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Original Article

Lymphocyte CD64 increased in patients with chronic HBV infection

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Abstract: CD64 was up-regulated in infection diseases, but there was no report about the change of CD64 in chronic hepatitis B virus (HBV) infection. The purpose of this study was to determine whether there was a dynamic change of CD 64 index and to judge the value to antiviral treatment. 96 CHB patients were enrolled and selected 33 healthy adults as control. We detected the level of CD64, found the level of CD64 were significantly increased in chronic HBV infection patients, especially the lymphocyte CD64 (8.12 ± 0.23 vs. 6.25 ± 0.27; $P < 0.001$). Further, we proved CD64 index was increased in various stages of chronic HBV infection. ROC curve analysis showed the level of lymphocyte CD64 had higher AUC value than neutrophil or monocyte. Then we monitor longitudinally the impact of the treatment with interferon-α and found that the suppression of viral replication induced by interferon-α resulted in a decrease in CD64 index. In conclusion, this study showed that CD64 index was increased in chronic HBV infection patients and changed with the course of disease, the therapy of interferon-α would correct it, and analysis prompted that the level of lymphocyte CD64 would be more suitable for as a biomarker to judge the condition of chronic HBV infection and the curative effect of interferon-α treatment.

Keywords: Hepatitis B, cirrhosis, hepatocellular carcinoma, CD64

Introduction

Hepatitis B is a global health problem caused by the hepatitis B virus (HBV). About 30% of the world's population showed serological evidence of HBV infection [1]. In 2006, the hepatitis B surface antigen (HBsAg) carrier rate was 0.96% among children under 5 years of age and estimated 84 million HBV carriers in china [2].

The elevation of inflammatory markers such as leukocyte, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) can contribute to diagnose bacterial infection, but it has few help for diagnose viral infection. The common markers used in clinical to judge the degree of HBV inflammation are alanine aminotransferase (ALT) and HBV-DNA, but those indicators cannot accurately describe the actual status, thus cause to some cases appeared hepatitis B-associated liver cirrhosis (HBV-LC) or hepatitis B-associated hepatocellular carcinoma (HBV-HCC) with the levels of normal ALT and undetected HBV-DNA.

CD64 [Fc gamma receptor 1 (FcγRI)] is a novel and promising biomarker used in predicting severe bacterial infection as neonatal sepsis [3], a meta-analysis showed the sensitivity of CD64 index was 79% (95%, confidence interval (CI) 70% to 86%) and the specificity is 91% (95%, CI 85% to 95%) [4]. CD64 index also up-regulated in autoimmune diseases as rheumatoid arthritis [5]. Increasing evidence demonstrated that CD64 index could be used in viral infection patients, as Human Cytomegalovirus [6], Epstein-Barr virus [7] or other DNA virus [8, 9]. But most studies were focused on bacterial or acute viral infection, and there was no report about chronic HBV infection.

As CD64 index would ascend at diseases of viral infection or autoimmune disorder, HBV mainly through immune response and part of the direct effect to damage liver cells. Therefore, we detected the CD64 index of peripheral blood in different stages of chronic HBV infection patients (CHB, HBV-LC and HBV-HCC), the pur-

Table 1. Summary of the characteristics of study participants

	HBV group	Control group	*P* value
Gender, No.	96	33	
Male	74	23	
Female	22	10	
Age, y (mean ± SD)	48.3 ± 15	43.5 ± 13.1	0.11

Chronic hepatitis B virus infection (HBV).

pose of this study was to determine whether there was a change of CD64 index and to judge the value of the indicator for clinic.

Since the only way to reduce morbidity and mortality from chronic HBV infection is antiviral treatment including nucleos(t)ide analogs (NAs) and interferon-α. Study showed that about 58% of HBV-infected patients had received or were receiving ongoing antiviral treatment in China [10]. So we selected part of CHB patients to treat with interferon-α to observe the change of CD64 during the treatment.

