Iran J Public Health, Vol. 50, No.8, Aug 2021, pp.1603-1612

Original Article

Efficacy Evaluation on the Color Doppler Ultrasound, Multislice Spiral CT Combined with Serum Markers in Diagnosis of Primary Hepatic Carcinoma

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(Received 22 Nov 2020; accepted 09 Dec 2020)

Abstract

Background: The efficacy of color Doppler ultrasound, multislice spiral CT combined with serum alpha-fetoprotein (AFP) and alpha-fetoprotein heterogeneity (AFP-L3) in the diagnosis of primary hepatic carcinoma was evaluated.

Methods: Seventy-nine patients with primary hepatic carcinoma (PHC group) and 50 patients with benign liver lesions (benign control group) admitted in Yantaishan Hospital (Yantai, China) from January 2016 to December 2018 were selected. The liver was scanned by color Doppler ultrasound and multiple multislice spiral CT. The serum AFP and AFP-L3 levels were detected by electrochemiluminescence. The value of color Doppler ultrasound, multislice spiral CT combined with serum AFP and AFP-L3 in diagnosis of primary liver cancer was retrospectively analyzed.

Results: The color Doppler flow imaging (CDFI) showed a high-speed and high-resistance spectrum. The serum AFP and AFP-L3 levels of patients with primary hepatic carcinoma were significantly higher than those of the benign control group were (U=138.000 and 155.500, *P*=0.000 and 0.000), *P*<0.01. The sensitivity, accuracy and negative predictive value of color Doppler ultrasound, multislice spiral CT combined with serum AFP and AFP-L3 examinations for diagnosis of primary hepatic carcinoma were 96.20, 90.70 and 93.18%, which was significantly improved compared with each single examination (X_2=27.888, 17.511 and 16.202, *P*=0.000, 0.002 and 0.003), *P*<0.01.

Conclusion: Color Doppler ultrasound, multislice spiral CT combined with AFP and AFP-L3 examination could significantly improve the diagnosis efficiency of primary hepatic carcinoma, which was beneficial to early clinical diagnosis and early treatment.

Keywords: Primary hepatic carcinoma; Ultrasound; Multislice Spiral CT; Alpha-fetoprotein receptor; Combination examination

Introduction

Primary hepatic carcinoma (PHC) was one of the most common malignant tumors in the world (1) and the sixth most common cancer in the world nowadays (2), for which the mortality rate ranks

as the second in China (3). Among them, primary hepatocellular carcinoma (HCC) was the main pathological type of PHC (it was counting for approximately 75% -85%) (4).

In recent years, with the change of lifestyle and the accelerating aging process, the morbidity and mortality of PHC have been increasing year by year-worldwide (5). The pathogenesis of primary hepatic carcinoma is relatively insidious, and most patients are diagnosed in the middle or advanced stages, therefore, they lost the best opportunities for radical treatments. Based on the statistics, the five-year survival rate of advanced liver cancer was less than 10% (6). Thus, early treatment was the key to affecting the prognosis of primary hepatic carcinoma (7).

The diagnosis of primary hepatic carcinoma rarely depends on pathological needle biopsy, therefore, the non-invasive diagnostic methods play a crucial role in the early diagnosis (8). Imaging examination (including ultrasound and CT) is a common method for diagnosis of primary hepatic carcinoma, which could display the liver morphology, lesion size and specific anatomical location of patient effectively. However, it is easy to misdiagnosis for small liver cancer (for those diameter<3 cm). At present, AFP is the most widely used as clinical diagnostic marker of PHC serum, but it could not ensure the high sensitivity and strong specificity, with some certain limitations (9), indicating that any singular examination method has limited application value.

We aimed to choose color Doppler ultrasound, multislice spiral CT combined with serum AFP and AFP-L3 to diagnose primary hepatic carcinoma, which intended to provide a reference for the early diagnosis of primary hepatic carcinoma.

Methods

Clinical materials

Seventy-eight patients with primary hepatic carcinoma (PHC group) and 50 patients with benign liver lesions (benign control group) were selected as the study subjects from January 2016 to December 2018 in Yantaishan Hospital（Yantai, China）. PHC was diagnosed according to histological classification criteria from WHO (2005) liver and intrahepatic bile duct tumor (10). There were 47 males and 32 females, 35-82 yr old, with an average age of (55.23±8.96). The staging criteria was in accordance with Union for International Cancer Control and American Joint Committee on Cancer (UICC/AJCC, 2010), and there were 11 cases in stage I, 27 cases in stage II, 31 cases in stage III, and 10 cases in stage VI.

