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Research on values of GDF-15 level in the diagnosis of primary liver cancer and evaluation of chemotherapeutic effect

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Abstract. – OBJECTIVE: To investigate the values of growth differentiation factor-15 (GDF-15) level in the diagnosis of primary liver cancer and evaluation of chemotherapeutic effect.

PATIENTS AND METHODS: 92 patients with liver cancer treated from June 2015 to May 2016 were selected as liver cancer group; 53 patients with benign liver lesion were selected as benign liver disease group, and 40 healthy subjects receiving physical examination were selected as healthy control group. Fasting venous blood was drawn from objects of study in the early morning at 1 d after admission and at the last day after chemotherapy (liver cancer group), and the serum GDF-15 level was measured.

RESULTS: The serum GDF-15 levels in patients in liver cancer group and benign liver disease group were significantly higher than those in healthy control group and benign liver disease group ($p<0.05$). The serum GDF-15 levels in patients with stage III and IV liver cancer were significantly higher than those in patients with stage I and II liver cancer, and the serum GDF-15 level in patients with stage IV liver cancer was significantly higher than that in patients with stage III liver cancer ($p<0.05$). There was no significant difference in serum GDF-15 level among patients with different clinical data ($p>0.05$). The ROC curve analysis showed that the threshold value of GDF-15 was 1573.23 ng/L, and the sensitivity, specificity, and accuracy were 81.23%, 83.99%, and 83.62%, respectively. The serum GDF-15 level in patients with progressive disease was significantly higher than those in patients with partial remission and stable disease, and the serum GDF-15 level in patients with stable disease was significantly higher than that in patients with partial remission ($p<0.05$).

CONCLUSIONS: The serum GDF-15 level has certain clinical values in the diagnosis of primary liver cancer and evaluation of chemotherapeutic effect.

Key Words:
GDF-15, Primary liver cancer, Diagnosis, Chemotherapeutic effect.

Introduction

Liver cancer is one of the most common malignant tumors in clinical practice, and its incidence rate is increased year by year. The early diagnosis of liver cancer is a key to improving the treatment effect on liver cancer and prolonging the survival time of patients[1]. At present, the clinical diagnosis of liver cancer is mainly based on the clinical symptoms and imaging examination results. However, there is no significant difference between early malignant lesion and benign lesion of the liver, so liver cancer cannot be diagnosed effectively through the early clinical symptoms and imaging examination results[2]. Therefore, liver cancer has often been in the advanced stage already when diagnosed, leading to poor treatment effect. Although cytology and pathological

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examination can be used to accurately diagnose liver cancer in clinic, they are traumatic and limited, and the repeated examination is not easy. With the rapid development of molecular biology of liver cancer, using biomarkers in the blood in the diagnosis of liver cancer has become a research hotspot. At present, alpha-fetoprotein is a biomarker in the serum detection of liver cancer, but its sensitivity and specificity in the diagnosis of liver cancer are unsatisfactory[3]. Growth differentiation factor-15 (GDF-15) is widely involved in the body's growth and development, inflammation and other pathophysiological processes[4,5]. Studies have shown that GDF-15 can be used as a tumor marker to predict tumor proliferation, migration, metastasis, drug resistance, etc., but there have been no reports on whether GDF-15, as a tumor marker of liver cancer, can be used to evaluate the diagnosis of liver cancer and chemotherapeutic effect. Therefore, in this study, patients with liver cancer treated in our hospital from June 2015 to May 2016 were taken as objects of study, to explore the values of serum GDF-15 level in the diagnosis of liver cancer and evaluation of chemotherapeutic effect. It is now reported as follows.

Patients and Methods

Patients

A total of 92 patients with primary liver cancer treated in our hospital from June 2015 to May 2016 were selected as liver cancer group, including 52 males and 40 females, aged 46-74 years old, with an average of (58.5±8.9) years old. According to the tumor-node-metastasis (TNM) staging of primary liver cancer, there were 18 cases in stage I, 24 cases in stage II, 31 cases in stage III, and 19 cases in stage IV among 92 patients with primary liver cancer. Another 53 patients with benign liver lesion treated in our hospital during the same period were selected as benign liver disease group, including 32 males and 21 females, aged 44-75 years old, with an average of (54.4±7.9) years old. Besides, 40 healthy subjects receiving physical examination were selected as a healthy control group, including 24 males and 16 females, aged 46-78 years old, with an average of (56.1±8.0) years old. Liver benign lesions and cancerous lesions were confirmed by surgery or by biopsy. This study was reviewed and approved by the Ethics Committee of our hospital.

