

## RESEARCH ARTICLE

# Clinical Significance of Joint Detection of Serum VEGF, SIL-2R and HGF in Patients with Primary Hepatocellular Carcinoma before and after Percutaneous Microwave Coagulation Therapy

Ji-Dong Chen<sup>1&</sup>, Yan-Qun Xiong<sup>2&</sup>, Ke Dong<sup>3</sup>, Jun Luo<sup>1\*</sup>, Lin-Xian Yue<sup>1&</sup>, Qin Chen<sup>1\*</sup>

## Abstract

**Objective:** To investigate the changes of serum vascular endothelial growth factor (VEGF), soluble interleukin-2 receptor (SIL-2R) and hepatocyte growth factor (HGF) contents in patients with primary hepatocellular carcinoma (HCC) before and after percutaneous microwave coagulation therapy (PMCT) and determine their clinical significance. **Materials and Methods:** Fasting venous blood (3 mL) from 81 patients with primary HCC diagnosed by pathology was collected in the mornings 1 day before PMCT, and 1 day, 7 days and 1 month after PMCT, and then the serum was separated and stored in -70°C. The contents of VEGF, SIL-2R and HGF were detected by enzyme linked immunosorbent assay (ELISA). **Results:** The serum VEGF, SIL-2R and HGF contents in 81 patients with primary HCC had obviously dynamic changes before and after PMCT. By comparison to 1 day after PMCT with pre-operation, there was no statistical significance regarding VEGF and SIL-2R contents ( $P>0.05$ ), but HGF content showed significant difference ( $P<0.01$ ). Compared with pre-operation, VEGF, SIL-2R and HGF contents 7 days and 1 month after PMCT all manifested significant differences ( $P<0.01$ ). By comparison to 7 days with 1 month after PMCT, there was no statistical significance regarding the VEGF content ( $P>0.05$ ), whereas SIL-2R and HGF contents showed significant change ( $P<0.01$ ). **Conclusions:** The contents of serum VEGF, SIL-2R and HGF have obviously dynamic changes in primary HCC before and after PMCT, and their joint detection is expected to be an effective hematologic evaluation index of PMCT for primary HCC.

**Keywords:** Primary HCC - percutaneous microwave coagulation therapy - VEGF - IL-2 receptor - HGF

*Asian Pac J Cancer Prev*, 15 (11), 4545-4548

## Introduction

Primary hepatocellular carcinoma (HCC) is one of the most commonly-encountered malignant tumors in the world (Abdelgawad et al., 2013; Berk et al., 2013; Chittmittrapap et al., 2013; Gupta et al., 2013; Norsa'adah et al., 2013; Hao et al., 2013; Zekri et al., 2013; Zhu et al., 2013; Cai et al., 2014; El Kassas et al., 2014; Fan et al., 2014; Ji et al., 2014; Jeng et al., 2014; Li et al., 2014; Tang et al., 2014; Wang et al., 2014; Zhang et al., 2014). Most small liver cancers (single and diameter  $\leq 5$  cm) are in the early stage of liver cancer development, which is crucial to treat the liver cancer in clinic. At present, percutaneous microwave coagulation therapy (PMCT) as a minimally-invasive treatment can technically inactivate the small liver cancer with the diameter  $\leq 5$  cm completely one time. Modern imaging examinations have been able to reflect the real situation of tumor tissue necrosis timely, but it still needs to find its hematologic monitoring index (Zhan et al., 2013). In clinic, we are hoping to find a good repeatable, convenient and effective hematologic evaluation index that can

reflect the efficacy of PMCT timely. Vascular endothelial growth factor (VEGF) is the most important angiogenesis factor in tumor tissues (Pircher et al., 2014). Studies have shown that both tumor tissue ischemia and necrosis can reduce the secretion of VEGF (Ahluwalia et al., 2012). Tumor occurrence and progression are closely associated with body cellular immunity function. Serum soluble interleukin-2 receptor (SIL-2R) is an important monitoring index which can reflect the body cellular immunity function (Kanazawa et al., 2006). Hepatocyte growth factor (HGF), a multifunctional cytokine, is the most important factor that can promote the liver regeneration. Hu et al. found that serum HGF concentration was increased significantly on day 1 after hepatectomy, suggesting that the process of early liver regeneration was launched after microwave treatment (Hu et al., 1999). This study detected the content changes of serum VEGF, SIL-2R and HGF in patients with primary HCC before and after PMCT by enzyme linked immunosorbent assay (ELISA) in order to provide some hematologic evidences for judging the efficacy of PMCT for primary HCC and prognosis.