Benign liver disease group

There were 29 males and 21 females, 33-81 yr old with an average age of (56.72±7.98) yr, including 20 cases with liver cirrhosis, 10 cases with hepatic hemangioma, 15 cases with hepatic cysts, 5 cases with hepatocellular adenoma.

The inclusion criteria of PHC group: ① They were diagnosed by puncture biopsy or surgical pathological examination, all of them had complete examination data of liver color Doppler ultrasound, multislice spiral CT, and serum AFP and AFP-L3; ② patients who were newly diagnosed without receiving tumor-related treatment before. ③ patients who signed the consent and cooperated in this study.

Exclusion criteria: ① patients who had other malignant tumors were excluded. ② patients who had incomplete study data. ③ patients who did not cooperate with researcher.

This study was approved by the hospital Ethics Committee, and the study subjects had signed an informed consent form.

Test

Color Doppler ultrasound examination

The diagnostic instrument was PHILIPS iU22, with the probe frequency of 3.5-5.0 MHz. The patient fasted for over 8 hours. In the examination, the lateral or supine position was taken, and the liver was scanned carefully to observe the location, number, size, shape, echo, intrahepatic bile duct, lumen of the lesion. Afterwards, the blood flow in the lesion area was measured by color Doppler ultrasound mode, and the peak systolic velocity (V_{max}) and resistance index (RI)

of each lesion were measured. The blood flow signal was divided into four levels: Grade 0 was defined as no blood supply in the tumor; Grade I was that there was blood supply with less amount in the tumor, with 1 to 2 punctiform blood flow; Grade II was that there was rich blood flow in the tumor, with 3 to 4 punctiform blood flow generally or 1 to 2 blood vessels; Grade III was that there was abundant blood flow in the tumor, with over 4 punctiform blood flow or more than 2 blood vessels.

Multislice spiral CT examination

The instrument was SIEMENS SOMATOM Definition flash spiral CT machine. The scanning parameters were as follows: tube voltage and current were 120 KV and 160 mA/s, screw pitch was 1.5 and layer thickness was 10 mm. During the examination, the patients were asked to take the supine position, and the scan ranged from the diaphragm to the lower edge of the pubic symphysis was performed. At first, the conventional CT scan was performed. Afterwards, the nonionic iodine-containing contrast agent iohexol was intravenous injected by a high-pressure syringe through the cubital vein of patients (Yangze River Pharmaceutical Group Co., Ltd., National Drug Permission NO.H10970322) in the dosage of 1.0 ml·kg^{-1}, with the injection speed as 3.0～4.0 mL/s, and the total hepatic three-phase dynamic enhancement scan in arterial-phases, portal vein phase and the hepatic artery phase were respectively performed at 20～25 s, 50～60 s and 120～180 s after the injection of contrast agent. The images were transferred to the workstation. More than 2 experienced physicians read the diagnostic images by using double-blind method.

Serum AFP, AFP-L3 examination

Four ml of venous blood were taken from patients in PHC group and benign control group on an empty stomach at 6.00-9.00 in the morning. After standing and self-coagulating, the sample was centrifuged at 2264 g (3500 r/min) for 15 min to separate the single serum for testing.

AFP-L3 affinity adsorption centrifuge tube, the required eluent were provided by Beijing Hotgen Biotech Co., Ltd. AFP-L3 was separated by a micro centrifugal column method. Agarose coupled with LCA was used as the affinity medium in the centrifugal column, which could specifically bind to AFP-L3. Overall, 400 ul serum of the research subject was add into 600 ul of cleaning solution and mixed, for which the 600 ul of the diluted serum dilution was taken into the agarose spin column coupled with LCA. The strong AFP heterogeneity bonded with LCA was left in the spin column, and the unbound AFP was washed away with the cleaning solution. After finally eluting with the special eluent, the processed sample was obtained, which contained the relative pure AFP-L3.

Determination of AFP and AFP-L3 method was electrochemiluminescence immunoassay, with the principle as the double antibody sandwich principle. The instrument ELecsys-2010 was provided by German Roche Company, and AFP and AFP-L3 diagnostic reagents were provided by Roche Diagnostics, to determine the original serum AFP and purified AFP-L3 levels. The operations were in strictly accordance with the operation manual.

