Serum hepatocyte growth factor and interleukin-6 levels can distinguish patients with primary or metastatic liver tumors from those with benign liver lesions

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Hepatocyte growth factor (HGF) is a potent stimulator of angiogenesis and cancer metastasis. Interleukin-6 (IL-6) is a pleiotropic cytokine that can act as an autocrine or paracrine growth factor in various tumor cells. In this study, we investigated the role of serum HGF and IL-6 levels to distinguish primary or metastatic liver tumors from benign liver lesions. Serum HGF and IL-6 levels were measured in 64 cancer patients and 12 healthy controls. Patients were divided into 5 groups: Group-1 (n=24): Breast cancer patients in complete remission without any liver lesion, Group-2 (n=8): Breast cancer patients in complete remission with benign liver lesion, Group-3 (n=10): Breast cancer patients with liver metastasis, Group-4 (n=11): Metastatic breast cancer patients without liver metastasis, Group-5 (n=11): Patients with hepatocellular carcinoma. Group-6 (n=12): Healthy controls. Serum HGF levels were found to be higher in group-5 (606.4 ± 255.8 pg/ml) than those in group-1 ($^{*}305.6 \pm 42.3$ pg/ml), group-2 ($^{*}293.9 \pm 44.8$ pg/ml), group-4 ($^{**}358.4 \pm 81.9$ pg/ml) and group-6 ($^{*}305.8 \pm 24.9$ pg/ml) ($^{*}p<0.001$, $^{**}p<0.05$). Patients in group-3 (448.9 ± 157.3 pg/ml) had higher serum HGF levels than those in group-1, group-2 and group-6 ($p<0.05$). Serum IL-6 levels were found to be higher in group-5 (54.9 ± 37.4 pg/ml) than those in group-1 (9.7 ± 6.4 pg/ml), group-2 (9.5 ± 4.8 pg/ml), group-4 (17.6 ± 19.6 pg/ml) and group-6 (12.6 ± 5.2 pg/ml, $p<0.05$). Patients in group-3 (32.5 ± 36.9 pg/ml) had higher serum IL-6 levels than those in group-1, 2 and group-6, but these were not statistically significant ($p>0.05$). This study showed that primer and metastatic liver tumors had higher serum HGF and IL-6 levels than other patients and controls. Measurements of these markers in serum may be used to distinguish patients with primer liver tumors or breast cancer patients with liver metastasis from those with benign liver lesions or non-metastatic patients.

Key words: hepatocyte growth factor, interleukin-6, breast cancer, hepatocellular carcinoma, liver tumors

Hepatocyte growth factor (HGF), also called as scatter factor, is the most potent mitogen and the most important growth factor for hepatocytes [20]. However, it has been reported that HGF has more diverse activities on a variety of cells including osteoclasts, endothelial cells, epithelium in lung and kidney other than hepatocytes in various physiological and pathological conditions [3, 6, 18, 26, 37, 38]. Recent studies have shown that HGF is involved in tumor progression mediated by promoting cell proliferation, motility, invasive potential of malignant cells and angiogenesis [1, 19, 21, 39, 40]. Serum HGF levels have been found to be associated with the pathological features and the progression of disease in some solid and hematologic malignancies including hepatocellular carcinoma (HCC) [5] and breast cancer [19, 37]. In breast cancer, serum HGF levels were found to be higher especially in patients with liver metastasis [19].

Interleukin-6 (IL-6), also known as hepatocyte-stimulating factor, is a multifunctional cytokine that can act as an autocrine or paracrine growth factor in various tumor cells [16, 29, 32, 34, 35]. It was suggested that IL-6 can up-regulate HGF synthesis in hepatocytes [23]. Increased mRNA expression of HGF and IL-6 was demonstrated in liver tissue damaged by tumor invasion [14]. In a study, serum IL-6 levels have been found to be increased in patients with HCC than healthy controls [7]. It was reported that patients with liver metastasis had higher serum IL-6 levels than those without liver metastasis in breast cancer [41].

In the present study, we investigated whether serum HGF and IL-6 levels may have a role to distinguish patients

with metastatic or primary liver tumors from those with benign liver lesions.

