L)	61.9±21.8	94.8±29.1 [†]	83.2±33.8 [†]	72.2±21.5
Total bilirubin (mg/dL)	0.9±0.3	0.8±0.3	1.1±0.7	1.5±1.0 [†]
Child-Pugh score	–	5.0±0.0	5.1±0.3	7.3±2.5 [†]
Daily alcohol intake (g/d)	–	58.1±8.9	60.4±11.3	68.0±16.2 [†]
Duration of alcohol intake (yr)	–	6.5±1.3	10.2±4.4	17.0±5.1 [†]
Interleukin-12 (pg/mL)	39.3±8.3	74.4±26.2 [†]	163.1±57.8 [†]	110.5±41.6 [†]

*Data presented as mean ± standard deviation or n; [†]p < 0.05 vs. the other 3 groups; [‡]p < 0.05 vs. controls. ALT=alanine aminotransferase (normal range, 0–40 U/L); AST=aspartate aminotransferase (normal range, 5–45 U/L); GGT=γ-glutamyltransferase (normal range, 8–60 U/L); Alk-P=alkaline phosphatase (normal range, 10–100 U/L). Total bilirubin normal range=0.2–1.6 mg/dL.

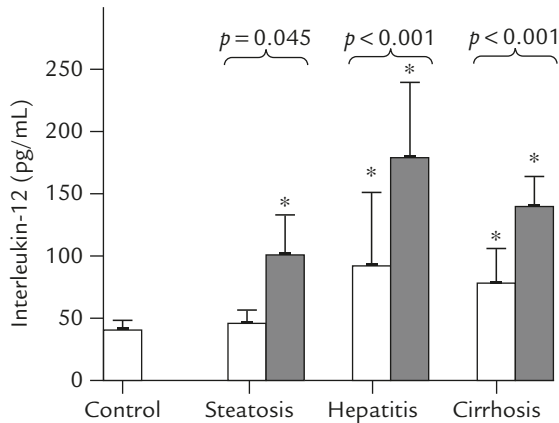


Figure 1. Serum interleukin-12 levels in patients who abstained from alcohol (white bars) and those who continued drinking (grey bars) as measured at the 9-month follow-up. *p < 0.002 vs. control patients.

If the hepatitis and cirrhosis groups were combined into 1 group with significant alcoholic liver disease, the patients who had abstained from alcohol had significantly lower serum IL-12 levels than those who did not abstain at the 3rd, 6th and 9th months (Figure 2). Furthermore, the difference in IL-12 levels between the alcohol non-abstinence and abstinence groups showed an increasing trend with time, from 9.0 pg/mL (95% confidence interval, –17.3–35.3) to 38.0 pg/mL (17.1–58.9), 70.8 pg/mL (51.1–90.6), and 83.2 pg/mL (61.1–105.3) at baseline, and the 3rd, 6th and 9th months, respectively (Figure 2). In the abstinence group, there was a significant reduction in IL-12 levels from baseline to the 3rd month, and from the 3rd month to the 6th month (p < 0.001), and a trend of IL-12

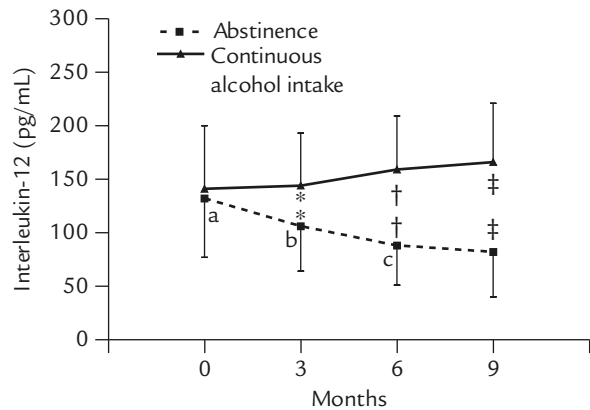


Figure 2. Difference in interleukin-12 levels between patients who continued drinking alcohol and those who abstained among the patients with alcoholic hepatitis and cirrhosis. The patients who continued drinking had higher mean serum interleukin-12 levels than those who abstained at the 3rd, 6th and 9th months of follow-up, respectively (*[†]p < 0.002). p_a vs. b < 0.001; p_b vs. c < 0.001.

reduction from the 6th month to the 9th month (p = 0.055). In contrast, there was no significant consecutive change in IL-12 levels in the non-abstinence group (Figure 2).

Table 2 denotes the diagnostic sensitivity and specificity of IL-12 for alcoholic liver disease after choosing the best cut-off value. IL-12 had good sensitivity and specificity in the diagnosis of overall alcoholic liver disease, or alcoholic hepatitis and cirrhosis, at the cut-off value of 54 pg/mL. However, IL-12 had relatively poor sensitivity (76.5%) for the detection of alcoholic steatosis. At the cut-off point of 94 pg/mL, IL-12 could differentiate alcoholic hepatitis from alcoholic steatosis with good sensitivity (91.9%) and acceptable specificity (82.3%) (Table 2).

