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Clinical Significance of Serum IL-18 and IL-18BP in Patients with Benign or Malignant Primary Liver Tumors

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OBJECTIVE To study the relationship between the serum levels of IL-18 and IL-18BP in the development and growth of primary liver cancer, benign liver tumors and liver cirrhosis and to determine the value of serum IL-18 and IL-18BP in the diagnosis of primary liver cancer.

METHODS The serum levels of IL-18 and IL-18BP in 36 patients with primary hepatic carcinoma (PHC) were detected. Eighteen patients were diagnosed with various benign liver tumors and 21 patients with cirrhosis of liver (LC), determined by using an ELISA assay. The serum levels of AFP in 36 patients with primary liver cancer were examined. The relationship among levels of serum IL-18, IL-18BP and AFP in the primary liver cancer was explored.

RESULTS The sIL-18 levels in PHC were significantly lower than in control group, the benign liver tumor group and the LC group. The sIL-18BP in PHC was significantly higher than that in control group, benign liver tumor group and LC group ($P < 0.001$). There was a close correlation between the levels of IL-18, IL-18BP and clinical stage in PHC: the later clinical stages had lower levels of IL-18 and higher levels of IL-18BP while the earlier clinical stages had higher levels of IL-18 and lower levels of IL-18BP. There was a negative correlation between serum levels of IL-18 and AFP in the PHC group ($r = -0.7152$, $n = 36$, $P < 0.01$), and there was a positive correlation between serum levels of IL-18 BP and AFP in the patients with PHC ($r = 0.6315$, $n = 36$, $P < 0.01$). The IL-18 and IL-18BP in the patients with various benign liver tumors or LC were significantly higher than those in control group. The differences were statistically significant ($P < 0.01$).

CONCLUSION Serum levels of IL-18 and IL-18BP can reflect the immune function of patients with primary liver cancer, with various benign liver tumors or with LC and can also be indicative of the clinic stage of primary liver cancer. It can be used to assist in making a diagnosis and in determining the clinical stage of PHC. Detecting AFP concurrently can help make the diagnosis of primary liver cancer more precise.

KEY WORDS: benign-malignant primary liver tumor, primary hepatic carcinoma (PHC), liver cirrhosis (LC), serum interleukin 18 (sIL-18), serum interleukin 18 link albumen (sIL-18BP)

Introduction

The body's immune system plays an important role in fighting against tumors. Changes in immune function can cause changes in levels of cytokines[1]. IL-18 was named as a new cytokine in 1996[2]. It can be induced by the proliferation of immune cells and enhance the activ-

ity of the immune cells, and furthermore, promote the production and secretion of cytokines. IL-18 enhances the apoptosis of tumor cells by Fas-FasL and simultaneously kills or restricts tumor cell growth by inhibiting tumor angiogenesis or through other mechanisms[3]. Therefore, it plays an important role in tumor immunity. IL-18 binding protein (IL-18BP) is a newly discovered glycoprotein[4]. Its expressed product can inhibit IL-18, inducing Th1 cells to produce IFN-γ. It can reduce the activation of IL-18 on NF-κB. Therefore, IL-18BP can be considered the antagonist of IL-18. At present, there are few reports on the serum levels of IL-18 and IL-18BP in patients with PHC in China or abroad. In this study, serum levels of IL -18 and IL-18BP were tested and analyzed to understand the changes in levels of serum IL-18 and IL-18BP in patients with benign or malignant primary liver tumors or with liver cirrhosis so as to explore the role of IL-18 and IL-18BP in the development and growth of PHC. The results of the research provide some theoretical basis for the prevention and treatment of PHC.

Materials and Methods

Clinical data

Control group

All indexes of 20 healthy examinees aged 17-53 years old from the Affiliated Hospital of Guangdong Medical College met the standards of the healthy subject as designated in our study criteria. Of the 20 participants, 12 were male and 8 were female.

Cirrhosis group

Twenty-five patients received treatment at the Affiliated Hospital of Guangdong Medical College from July 2005 to June 2007. The diagnosis and staging of the 25 patients met the revised diagnostic criteria and clinical stage criteria confirmed at the National Infectious and Parasitic Diseases Conference in 1995. The 25 cases were proved by microscopic examination as cirrhosis. Sixteen were male and 9 female with the age ranging from 31-63 years old.

Benign liver tumor group

Eighteen cases were diagnosed by pathologic examination as benign liver tumor, including hepatocellular adenoma in 6 patients, hepatic hemangioma in 10 patients and hepatic cysts in 2 patients.