Result determination

CT, ultrasound and pathological diagnosis of PHC group and benign control group in conformity were defined as true positive or true negative, and non-conformity was defined as false positive or false negative. AFP and AFP-L3 exceeded the critical value was defined as positive, the positive result in one or more than one joint examination was defined as positive, and all negative results were defined as negative.

Statistical methods

The data were analyzed by using SPSS 23.0 statistical software (Chicago, IL, USA). The measurement data were non-normally distributed, expressed as median and quartile [M (P_{25}, P_{75})], and comparison between the two groups was performed by Wilcoxon rank sum test; the count data were expressed by the rate (%), X^2 test was performed, and P<0.05 should be considered as

statistical significance. The curve (AUC) value under AFP and AFP-L3 area was obtained by the analysis of receiver operating characteristic curve of AFP and AFP-L3 (ROC curve), the best threshold of AFP and AFP-L3 diagnosed PHC was obtained according to the maximum Youden index.

Results

The characteristics of two-dimensional ultrasound imaging of primary hepatic carcinoma: the shape of the lesion was round, round-like, irregular, with the uniform density, as shown in Fig. 1 (A). The blood flow could be seen entering from the periphery into the tumor by color Doppler flow imaging (CDFI) (Table 1).

Table 1: Comparison of blood flow grading in different lesions of PHC [n (%)]

Size	*Number of lesions*	*Grade 0*	*Grade I*	*Grade II*	*Grade III*
≤5 cm	45	6 (13.33)[a]	9 (20.00)[a]	19 (42.22)[a]	11 (24.45)[a]
>5 cm	42	0 (0)	2 (4.76)	9 (21.42)	31 (73.82)
X^2		6.015	4.567	4.304	21.201
P		0.014	0.033	0.039	0.000

Note: [a]$P<0.05$

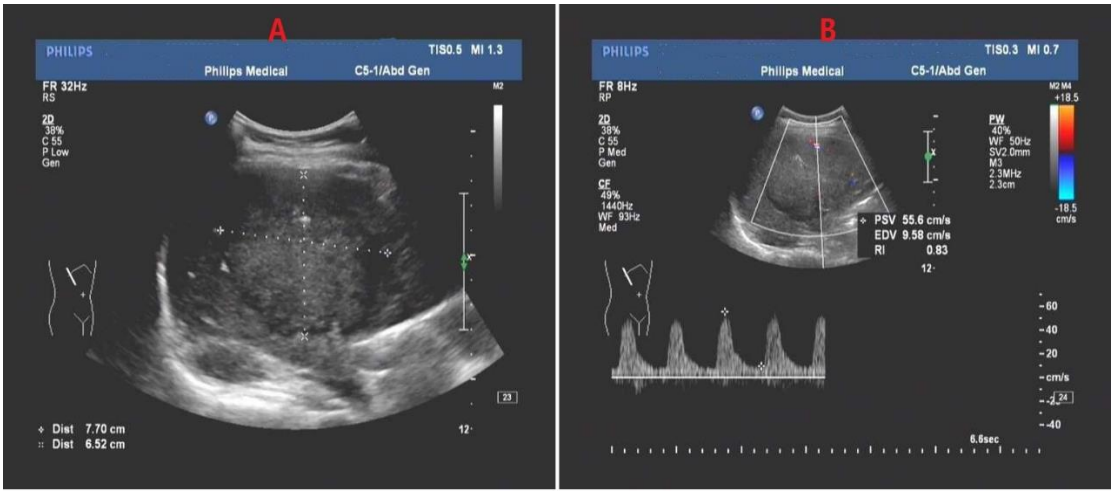

Fig. 1: A: Two-dimensional ultrasound was shown an image of a tumor in the right lobe of the liver, with a size of about 7.7×6.52 cm, a round shape, low echo inside, and uneven distribution. The edges were relative clear, with the low halo surrounded.
B: Color doppler flow imaging (CDFI): it was shown blood flow signals around and inside the tumor, RI: 0.83

CDFI showed a high-speed and high-resistance spectrum, and the flow velocity and flow rate increased, as shown in Fig. 1 (B). Taking pathological examination as the gold standard, in 79 cases with PHC, the ultrasound results of 61 cases were consistent with pathological diagnosis (true positive), and 18 cases with inconsistency (false negative); while the ultrasound results were consistent with pathological diagnosis (true negative) in 45 cases among 50 cases with benign liver lesions, and there were 5 cases with inconsistency (false positive).There were 87 lesions detected among 79 PHC patients (Table 2).

