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Translation of the article

THE FIRST EXPERIENCE OF CONTRAST-ENHANCED ULTRASOUND IN EPITHELIAL RECTAL TUMORS

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AIM: to evaluate the diagnostic capabilities of contrast-enhanced ultrasound for the diagnostics of epithelial rectal tumors.
PATIENTS AND METHODS: the study included 15 patients, who underwent endorectal ultrasound and transvaginal contrast-enhanced ultrasound. All the patients underwent surgery.
RESULTS: morphology revealed adenomas in 9 cases, and adenocarcinomas with different invasion depth in 6 cases.
When analyzing the tumors contrast, significant differences in arrival time of contrasting between adenomas and adenocarcinomas were obtained (p = 0.041), and the contrast enhancement of adenocarcinomas was faster (p = 0.036).
Negative correlations of peak intensity of contrast enhancement of hypoenhanced zones in adenocarcinoma with indices T (rxy =-0.781; p=0.001) and N (rxy=-0.519; p=0.047) and a positive correlation with the tumor differentiation degree (rxy=0.742; p=0.002) were established. Also, the negative correlation of the arrival time with the index T (rxy=-0.552; p=0.033) was found.
CONCLUSION: contrast-enhanced ultrasound is an imaging technique that allows real-time qualitative and quantitative assessment of tumor tissue perfusion. The method is not standardized, but it can be a useful non-invasive method for assessing the blood supply of rectal tumors at the preoperative stage, and also has the potential to assess risk factors for lymphogenic metastasis.

[Key words: contrast-enhanced ultrasound, rectal cancer, rectal adenoma]

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INTRODUCTION

Contrast-enhanced ultrasound (CEUS) is a new method for evaluating the vascularization of tumors of various localization, based on the use of stabilized gas microbubbles and the dependence of the image intensity on the concentration of bubbles in the vascular network.

The first contrast agent officially registered in 1982 was Echovist (Schering, Berlin, Germany), which had a low stability of microbubbles and was used mainly in echocardiography.

Further, drugs with a longer period of action were developed and introduced into practice, which are now used for the study of organs of the abdominal cavity and pelvis, breast and thyroid glands, as well as used in the study of transplants and abdominal injuries [1]. In addition, there are single reports of the use of CEUS in the diagnosis of neoplasms of the liver, pancreas and breast, as well as other organs [2-5].

Currently, the most widely used contrast agent of the second generation is SonoView® (Bracco, Milan, Italy), consisting of per fluorocarbon gas (sulfur hexafluoride) stabilized with surfactants.

Micro-bubbles of gas are formed by mixing saline solution with lyophilizate and remain stable in the vial for several hours. The gas fraction in the vial is sulfur hexafluoride, and the lyophilized powder is made from a combination of pharmaceutically high-quality polyethylene glycol, phospholipids and palmitic acid.

Due to this combination, the drug has a low frequency of side effects [6]. The development of drugs with a more stable microbubble membrane continues [7].

The use of contrast agents in the diagnosis of colorectal cancer has not been carried out yet.

AIM

The aim of our study is to evaluate the diagnostic capabilities of contrast-enhanced ultrasound in the diagnosis of rectal epithelial tumors.

PATIENTS AND METHODS

Fifteen females at an average age of 65±11 (M±σ) years with histologically confirmed glandular tumors of the rectum were included in the pilot study.

In 3/15 (20%) patients, the tumor was in the low rectum, in 9/15 (60%) – in the middle rectum and in 3/15 (20%) – in the upper ampullary rectum. The average distance of the tumor from the anal verge (M±SD) was 9.6±4.1 cm, and the average size of the tumor (M±SD) was 44±15 mm in diameter.

All the patients at the preoperative stage underwent multiparametric endorectal ultrasonography (ERUS) and transvaginal ultrasonography (US) with contrast enhancement. The exclusion criteria were the recurrent tumor and chemoradiotherapy at the preoperative stage.

All the patients gave their written consent to participate in the study and to have their personal data processed.

Ultrasound was performed on a Hi Vision Preirus device (Hitachi®, Japan) with pre-installed software for contrast-enhanced studies. ERUS was performed using multifrequency biplane rectal sensor with a frequency of 5-10 MHz, transvaginal ultrasound - with the help of intracavitary miniconvex sensor with a frequency of 4-8 MHz.