Materials and methods

Patients

Ninety-six patients of chronic HBV infection (HBV group), median age was 48.27 years (rang: 23-72 y) with 74 males (77.1%) and 22 females (22.9%), including 43 patients of CHB (CHB group), 29 patients of HBV-LC (HBV-LC group), 24 patients of HBV-HCC (HBV-HCC group) were enrolled in this study. The diagnosis was based on the guidelines for chronic hepatitis B diagnosis of the American Association for the Study of Liver Diseases (AASLD) [11]. Thirty-three healthy individuals were enrolled as normal controls (Control group) shown as **Table 1**. The exclusion criteria included: (1) used antiviral or immunomodulating treatment in the past six months, (2) positive pregnancy test in female, (3) alcoholic liver disease, (4) subjects with bacterial infections (5) with hepatitis C virus, hepatitis D virus or human immunodeficiency virus co-infection, (6) accompaniment of diabetes, thyroid dysfunction, autoimmune diseases and psychological issues were excluded from the study.

After the enrollment, 22 CHB patients were subcutaneously administered with interferonα-1b (Recombinant Human Interferon α1b for Injection, Shengzhen Kexing Biotech Co, CHN) 5MIU every other day for 48 weeks. All subjects accepted physical examination and serum analysis at week 0, 12, 24 and 48.

The study protocol was approved and monitored by the ethics committee of Nanjing Jiangbei Peoples' Hospital, and written informed consent was obtained from the patients.

Flow cytometric analysis

CD45 APC, CD64 PE, Hemolysin were purchased from BD PharMingen (San Diego, CA). Whole blood samples (100 μl) were stained with CD45 APC, CD64 PE in dark, then added hemolysin after 15 min, then tested on the Flow Cytometry within 3 h and counted neutrophil, monocyte and lymphocyte CD64 index, respectively. Three-color flow cytometric analyses were performed using a FACSCalibur and CellQuest software (Becton Dickinson, San Jose, CA). Three-color flow cytometric analyses were performed using FACSCalibur and CellQuest software (Becton Dickinson, San Jose, CA).

HBV-DNA assessment

HBV-DNA levels were determined by real-time polymerase chain reaction (Roche). The threshold of the HBV- DNA detection limit was 250 IU/ml.

Statistical analysis

Results were reported as means ± SD for percentages. Statistical comparisons between two groups used independent samples T-test. The one-way ANOVA and Scheff method were used for multiple comparisons. Data analysis was done by using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL). Confidence interval was 95%.

Results

Peripheral blood CD64 index increased in HBV group

Baseline characteristics of patients and healthy individuals were summarized in **Table 1**. By the experimental results, compared to control group, the level of neutrophil CD64 in HBV group was significantly increased (63.47 ± 5.02 vs. 34.27 ± 4.42; P = 0.0016; **Figure 1A**) and same as lymphocyte CD64 (8.12 ± 0.23 vs.

