The role of contrast enhanced ultrasound for the characterization of hepatocellular carcinoma

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Summary

Objective: To analyze the hepatocellular carcinoma (HCC) characterizations on contrast enahanced ultrasound and the correlation between these characterizations with tumor size and tumor differentiations. *Subject and method:* This study included 135 patients with 135 HCC nodules confirmed by histopathology between February 2014 and May 2016. All patients were evaluated by contrast enhanced ultrasound with contrast agent SonoVue (Bracco Company, Netherlands), using US machine (Philips EPIQ 5G, US) that has contrast programe including contrast auto-tracking software ROI, following the Guideline of WFUMB and EFSUMB 2012. *Result:* Of 135 nodules, 134 nodules (99.3%) presented full enhancement pattern, 01 nodule (0.7%) with non-enhancement pattern, 53 nodules (39.3%) with incomplete enhancement pattern, 65 nodules (48.1%) with heterogeneous enhancement, 43 nodules (31.9%) with necrosis. There were 99.3% of HCCs with enhancement pattern in the arterial phase, 61.5% of HCCs wash-out in the portal phase and 85.9% of HCCs wash-out in the late phase. There were differentiated between HCCs with level's size (under 20mm, from 20 upto 40mm, from 40mm) and between the well-differentiated HCCs and the moderately to poorly HCCs from hypo-enhancement in the portal phase with statistically significant (p=0.0002 and p=0.0001). *Conclusion:* The HCCs nodules showed a "fast-in" and "fast-out" enhancement pattern. The wash-out phase correlates with HCCs' size and differentiations, that plays an important role in predicting the differentiation of HCCs.

Keywords: Contrast enhanced ultrasound, hepatocellular carcinoma.

1. Background

Hepatocellular carcinoma (HCC) is the most common primary malignancy tumor of the liver and is the main cause of death in patient with cirrhosis. Therefore, it is important in diagnosis and treatment hepatocellular carcinoma at an early stage.

Sonographic characterization of hepatic lesions is based on the gray scale morphologic features and on vascular information from Doppler imaging. However, considerable overlapping exists between the appearances of benign and malignant lesions, making reliable differential diagnosis impossible with conventional sonographic techniques. There are sequential changes in the supplying vessels during this procedure. As dysplastic nodules undergo malignant transformation, abnormal neoplastic arteries increase, whereas normal arterial and portal supplies decrease [10]. A typical hepatocellular carcinoma is exclusively supplied by the hepatic artery and lacks a portal venous supply [8].

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Currently with the development of the new generation of contrast agents as SonoVue (Bracco, Milan, Italy) is widely available in Europe and Asia (China, Korean…), replacing Levovist for radiology application. So that Contrast enhanced ultrasound (CEUS) is the diagnostic imaging method that using common.

The using of CEUS in dagnosis of hepatocellular carcinoma has been suggested in guidelines in the World Health Organization's Guidelines for the diagnosis and treatment of hepatologists and associations in the world, such as the Japan Society of Hepatology - 2014 (JSH) [1], the Asia Pacific Association for the Study of Liver Disease - 2010 (APASL) [14]. The CEUS is considered a diagnostic imaging method of hepatocellular carcinoma, particularly in the lesions with avascular on CT scanner and MRI images.

The aim of this study was to analyze the hepatocellular carcinoma (HCC) characterizations on contrast enahanced ultrasound with SonoVue and the correlation between these characterizations with tumor size and tumor differentiations.

2. Subject anh method

2.1. Subject

From February 2014 to May 2016, 135 patients were included in this study. All the patients were prospectively selected on the basic of ultrasound and histopathology examination. Written informed consent was obtained from all patients, and the study was approved by the Ethical Commitee of the 108 Military Central Hospital. Of these 135 patients, 95 had solitary nodule (70.4%), 40 had multiple nodules (29.6%). When a patient had multiple nodules, only the largest nodules on the basic ultrasonographic was evaluated, thus, 135 HCCs in 135 patients constituted in the study group. The maximum diameters of the nodules ranged from 14mm to 120mm (mean, 43.7 ± 21.0mm) and divided into 3 size's groups: Under 20mm (15 nodules), from 20mm to under 40mm (50 nodules) and from 40mm (70 nodules). The diagnosis of HCC for all these 135 nodules were confirmed by histopathologic examination with the specimens obtained by ultrasonography guided biopsy (n = 97) or surgical resection (n = 38).