Table 2: Intrahepatic lesion size and distribution showed on the CT images of 87 lesions in 79 patients with primary hepatic carcinoma

Size of lesion (cm)	***Number (n)***	***Distribution of lesions (n/n)***			
		Left lateral lobe of liver	Left medial lobe of liver	Right anterior lobe of liver	Right posterior lobe of liver
<1	8	0(0/10)	10.53(2/19)	8.33(1/12)	10.87(5/46)
1-3	15	10.00(1/10)	15.79(3/19)	16.67(2/12)	19.57(9/46)
3-5	22	20.00(2/10)	26.32(5/19)	33.33(4/12)	23.91(11/46)
>5	42	70.00(7/10)	47.36(9/19)	41.67(5/12)	45.65(21/46)

The lesions should be shown as oval or round-shaped shadows and some lesions were irregular or lobulation with low and medium density mainly in CT plain scan images, as shown in Fig. 2 (A); the enhanced scanning lesions were obviously unevenly enhanced in the arterial phase, as shown in Fig. 2 (B); the degree of enhancement in the portal venous phase decreased significantly, as shown in Fig. 2 (C); the degree of enhancement in the delayed phase continued to decrease, as shown in Fig. 2 (D).

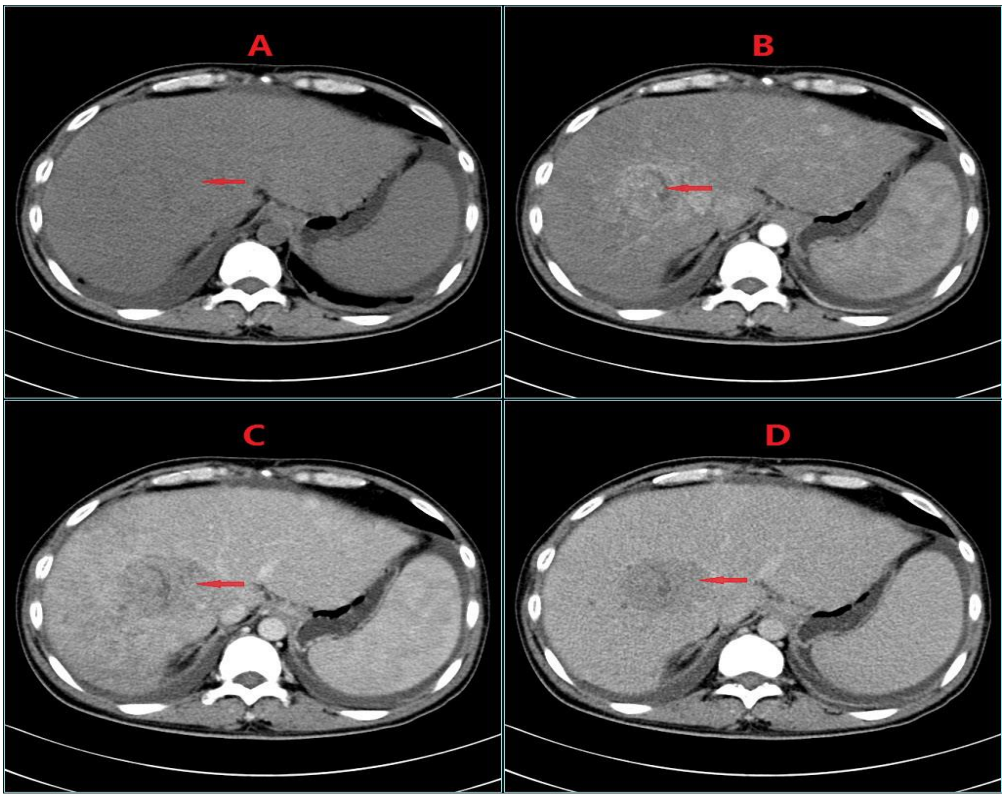

Fig. 2: A: the arrow in the plain CT scan was pointed out an oval heterogeneous low-density lesions in the right lobe of the liver, with a diameter as about 6 cm; B: the arrow in the enhanced scan of the lesion in the arterial phase was pointed out the obvious uneven enhancement; C: the arrow was pointed out that the degree of enhancement at the portal venous phase decreased significantly; D: the arrow was indicated that the degree of enhancement during the delayed phase continued in declination

In 79 PHC patients, the CT results were consistent with pathological diagnosis in 60 cases (true positives), and there were 19 cases with inconsistency (false negatives); while 50 cases of benign lesions were consistent with pathological diagnosis in 46 cases (true negatives), there were 4 cases with inconsistency (false positives).