Patients and methods

Patients. A total of 64 patients with histologically confirmed breast carcinoma and HCC were enrolled in this study. Patients were divided into 6 groups: Group-1 (n=24): Breast cancer patients in complete remission at least for 3 years after radical surgery. They had no benign or malignant liver lesion in their, routine ultrasonography of the abdominal region. Group-2 (n=8): Breast cancer patients in complete remission at least for 3 years after radical surgery. They had benign liver lesions which were initially thought as a metastasis in their routine ultrasonography of the abdominal region. However, the diagnoses were confirmed with dynamic computerized tomography of the abdominal region and clinical follow-up of patients at least for 2 years. Of the 8 patients, 6 had a benign cystic lesion and 2 had a hemangioma. Group-3 (n=10): Breast cancer patients with liver metastasis. Metastatic site was only liver in 3 patients, liver and lung in 4 patients and liver and bone in 3 patients. Group-4 (n=11): Metastatic breast cancer patients without liver metastasis. Of the 11 patients, 5 had only bone metastasis and 6 had bone and lung metastasis. Group-5 (n=11): Patients with hepatocellular carcinoma. Seven of these patients were male. Six patients had chronic hepatitis, 2 patients had alcoholic liver disease and one patients had postnecrotic cirrhosis in this group. Group-6 (n=12): Healthy controls. Ultrasonography of abdominal region was normal in this group. None of the patients suffered from infectious, renal or other systemic diseases. Patients with hepatitis or cirrhosis were also excluded from the groups (except group-5). All serum samples were taken after informed consent was given.

Statistical analysis. The results were presented as mean $\pm$ SD. Mann Whitney U test, Kruskall Wallis test and Pearson's correlation analysis were used in statistical analysis. p values less than 0.05 were accepted as significant.

HGF and IL-6 measurements. Serum samples were collected from the patients and were stored at –20 ˚C until HGF and IL-6 assays. The concentrations of HGF and IL-6 in the serum were determined by using solid phase sandwich enzyme linked immunosorbent assay (ELISA) kits (Biosource International, California, USA) according to the manufacturer's recommended protocol. The detectable ranges of HGF and IL-6 concentrations of this ELISA kits were 10 to 3830 pg/ml and 2 to 222 pg/ml, respectively [8, 31].

Results

There was no significant difference among 6 groups in terms of age (p>0.05) (Tab. 1). Serum HGF levels were found to be higher in group-5 (606.4 ± 255.8 pg/ml) than those in group-1 ($^{*}305.6 \pm 42.3$ pg/ml), group-2 ($^{*}293.9 \pm 44.8$ pg/ml), group-4 ($^{**}358.4 \pm 81.9$ pg/ml) and group-6 ($^{*}305.8 \pm 24.9$ pg/ml) ($^{*}p<0.001$, $^{**}p<0.05$). Patients in group-3 (448.9 ± 157.3 pg/ml) had higher serum HGF levels than those in group-1, group-2 and group-6 (p<0.05). Serum levels of HGF were found to be higher in group-5 than those in group-3, but this was not statistically significant (p<0.05). The results are shown in Table 1 and Figure 1.

Serum IL-6 levels were found to be higher in group-5 (54.9 ± 37.4 pg/ml) than those in group-1 (9.7 ± 6.4 pg/ml), group-2 (9.5 ± 4.8 pg/ml), group-4 (17.6 ± 19.6 pg/ml) and group-6 (12.6 ± 5.2 pg/ml, p<0.05). Patients in group-3 (32.5 ± 36.9 pg/ml) had higher serum IL-6 levels than those in group-1, 2 and group-6, but these were not statistically significant (p>0.05). Serum levels of IL-6 were found to be higher in group-5 than those in group-3 (p=0.053). The results are shown in Table 1 and Figure 2.

Table 1. Characteristics of patients (mean $\pm$ SD)

Groups	n	Age (years)	HGF (pg/ml)	IL-6 (pg/ml)
Group-1	24	51.5 ± 13.3	305.6 ± 42.3	9.7 ± 6.4
Group-2	8	50.8 ± 5.3	293.9 ± 44.8	9.5 ± 4.8
Group-3	10	49.1 ± 10.8	448.9 ± 157.3	32.5 ± 36.9
Group-4	11	50.1 ± 10.2	358.4 ± 81.9	17.6 ± 19.6
Group-5	11	60.8 ± 10.8	606.4 ± 255.8	54.9 ± 37.4
Group-6	12	50.3 ± 9.9	305.8 ± 24.9	12.6 ± 5.2

In group-4, serum HGF and IL-6 levels were significantly correlated with serum Alanine Amino Transferase (ALT) (r=0.758 for HGF, r=0.80 for IL-6) and Aspartate Amino Transferase (AST) levels (r=0.758 for HGF, r=0.717 for IL-6) (p<0.05). These levels were not correlated with serum hemoglobulin, albumin and total bilirubin levels (p>0.05). In group-3, there was a positive correlation between serum levels of HGF and IL-6 in this group (r=0.780, p<0.05). In other groups, neither HGF nor IL-6 were correlated with each others or serum levels of hemoglobulin, albumin, total bilirubin, ALT and AST levels (p>0.05). There was no correlation between HGF and IL-6 levels and tumor markers, CA-15-3 for breast cancer and alpha-fetoprotein for HCC patients (p>0.05).