Conditions of Patients

Inclusion criteria: (1) Pathological examination results showed that all patients in liver cancer group had primary liver cancer without canceration in other tissue organs, (2) patients in benign liver disease group suffered from cancer, (3) none of research objects received any treatment within 1 week before blood collection in our hospital, (4) none of research objects had diseases of the blood system, (5) none of the patients (except healthy control group) had lesions other than those in the liver, and (6) all patients were informed of this study and signed the informed consent.

GDF-15 Detection

Five mL fasting venous blood was drawn from all objects of study in the early morning at 1 d after admission and at the last day after chemotherapy (liver cancer group), and centrifuged at 3000 rpm for 10 min. The supernatant was isolated and collected. The serum GDF-15 level was measured by using a human GDF-15 serum detection kit manufactured by R&D Systems (Minneapolis, MN, USA). The ROC curve analysis showed that the threshold value of GDF-15 was 1573.23 ng/L, and the sensitivity, specificity, and accuracy were 81.23%, 83.99%, and 83.62%, respectively.

Collection of Clinical Data

General clinical data of patients, including hepatitis B virus (HBV) infection, drinking, smoking, body mass index, dietary habit of moldy pickled food, character, hemoglobin, prealbumin and lactate dehydrogenase, were collected. Dietary habit of moldy pickled food referred to the eating frequency ≥1 time per week. The normal value of hemoglobin is 120-165 g/L in male and 110-150 g/L in female, that of prealbumin is 180-390 mg/L, and that of lactate dehydrogenase is 109-245 U/L.

Treatment and Therapeutic Evaluation

According to different pathological types and physical conditions of patients in liver cancer group, chemotherapy was performed based on the internationally-recommended first-line chemotherapy regimen; one chemotherapy cycle for all patients lasted for 3 weeks, and the curative effect on all patients was evaluated after 2 cycles of chemotherapy. The therapeutic evaluation was based on the modified response evaluation criteria in solid tumors (RECIST) proposed in 2009. In RECIST, the curative effect is divided into complete remission (the enhancement regions of all target

lesions in the arterial phase disappear), partial remission (the diameter of enhancement region of target lesion is reduced by ≥30.0%), stable disease (between partial remission and progressive disease), and progressive disease (the diameter of enhancement region of target lesion is increased by ≥20.0%, or new lesions emerge).

Statistical Analysis

Data collected in this study were statistically processed by using Statistical Product and Service Solutions (SPSS) 19.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). Measurement data were presented as ($\bar{x}\pm s$). The *t*-test was used for the statistical analysis of intergroup difference in measurement data, and the chi-square test was used for the statistical analysis of intergroup difference in enumeration data. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparison of Serum GDF-15 Level Among Different Groups

The serum GDF-15 level was compared among patients in liver cancer group, benign liver disease group, and healthy control group. Results showed that the serum GDF-15 levels in patients in liver cancer group and benign liver disease group were significantly higher than that in healthy control group ($p<0.05$), and the serum GDF-15 level in patients in liver cancer group was also significantly higher than that in benign liver disease group ($p<0.05$) (Table I).

Comparison of Serum GDF-15 Level Among Patients in Different TNM Staging

The serum GDF-15 level was compared among patients with primary liver cancer in different TNM staging. Results showed that there was no significant difference in serum GDF-15 level between patients with stage I liver cancer and those with stage II liver cancer ($p>0.05$); the serum GDF-15 levels in patients with stage III and IV liver cancer were markedly higher than those in patients with stage I and II liver cancer ($p<0.05$), and the serum GDF-15 level in patients with stage IV liver cancer was markedly higher than that in patients with stage III liver cancer ($p<0.05$) (Table II).

Table I. Comparison of serum GDF-15 level among patients in liver cancer group, benign liver disease group and healthy control group.

Group	n	GDF-15 (ng/L)
Healthy control group	40	579.43±120.76
Benign liver disease group	53	1120.52±189.39*
Liver cancer group	92	1607.32±230.48*#

Note: *$p<0.05$ *vs.* healthy control group, #$p<0.05$ *vs.* benign liver disease group.