Table 2. Diagnostic value of interleukin-12 in alcoholic liver disease

	Best cut-off value of IL-12 (pg/mL)	Sensitivity (%)	Specificity (%)
All ALD vs. controls	54.0	90.4	97.1
Hepatitis or cirrhosis vs. controls	54.0	93.5	97.1
Steatosis vs. controls	54.0	76.5	97.1
Hepatitis vs. steatosis	94.0	91.9	82.4
Hepatitis vs. cirrhosis	128.5	81.1	67.5

IL = interleukin; ALD = alcoholic liver disease.

Discussion

The present study showed that serum IL-12 levels were elevated in patients with alcoholic steatosis, alcoholic hepatitis, and alcoholic cirrhosis; patients with alcoholic hepatitis had the highest serum IL-12 levels. Patients' serum levels progressively decreased if they abstained from alcohol. Most of our results are compatible with Laso et al's findings.⁶

In patients with chronic alcoholism without significant liver disease, both pro- and anti-inflammatory cytokines may be produced and maintain a subtle balance.² However, when gut permeation is changed by continuous alcohol intake, endotoxemia may develop.² IL-12 can be produced by Kupffer cells upon stimulation by endotoxin. IL-12 production may thereafter outnumber the production of anti-inflammatory cytokines, with the subsequent production of interferon- γ and maintenance of T helper 1 immune response.⁷ Serum IL-12 then induces the expression of interferon- γ in T cells and natural killer cells, which leads to macrophage activation and further inflammation.^{8,9}

On the other hand, progression to alcoholic cirrhosis requires the activation of other fibrogenic cytokines such as transforming growth factor- β . It has been shown that transforming growth factor- β can inhibit the production of IL-12.¹⁰ This may partly explain why people with alcoholic cirrhosis had lower serum IL-12 levels than alcoholic hepatitis patients.

In this study, serum IL-12 levels were found to decrease after alcohol abstinence in patients with alcoholic hepatitis and cirrhosis. But these levels were still higher than those of controls. In contrast, Laso et al demonstrated that serum IL-12 levels returned to within normal limits after 1 year of alcohol abstinence.⁶ Although our study had a similar finding, we only followed serum IL-12 levels to the 9th month after alcohol abstinence. It is probable that serum IL-12 levels would have decreased to normal range if we had followed-up our patients for longer.

Patients with chronic alcohol consumption may hesitate to admit their habit, and it is difficult for us to determine whether or not patients abstained from alcohol following our advice and education. The clinical significance of decreasing IL-12 levels after abstinence may help us to evaluate the real alcohol drinking status of patients, which would be helpful in the management of patients and prediction of prognosis.

We found that serum IL-12 levels decreased to normal range in patients with alcoholic steatosis after alcohol abstinence. This may be a reflection of the reversible nature of alcoholic fatty liver itself. It is well known that the appearance of steatohepatitis is an important rate-limiting step in the development of progressive alcoholic liver disease. When cirrhosis has developed, alcohol abstinence may not halt the progression of cirrhosis.¹

Since the clinical manifestations of alcoholic hepatitis and alcoholic steatosis are similar, it is very difficult to differentiate between these 2 entities without confirmation from liver pathology. In this study, if we chose 94.0 pg/mL as the cut-off point, the discriminative sensitivity and specificity between these 2 alcoholic liver diseases were more than 82% (Table 2). To the best of our knowledge, there is no other biomarker with such diagnostic accuracy. IL-12 has the potential to discriminate steatosis from hepatitis. However, further large-scale studies are warranted to establish the true diagnostic role of IL-12 in this setting.

Since the discovery of IL-12, much has been learned about its biological activity and signal transduction pathway. A better understanding of the pathogenesis of alcoholic liver disease and the biological activities of IL-12 will lead to an exploration of agents for the treatment of alcoholic liver disease. Inhibitors of IL-12 production or activity have potential for the treatment of alcoholic liver disease.¹¹ These agents act through the inhibition of nuclear factor- κ B, modulating intracellular cAMP, post-translational inhibition of IL-12 production or inhibition of IL-12 signal transduction pathway.^{12,13} Of these, pentoxifylline has been used in the

treatment of severe acute alcoholic hepatitis with promising results.^{14,15}

Alcoholic and non-alcoholic steatohepatitis (NASH) share similar clinical manifestations and pathological features. Patients with NASH have dysregulated cytokine metabolism similar to but less pronounced than the abnormalities documented in alcoholic hepatitis.¹ Further studies are necessary to evaluate the role of cytokines in the pathogenesis of NASH.

In conclusion, our study demonstrated that serum IL-12 levels reflect the different stages of alcoholic liver disease and can represent the status of continuous alcohol consumption. It has the potential to become a biomarker of alcoholic liver disease.

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