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 (X²=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; 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 strict accordance with the operation manual.

**Result determination**

CT, ultrasound and pathological diagnosis of PHC group and benign control group in conformation 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 (%), X² 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

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) | 9 (20.00) | 19 (42.22) | 11 (24.45) |
| >5 cm| 42                | 0 (0)   | 2 (4.76) | 9 (21.42) | 31 (73.82) |
| Χ²   | 6.015             | 4.567   | 4.304   | 21.201   |
| P    | 0.014             | 0.033   | 0.039   | 0.000    |

Note: *P*<0.05

[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](http://ijph.tums.ac.ir)

**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    | 79  | 101.12 (15.75, 121.23)$^b$| 19.50 (6.63, 23.42)$^b$|
| Benign lesion                | 50  | 13.23 (8.21, 16.42) | 4.01 (2.26, 6.47) |

Note: $^bP<0.01$

**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 exami-   | 96.20(76/79)$^e$| 82.00(41/50)| 90.70(117/129)$^e$ | 89.41(76/85) | 93.18(41/44)$^e$ |
| nation              |             |             |          |                          |                             |

Note: $^eP<0.01$

**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 grade 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 in-
creasing 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 un-
satisfactory.
Significance of combination examination: imag-
ing could be visually observed the location, size,
number, echo and blood supply of PHC lesions.
The detection of serum tumor markers was pros-
spective for the early diagnosis of PHC, but the
value of single examination was limited. The re-
results of this study showed that color Doppler
ultrasound, multislice spiral CT combined with
serum AFP and AFP-L3 examinations could com-
plement 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 di-
agnosis could be reducing.

Conclusion
Color Doppler ultrasound, multislice spiral CT
combined with serum AFP, AFP-L3 examination
could significantly improve the diagnosis effi-
ciency 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 fals-
sification, 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.

References
1. Colombo M (1992). Hepatocellular carcinoma. J
Hepatol, 15(1-2): 225-236.
2. Bray F, Ferlay J, Soerjomataram I, et al (2018).
Global Cancer Statistics 2018: GLOBOCAN
Estimates of Incidence and Mortality World-
wide for 36 Cancers in 185 Countries. CA Can-
er J Clin, 68(6): 394-424.
3. El-Serag HB (2012). Epidemiology of viral hepatitis
and hepatocellular carcinoma. Gastroenterology,
142(6): 1264-1273.
4. European Association for the Study of the Liver
(2018). EASL Clinical Practice Guidelines:
Management of Hepatocellular Carcinoma. J
Hepatol, 69(1): 182-236.
5. Torre LA, Bray F, Siegel RL, et al (2015). Global
cancer statistics, 2012. CA Cancer J Clin, 65(2):
87-108.
6. Trinchet JC, Alperovitch A, Bedossa P, et al (2009).
Epidemiology, prevention, screening and diag-
nosis of hepatocellular carcinoma. Bull Cancer,
96(1): 35-43.
7. Dong Y, Wang WP, Mao F, et al (2016). Application
of imaging fusion combining contrast-
enhanced ultrasound and magnetic resonan-
ceimaging in detection of hepatic cellular carci-
nomas undetectable by conventional ultrasound.
J Gastroenterol Hepatol, 31(4): 822-828.
8. van Meer S, de Man RA, Sierssema PD, et al (2013).
Surveillance for Hepatocellular Carcinoma in
Chronic Liver Disease. Evidence and Contro-
versies. World J Gastroenterol, 19(40): 6744-6756.
9. Chen GG, Ho RL, Wong J, et al (2007). Single nucleotide polymorphism in the promoter region of human alpha-fetoprotein (AFP) gene and its significance in hepatocellular carcinoma (HCC). *Eur J Surg Oncol*, 33(7): 882-826.

10. Bosman PI, Carneiro F, Hruban RH, et al. WHO Classification of Tumours of the Digestive System(4th[M]). Lyon, France: IACR Press, 2010, 425-440.

11. Shimazaki H (2016). Inspection of Hepatocellular Carcinoma 1: Ultrasoundography. *Nihon Hoshasen Gijutsu Gakkai Zasshi*, 72(3): 281-289.

12. Kong S, Yue X, Kong S, et al (2018). Application of contrast-enhanced ultrasound and enhanced CT in diagnosis of liver cancer and evaluation of radiofrequency ablation. *Oncol Lett*, 16(2): 2434-2438.