2.2. Equipment

The ultrasonographic equipment used in this study was an EPIQ 5G machine (Philips, US) with a convex transducer with frequency of 3.5MHz, and the contrast-specific software with the low mechanic index (MI = 0.06) in the system. The contrast agent used in this study was SonoVue (Bracco, Amsterdam, Netherlands), a sulfur hexafluode-filled microbubble contrast agent. A volume of 2.4mL of SonoVue was injected into the antecubital vein in a bolus fashion (within 1 - 2 seconds) through a 20-gauge intravenous cannula, folowed by a flush of 10mL of 0.9% normal saline solution.

2.3. Ultrasonographic examination

The scanning was performed by one investigator, who had experience in CEUS. Before administration of the contrast agent, baseline ultrasonography was performed to scan the liver thoroughly. All nodules were located less than 10cm from the body surface. When the target lesion was located, the imaging settings such as gain, depth, and focal zone were optimized. Generally, a single focal zone was placed at the bottom of the nodules to be assessed. The maximum diameter of each nodules was measured, and the liver segment location was determined according to the Couinaud [7] and Bismuth [1] classification systems. Digital cine clips were stored on the personal computer that connected to the US machine by way of a high performance, hardware-based and real-time. The CEUS's processing was divided into 3 phases following

the “Guidelines and Good Clinical Practice Recommendations for Contrast Enhanced Ultrasound (CEUS) in the Liver - Update 2012” [6], including arterial phase (start: From 10 to 20 seconds, end: From 30 to 45 seconds), portal phase (start: from 30 to 45 seconds, end: At 120 seconds) and late phase (over 120s). The enhancement patterns in the 3 phases were subdivided into hypo-enhancement, iso-enhancement, and hyper-enhancement compared with the surrounding liver parenchyma.

2.4. Histopathologic evaluation

All histopathologic specimens were reviewed by a single pathologist who was blinded to the contrast-enhanced sonographic findings. The degree of cellular differentiation (well, moderate, or poor) was categorized according to the World Health Organization Classification [9]. Of these 135 HCCs, 49 nodules had well-differentiated (36.3%), 68 nodules had moderately differentiated (50.4%) and 18 nodules had poorly differentiated (13.3%). All the nodules were divided into 2 groups: Group 1 (well-differentiated hepatocellular carcinomas) and group 2 (moderately to poorly differentiated carcinomas).

2.5. Statistical analysis

A Student t test were used to compare the differences in perfusion parameters between the groups. A χ^2 test was used to compare the enhancement patterns between the groups. Quantitative data are expressed as mean ± SD. $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 16.0 for Windows software.

3. Result

Most of HCCs showed fast-in by the enhanced pattern in the arterial phase (134 of 135 nodules), only one nodule showed slow-in. Of 135 nodules, had 23 nodules (17.0%) showed hyper-enhancement in the arterial phase and iso-enhancement in the portal phase, had 93 nodules (68.9%) showed hyper-enhancement in the arterial phase and hypo-enhancement in the portal phase. Thus, the ratio of HCCs showed fast-in and fast-out were 85.9% by the enhancement in the arterial phase and iso-/ hypo-enhancement in the portal and the late phase. On the contrary, there were 19 nodules (14.1%) of HCCs with fast-in and slow-out that expression by the iso-enhancement pattern in the late phase (Table 1).

Table 1. Enhanced pattern US in phases

Phases	Enhanced Pattern, n (%)		
	Hyper-enhancement	Iso-enhancement	Hypo-enhancement
Arterial phase	134 (99.3)	0 (0.0)	1 (0.7)
Portal phase	0 (0.0)	52 (39.5)	83 (61.5)
Late phase	0 (0.0)	19 (14.1)	116 (85.9)

HCCs were found 53 nodules with incomplete enhancement pattern (39.3%), 65 nodules with heterogeneous enhancement (48.1%), 43 nodules (31.9%) with necrosis. There is a correlation between these features of HCCs and tumor size and cell differentiation: HCCs with size under 20mm had 1 nodule with heterogeneous (6.7%), 1 nodule with incomlpete enhanced pattern (6.7%) and zero with necrosis. These characteristics were higher in the tumor group of 20mm to 40mm and from 40mm or more with the ratio of 18.0% and 78.6% (heterogeneous enhancement), 12.0% and 65.7% (incomlplete enhanced pattern), 2.0% and 60.0% (necrosis) (Table 2).

