

ORIGINAL ARTICLE

Correlations of p53 expression with transvaginal color Doppler ultrasound findings of cervical cancer after radiotherapy

Pingping Wang¹, Wenqing Sun¹, Longxia Wang², Jie Gao³, Jie Zhang¹, Ping He²

¹Department of Ultrasound, Beijing Luhe Hospital Affiliated to Capital Medical University, Beijing 101149, China; ²Department of Ultrasound, Chinese PLA General Hospital, Beijing 100853, China; ³Department of Obstetrics and Gynecology, Beijing Luhe Hospital Affiliated to Capital Medical University, Beijing 101149, China

Summary

Purpose: To investigate the correlations of p53 expression with transvaginal color Doppler ultrasound findings of cervical cancer after radiotherapy (RT).

Methods: A total of 78 patients with cervical cancer (stage II and III) treated in the Oncology Department of our hospital from March 2011 to September 2017 were enrolled, and another 10 normal cervical tissue specimens were taken from the Pathology Department as controls. RT was performed to the 78 enrolled patients. Morphological features of tumor tissues after RT were detected via hematoxylin-eosin (HE) staining, the mutant p53 protein level was detected via immunohistochemistry (IHC), and imaging signs and blood flow resistance index (RI) of cervical cancer were detected via transvaginal color Doppler ultrasound. Finally, the correlations of p53 protein with transvaginal color Doppler ultrasound findings were analyzed.

Results: After RT, most cervical cancer tissues showed nuclear degeneration, karyolysis, cytoplasmic keratosis (vacuolization), and regeneration and fibrosis of cancer tissues. The expression of p53 was negative in normal cervix, while there were 48 p53-positive cases (61.54%) and 30 p53-nega-

tive cases (38.46%) in patients with cervical cancer ($p < 0.05$). No echo was detected in 2 out of 78 patients, and there were 4 cases of equal echo, 36 cases of low echo and 36 cases of high echo. Results of χ^2 test showed that the positive rate of p53 protein was significantly correlated with cervical space-occupying lesion and mass diameter shown in transvaginal color Doppler ultrasound ($p < 0.05$), but it had no significant correlation with pelvic lymph node metastasis ($p > 0.05$). The p53 protein expression level was significantly correlated with color Doppler flow imaging (CDFI) grading and RI ($p < 0.05$).

Conclusions: The p53 protein expression in cervical cancer after RT is significantly correlated with cervical space-occupying lesion and tumor size shown in transvaginal color Doppler ultrasound, CDFI grading and RI. p53 level and transvaginal color Doppler ultrasound can provide certain valuable clinical information for the treatment and monitoring of cervical cancer.

Key words: cervical cancer, p53, radiotherapy, transvaginal color Doppler ultrasound

Introduction

Cervical cancer is the second major malignant tumor in females. There are about 100,000 new cases each year in China, and its incidence rate is increased year by year, showing a trend for younger women [1]. However, the prevalence rate of cervical cancer has declined by more than 50% in developed countries since the comprehensive imple-

mentation of cervical cancer screening for 30 years [2]. In the early stages of cervical cancer, radical surgery is the preferred treatment method, but the preferred treatment means is radiotherapy (RT) for most patients in stage II and III [3]. One of the major mechanisms of radiation effect is induction of apoptosis, thereby inhibition of tumor progression [4].

p53, an important tumor suppressor gene, is located on chromosome 17p and encodes 53 kDa proteins [5]. p53 plays an important role in cell cycle regulation, which is also closely related to RT sensitivity and radiation-induced apoptosis [6]. Many researchers have clarified the prognostic significance of p53 in patients with various malignant tumors [7]. There are few studies on the correlation between p53 gene mutation after RT of cervical cancer and RT effect on cervical cancer.

In this study, the expression of p53 protein after RT of cervical cancer was detected using immunohistochemistry, the signs after RT were explored by transvaginal color Doppler ultrasound, and correlations of p53 expression with signs of transvaginal color Doppler ultrasound after RT were analyzed, so as to provide new ideas for the clinical treatment and monitoring of cervical cancer.

