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● *Original Contribution*

THE RELATIONSHIP BETWEEN ENHANCED INTENSITY AND MICROVESSEL DENSITY OF GASTRIC CARCINOMA USING DOUBLE CONTRAST-ENHANCED ULTRASONOGRAPHY

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Abstract—The purpose of this study was to assess the value of double contrast-enhanced ultrasound (DCUS), in which intravenous microbubbles are used together with an oral contrast agent as a method to evaluate the microvascular density (MVD) of gastric cancer, and its relationship with the contrast-enhanced intensity (EI) in gastric carcinomas. Sixty-nine patients with gastric cancer were examined preoperatively using DCUS. The arrival time (AT), time-to-peak (TTP), peak (PI) and baseline (pre-injection) intensities (BI) of gastric carcinoma and normal gastric wall were measured. Contrast-enhanced intensity (PI minus BI) was calculated. A monoclonal antibody against CD34 was used to display vascular endothelial cells in the resected gastric carcinoma specimens and in normal gastric mucosal tissues, and MVD was calculated by counting CD34-positive vascular endothelial cells. The differences in AT, TTP, EI and MVD between gastric carcinoma specimens and normal gastric wall tissues were evaluated using Student's *t*-test. The relationships between EI and MVD in gastric cancer were analyzed by Spearman rank correlation analysis. Both EI and MVD were significantly higher in gastric carcinomas than in normal gastric wall ($p < 0.001$). However, AT and TTP showed no significant differences between gastric carcinoma specimens and normal gastric wall tissues ($p > 0.05$). There was a strong positive linear correlation between EI and MVD in gastric carcinoma ($r = 0.921$, $p < 0.001$). Double contrast-enhanced ultrasound is a useful method for evaluation of the MVD in gastric carcinomas *in vivo*. Contrast-enhanced intensity has a strong positive linear correlation with MVD and could form a new index for assessing angiogenesis and the biological behavior of gastric carcinomas. (E-mail: huangpintong@126.com) © 2009 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Microvessel density, Gastric carcinoma, Contrast-enhanced ultrasonography.**

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide. Approximately 600,000 new cases are diagnosed each year and almost two thirds of these patients will die of their disease (Ferlay et al. 2004). Most cases (65 to 75%) of gastric cancer occur in developing countries. In many regions of Eastern Europe and East Asia, gastric cancer is the leading cause of cancer death. The countries with the highest incidence rates (more than 40 new diagnoses per 100,000 individuals per year) are Korea, Japan, Chile, Belarus, Kazakhstan, China and Costa Rica (Corella and Guillen 2001; Parkin et al. 2002). Despite recent advances in diagnostic techniques for early detection and improved surgical treatment, gastric carcinoma remains the second most common cause of death from cancer worldwide (Parkin et al. 2005).

Tumor angiogenesis is necessary for tumor growth because it is the means of supplying oxygen and nutrients, and it is important for metastasis because it increases the opportunity for tumor cells to enter the circulation (Folkman 1990). Many earlier studies have documented a positive correlation between tumor microvessel density (MVD) and tumor aggressiveness in malignancies (Albo et al. 1994; Porschen et al. 1994; Williams et al. 1994).

Conventional transabdominal ultrasound is considered unsuitable for the detection and staging of early gastric lesions and it has been limited to the evaluation

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of T and N staging (Nuernberg et al. 2007). Ultrasonography with an oral contrast agent has been used widely to detect gastric cancer in China. In the present study, we sought to assess tumor MVD in gastric carcinoma specimens *in vivo* using double contrast-enhanced ultrasonography (DCUS), in which an oral ultrasonic contrast agent is combined with an intravenous contrast agent.

MATERIALS AND METHODS

Patients

Eighty patients with gastric cancer proven by pathology were examined with DCUS preoperatively between October 2006 and December 2008. Six cases were excluded because of unresectable lesions and another five cases because the goodness of fit (GOF) of the time-intensity curves was less than 0.75. The remaining 69 patients were enrolled in the study. Surgical resections were performed within 3–5 d after the DCUS examination. None of the patients in this study received nonsteroidal antiinflammatory drugs, chemotherapy, radiotherapy or immunotherapy before surgery. Informed consent was obtained from all patients before their examination, and the hospital ethics committee approved this study (Ethics No.2006-02).