<sup>1</sup>Department of Interventional Ultrasound, <sup>2</sup>Department of Operating Room, <sup>3</sup>Department of General Surgery, Sichuan Provincial People's Hospital, Chengdu, China <sup>&</sup>Equal contributors \*For correspondence: 1718686103@qq.com; Milton-lj@hotmail.com

**Table 1. Changes of Serum VEGF, SIL-2R and HGF Contents at Different Time Points before and after PMCT(x±s, pg/ml)**

Indexes	Before PMCT	1 day after PMCT	7 days after PMCT	1 month after PMCT
VEGF	270.12±66.87	254.73±73.45	165.05±77.40**	171.92±76.10**
SIL-2R	1204.24±77.73	1183.43±90.77	676.40±99.03**	784.04±104.45***
HGF	771.84±277.60	987.06±309.15**	1178.51±328.07**	802.05±330.09***

Compared with before PMCT, \*\* $P<0.01$ ; Compared with 7 days after PMCT, \*\*\* $P<0.01$

## Materials and Methods

### General data

A total of 81 patients with primary HCC and undergoing PMCT in our hospital from Nov., 2011 to Oct., 2013 were selected, in which the males and females at the age of 35~84 years were respectively 68 and 13 cases, with the median age of (53.25±11.67) years. All the patients were diagnosed by clinical imaging and serum alpha fetal protein (AFP) detection, and finally confirmed by pathological section. There were no surgical indications or patients to refuse the surgery. According to the standards made in China liver cancer meeting in 2001, the clinical staging of all patients pertain to phase II. There were no patients with portal venous bronchi, hepatic veins or bile duct carcinoma, and all liver functions were Child A. According to CT measurement, the average HCC nodule was (3.78±1.22) cm. All the patients did not receive any other therapies before PMCT, and fill the application form and signed by themselves or their relatives before biopsy and treatment. They did not receive any other therapies during microwave treatment and research.

### Methods

Specimen collection: Fasting venous blood (3 mL) from all patients was collected in the morning respectively on 1 day before PMCT, 1 day, 7 days and 1 month after PMCT, and then the serum was separated and stored in -70°C.

Treatment methods: First, apply American-made Bard18G automatic biopsy gun to penetrate into the liver tumor site percutaneously under ultrasound before PMCT, and then take 2~3 pieces of mass and put them in 40% formalin for fixation, finally, send them to inspect; Second, apply KY-2000 type microwave coagulation therapy apparatus (Nanjing Kangyou Company, China) with 2450MHZ of microwave frequency and 100 W of maximum output power; Third, routinely sterilize the local skin and shop the towel, and then puncture the microwave therapeutic needle into the liver tumor percutaneously under ultrasound after application of 2% lidocaine for local anesthesia, at last, turn on the power supply to start the treatment after determining the location. According to the tumor size and the surrounding tissue, the specific treatment power and time were set. For the patients with tumor diameter < 3 cm, the single ablation needle with 60-70 W was used for 500~700 s; for those with tumor diameter being 3~5 cm, two ablation needles with 60~70 W were applied for 700~900 s to kill the tumor tissues in situ conformally one time. Ultrasound imaging or enhanced MRI was conducted to examine and judge the degree of tumor necrosis 1 month after the microwave

treatment.

Determination of serum VEGF and SIL-2R: Double antibody sandwich ELISA, VEGF and SIL-2R kits (ADL Company, America) were adopted.

Determination of serum HGF: ELISA kit (R&D Company) was used to detect the serum HGF concentration in the patients with small liver cancer, and the testing steps were carried out strictly in accordance with the kit instructions. The OD value was red at a wavelength of 492nm on the enzyme mark instrument. The standard curve was drawn with OD value and standards respectively as the ordinate and abscissa. Finally, the concentration was inquired according to the OD value of serum samples.

### Statistical data analysis

SPSS 13.0 statistical software was used to analyze the laboratory data expressing with the mean ± standard deviation (x±s). q test was applied for pairwise comparison, one-way analysis of variance with repeated measurements for comparison among groups.  $P<0.05$  was considered to be statistically significant.

## Results

### Degree of tumor necrosis

The ultrasound imaging or enhanced MRI was conducted in 81 patients 1 month after microwave treatment, and the results showed that all of them had no residual carcinoma and recurrence of liver inside and outside.