PHC group

Thirty-six patients were clinically diagnosed with PHC based on the diagnostic criteria revised by the National Cooperation Conference of whose sole purpose is to prevent liver cancer. In the 36 patients, 7, 15 and 14 cases were clinically diagnosed as stage I, II and III, respectively according to the clinical stage criteria revised by the Fourth National Hepatocarcinoma Academy in 1999. Twenty-one were male and 15 female with the age ranging from 25-68 years old.

Experimental methods

In the morning, 2ml of the blood was taken from every subject of the 4 groups before having breakfast, and then the plasma was separated and distilled. Subsequently, the processed plasma was preserved in a -20°C refrigerator. No hemolysis or lipid turbidness was present in the serum. The concentrations of IL-18 and IL-18BP in the serum were measured using ELISA after gathering the samples. Standard curves were made according to the concentration and absorbency of standard sample measured. Then the concentration of IL-18 and IL-18BP were calculated by the absorbance of standard samples according to the standard curves. A cytokine enzyme-linked immunosorbent assay kit was purchased from Shanghai Zuokang Company.

Statistical analysis

All data were measured by mean ± SD. The SPSS11.5 statistic procedure was adopted to analyze the data. The data were examined by *t*-test.

Results

The serum levels of IL-18 and IL-18 BP

Serum levels of IL-18 and IL-18 BP in each group are shown in Table 1. The serum levels of IL-18 and IL-18 BP in the PHC group were significantly lower than those in the control group, the benign liver tumor group and the LC group ($P < 0.05$). Serum levels of IL-18BP in the PHC group were significantly higher than those in the control group, the benign liver tumor group and the LC group ($P < 0.01$). Serum levels of IL-18 in the benign liver tumor group, the LC group were significantly higher than that in the control group. Serum IL-18BP levels in the LC group were significantly higher than in the control group. All the differences among the groups were statistically significant ($P < 0.001$).

The relationship between serum levels of IL-18, IL-18 BP and clinical stage in the patients with PHC

Table 2 shows that different clinical stages were accompanied by certain serum levels of IL-18 and IL-18 BP. Serum levels of IL-18 in the patients with stage I, stage II, and stage III were gradually lower. But serum levels of IL-18BP in the patients with stage I, stage II, and stage III were gradually higher. The differences were statistically significant ($P < 0.05$ or $P < 0.01$).

The relationship between serum levels of IL-18, IL-18 BP and AFP in the patients with PHC

There was a negative correlation between the serum levels of IL-18 and AFP in the PHC group. The higher the concentration of AFP was, the lower the serum levels of

IL-18 (r = -0.7152, n = 36, $P < 0.01$). There was a positive correlation between the serum levels of IL-18 BP and AFP in the patients with PHC. The higher the concentration of AFP was, the higher the serum level of IL-18BP (r = -0.6315, n = 36, $P < 0.01$). See Table 3.

Table 1. The levels of serum IL-18 and IL-18BP (mean ± SD).

Group	*n*	IL-18 (pg/ml)	IL-18BPa (μg/L)
PHC [a]	36	50.16 ± 28.56	15.12 ± 4.13
Benign liver tumor [b]	18	701.23 ± 186.02	9.13 ± 3.02
LC [c]	25	718.52 ± 190.28	8.86 ± 2.86
Normal control [d]	20	90.12 ± 28.89	2.06 ± 0.65

a compared with b, c, d and b compared with d, c compared with d, $P < 0.001$; 2 compared with 3, $P > 0.05$.

Table 2. The relationship between the serum levels of IL-18, IL-18 BP and clinical stage in the patients with PHC (mean ± SD).

Clinical stage	*n*	IL-18 (pg/ml)	IL-18BP (μg/L)
I	7	66.26 ± 20.36	10.32 ± 4.38
II	15	49.12 ± 13.42	14.52 ± 4.65
III	14	37.27 ± 14.65	23.24 ± 5.21

The differences among each group $P < 0.05$ or $P < 0.01$.

Table 3. The relationship between serum levels of IL-18, IL-18BP and AFP in patients with PHC (mean ± SD).