Previously, the intestinal lumen was cleansed with low-volume enemas.

The first stage was multiparametric ERUS, which included the assessment of the tumor in B-mode, energy dopplerography, and compression elastography.

At this stage, the site, structure and shape of the tumor, the depth of invasion, the preservation of angioarchitectonics, as well as a qualitative and quantitative assessment of the stiffness of the neoplasm tissue were evaluated.

At the next stage, the transvaginal ultrasound of the tumor with intravenous contrast-enhanced agent of sulfur hexafluoride lyophilisate was performed.

Through a cannula, 2.4 ml of SonoVue® contrast agent (Bracco, Milan, Italy) was bolus-injected into the cubital vein, followed by 10 ml of saline.

The device settings were identical for each patient with a frame rate of 15 frames per second and a mechanical index of 0.07.

Two video loops were recorded for 2 minutes each: recording the periods of accumulation and washing out the contrast agent and recording the study with sequential activation of the "flash" and "maximum intensity" modes (IMAX). The resulting video loops were stored on the hard disk of the device for subsequent qualitative and quantitative analysis of tumor contrast at the end of the study.

Time was measured from the moment of drug injection; contrast (signal amplification) was observed in real time. When analyzing the periods of accumulation and washing out of the contrast agent, the regions of interest (ROI) were selected manually for subsequent construction of the time-intensity curve (TIC) and calculation of the tumor contrast parameters: peak intensity (PI), time before contrasting - arrival time (AT), time to peak (TTP), accumulation time - wash-in-time (WIT) (Fig.1,2).

Ultrasound was performed for analysis of the study recording using the Flash and IMAX modes. The tumor contrast was evaluated by degree (hypo- or hypercontrast enhancement) and homogeneity (homogeneous or heterogeneous contrast enhancement). The Flash mode causes simultaneous destruction of microbubbles and subsequent re-distribution of the contrast agent through the vessels, which was later used to apply the peak intensity mode. The IMAX mode allows summation of signals from the contrast agent during the time period when this mode is enabled.

All the patients were operated on depending on the tumor site. In 7/15 (46.7%) cases, radical transabdominal procedures were performed (low anterior resection). Eight patients (53.3%) underwent organ-preserving procedures: endoscopic dissection in the submucosal layer (ESD) or transanal endomicrosurgical removal (TEM).

Statistical data processing was performed using IBM SPSS v.23.0 software. Two-way exact Fisher test was used to compare binary parameters, and Mann-Whitney U-test was used to compare medians.

In order to reveal the correlation between the parameters presented by quantitative data, the Spearmen correlation test was done.

The obtained differences were considered significant at $p<0.05$.

RESULTS

The pilot study included 15 females whose gender restrictions were related to technical support and the ability to perform ultrasound with contrast enhancement only using a transvaginal miniconvex sensor.

Preoperative biopsy revealed signs of adenomas in 9 cases, and signs of glandular cell atypia characteristic of adenocarcinomas in 6 cases.

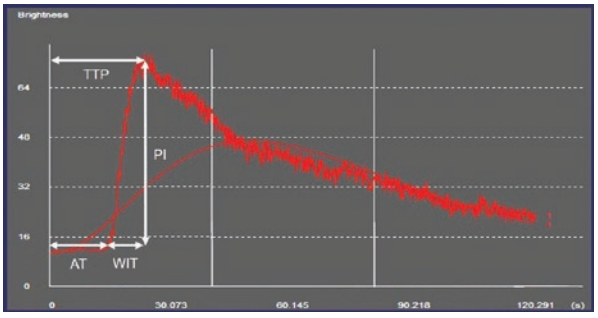

Figure 1. *Time-intensity curve (TIC) from region of interest (ROI) within the tumor and the ultrasound TIC parameters. Peak intensity (PI), Arrival time (AT), time to peak enhancement (TTP), wash-in-time (WIT)*