### Changes of serum VEGF, SIL-2R and HGF contents at different time points before and after PMCT

The serum VEGF, SIL-2R and HGF contents in 81 patients with primary HCC had more obviously dynamic changes before and after PMCT. By comparison to 1 day after PMCT with pre-operation, there was no statistical significance regarding VEGF and SIL-2R contents ( $P>0.05$ ), but HGF content showed significant difference ( $P<0.01$ ). Compared with pre-operation, VEGF, SIL-2R and HGF contents 7 days and 1 month after PMCT were all manifested significant differences ( $P<0.01$ ). By comparison to 7 days with 1 month after PMCT, there was no statistical significance regarding the VEGF content ( $P>0.05$ ), whereas SIL-2R and HGF contents showed significant difference ( $P<0.01$ ).

## Discussion

As a minimally-invasive treatment method for liver cancer, PMCT has achieved a very good therapeutic effect in clinic after almost 20-year development (Xiong et al.,

2013; Eisele et al., 2014). It can not only inactivate the small liver cancer with the diameter  $\leq 5$  cm completely one time, but also activate the body immunity (Dong et al., 2002). The further studies on the influence of growth factors on promoting the tumor growth, metastasis and recurrence after PMCT have the profound significance to promote the microwave treatment.

VEGF is a currently-known tumor-related angiogenesis factor with the strongest activity for promoting the proliferation and division of vascular endothelial cells in the body (Ahluwalia et al., 2012). It is highly expressed in liver cancer tissue together with vascular endothelial growth factor receptor (VEGFR). After PMCT, a large number of liver cancer cells were killed, which can affect the change of VEGF content by decreasing VEGF secretion (Cui et al., 2005). The research results in this study showed that PMCT could effectively kill the tumor cells, thereby eliminating the sources of VEGF, inhibiting the proliferation of tumor tissue and blood vessels. After PMCT, the change of serum VEGF content could reflect the necrosis of liver tissue independently, and it is a simple and effective biological indicator. However, the reason why VEGF content 1 month after PMCT goes up still needs to be studied.

SIL-2R is produced by the activation of T lymphocytes and monocytes. Recent studies have shown that SIL-2R may be increased significantly in the serum of patients with autoimmune diseases and tumors, and is closely related to the course, efficacy and prognosis (Dong et al., 2002; 9.). Gustafsson et al. adopted the infusion heating therapy to treat the limb tumors, which could not only heal the primary lesions, but also decrease the metastatic rate of tumors obviously (Gustafsson et al., 2010). He thought that it may be related with the antitumor immune response produced by tumor antigens stimulating the body during heating treatment. SIL-2R, a chain of IL-2R, is an important immune inhibitor with the relative molecular mass (Mr) of 4 500.

It can bind to IL-2R with the SIL-2 on the cell membrane competitively, consume IL-2 around the activated T cells, and inhibit the differentiation and proliferation of activated T cells. High SIL-2R level in patients with tumors can inhibit the proliferation of T cells. The proliferation of T cells will be reinforced if serum SIL-2R level is reduced (Eller et al., 2009). The research results in this study revealed that serum SIL-2R level went down significantly after effective treatment, suggesting that microwave treatment can improve the body immune function except that the thermal effect damages the tumor cells. Serum SIL-2R level in patients with primary HCC 7 days after PMCT was decreased markedly by comparison to PMCT before, considering that the reduced tumor load after treatment decreases the number of activated lymphocytes induced by tumors *in vivo*, weakens the number of infiltrative lymphocytes inside tumors and lowers SIL-2R release, consequently leading to reduction of SIL-2R level, which can indirectly reflect the disease outcome. It can be concluded that detection of serum SIL-2R level is conducive to evaluating the efficacy. The serum SIL-2R level 1 month after treatment was notably higher than 7 days after treatment, which might be related

to the inhibition of IL-2 function caused by the existence of cancer-induced abnormal immune reaction after treatment (Hombach et al., 2012), suggesting that the comprehensive treatment like combined immunotherapy may be obtain a better therapeutic effect.