Discussion

It is often important to distinguish metastatic or primary liver tumors from benign lesions of the liver in the follow-up of patients with many solid tumors. This is particularly necessary to begin effective and suitable therapy immedia-

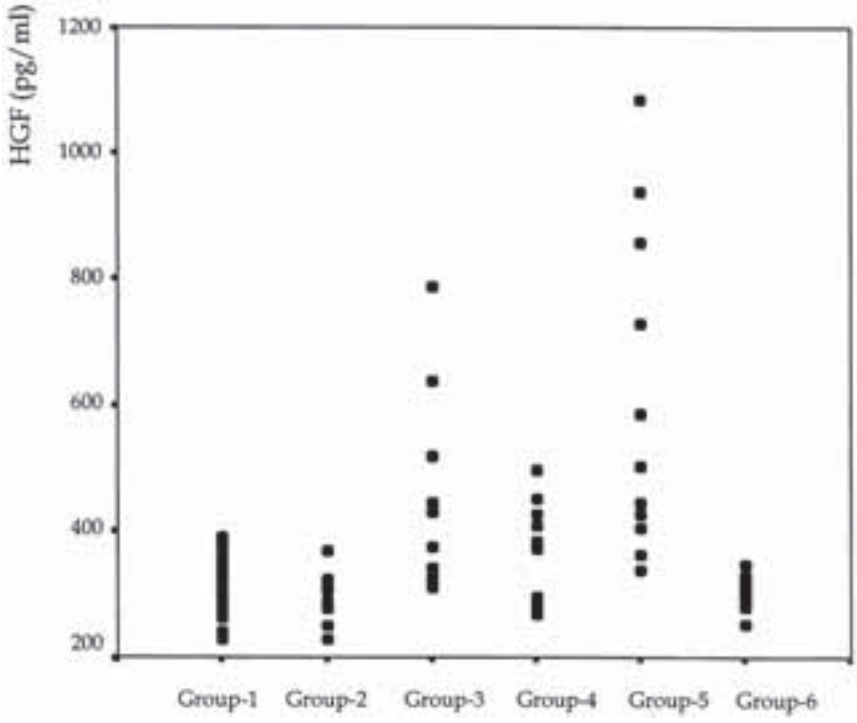

Figure 1. Individual serum HGF levels in patients and controls.

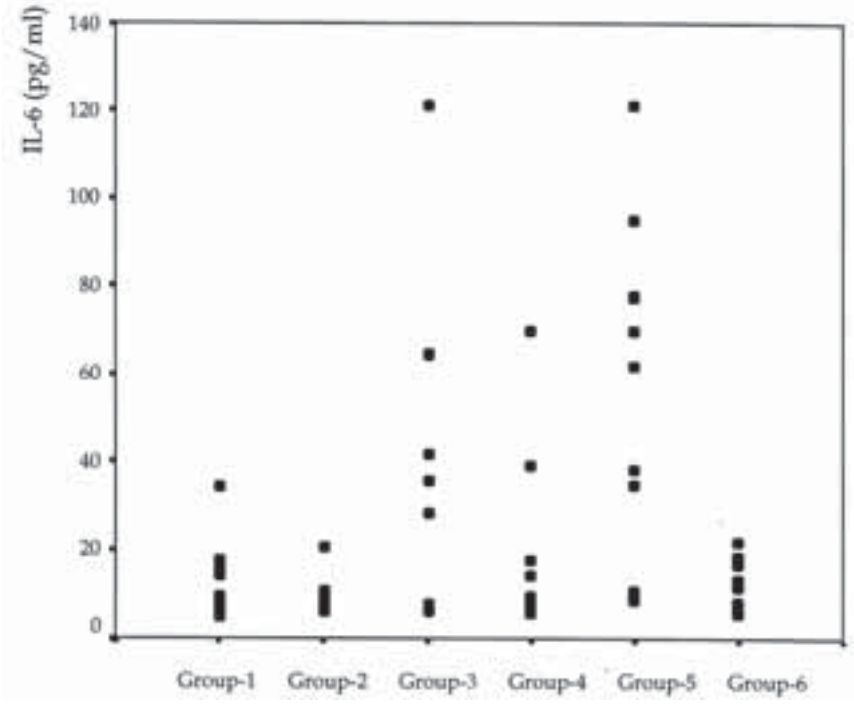

Figure 2. Individual serum IL-6 levels in patients and controls.

telly. Sometimes, well-known radiological or biochemical methods such as ultrasonography, tomography or tumor markers may not be sufficient to distinguish these lesions and invasive methods, such as liver biopsy can be contraindicated for these patients because of their inappropriate liver functions. Therefore, a practical and non-invasive method is still required in clinical practice of many physicians.