The expression levels of serum AFP and AFP-L3 in primary hepatic carcinoma were significantly higher than those in benign lesion group were (U=138.000 and 155.500, *P*=0.000 and 0.000), *P*<0.01 (Table 3).

According to the AFP and AFP-L3 ROC curves (Fig. 3 and Fig. 4), maximum Youden indexes of AFP and AFP-L3 (0.650 and 0.633) were selected.

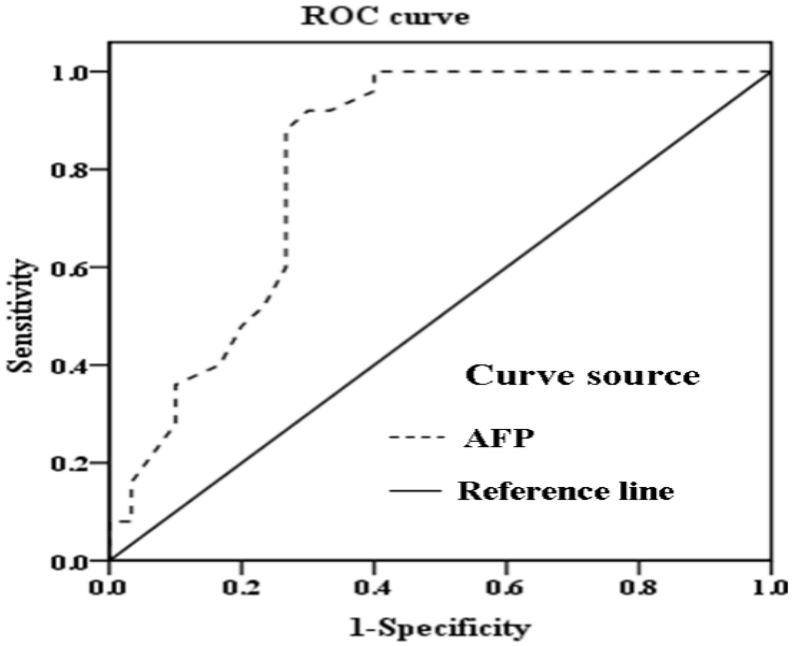

Fig. 3: ROC curve of PHC by AFP diagnosis

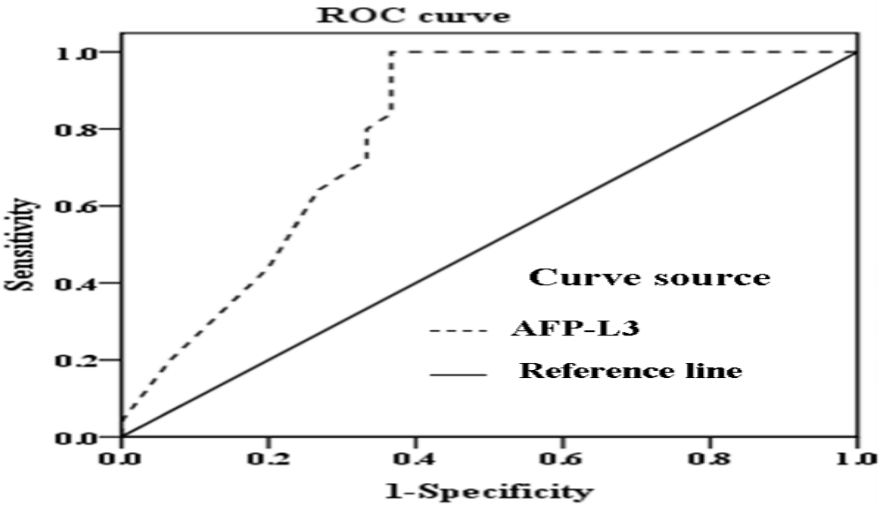

Fig. 4: ROC curve of PHC by AFP-L3 diagnosis

The sensitivity, accuracy and negative predictive value of color Doppler ultrasound, multislice spiral CT combined with serum AFP and AFP-L3 examinations on the diagnosis of primary hepatic carcinoma were significantly improved compared with each single examination (x^2 =27.888, 17.511 and 16.202, *P*=0.000, 0.002 and 0.003), *P*<0.01 (Table 4).