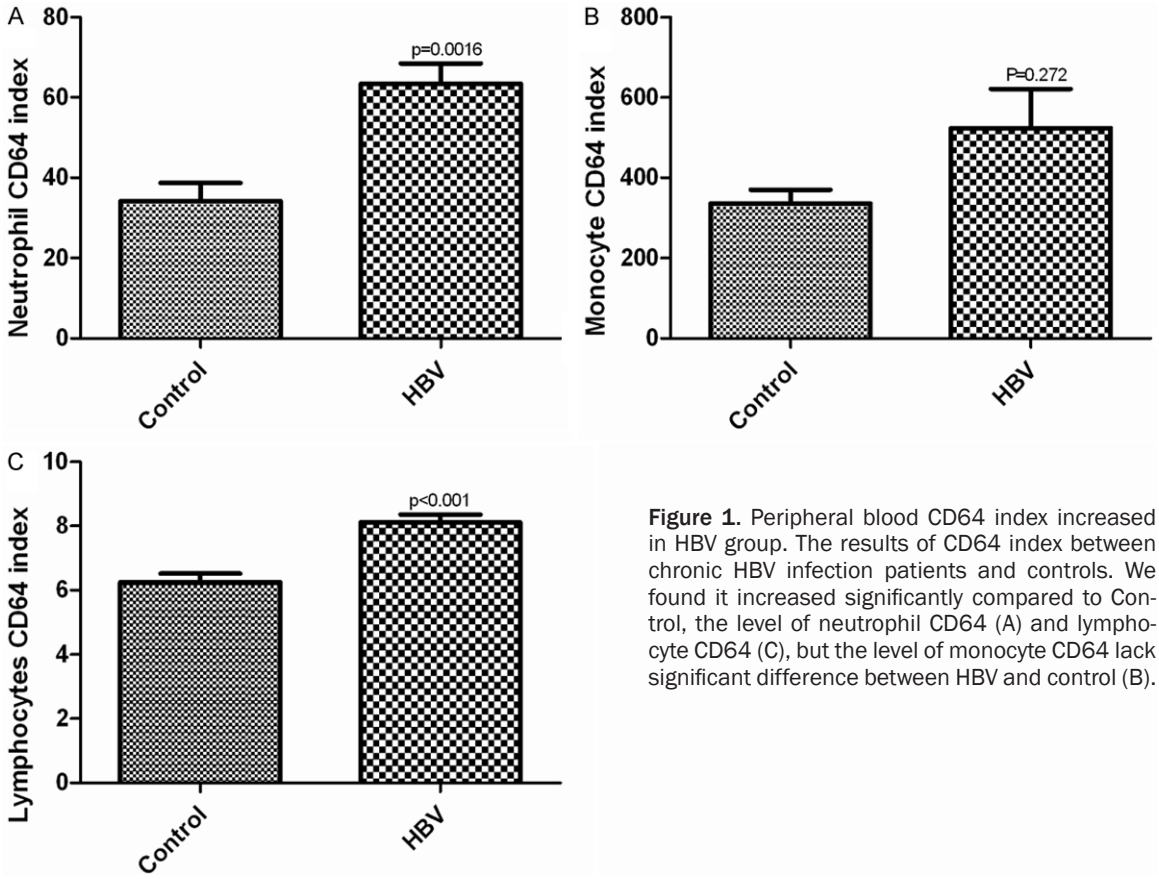

Figure 1. Peripheral blood CD64 index increased in HBV group. The results of CD64 index between chronic HBV infection patients and controls. We found it increased significantly compared to Control, the level of neutrophil CD64 (A) and lymphocyte CD64 (C), but the level of monocyte CD64 lack significant difference between HBV and control (B).

6.25 ± 0.27; P < 0.001; **Figure 1C**), but to monocyte CD64, there was not significant difference between HBV and control groups (523.82 ± 97.84 vs. 336.06 ± 33.99; P = 0.2719; **Figure 1C**).

Peripheral blood CD64 index increased in different stages of chronic HBV infection

To determine whether there was dynamic change of CD64 index in different stages of chronic HBV infection, we divided HBV group into three groups (CHB, HBV-LC and HBV-HCC) according to the stage of chronic HBV infection. Analysis showed that all of CD64 index of HBV-HCC group (neutrophil vs. lymphocyte vs. monocyte; P = 0.0001 vs. P < 0.001 vs. P = 0.05) were higher than control. The level of lymphocyte CD64 had most significantly difference between the HBV groups and control (P = 0.006; **Figure 2C**) and the level of monocyte CD64 had most significant difference between the HBV-HCC and others (P < 0.001; **Figure 2B**). To the level of neutrophil CD64, there was only significant difference between HBV-HCC and control group (P = 0.001; **Figure 2A**).