HGF is a pleiotropic polypeptide growth factor that was suggested to be involved in the process of tumor growth and metastasis because of its some biological functions including cell proliferation [25, 30], movement [11, 24], invasiveness [24, 30, 36], morphogenesis [4, 22] and angiogenesis [12]. It was reported that increased serum HGF levels are associated with progression of various types of solid and hematologic tumors including breast cancer [19, 37], esophageal cancer [33], colorectal cancer [10], multiple myeloma [17] and HCC [5]. Similar to HGF, serum levels of IL-6 have also been found to be increased in several tumors such as gynecological tumors [27], renal carcinoma [2], breast carcinoma [41] and HCC [7].

In the present study, patients with primary or metastatic liver tumors showed higher serum HGF levels when compared to the patients with benign liver lesions ($p>0.05$). Patients with HCC showed higher serum IL-6 levels than patients with benign liver lesions ($p<0.05$). Serum IL-6 levels were also found to be higher in breast carcinoma patients with liver metastasis than those with benign liver lesions, but this was not statistically significant ($p>0.05$). Both serum HGF and IL-6 levels were not correlated with tumor markers in breast cancer and HCC patients. It can be considered that measurements of HGF and IL-6 in serum may be useful markers to distinguish patients with primary or metastatic liver tumors from those with benign liver lesions. In this study, breast cancer patients have been selected as a model for metastatic liver disease. However, these paremeters can be studied in other solid tumors with liver metastasis and in further studies with larger patient's groups, a cut-off level for HGF and IL-6 can be estimated to be used in clinical practice of physicians.

It was reported that the liver plays an important role in the clearance of HGF and IL-6 [28] and various liver dis-

eases such as acute hepatitis, fulminant hepatitis, alcoholic liver disease and liver cirrhosis have been reported to represent increased serum HGF and IL-6 levels because of their decreased hepatic clearance [9, 13, 15, 28]. In our study, despite statistical insignificance, serum HGF and IL-6 levels were found to be higher in patients with HCC than in patients with liver metastasis. This extra increase in serum HGF and IL-6 levels for HCC patients can be explained by their underlying liver disease which can cause to decrease in hepatic clearance for HGF and IL-6. Because most of the patients in HCC group had a history of chronic hepatitis, alcoholic liver disease or liver cirrhosis. Supporting to these findings, in HCC group (group-4), serum HGF and IL-6 levels were found to be correlated with serum ALT and AST levels. This correlation was not observed in other groups.

It was demonstrated that recombinant human IL-6 administration resulted in a increase in serum HGF levels [8]. IL-6 induced liver regeneration has been shown to be mediated by an increase in serum HGF levels [8]. In our study, there was a positive correlation between serum HGF and IL-6 levels only in patients with liver metastasis (group-3). The lack of this correlation in HCC group (group-4) may be due to different clearance mechanisms of HGF and IL-6 in the liver. It is not suprising that we found no correlation between serum HGF and IL-6 levels in other groups, because these patients had not increased hepatic stimulation, as expected and it may be that IL-6 cannot stimulate HGF at these levels.

MAEMURA et al [19] reported that patients with liver metastasis showed a mild increase in serum HGF levels than patients with other metastasis in breast cancer. Similar to HGF, ZHANG et al [41] reported that serum IL-6 levels were higher in patients with liver metastasis than in patients without liver metastasis in breast cancer. Our study confirms the results of these two studies. In our study, patients with liver metastasis had higher serum HGF and IL-6 levels than patients with other metastasis.

In conclusion, our study demonstrated that patients with HCC and metastatic liver tumors had higher serum HGF and IL-6 levels, when compared to non-metastatic patients with and without benign liver lesion, other metastatic patients and healthy controls. Serum levels of HGF and IL-6 can be used to distinguish patients with primary liver tumors or breast cancer patients with liver metastasis from those with benign liver lesions or non-metastatic patients. Although in this study we used breast carcinoma and HCC as a model for liver tumors, future studies may be focused on other solid tumors with liver metastasis.

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