AFP (μg/L) concentration	*n*	IL - 18 (pg/mL)	IL - 18BP (μg/L)
< 200	3	63.32 ± 15.63	11.23 ± 3.88
200-400	8	51.25 ± 13.77	15.55 ± 4.02
> 400	25	38.63 ± 12.32 9	25.03 ± 4.85
Total	36	50.16 ± 28.56	15.12 ± 4.13

Discussion

IL-18 is a multifunctional cytokine produced by activated macrophages. It was first isolated by Okamura[5] in 1995. Its structure is similar to that of IL-1 and its function is similar to that of IL-12. The most obvious characteristic is the regulation of T cells, especially the activities of TH1 cells and NK cells. One study on mice models with liver injury[6] has shown that IL-18 can cause liver damage by strongly inducing IFN-r. Endogenous IFN-r can enhance the secretion of IL-18 andIL-12, and as a result, increase liver damage. In addition, IL-18 can induce the expression of IL-12 mRNA, IFN-γ mRNA, FasL mRNA, and TNF-sa mRNA. IL-18 can block the production of IFN-γ, TNF-a and Fasl by applying IL-18BP before LPS is sensitized in order to completely inhibit liver injury. IL-18BP can inhibit the emergence of IFN-γ and induce the secretion of PGE2 in peripheral blood cells. PGE2 also can inhibit the T cells of TH1-related cytokines and reduce the expression of IFN-γ[7]. Another study showed[8,9] that IL-18 can inhibit a variety of tumors, the production of malignant ascites, and can prevent tumors from metastasizing to the lung or from infiltrating into surrounding tissues, and, as a result, can extend the survival time. Furthermore, the anti-tumor activity of IL-18 is not dependent on internal factors of IL-12 and IFN-γ[10]. The IL-18 receptor is obtained from the purified lymphoma cell strains (IL-1Rrp). IL-18 receptor is the marker distinguishing TH1 cells and TH2 cells in selective reactions between TH1 cells and IL-18. IL-18BP is the antagonist of IL-18. The expression of IL-18BP can inhibit IL-18 to induce the production of IFN-γ through Th1 cells. It can reduce the NF-κB activation via IL-18.

This study demonstrates the following points. *i*) IL-18 in peripheral blood of patients with PHC was significantly lower than that in the control group, the LC group and the benign liver tumor group. IL-18BP in peripheral blood of the patients with PHC was significantly higher than that in the control group, the LC group and the benign liver tumor group ($P < 0.001$), suggesting that decreased levels of IL-18 may be an important factor that inhibits the immune functions in patients with PHC. *ii*) IL-18 in peripheral blood taken from patients with LC and patients with benign liver tumors was significantly higher than that in the patients from the control group and the PHC group, but IL-18BP in peripheral blood taken from the patients with LC and from the patients with benign liver tumors was lower than the PHC group and higher than the normal group ($P < 0.001$). This is similar to the results reported by Ludwiczek[11] showing that IL-18 was excessive and IL-18BP was relatively insufficient. Serum IL-18BP levels in patients with LC and benign liver tumors are relatively low. IL-18 should not be neutralized as there may be substantial, free IL-18 in blood. Activation of Th1 cytokines causes a strong reaction, eventually leading to severe immune hepatic injury and diffuse hepatocyte necrosis. *iii*) Different clinical stages of patients with PHC were accompanied by certain serum levels of IL-18 and IL-18BP. Serum levels of IL-18 in the patients with stage I, stage II, and stage III were gradually lower. But serum levels of IL-18BP in the patients with stage I, stage II, and stage III were gradually higher ($P < 0.05$ or $P < 0.01$). This is similar to the results reported by Spanish researcher[12] showing that serum levels of IL-18 in LC patients (different Child-Pugh class) with liver function graded as C was significantly higher than that in Child-Pugh grade A and B groups. Serum levels of IL-18BP in the Child-Pugh grade C group was lower than that in the Child-Pugh grade B. A significant quantity of free IL-18 can be detected in plasma. *iv*) Serum levels of IL-18 in patients with PHC were negatively correlated with AFP. Serum levels of IL-18BP were positively correlated with AFP.

This means that the higher the AFP, the worse the patient's immune function. This is why the higher the AFP is in patients, the worse the effects of the treatment.

IL-18 can enhance immune function. Direct application of IL-18 in the treatment of tumors in animal experiments has proven successful, Serum levels of IL-18 in patients with PHC were low, while serum levels of IL-18BP were high, and it may be that the immune function of patients with PHC is inhibited. Therefore, increasing serum levels of IL-18 in patients with PHC can induce the body to produce more anti-tumor cytokines and increase the activity of immune cells, so as to effectively kill neoplastic hepatocytes cells, and as a result, achieve a better therapeutic effect. This may be a new approach of treatment for patients with PHC.

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