The level of lymphocyte CD64 is more suitable for as chronic HBV infection -specific marker

To examine the selectivity of CD64 index for chronic HBV infection, the data were subjected to ROC curve analysis. As is understood from the data of **Figure 3**, the AUC (area under curve) value of neutrophil CD64 (**Figure 3A**) was 0.527 with low sensitivity (24.2%) and high specificity (90.3%). To the level of monocyte CD64 (**Figure 3B**), the AUC value was 0.621, also with low sensitivity (29%) and high specificity (96.8%). But when we analyzed the lymphocyte CD64 (**Figure 3C**), we found the AUC value was above 0.75 (0.756), with high sensitivity (71.6%) and specificity (75%), respectively. The results indicated that the level of lymphocyte CD64 was more useful than neutrophil or monocyte CD64 as chronic HBV infection -specific markers.

CD64 index decreased during interferon-α treatment

Twenty-two of total CHB patients received an interferon-α treatment of 48 weeks course, and detected the level of lymphocyte CD64, HBV-DNA and ALT at baseline, 12, 24 and 48

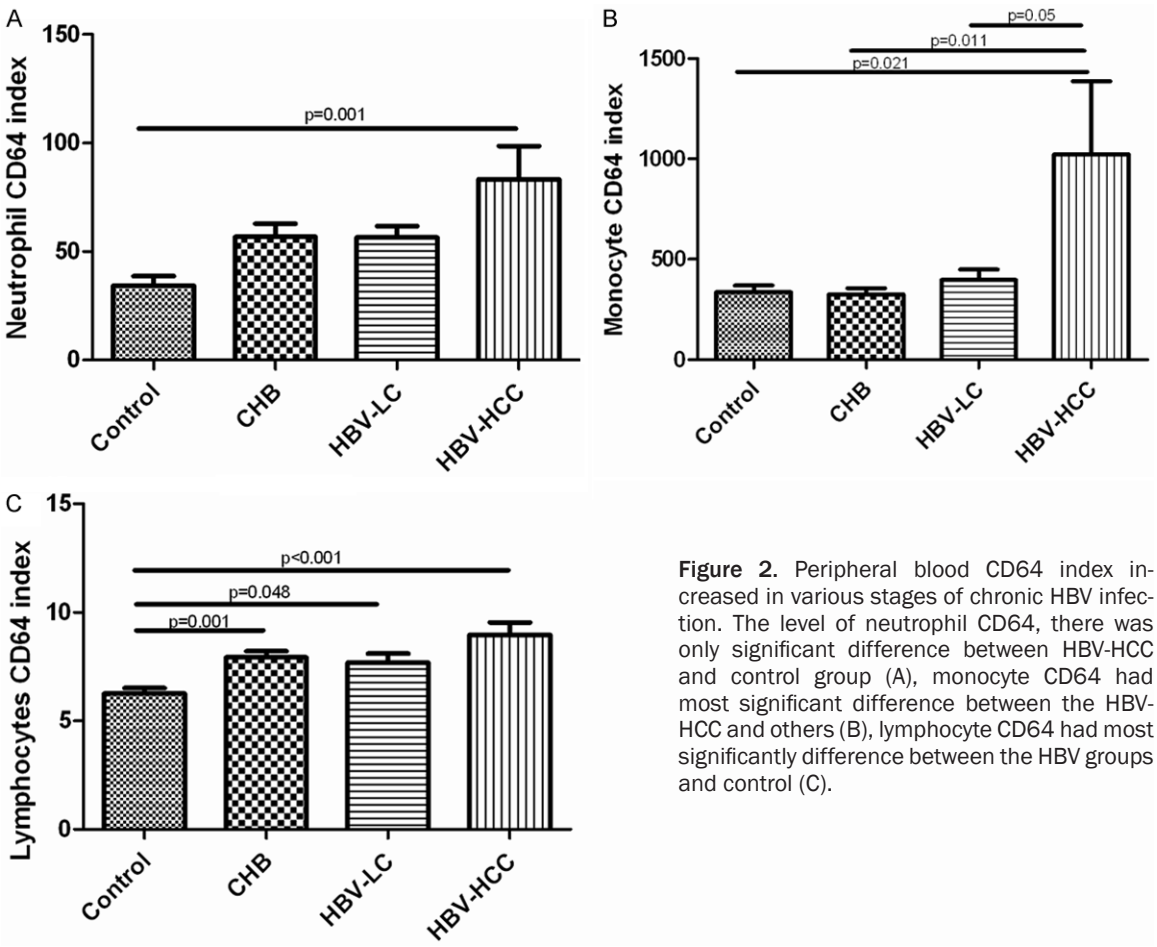

Figure 2. Peripheral blood CD64 index increased in various stages of chronic HBV infection. The level of neutrophil CD64, there was only significant difference between HBV-HCC and control group (A), monocyte CD64 had most significant difference between the HBV-HCC and others (B), lymphocyte CD64 had most significantly difference between the HBV groups and control (C).