Methods

Clinical data

A total of 78 patients diagnosed with cervical cancer via cervical cytological examination treated in the Oncology Department of our hospital from March 2011 to September 2017 were enrolled, and they were aged 30-58 years with an average age of 50 years. There were 10 cases of contact bleeding, 10 cases of postmenopausal vaginal bleeding and 20 cases of vaginal discharge. Another 10 normal cervical tissue specimens were taken from the Pathology Department as controls. Preoperative staging and evaluation of cervical cancer included routine blood and biochemical tests, renal function examination, chest radiography, intravenous pyelography, cystoscopy and abdominopelvic CTs. Cervical cancer of all patients was staged according to the staging system of International Federation of Gynecology and Obstetrics (FIGO). There were 39 cases in stage II (IIa + IIb) and 39 cases in stage III. After biopsy, according to the pathological examination results, there were 38 cases of cervical squamous cell carcinoma and 40 cases of adenocarcinoma. All patients underwent intracavity brachytherapy and external RT in the Radiotherapy Department, followed by biopsy.

RT regimen

RT was performed for 6 weeks. In the first 4 weeks, the entire pelvis received external irradiation at a dose of 45 Gy in 20 fractions (5 fractions per week). Within 2 weeks after external irradiation, intracavity RT was performed using the high-dose-rate breach-loading afterloader (Nucletron, Netherlands) at a dose of DT1200 Gy/2 times, once a week.

Transvaginal color Doppler ultrasound

Acuson (Aspen) and Agilent ImagePoint HX ultrasonic diagnostic apparatus was used with the transvagi-

nal probe frequency of 5.0-7.5 MHz. In a bladder lithotomy position, the probe was inserted into the dome via the vagina, and the two-dimensional ultrasonic examination was performed first to observe the ovaries. Scanning was carefully conducted from the cervical outer opening to the inner opening and parametrial area to observe the size of the cervix, internal echo, cervical mucosa and partial vagina. Then, the blood flow volume was observed via color Doppler flow imaging (CDFI), and the resistance index (RI) was measured.

Hematoxylin-eosin (HE) staining and immunohistochemical (IHC) detection

A total of 78 cervical cancer biopsy specimens were fixed with 4% formaldehyde, dehydrated routinely and embedded into paraffin blocks. Paraffin blocks were sliced at 4 μ m-thick sections, followed by HE staining and histopathological observation.

Streptavidin-peroxidase (SP) staining was performed in IHC detection: Sections were dewaxed with xylene and dehydrated in gradient ethanol concentrations, followed by antigen retrieval using sodium citrate buffer solution via microwave. Afterwards 3% H₂O₂ was used to block peroxidase, sections were sealed via 10% donkey serum, and dropwise added with primary antibody (p53, Abcam, USA, diluted at 1:200) for incubation in a wet box at 4°C overnight. On the next day, sections were washed with phosphate buffered saline (PBS) for 3 times, and incubated with the ready-to-use universal secondary antibody, followed by color development via diaminobenzidine (DAB) and they were photographed under a microscope. Those with brown and dark-brown nuclei were positive cells, and the number of positive cells was counted. A ratio of the number of positive cells to the total number of cells in the field of view >5% indicated positive expression.

Statistics

All data were statistically analyzed using SPSS 10.0 software package (Chicago, IL, USA). Student's t-test was used to compare the difference in p53-positive cases between squamous cell carcinoma and adenocarcinoma. Chi-square test was used to compare the difference in p53 protein expression between the two groups, and to detect the relationships of p53 with ultrasound indexes. $p < 0.05$ suggested that the difference was statistically significant.

Results

Morphological characteristics of normal cervical tissues and cervical cancer tissues after RT detected via HE staining

No tissue atypia was found in normal cervical tissues under the microscope after HE staining. After RT, however, most cervical cancer tissues showed nuclear degeneration, karyolysis, cytoplasmic keratosis (vacuolization), and regeneration and fibrosis of cancer tissues (Figure 1).

p53 protein expressions in normal cervical tissues and cervical cancer tissues detected via IHC

As shown in Table 1 and Figure 2, p53 expression was deleted in normal cervical tissues. Among 78 patients with cervical cancer, there were 48 (61.54%) p53-positive cases and 30 (38.46%) p53-negative cases. The difference in p53 expression was statistically significant ($p < 0.05$). Among 48 p53-positive cases, there were 27 (56.25%) p53-positive cases in squamous cell carcinoma and

21 (43.75%) p53-positive cases in adenocarcinoma, the difference being not significant ($t = 0.51$, $p > 0.05$).