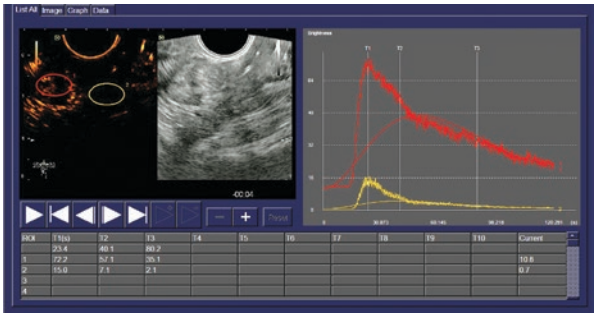

Figure 2. *Echogram of rectal adenocarcinoma. Time-intensity curves of the selected contrast zones: ROI 1 is highlighted in red, and ROI 2 is highlighted in yellow. The table shows the values of enhancement at various points of the study – the peak intensity at 23.4 seconds (PI ROI1 72.2 dB, PI ROI2 15.0 dB)*

The final pathomorphology of removed specimens detected adenomas in 9/15 (60%) with moderate (6/9 (66.7%) or severe (3/9 (33.3%) epithelial dysplasia.

6/15 (40%) female patients showed adenocarcinomas, which had moderate differentiation in 5/6 (83.3%) cases and high differentiation in only 1(16.7%). In 2/6 (33.3%) cases, histology of the specimens revealed the presence of tumor necrosis sites.

When multiparametric ERUS was performed, in 11/15 (73.3%) cases a conclusion was made about the presence of a malignant tumor, and the final pathomorphological study confirmed the presence of adenocarcinoma in 6/15 (40%) cases. Thus, the results of preoperative ultrasound and postoperative pathomorphology did not coincide in 33.3% of cases.

When analyzing the nature of tumor contrast in the maximum enhancement mode, 5 (83.3%) of the six adenocarcinomas had heterogeneous contrast with the presence of a hyperenhanced contrast peripheral zone and a hypoenhanced areas of various sizes located centrally (Fig.3). In 1/6 (16.7%) cases, the tumor had homogeneous contrast. In contrast, in 7(77.8%) of 9 patients with adenomas, the tumor had a homogeneous hyperenhanced contrast image, in 2/9 (22.2%) cases, heterogeneous contrast was obtained with the presence of a small hypoenhanced contrast area in the lower pole (Fig.4). When comparing adenomas and adenocarcinomas, significant differences were obtained depending on the contrast pattern (p=0.041).

In 7/9 adenomas (77.8%), a hyperenhanced central vessel was visualized, which is well differentiated against the background of the surrounding hyperenhanced contrast tumor tissue (Fig.5). Among the cases of adenocarcinomas, this pattern of contrast enhancement was noted in no cases. When comparing the data obtained, significant differences were revealed (p=0.007).

When analyzing quantitative indicators of contrast enhancement, due to the pronounced heterogeneity of adenocarcinoma contrast, the peak intensity (PI) for the latter was measured twice - in the peripheral hyperenhanced zone (region of interest 1) and additionally in the central hypoenhanced zone (region of interest 2). The data obtained are presented in table 1.

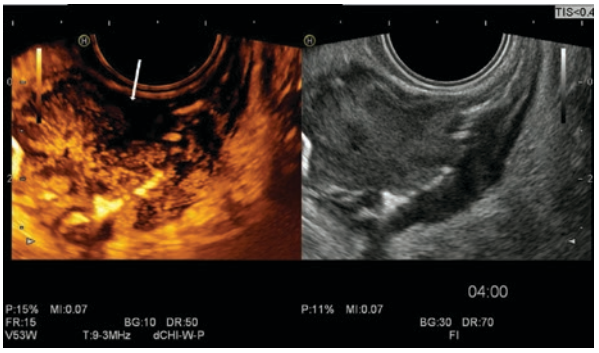

Figure 3. *Echogram of rectal adenocarcinoma with contrast-enhanced ultrasound in IMAX mode. Heterogeneous contrast was observed with the hypoenhanced central zone (arrow) and the hyperenhanced peripheral areas of the tumor*

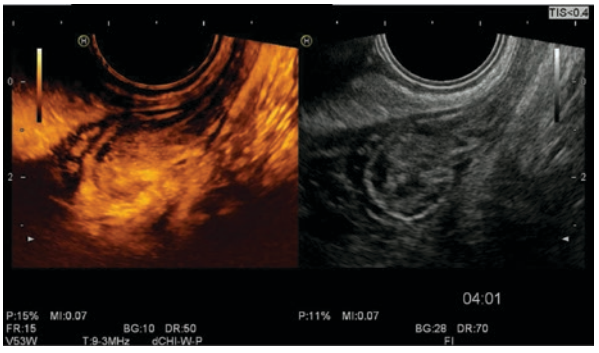

Figure 4. *Echogram of rectal adenoma with contrast-enhanced ultrasound in IMAX mode, hyper contractive homogeneous enhancement of the tumor marked*