weeks. The HBV replication was considerably lower than that at baseline in all patients, the level of HBV DNA was from 5.58 ± 1.96 log10 IU/ml reduced to 0.71 ± 1.22 log10 IU/ml at week 48 (**Figure 4A**), at the end of treatment, HBV DNA was undetectable in 20 patients (91%) and reduced over 2 log10 IU/mL in 2 patients, and this decrease was paralleled in the serum ALT levels (from 187.5 ± 128.2 U/L to 27.53 ± 4.08 U/L) (**Figure 4B**). Fit to our hypothesis, the level of lymphocyte CD64 results showed it normalized since week 12 and last till week 48 (**Figure 4C**).

Discussion

Increasing evidence demonstrated that HBV could be bind to the components above the liver cell membrane and lead to cellular and humoral immune response, stimulate autoimmune response and immune dysfunction, then damaged the liver cells directly, or through mediate antibody-dependent cytotoxicity (ADCC) to cause liver injury [8, 12], appeared the imbalance of ratio of T helper cells [13-15], and produced autoantibodies. These antibodies like as anti-liver cell specific protein (anti-LSp) were IgG, CD64 as the receptor of IgG Fc fragment would be changed in HBV infection patients.

Our study found that the level of CD64 was higher in the patients with chronic HBV infection than healthy subjects, suggested that as we expected, CD64 index would be used as the indicator of chronic HBV infection, but unlike reports about the level of neutrophil CD64 was increased in bacterial or acute viral infection, ROC curve analysis showed the level of lymphocyte CD64 had higher AUC value than neutrophil or monocyte.

Further, we found in pace with the duration of chronic HBV infection, the level of CD64 also increased significantly in lymphocyte than others. When to HBV-HCC group, all had significant