Ultrasonography

Double contrast-enhanced ultrasonography was performed after fasting for at least 6 h; atropine sulfate (0.05 mg/kg) was administered *via* intramuscular injection 30 min before the examination to inhibit gastric peristalsis. An Acuson Sequoia 512 system (Siemens, Mountain View, CA, USA), equipped with a 4V1 vector™ transducer (frequency 1.0 to 4.0 MHz) and contrast pulse sequencing (CPS) technology with auto-tracking contrast quantification (ACQ) software was used (Phillips and Gardner 2004).

The oral contrast agent Xinzhang® (Huqingyutang, HangZhou, China) was supplied as a powder composed of a type of soya derivative (48 g per package); it was reconstituted by adding 500 mL of boiling water and gently agitating the water by hand to form a homogeneous suspension. It dilates the stomach and displaces the air it contains so that the lumen appeares as a homogenous acoustic window that lasts for 60 min (Fig. 1).

The distal esophagus and the cardia of all patients were observed in real time while the patients ingested the oral agent. Then the remaining parts of the stomach and the duodenal bulb were examined in turn with the patient in a variety of body positions. When a suspected lesion was identified, it was measured routinely. If the lesion was smaller than 5 cm in maximum dimension, the scanner's zoom control was used to improve the spatial resolution (Fig. 2). After the B-mode examination,

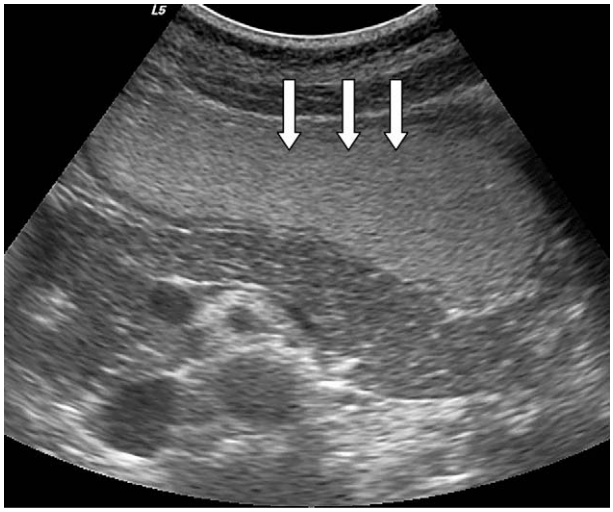

Fig. 1. The gastric lumen appeares as a homogenous acoustic window (arrows) after taking Xinzhang® oral contrast agent in normal case.

dynamic real-time contrast-enhanced sonography was performed using the CPS mode at a frequency of 1.5 MHz and an acoustic power of –15 to –21 dB. This resulted in a low mechanical index (0.20), which minimized microbubble disruption.

Contrast-enhanced sonographic studies were performed after the administration of 2.4 mL of SonoVue (Bracco SpA, Milan, Italy) as a bolus injection *via* a 19-gauge peripheral venous cannula, followed by a 10-mL saline flush. A timer on the sonography unit was activated at the beginning of the injection, and the entire movie sequence (at least 5 min) was stored on magnetic optical disks for analysis. The contrast study could be repeated a second time if necessary.

Image analysis

The cine loops of the 69 lesions were reviewed retrospectively by two independent radiologists who are experts in sonography and microbubble contrast agents. These two reviewers were unaware of the definitive diagnosis and other imaging information at the time of the analysis. The clip loop of each patient was automatically divided into four segments, and the first segment (during the arterial phase) was used. Regions-of-interest (ROI) of similar size were drawn by each observer on the tumor and the normal gastric wall, taking care to avoid the lumen and perigastric tissues (Fig. 3). To allow for the effects of movement from the patient's breathing, the ROI was adjusted manually frame by frame as necessary, and a goodness-of-fit above 0.75 was considered acceptable. The arrival time (AT), time-to-peak (TTP), basal (BI) and peak intensities (PI) within the tumors and the normal gastric wall were computed by the built-in software (Fig. 4). The enhanced intensity (EI) was calculated as PI minus BI.