When comparing groups by quantitative parameters, significant differences were found when comparing the level of peak intensity of contrast between adenomas and a similar indicator in the additional region of

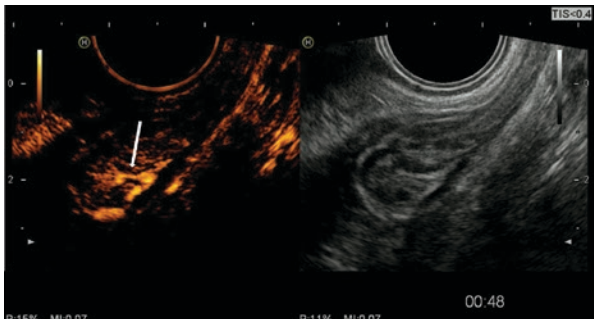

Figure 5. *Echogram of rectal adenoma with contrast-enhanced ultrasound. The arrow marks the contrasted central vessel in the tumor tissue*

interest of adenocarcinomas (p<0.001).
There were also significant differences between the groups when they were compared in terms of AT (p=0.036). Adenocarcinoma contrast occurred faster than adenoma contrast (medians were 15 and 23 seconds, respectively). There were no significant differences in WIT and TTP between adenomas and adenocarcinomas, but the lack of significance is probably due to a small sample of patients.
Significant negative correlations of peak contrast intensity in the additional region of interest were found with the indices T (p=-0.781; p=0.001) and N (p=- 0.519; p=0.047), and a direct correlation with the degree of tumor differentiation (p=0.742; p=0.002) (Table 2).
The greater the depth of invasion and the lower the degree of differentiation of adenocarcinomas, the sparser contrast is in the central hypoenhanced contrast areas of the tumor.
Also, a significant negative correlation was found between the arrival time of the tumor contrast (AT) and the index T (p=-0.552; p=0.033), that is, the greater the depth of invasion, the earlier the tumor tissue contrast occurs.
No reliable correlations were obtained for other parameters.

DISCUSSION

Angiogenesis is a critical process of tumor growth and metastasis resulting from the interaction of numerous growth factors and signaling molecules that lead to activation of endothelial cells and the formation of new blood vessels.
Currently, the standard method for quantifying angiogenesis is immunofluorescence analysis of intra tumor microvessel density, which determines the number of vessels per unit volume.
Later, using contrast-enhanced ultrasound, it became possible to quantify perfusion based on the analysis of time-intensity curves (TIC), which reflect changes in the signal intensity of the region of interest over time [8].
Methodologically, contrast-enhanced ultrasound can be performed in several ways:
1. After bolus injection of a contrast agent, a video recording of the image of the object under study is made in one plane during the entire period of contrast.

Table 1. *Quantitative parameters of contrast-enhanced ultrasound in the study groups*

Parameters of CEUS	adenocarcinoma n = 6	adenoma n = 9	p
	Me (Q1:Q3)*	Me(Q1:Q3)*	
Peak intensity of ROI 1. dB	66.3 (39.2:72.1)	47.0 (35.0:65.9)	0.6
Peak intensity of ROI 2. dB	14.9 (9.9:15.2)		<0.001
AT (arrival time). s	15.0 (14.3:18.0)	23.0 (22.0:23.0)	0.04
WIT (wash-in-time). s	15.2 (12.4:17.4)	14.0 (10.0:24.0)	0.9
TTP (time-to-peak). s	29.7 (25.6:35.4)	39.0 (33.0:47.0)	0.1

**Me-median. Q1-Q3 - 1 and 3 quartiles*

Table 2. *Correlations time-intensity curve parameters with histologic grade. T and N stage*