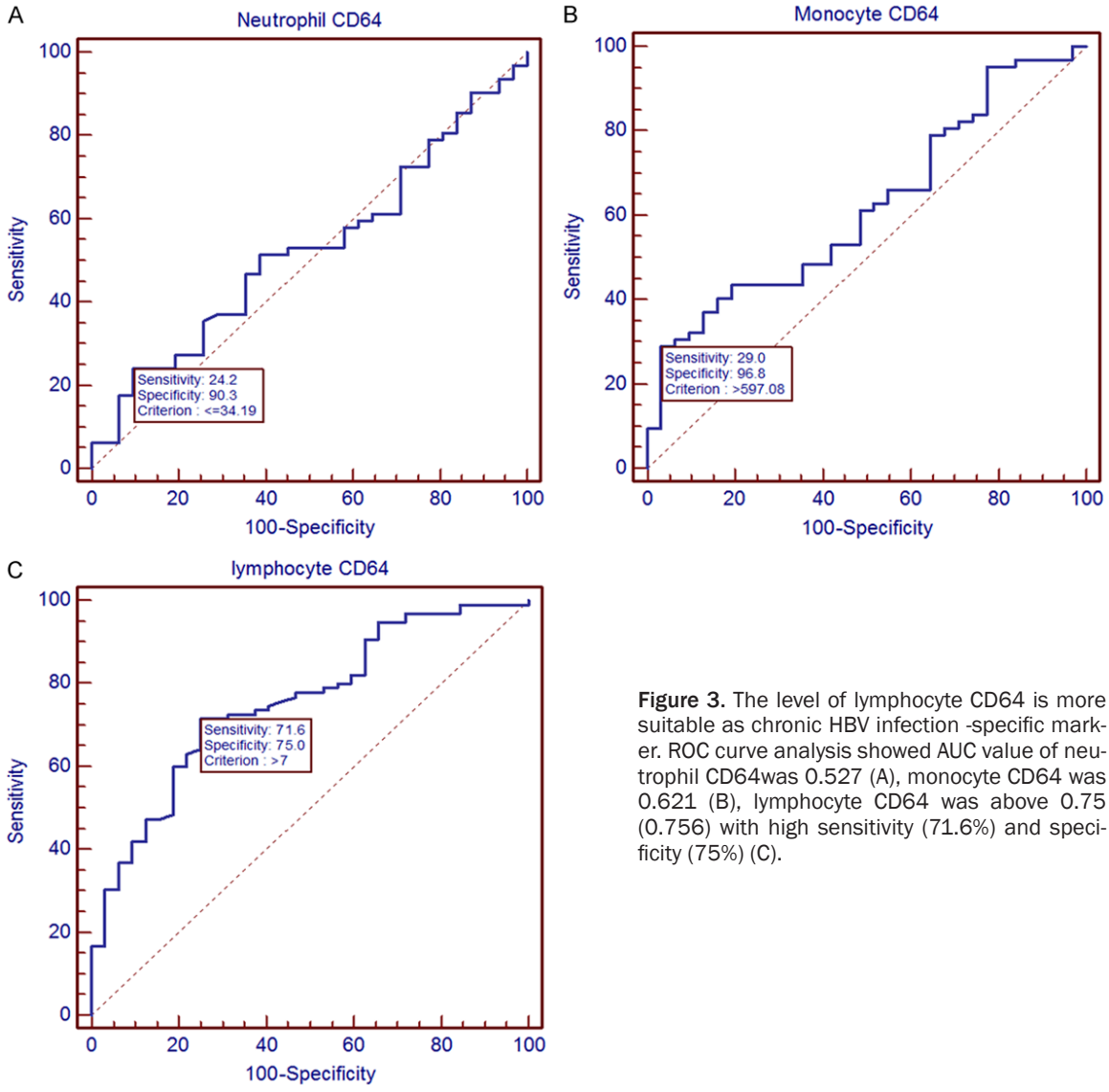

Figure 3. The level of lymphocyte CD64 is more suitable as chronic HBV infection -specific marker. ROC curve analysis showed AUC value of neutrophil CD64was 0.527 (A), monocyte CD64 was 0.621 (B), lymphocyte CD64 was above 0.75 (0.756) with high sensitivity (71.6%) and specificity (75%) (C).

different compared to control group, which maybe suggest that there were more intense inflammation and immune activity in patients with HBV-HCC.

Interferon-α was widely used for the effect of both anti-HBV and immunoregulation dual functions in past decade [16, 17], we observed the dynamic change of lymphocyte CD64 during the antiviral treatment of interferon-α, and found paralleled by response to the therapy, the level of lymphocyte CD64 from baseline normalized at week 12 and last to the end, which may attribute it to interferon-α inhibited inflammation and regulated immune.

In conclusion, this study showed that CD64 index was increased in chronic HBV infection patients and changed with the course of disease, the therapy of interferon-α would correct it, and analysis prompted that the level of lymphocyte CD64 would be more suitable for as a biomarker to judge the condition of chronic HBV infection and the curative effect of interferon-α treatment. It could be served for determine the degree of liver damage. However, CD64 index has high application value to clinic for it can be detected easy with high sensitivity and specificity.