Table 3: Comparison of serum expression levels of AFP and AFP-L3 in primary hepatic carcinoma and benign lesion groups [M (P_{25}, P_{75})]

Group	*n*	*AFP (ng/ml)*	*AFP-L3 (ng/ml)*
Primary hepatic carcinoma Group	79	101.12 (15.75, 121.23)[b]	19.50 (6.63, 23.42)[b]
Benign lesion group	50	13.23(8.21, 16.42)	4.01 (2.26, 6.47)
U		138.000	155.500
P		0.000	0.000

Note: [b]*P*<0.01

Table 4: Efficacy evaluation on the ultrasound, CT, serum AFP and AFP-L3 single examination and combined examination for diagnosis of PHC [%(n/n)]

Detection Indicator	*Sensitivity*	*Specificity*	*Accuracy*	*Positive predictive value*	*Negative predictive value*
Ultrasound	77.22(61/79)	90.00(45/50)	82.17(106/129)	92.42(61/66)	71.43(45/63)
CT	75.95(60/79)	92.00(46/50)	82.17(106/129)	93.75(60/64)	70.77(46/65)
AFP	65.82(52/79)	84.00(42/50)	72.87(94/129)	86.67(52/60)	60.87(42/69)
AFP-L3	64.56(51/79)	88.00(44/50)	73.64(95/129)	89.47(51/57)	61.11(44/72)
Combination examination	96.20(76/79)[c]	82.00(41/50)	90.70(117/129)[c]	89.41(76/85)	93.18(41/44)[c]
X^2	27.858	3.082	17.511	2.246	16.202
P	0.000	0.544	0.002	0.691	0.003

Note: [c]*P*<0.01

Discussion

Imaging examination was an important method for screening and diagnosing primary hepatic carcinoma. Among them, color Doppler ultrasound, a simple, fast, non-invasive and safe method of examination, was the preferred method for the examination of primary hepatic carcinoma (11). Liver occupying lesions could be found, and the blood flow signal parameters in the occupying lesions could be used to observe the blood supply of the tumor (12). Conventional two-dimensional ultrasound could display the location, size, number and internal echo of the lesion, the relationship between the lesion and the surrounding tissue.

Increased secretion of angiogenic factors within the tumor of the primary hepatic carcinoma could cause the increments of neovascularization within and around the tumor (13,14), resulting in the increment of peak systolic velocity (V_{max}) of the arterial systolic phase and increment of resistance index (RI) within the tumor (15). The results showed that the V_{max} and RI of primary hepatic carcinoma were (108.52±16.24) cm/s and (0.78±0.08) in the study, which were significantly higher than those of benign lesions (61.22±6.23) and (0.38±0.03), *P*<0.01. The blood flow grade of cancer lesion ≤5 cm in diameter was significantly lower, which was indicating the tumor within the early stage of invasion, while the blood flow grade of lesions > 5 cm in diameter was significantly improved, with the blood flow of grade

III (71.43%) mainly, indicating that the tumor was in relatively vigorous growth and invasion process, with the rapid disease progression, which was entering the active stage of invasion (16).

However, there were certain limitations of ultrasound examination, for which the acoustic imaging characteristics of the lesions along with thick fatty liver, abdominal wall and visceral fat were not obvious. Moreover, for other liver-occupying lesions, such as nodular cirrhosis and small hepatic hemangioma, single liver metastatic cancer and other lesions were prone to misdiagnosis. In addition, the small liver cancer lesions with insufficient early blood supply had the low detection rate, and the breathing of patient may affect the image imaging quality, which was easy to cause missed diagnosis. The results in this study were shown that the sensitivity of PHC was 77.22% with the accuracy of 82.17% in color Doppler ultrasound diagnosis, compared with the pathological results.