The regenerative process of liver cells is influenced by multiple factors, including the factors respectively promoting and inhibiting the liver cell regeneration to maintain the starting and ending balance of liver regeneration (Fulop et al., 2001). HGF is the most important growth factor that promotes the liver regeneration in the process of liver regeneration. It has an effect of promoting mitosis on the liver cells when its concentration reaches as small as 1 ng/ml, and this effect comes up to the largest when the concentration is 5~10 ng/ml. It can be activated rapidly in the repair of liver injury. Miyata et al. measured the serum HGF concentrations in 43 patients who accepted partial liver resection before and after treatment, and compared with the weight of removed liver tissue. The results confirmed that HGF immediately went up after the liver resection, and then went down. Besides, the serum maximum HGF concentration was associated with the weight of resected liver tissue (Miyata et al., 1996). HGF can not only promote the liver regeneration, but also can promote the movement of liver cancer cells and make tumor cells tend to spread. It can stimulate the formation of tumor blood vessels in the body and promote tumor growth and diffusion by promoting epithelial hyperplasia and activities. Meanwhile, HGF can also play the role through its receptor c-Met. C-Met as the product coded by oncogenes can exert the effect of simulating the liver cell growth after in combination with HCG. The results in this study revealed that the serum HGF concentration 1 day after PMCT went up significantly compared with pre-operation, and reached the peak 7 days after PMCT, with statistical significance, illustrating that the process of early liver regeneration starts after microwave treatment, and mesenchymal cells secrete HGF for repair. This repair 1 month after treatment can make it restore to the level before treatment. However, increased HGF concentration may accelerate the metastasis and recurrence of liver cancer if micrometastasis is accompanied. The reason lies in HGF receptor c-Met has a high expression rate in the liver cell tissue of liver cancer (Aydemir et al., 2012), and a small amount of HGF can stimulate the growth of micrometastatic lesions.

In a word, how to reduce or eliminate high VEGF and SIL-2R levels in serum of patients with tumors and make the body immune system perform the normal function may be a new way for the prevention and control of malignant tumors. Hence, monitoring the serum VEGF and SIL-2R can provide some evidences for judging the efficacy of PMCT. After microwave treatment, the effective control of HGF concentration and sealing its receptor c-Met to weaken the biological effect after the HGF in combination with c-Met may have certain significance in inhibiting the early metastasis and recurrence of HCC. This study is of great importance in investigating the weakened immune function of patients with tumors, mechanism of tumor occurrence and progression, as well as its immune diagnosis, disease assessment and therapeutic effect.