Table 2. The correlation between size, pathologic differentiation and enhanced pattern

HCC	n	Contrast enhanced ultrasound, n (%)		
		Heterogeneous enhancement	Incomplete enhanced pattern	Necrosis
Total	135	65 (48.1)	53 (39.3)	43 (31.9)
Size				
< 20mm	15	1 (6.7)	1 (6.7)	0 (0.0)
20 - < 40mm	50	9 (18.0)	6 (12.0)	1 (2.0)
≥ 40mm	70	55 (78.6)	46 (65.7)	42 (60.0)
Differentiated				
Well	49	19 (34.7)	16 (32.7)	19 (18.4)
Moderately	68	35 (51.5)	26 (38.2)	25 (36.8)
Poorly	18	13 (72.2)	11 (61.1)	9 (50.0)

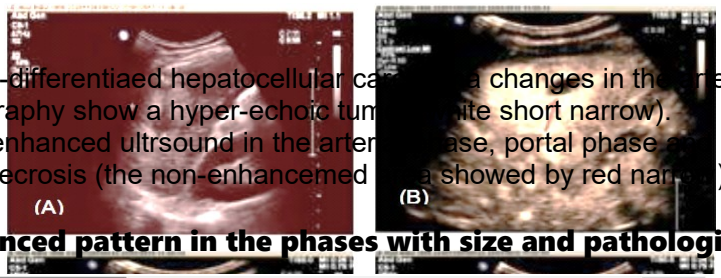


Figure 1. The well-differentiaed hepatocellular ca[illegible]a changes in th[illegible]erial and portal phase.
A: Baseline sonography show a hyper-echoic tum[illegible]ite short narrow).
B, C, D: Contrast enhanced ultrsound in the arter[illegible]ase, portal phase [illegible] late phase: The tumor with intratumoral necrosis (the non-enhancemed [illegible]a showed by red nar[illegible]).

Table 3. Enhanced pattern in the phases with size and pathologic differentiation

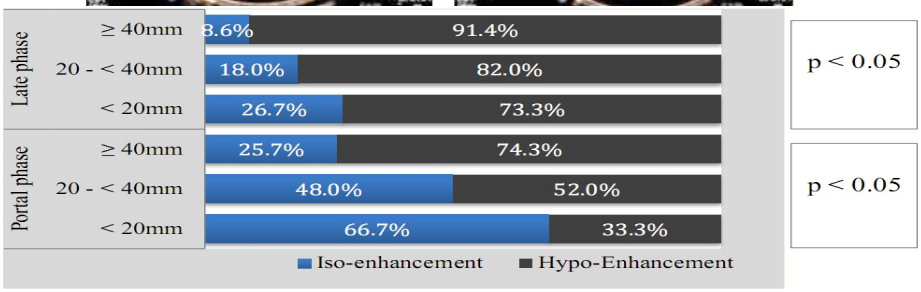
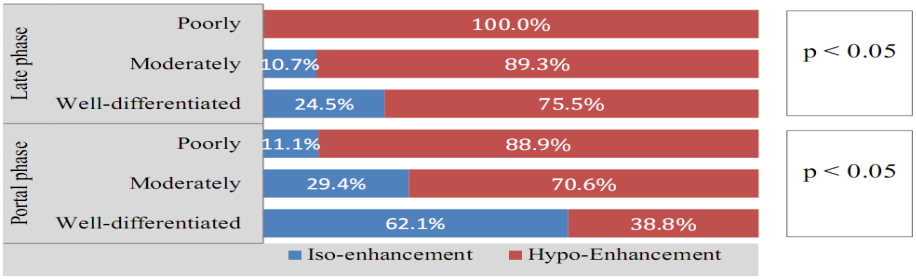


Table 4. Enhanced pattern in the phases with pathologic differentiation



Studying of the correlation between the HCCs group's size and enhancement in the phases. In the portal phase, the ratio of the nodules with hypo-enhancement pattern in the size under 20mm, from 20 to under 40mm and from 40mm or more were 33.3%, 52.0% and 74.3%. There were the differentiation between HCCs with level's size from hypo-enhancement pattern in the portal phase with statistically significant (p=0.0002) (Table 3). The similar to the group of HCCs with diferentiated (well, moderlately and poorly), the ratio of hypo-enhancement pattern in the well-differentiated, moderately and poorly were 38.8% (19 of 49 nodules), 70.6% and 88.9% (p=0.0001) (Table 4).