Table II. Comparison of serum GDF-15 level among patients in different TNM staging.

Group	n	GDF-15 (ng/L)
Stage I	18	1489.56±156.35
Stage II	24	1510.39±163.25
Stage III	31	1645.20±166.35*
Stage IV	19	1719.35±146.28*#

Note: *$p<0.05$ vs. patients in stage I and II, #$p<0.05$ vs. patients in stage III.

Comparison of Serum GDF-15 Level Among Patients With Different Clinical Data in Liver Cancer Group

The serum GDF-15 level was compared among patients with different clinical data, such as HBV infection, drinking, smoking, body mass index, dietary habit of moldy pickled food, character, hemoglobin, prealbumin and lactate dehydrogenase. Results revealed that there was no significant difference in serum GDF-15 level among patients with different clinical data ($p>0.05$) (Table III).

Comparison of Serum GDF-15 level Among Liver Cancer Patients With Different Curative Effects After Chemotherapy

The curative effect after chemotherapy was evaluated, and 80 out of 92 patients completely received chemotherapy, and the remaining 12 patients failed to receive chemotherapy throughout the treatment. Among the 80 patients, there were 22 cases of partial remission, 38 cases of stable disease, and 20 cases of progressive disease. The serum GDF-15 level in patients with progressive disease was significantly higher than those in patients with partial remission and stable disease ($p<0.05$), and the serum GDF-15 level in patients with stable disease was significantly higher than that in patients with partial remission ($p<0.05$) (Table IV).

Table III. Comparison of serum GDF-15 level among patients with different clinical data in liver cancer group.

Clinical data	n	GDF-15 (ng/L)
HBV infection	Yes	1508.44±178.04
	No	1610.39±169.41
Smoking	Yes	1587.30±181.24
	No	1573.26±177.46
Drinking	Yes	1611.28±183.56
	No	1596.46±175.04
Body mass index (kg/m^2)	≥24	1653.53±180.40
	<24	1590.16±178.33
Dietary habit of moldy pickled food	Yes	1580.44±201.53
	No	1601.50±166.46
Character	Extrovert	1577.04±173.53
	Introvert	1600.45±169.44
Hemoglobin (g/L)	Normal	1611.04±186.34
	Below normal	1603.67±163.67
Prealbumin (mg/L)	Normal	1603.52±168.93
	Below normal	1610.49±178.49
Lactate dehydrogenase (U/L)	Normal	1599.35±193.01
	Above normal	1603.57±183.57
	No	1573.26±177.46
Drinking	Yes	1611.28±183.56
	No	1596.46±175.04
Body mass index (kg/m^2)	≥24	1653.53±180.40
	<24	1590.16±178.33
Dietary habit of moldy pickled food	Yes	1580.44±201.53
	No	1601.50±166.46
Character	Extrovert	1577.04±173.53
	Introvert	1600.45±169.44
Hemoglobin (g/L)	Normal	1611.04±186.34
	Below normal	1603.67±163.67
Prealbumin (mg/L)	Normal	1603.52±168.93
	Below normal	1610.49±178.49
Lactate dehydrogenase (U/L)	Normal	1599.35±193.01
	Above normal	1603.57±183.57

Note: $^{*}p<0.05$ *vs.* healthy control group, $^{\#}p<0.05$ *vs.* benign liver disease group.

Discussion

Primary liver cancer has a very high incidence rate in China, and it occurs in any age group, whose mortality rate ranks third among all malignant tumors[6]. At present, the clinical diagnosis of primary liver cancer is mainly based on ultrasound, computed tomography (CT), and other imaging examination and pathological examination. Specificity and sensitivity of ultrasound, CT, and other imaging examinations are not high, and pathological examination results are golden standards for the diagnosis of primary liver cancer, but the sampling process is traumatic[7,8]. In the growth and development processes of tumors, a variety of biomarkers are produced and secreted into the blood or tissue fluid, and these biomarkers include proteins, peptides, oncogene products, polyamines, and hormones[9]. These biomarkers can effectively reflect the presence and growth status of tumors accurately. In recent years, using various biomarkers as bases for the diagnosis and therapeutic evaluation of tumors has been a research hotspot. At present, a variety of biomarkers have been used clinically as bases for the diagnosis and therapeutic evaluation of tu-

Table IV. Comparison of serum GDF-15 level among liver cancer patients with different curative effects after chemotherapy.