The limited sample population and duration of follow-up in study precluded us from further refinement, as CHB including mild, moderate and severe CHB, HBV-LC including compensated and decompensate LC. Large-scale multicenter studies are needed to prove the further

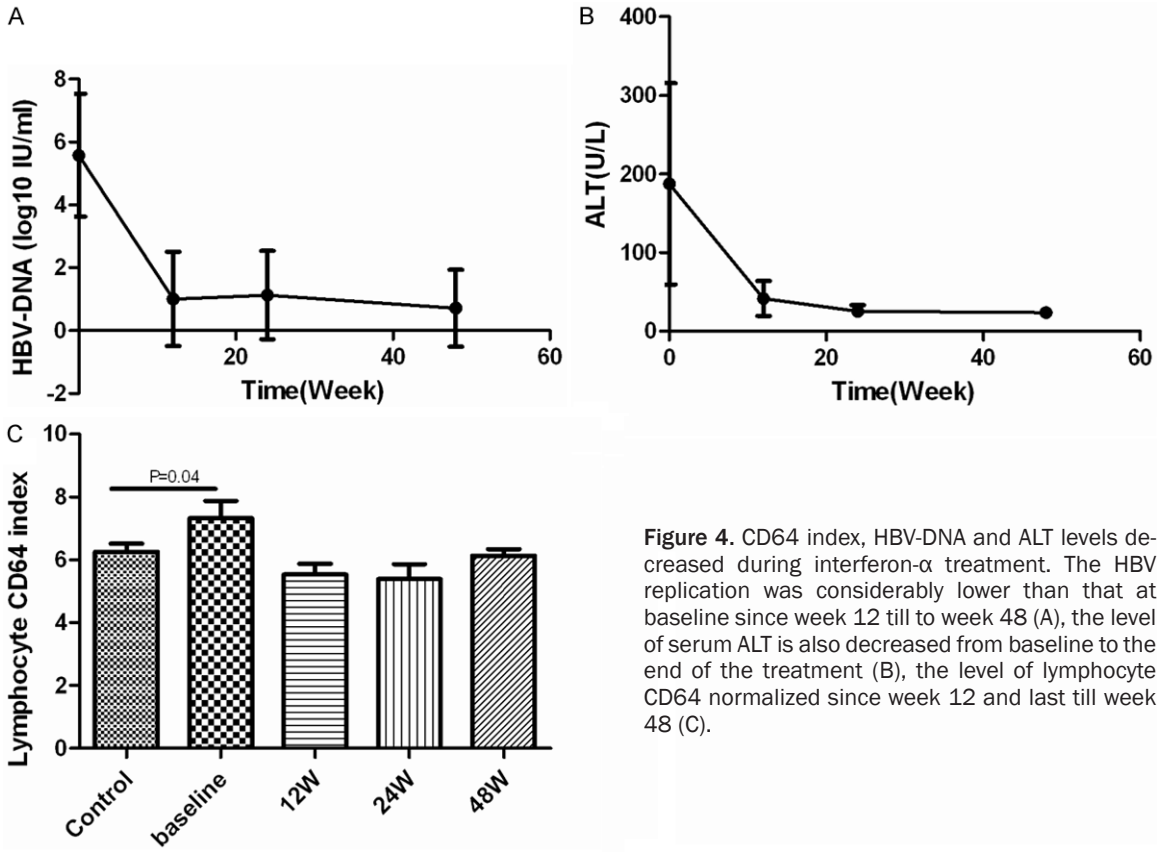

Figure 4. CD64 index, HBV-DNA and ALT levels decreased during interferon-α treatment. The HBV replication was considerably lower than that at baseline since week 12 till to week 48 (A), the level of serum ALT is also decreased from baseline to the end of the treatment (B), the level of lymphocyte CD64 normalized since week 12 and last till week 48 (C).

association of CD64 index with chronic HBV infection.

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Disclosure of conflict of interest

None.

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