So that, using the hypo-enhancement pattern in the portal phase to assess the prognosis

of hepatocellular carcinoma malignancy in the study, the moderately and poorly differentiated were 64/86 (74.4%), the well differentiated hepatocellular carcinoma were not excluded in intravenous phase with homogeneous images of 30/49 nodules (61.2%).

4. Discussion

Hepatocellular carcinomas is a hypervascular tumor, so the contrast agents will rapidly infiltrate in the arterial phase after intravenous injection. The rate of HCCs with presented contrats agents in this study were 99.3%, both in the central and peripheral areas, only one nodule had non-enhanced pattern (0.7%). In this study, the nodules had non-enhanced pattern that confirm hepatocellular carcinoma with well-differentiated by histopathology, in previous studies showed this features. Blondi et al reported that almost all hepatocellular carcinoma showed intratumoral enhancement in the arterial phase, however some of them showed hypovascular. Blondi et al found 4 of 43 nodules with the size under 20mm (9.3%) showed non-enhancemend in the arterial phase and this rate decreased to 0% when the tumor increased over 20mm [2]. On pathology, Choi et al had found that the tumor vascular involved in tumor size and developmental stage [5].

In this study we found that the HCCs enhanced heterogeneous with 48.1%, incomplete enhanced pattern with 39.3% and the hepatocellular carcinoma with necrosis were 31.9%. Researchs' pathology of hepatocellular carcinoma demonstrated that hepatocellular carcinoma's enhancement with heterogeneous, incompletely and necrosis related to fibrosis, haemorrhage and necrosis and these characteristics changed according to the development of the tumor shown by the tumor size and the differentiations. The tumor with necrotic and haemorrhagic areas is not present so the contrast agents were not presented, and the fibrotic areas are less vascular than other areas are hypervascular so that the intensive of contrats agents less density than others [3], [4]. The ratio of hepatocellular carcinoma with heterogeneous, incomplete and necrotic increased by the tumor size and the differentiaed cells (Table 2), this issues was also mentioned by Choi et al [5].

Hepatocellular carcinoma showed enhancement fast-in and fast-out by the hyper-enhancement in the arterial phase (99.3%) and hypo-enhancement in the portal phase (62.5%) and in the late phase (85.9%) (Table 1). By Mohamed AM, this result is due to the hepatocellular carcinoma being supplied mainly by the arteries and the intratumoral abnomal arteries as: Morphology, vascular structure, flow's velocity, resistance, intratumoral arteriovenous shunt… [12]. So that hepatocellular carcinoma enhanced faster and earlier than parenchymal liver. The fast wash-out of the contrast agents in the hepatocellular carcinoma related to the tumor size and differntiation cells expressed by difference in hypo-enhancement in the portal phase (Table 3, Table 4). The tumor with large size have more malignant and its ratio of intratumoral arteriovenous shunt is higher than small tumor, this is one of the causes made the big hepatocellular carcinoma wash-out faster. According to Mohamed the intratumoral arteriovenous shunt rate increases in proportion to the degree of malignancy [12]. Besides, the role of Kupffer cell also affects the wash-out, following by Inoue et al showed that the tumors with iso-enhancement had more Kupffer cells than the tumors with hypo-enhancement in portal phase [10].

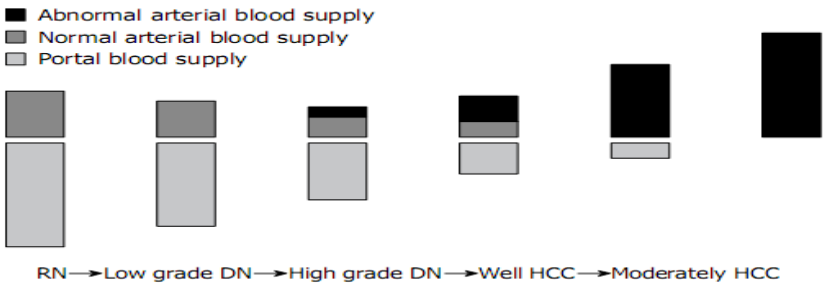


Figure 2. The changes in intranodular blood supply with the progression of hepatocarcinogenesis from dysplastic nodule to hepatocellular carcinoma (RN: Regenerative nodule, DN: Dysplastic nodule, HCC: Hepatocellular carcinoma).