In recent years, with the continuous development of imaging diagnostic technology, multislice spiral CT has brought a major breakthrough in the diagnosis of primary hepatic carcinoma (17), which could simultaneously complete a one-time scan for the three phases as hepatic arterial phase, equilibrium phase and delayed phase to obtain the rich and clear hemodynamic images of tumor, for which could accurately reflect the blood supply characteristics of tumor, improve the early diagnosis rate of liver lesions and have a better effect for diagnosis of PHC (18). The multislice spiral CT not only could shorten the scanning time interval significantly and greatly improve the resolution, but also could significantly improve the scanning speed, which could reduce the influence of motion artifacts on the display results; at the meanwhile, the analysis of layer thickness and image reconstruction could be arbitrarily selected within a certain range to show the small lesions in the liver clearly (19).

Contrast injection with liver-enhanced CT examination not only could show the morphological characteristics of the tumor, but also reflect the characteristics of bleeding supply (20). In the three-phase enhancement scan, the density of lesions showed the changes as "High-Low-Low", that is, the arterial phase enhanced significantly after entering the portal venous phase, the density of lesion decreased rapidly in the portal venous phase, and the delayed phase was shown a continuous decline in density, with the imaging features of "Fast-in and Fast-out" (21).

This study showed that 87 lesions were detected among 79 cases with primary hepatic carcinoma, which were mainly found as solitary lesions. The lesions were mainly concentrated in the left medial lobe of liver (19 lesions) and the right posterior lobe of the liver (46 lesions). Compared with pathological diagnosis, the sensitivity of multislice spiral CT in the diagnosis of primary hepatic carcinoma was 75.95% with the accuracy as 82.17%. It was worth to note that because CT enhanced scanning imaging was intermittent with the short fixed time, therefore, it was easy to be misdiagnosed and missed diagnosis for the lesions with low blood supply, moreover, it should not be repeated in the short period for its examination with radiation, and the contrast agents may affect renal function, so the examination had certain limitations.

With the rapid development of tumor molecular immunology, tumor markers had been played an indispensable role in the screening and diagnosis of primary hepatic carcinoma. AFP was a glycoprotein, which always was a classic marker for the diagnosis of PHC, however, 30%-40% of PHC patients were negative or in low level, and the phenomenon of elevating AFP levels also appears in serum under pregnancy physiological conditions or patients with benign diseases such as severe hepatitis, cirrhosis (22). AFP for diagnose PHC was only shown the sensitivity as 67.5% (23). AFP electrophoresis had different mobility in 1970, and proposed the concept of AFP heterogeneity (24). Subsequently, Okuyama et al (25) had classified AFP and lens culinaris agglutinin (LCA) according to their different binding abilities, which were divided into LCA non-bound type (including AFP-L1 and AFP-L2) and LCA-bound type (AFP-L3). AFP-L3 was a specific secretion of liver cancer cells (26), which

had a higher specificity than AFP, called as a new generation of liver cancer markers (27). However, AFP-L3 did not increase in 15-30% patients with AFP-positive liver cancer, i.e., the low value of AFP-L3 could not exclude the presence of liver cancer (28). In some patients with early-stage liver cancer who were finally diagnosed, these liver cancer tumor markers could show an increasing trend as early as six months before the diagnosis of liver cancer, which was indicating that tumor markers were prospective for early screening of PHC (29). The results showed that when the critical value of PHC diagnosis of AFP and AFP-L3 was 20.00 ng/ml and 8.00 ng/ml respectively in this study according to the area of ROC curve, the sensitivity of PHC diagnosis was 65.82 and 64.56% respectively, indicating that the diagnostic value of single detection was still unsatisfactory.

Significance of combination examination: imaging could be visually observed the location, size, number, echo and blood supply of PHC lesions. The detection of serum tumor markers was prospective for the early diagnosis of PHC, but the value of single examination was limited. The results of this study showed that color Doppler ultrasound, multislice spiral CT combined with serum AFP and AFP-L3 examinations could complement and confirm each other, and the sensitivity, accuracy, and the negative predictive value were significantly improved compared with each single examination as 96.20, 90.70 and 93.18%, thereby the misdiagnosis and missed diagnosis could be reducing.

Conclusion

Color Doppler ultrasound, multislice spiral CT combined with serum AFP, AFP-L3 examination could significantly improve the diagnosis efficiency of primary hepatic carcinoma, which was conducive to early clinical diagnosis and early clinical intervention. In the future, the sample size of the study should be increased, and the dynamic follow-up after treatment needs to be further strengthened.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received in this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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