Group	n	GDF-15 (ng/L)
Partial remission	22	1214.23±125.42
Stable disease	38	1560.24±158.05*
Progressive disease	20	1703.51±178.55$^{*\#}$

Note: $^{*}p<0.05$ *vs.* patients with partial remission, $\#p<0.05$ *vs.* patients with stable disease.

mors. For example, carcinoembryonic antigen is clinically used to diagnose lung adenocarcinoma. At present, some scholars have taken biomarkers, such as alpha-fetoprotein, carbohydrate antigen (CA)199, and glutamyl transpeptidase, as bases for the diagnosis and therapeutic evaluation of primary liver cancer, and have used them in clinical practice. However, it is found in the application that their sensitivity and accuracy are not high[10]. Therefore, it is still needed to search new biomarkers for the diagnosis and therapeutic evaluation of primary liver cancer.

GDF-15 is also known as non-steroidal anti-inflammatory drug activating gene-1, macrophage inhibitor-1, placental bone morphogenetic protein, placental transforming growth factor, and prostate-derived factor. GDF-15 is a member of the transforming growth factor-β superfamily. It participates in inflammatory regulation and apoptosis pathways in organ damage, while hepatocyte inflammation and hepatocyte apoptosis are the major pathologies of viral hepatitis, chronic hepatitis, and cirrhosis. GDF-15 promotes the pathological process of viral hepatitis, chronic hepatitis, and cirrhosis by promoting hepatocyte inflammation and hepatocyte apoptosis. Its encoding gene contains one intron and two exons, and a total of 308 amino acids constitute the protein precursor[11]. The N-terminal signal peptide sequence of protein precursor is cut off during the hydrolysis, and disulfides bond into the dimmer. After the endoplasmic reticulum is correctly folded, the dimer is cut by the proprotein convertase, and then secreted into the extracellular medium. It has been found currently that the serum GDF-15 levels in patients with breast cancer, prostate cancer, colon cancer, liver cancer, stomach cancer, etc., are significantly higher than that in non-cancer people, and the serum GDF-15 level is closely associated with cancer prognosis and survival time[5]. Si et al[12] have shown that GDF-15 can be induced by hepatitis C virus infection to regulate hepatocellular oncogenes. Liu et al[13] found that GDF-15 expression is associated with the hepatitis-related liver disease. Shnaper et al[14] found that the serum GDF-15 level in patients with prostate cancer is closely related to bone metastasis. Wallin et al[15] and Staff et al[16] also observed that the serum GDF-15 level in patients with endometrial cancer is closely related to bone metastasis. Besides, Brown et al[17] demonstrated that the serum GDF-15 level is gradually increased during the malignant transformation caused by benign colon lesion. In this study, the serum GDF-15 level in patients with primary liver cancer was significantly higher than those in benign liver disease group and healthy control group, and it was increased gradually with the progression of primary liver cancer. The serum GDF-15 levels in patients with different clinical data were investigated, and it was found that the serum GDF-15 level was different due to different HBV infections and hemoglobin levels, indicating that the GDF-15 level can be used as a diagnostic criterion for primary liver cancer without being affected by the differences in general clinical data.

Currently, the clinical evaluation of chemotherapeutic effect is based on RECIST. In RECIST, the curative effect is evaluated generally through the comprehensive analysis of imaging examination results, such as enhanced CT, B-mode ultrasound, and enhanced magnetic resonance imaging (MIR). Although the evaluation result of curative effect is more accurate, this method is expensive and time-consuming. However, evaluating the chemotherapeutic effect by using tumor biomarkers is cheap and convenient[18]. It was found in this study that the serum GDF-15 level was significantly different in patients due to different curative effects after chemotherapy, so that the serum GDF-15 level can be used as an evaluation index of curative effect on patients with primary liver cancer after chemotherapy.

Conclusions

We showed that the serum GDF-15 level has certain clinical values in the diagnosis of primary liver cancer and evaluation of chemotherapeutic effect.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Eastern Hepatobiliary Hospital. Signed written informed consents were obtained from the patients and/or guardians.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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