Parameters of CEUS	Differentiation degree		pT		pN	
	*p**	p	*p**	p	*p**	p
Peak intensity of ROI 1. dB	- 0.179	0.522	0.097	0.731	0.440	0.101
Peak intensity of ROI 2. dB	0.742	0.002	- 0.781	0.001	- 0.519	0.047
AT. s	0.358	0.190	- 0.552	0.033	- 0.480	0.070
WIT. s	0.160	0.570	- 0.166	0.554	- 0.228	0.414
TTP. s	0.413	0.126	- 0.501	0.057	- 0.445	0.097

p – Spirmen correlation coefficient*

Next, the parameters of the time-intensity curve (TIC) of one region of interest are analyzed [9].
2. The technique of contrast is similar to the first one, but in addition to the main one, the second region of interest is highlighted, which is exposed to the "control" tissue for subsequent comparison of quantitative parameters [10].
3. Intravenous administration of the drug with the analysis of contrast agent replenishment of the region of interest: the drug is administered using a pump or drip for 5-20 minutes.
First, the contrast of the object under study is displayed at the initial settings of the device with a low value of the mechanical index (MI), then MI increases briefly, causing the destruction of bubbles (Flash mode).
Immediately after this, the MI level returns to the initial level to observe the replenishment of the region of interest with a contrast agent [11].
We found the most informative study of the object image in one plane during the contrast period in combination with the study in the Flash and IMAX modes.
The first part of the study allows you to get quantitative indicators of contrast, the second-to assess its nature in detail (to get qualitative indicators).
It is important to note that the method is not standardized, since at the moment there are only few-publications about its use in the diagnostics of rectal cancer.
Most often, in the corresponding studies, the authors study only the most contrasting areas of the tumor, the analysis of the contrast parameters of hypoenhanced zones was not performed [12,13].
In a single-center prospective study by Lu M. et al., which included 172 patients with adenocarcinomas and 45 patients with adenomas, double-contrast ERUS (intraluminal and intravenous) were performed [12]. The results of the study showed that adenocarcinomas have a faster arrival time of contrast and a greater intensity of contrast compared to adenomas ($p<0.001$), which coincides with the results obtained and indicates the reproducibility of the study.
However, the authors interpreted hypoenhanced contrast areas as zones of necrosis without specifying the percentage of necrotic changes detected during the final pathomorphological study.
In our study, the presence of central hypoenhanced contrast zones was noted in 83.3% of adenocarcinomas, while necrosis zones were confirmed by histology only in a third of cases, which can be explained by the proportion of patients with advanced tumors.
Wang Y. et al. also analyzed the results of the most contrasting areas of adenocarcinomas in 66 patients and found a direct correlation between peak intensity and microvascular density (r=0.295, p=0.016) and an negative correlation with the degree of differentiation (r=-0.264, p=0.007) [13].
In the course of our pilot study, we analyzed the contrast indicators of both hyperenhanced peripheral zones and hypoenhanced central areas of the tumor.
Negative correlations of peak contrast intensity in hypocontrasted central areas (additional regions of interest) with the indices T (p=-0.781; p=0.001) and N (p=-0.519; p=0.047) and a direct correlation with the degree of tumor differentiation (p=0.742; p=0.002) were established. The lack of patterns for hyperenhanced areas is more likely due to a small sample of patients, which is the main limitation of our study.
Comparison of contrast parameters with markers of tumor angiogenesis obtained from immunohistochemical and genetic analysis is promising. In the study by Cârțână E.T. et al., which included 42 patients with adenocarcinomas, positive correlations were found between the parameters of the time-intensity curve and metastasis to locoregional lymph nodes ($p<0.05$) [14]. No other correlations were found between the CEUS parameters and the depth of invasion or the degree of tumor differentiation. In addition, no correlation was found between the calculated curve parameters and the expression of the VEGFR1 or VEGFR2 gene.
However, the authors found a positive correlation between the parameters of WIT and TTP with the density of the vasculature in the tumor, which was assessed using CD31 immunohistochemical staining with the calculation of vascular area (rxy=0.415 and rxy=0.421, respectively, $p<0.05$).

CONCLUSIONS

Contrast-enhanced ultrasound is an imaging technique that allows real-time qualitative and quantitative assessment of tumor tissue perfusion.
The method is not standardized. However, it can become a useful non-invasive method for assessing blood supply to rectal tumors at the preoperative stage, and also has the potential to assess risk factors for lymphogenic metastasis.
We consider it promising to further study the effectiveness of contrast-enhanced ultrasound in assessing tumor vascularization as a factor of regional and long-term metastasis that affects the surgical approach for rectal tumors.

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Statistical processing: *Bogdanova E.M., Khomyakov E.A.*
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