## References

- Abdelgawad IA, Mossallam GI, Radwan NH, et al (2013). Can Glypican3 be diagnostic for early hepatocellular carcinoma among Egyptian patients? *Asian Pac J Cancer Prev*, **14**, 7345-9.
- Ahluwalia A, Tarnawski AS (2012). Critical role of hypoxia sensor--HIF-1 $\alpha$  in VEGF gene activation. Implications for angiogenesis and tissue injury healing. *Curr Med Chem*, **19**, 90-7.
- Aydemir TB, Sitren HS, Cousins RJ (2012). The zinc transporter Zip14 influences c-Met phosphorylation and hepatocyte proliferation during liver regeneration in mice. *Gastroenterology*, **142**, 1536-46.
- Berk V, Kaplan MA, Tonyali O, et al (2013). Efficiency and side effects of sorafenib therapy for advanced hepatocellular carcinoma: a retrospective study by the anatolian society of medical oncology. *Asian Pac J Cancer Prev*, **14**, 7367-9.
- Cai XY, Wang JX, Yi Y, et al (2014). Low counts of  $\gamma\delta$  T cells in peritumoral liver tissue are related to more frequent recurrence in patients with hepatocellular carcinoma after curative resection. *Asian Pac J Cancer Prev*, **15**, 775-80.
- Chittmitrapap S, Chieochansin T, Chaiteerakij R, et al (2013). Prevalence of aflatoxin induced p53 mutation at codon 249 (R249s) in hepatocellular carcinoma patients with and without hepatitis B surface antigen (HBsAg). *Asian Pac J Cancer Prev*, **14**, 7675-9.
- Cui J, Dong BW, Liang P, et al (2005). Construction and clinical significance of a predictive system for prognosis of hepatocellular carcinoma. *World J Gastroenterol*, **11**, 3027-33.
- Dong BW, Zhang J, Liang P, et al (2002). Influencing factors of local immunocyte infiltration in hepatocellular carcinoma tissues pre- and post-percutaneous microwave coagulation therapy. *Zhonghua Yi Xue Za Zhi*, **82**, 393-7.
- Eisele RM, Denecke T, Glanemann M, et al (2014). Minimal-invasive microwave coagulation therapy for liver tumours: laparoscopic and percutaneous access. *Zentralbl Chir*, **139**, 235-43.
- Eller T, Aluoja A, Maron E, et al (2009). Soluble interleukin-2 receptor and tumor necrosis factor levels in depressed patients in Estonia. *Medicina*, **45**, 971-7.
- El Kassas HY, Attia AA (2014). Bactericidal application and cytotoxic activity of biosynthesized silver nanoparticles with an extract of the red seaweed *Pterocladia capillacea* on the HepG2 cell line. *Asian Pac J Cancer Prev*, **15**, 1299-306.
- Fan FT, Shen CS, Tao L, et al (2014). PKM2 regulates hepatocellular carcinoma cell epithelial-mesenchymal transition and migration upon EGFR activation. *Asian Pac J Cancer Prev*, **15**, 1961-70.
- Fulop AK, Pocsik E, Brozik M, et al (2001). Hepatic regeneration induces transient acute phase reaction: systemic elevation of acute phase reactants and soluble cytokine receptors. *Cell Biol Int*, **25**, 585-92.
- Gupta SP, Mittal A, Sathian B, et al (2013). Elevated serum insulin is an independent risk factor for hepatocellular carcinoma: a case control study from Nepal. *Asian Pac J Cancer Prev*, **14**, 7331-3.
- Gustafsson A, Andersson M, Lagerstedt K, et al (2010). Receptor and enzyme expression for prostanoid metabolism in colorectal cancer related to tumor tissue PGE2. *Int J Oncol*, **36**, 469-78.
- Hao YX, Wang JP, Zhao LF (2013). Associations between three common microRNA polymorphisms and hepatocellular carcinoma risk in Chinese. *Asian Pac J Cancer Prev*, **14**, 6601-4.
- Hombach AA, Abken H (2012). Antibody-IL2 fusion proteins for tumor targeting. *Methods Mol Biol*, **907**, 611-26.
- Hu RH, Lee PH, Yu SC, et al (1999). The serum hepatocyte growth factor before and after resection for hepatocellular carcinoma. *Hepatogastroenterology*, **46-48**, 1842-7.
- Ji D, Lu ZT, Li YQ, et al (2014). MACC1 expression correlates with PFKFB2 and survival in hepatocellular carcinoma. *Asian Pac J Cancer Prev*, **15**, 999-1003.
- Jeng JE, Tsai MF, Tsai HR, et al (2014). Impact of chronic hepatitis B and hepatitis C on adverse hepatic fibrosis in hepatocellular carcinoma related to betel quid chewing. *Asian Pac J Cancer Prev*, **15**, 637-42.
- Kanazawa S, Yamaguchi K, Kinoshita Y, et al (2006). Elevation of soluble interleukin-2 receptor in patients with non-small cell lung cancer treated with gefitinib. *J Cancer Res Clin Oncol*, **132**, 719-25.
- Li T, Xu WS, Wu GS, et al (2014). Platycodin D induces apoptosis, and inhibits adhesion, migration and invasion in HepG2 hepatocellular carcinoma cells. *Asian Pac J Cancer Prev*, **15**, 1745-9.
- Miyata K, Taniguchi H, Takeuchi K, et al (1996). Weight of resected liver is positively correlated with serum hHGF level. *Hepatogastroenterology*, **43**, 1589-93.
- Norsa'adah B, Nurhazalini-Zayani CG (2013). Epidemiology and survival of hepatocellular carcinoma in north-east Peninsular Malaysia. *Asian Pac J Cancer Prev*, **14**, 6955-9.
- Pircher A, Wellbrock J, Fiedler W, et al (2014). New antiangiogenic strategies beyond inhibition of vascular endothelial growth factor with special focus on axon guidance molecules. *Oncology*, **86**, 46-52.
- Tang YH, Zhu WJ, Wen TF (2014). Influence of clinically significant portal hypertension on hepatectomy for hepatocellular carcinoma: a meta-analysis. *Asian Pac J Cancer Prev*, **15**, 1649-54.
- Wang NY, Wang C, Li W, et al (2014). Prognostic value of serum AFP, AFP-L3, and GP73 in monitoring short-term treatment response and recurrence of hepatocellular carcinoma after radiofrequency ablation. *Asian Pac J Cancer Prev*, **15**, 1539-44.
- Xiong YQ, Chen JD (2013). The surgical care of Ultrasound-guided percutaneous microwave coagulation therapy (PMCT) of liver cancer. *Pract J Clin Med*, **10**, 146-7.
- Zekri Ael-R, Nassar AA, El-Din El-Rouby MN, et al (2013). Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with increasing DNA promoter methylation. *Asian Pac J Cancer Prev*, **14**, 6721-6.
- Zhan P, Qian Q, Yu LK (2013). Prognostic significance of vascular endothelial growth factor expression in hepatocellular carcinoma tissue: a meta-analysis. *Hepatobiliary Surg Nutr*, **2**, 148-55.
- Zhang SC, Liang MK, Huang GL, et al (2014). Inhibition of SMP30 gene expression influences the biological characteristics of human Hep G2 cells. *Asian Pac J Cancer Prev*, **15**, 1193-6.
- Zhu WJ, Huang CY, Li C, et al (2013). Risk factors for early recurrence of HBV-related hepatocellular carcinoma meeting milan criteria after curative resection. *Asian Pac J Cancer Prev*, **14**, 7101-6.