Hepatocellular carcinoma usually occurs with liver cirrhosis. Carcinogenesis is often a multistep progression in cirrhosis, from a cirrhotic nodule to a macroregenerative nodule, to a high-grade dysplastic nodule, and to a low-grade dysplastic nodule then to frank hepatocellular carcinoma [8]. Progression along the multistep pathway is characterized by cytologic and architectural changes [5].

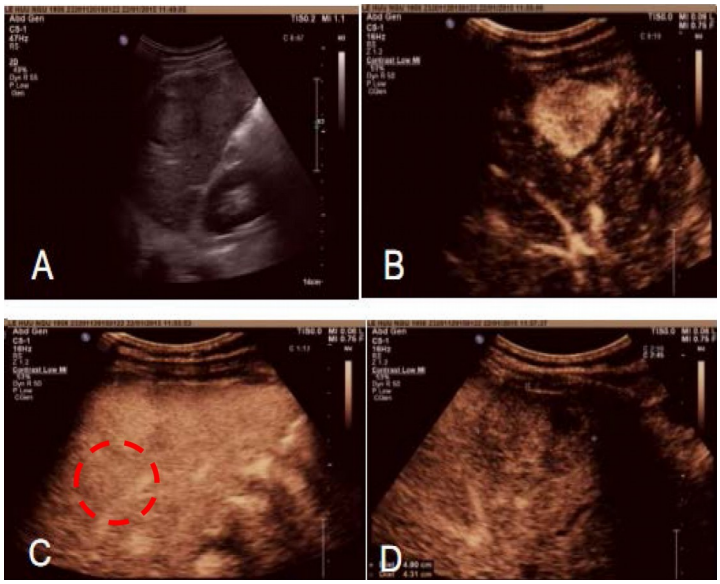


Figure 3. The well differentiated hepatocellular carcinoma changes in the arterial and portal phase.
A: Liver tumor with conventional US.
B: Contrast enhanced ultrsound in the arterial phase: The tumor with hyper-enhancement.
C: Contrast enhanced ultrsound in the portal phase: The tumor iso-enhancement bordered by dotted red circle.
D: Contrast enhanced ultrsound in the portal phase: The tumor hypo-enhancement.

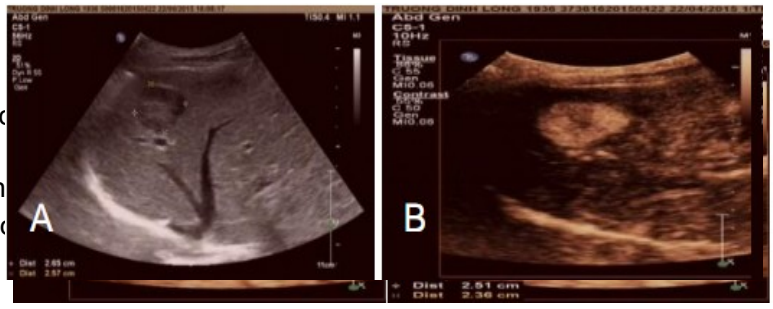


Figure 4. The mod arterial and portal phase
A: Liver tumor with
B: Contrast enhanc ncement (short narrow).

C, D: Contrast enhanced ultrasound in the portal phase: The tumor hypo-enhancement.

Results showed that the excretory of moderately and poorly differentiated HCCs was faster than that of well differentiated HCCs (p=0.001). Using this feature for evaluating the level of malignancy of HCC in order to predict (distinguish between high-grade and low-grade HCC), we found that the value of the diagnosis with the sensitivity was 74.4%, the specificity was 62.1% and the positive predictive value was 77.1%

So that, the grade of tumor cell differentiation is an important prognostic factor for hepatocellular carcinoma, and histopathologic examination is the only way to clarify the differentiation. With the development of second generation sonographic contrast agents and contrast specific imaging techniques, real-time contrast-enhanced sonography can be used to visualize lesion perfusion.

Contrast enhanced sonography has become a useful and less invasive method for characterizing and evaluating the intranodular blood supply of focal liver lesions, and it can indicate pathologic features of hepatocellular carcinomas preoperatively.

5. Conclusion

The HCCs nodules showed a “fast-in” and “fast-out” enhancement pattern. The wash-out phase correlates with HCCs’ size and plays an important role in predicting the differentiation of HCCs.

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