13. Lin W, Zhao J, Cao Z, et al (2014). Livistona chinensis seeds inhibit hepatocellular carcinoma angiogenesis in vivovia suppression of the Notch pathway. *Oncol Rep*, 31(4): 1723-1728.

14. Zhan P, Qian Q, Yu LK (2013). Prognostic significance of vascular endothelial growth factor expression inhepatocellular carcinoma tissue: a meta-analysis. *Hepatobiliary Surg Nutr*, 2(3): 148-155.

15. Lassau N, Charni L, Chebil M, et al (2011). Dynamic contrast-enhanced ultrasonography (DCE-US) and anti-angiogenic treatments. *Dis Sec Med*, 11(56): 18-24.

16. Yang X, Zhu H, Hu Z (2010). Dendritic cells transduced with TEM8 recombinant adenovirus prevent hepatocellular carcinoma angiogenesis and inhibits cells growth. *Vaccine*, 28(43): 7130-7135.

17. Lee YJ, Lee JM, Lee JS, et al (2015). Hepatocellular carcinoma: diagnostic performance of multisector CT and MR imaging-a systematic review and meta-analysis. *Radiology*, 275(1): 97-109.

18. Hinrichs JB, Shin HO, Kaecher D, et al (2016). Parametric response mapping of contrast-enhanced biphasic CT for evaluating tumour viability of hepatocellular carcinoma after TACE. *Eur Radiol*, 26(10): 3447-3455.

19. Ladd LM, Tirkes T, Tann M, et al (2016). Comparison of hepatic MDCT, MRI, and DSA to explain pathology for the detection and treatment planning of hepatocellular carcinoma. *Clin Mol Hepatol*, 22(4): 450-457.

20. Ogul H, Kantarcı M, Genç B, et al (2014). Perfusion CT imaging of the liver: review of clinical applications. *Diagn Imaging Radiol*, 20(5): 379-389.

21. Dong A, Dong H, Zuo C, et al (2015). Diffuse Infantile Hepatic Hemangioendothelioma With Early Central Enhancement in an Adult: A Case Report of CT and MRI Findings. *Medicine (Baltimore)*, 94(51): e2353.

22. Lim TS, Kim DY, Han KH, et al (2016). Combined use of AFP, PIVKA-II, and AFP-L3 as tumor markers enhances diagnostic accuracy for hepatocellular carcinoma in cirrhotic patients. *Saud J Gastroenterol*, 51(3): 344-353.

23. Seo SJ, Kim HS, Kim WJ, et al (2015). Diagnostic value of PIVKA-II and alpha-fetoprotein in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol*, 21(13): 3928-3935.

24. Abdel-Aziz MM, Elshafy MF, Abbas AT, et al (2016). Comparison of AFP-L3 and p53 Antigen Concentration with Alpha-Fetoprotein as Serum Markers for Hepatocellular Carcinoma. *Clin Lab*, 62(6): 1121-1129.

25. Okuyama M, Ueno H, Kobayashi Y, et al (2016). Target-selective photo-degradation of AFP-L3 and selective photo-cytotoxicityagainst HuH-7 hepatocarcinoma cells using an anthraquinone-PhoSL hybrid. *Chem Commun (Camb)*, 52(10): 2169-2172.

26. Subwongcharoen S, Leelawat K, Treepongkaruna SA, Narong S (2011). Serum AFP and AFP-L3 in clinically distinguished hepatocellular carcinomafrom patients with liver masses. *J Med Assoc Thai*, 94 Suppl 2: S46-51.

27. Hiraoka A, Ishimaru Y, Kawasaki H, et al (2015). Tumor Markers AFP, AFP-L3, and DCP in Hepatocellular Carcinoma Refractory to Transcatheter Arterial Chemoembolization. *Onkology*, 89(3): 167-174.

28. Zhao J, Guo LY, Yang JM, et al (2015). Sublingual vein parameters, AFP, AFP-L3, and GP73 in patients with hepatocellular carcinoma. *Genet Mol Res*,14(2): 7062-7067.

29. Choi J, Kim GA, Han S, et al (2019). Longitudinal Assessment of Three Serum Biomarkers to Detect Very Early-Stage Hepatocellular Carcinoma. *Hepatology*, 69(5): 1983-1994.