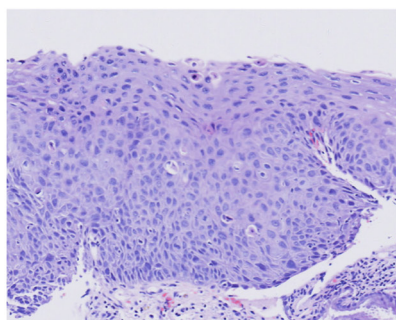
Transvaginal color Doppler ultrasound features of cervical cancer

Among 78 patients, there were 2 cases of no echo, 4 cases of equal echo (the shape was hard to be defined, the tumor echo intensity was close to that of the surrounding tissues, and the internal echo was homogeneous), 36 cases of low echo (the cervical echo was lower than that of the uterine body, mostly homogeneous, the edge of lesion was clear, and strong echo could be occasionally seen in cervical solid mass) and 36 cases of high echo (the shape was irregular, light spots were less enhanced, the edge was clear and the distribution was slightly uneven). Ultrasonic examination showed that the cervixes of the majority of patients were increased irregularly, some had shoe-like hypertrophy, and the internal echo was heterogeneous. The local cervical edge was irregular, the border

Table 1. Differences in p53 protein expression between normal cervical tissues and cervical cancer tissues

| Group | n | p53 | |
|-------------------------|----|--------------|--------------|
| | | Positive (%) | Negative (%) |
| Normal cervical tissues | 20 | 0 (0) | 20 (100) |
| Cervical cancer tissues | 78 | 48 (61.54) | 30 (38.46) |
| χ^2 | | 7.18 | |
| p | | 0.014 | |

Normal cervical tissues



Cervical cancer tissues

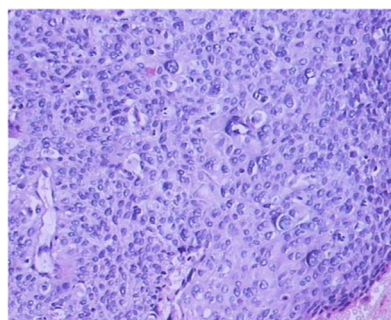


Figure 1. Morphological characteristics of normal cervical tissues and cervical cancer tissues after RT. No tissue atypia was found in normal cervical tissues. After RT, cervical cancer tissues showed nuclear degeneration, karyolysis, cytoplasmic keratosis (vacuolization), and regeneration and fibrosis of cancer tissues.

Normal cervical tissues

Cervical cancer tissues

p53-negative

p53-negative

p53-positive

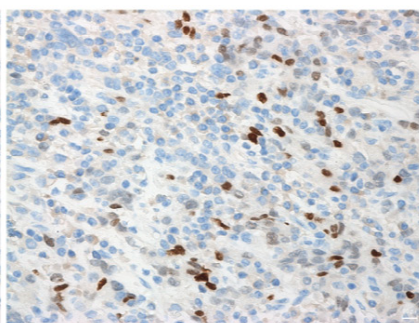
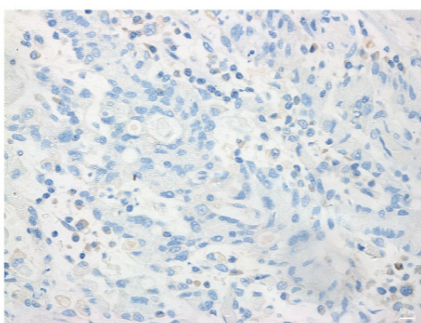
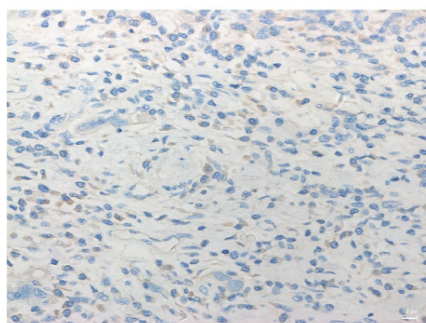


Figure 2. p53 protein expressions in normal cervical tissues and cervical cancer tissues detected via IHC. P53 expression was deleted in normal cervical tissues among 78 patients with cervical cancer. There were 48 (61.54%) p53-positive cases and 30 (38.46%) p53-negative cases.

was not clear, and sometimes specific nodules showed hyperechoic solid mass or flake plaque. Cervical canals were not shown clearly, a little tumor infiltration led to the obstruction of cervical canals, and there might be irregular anechoic areas with poor acoustic transmission (empyema or effusion) in the pelvic cavity. In CDFI, abundant blood flow signals could be seen inside the mass, often

showing high-speed low-resistance arterial blood flow spectrum.

A female patient aged 64 years suffered from lower abdominal pain accompanied with contact bleeding for 6 months. Color Doppler ultrasound showed cervical hypertrophy, messy muscular echo, visible strong flake echo and unclear cervical canal structure, and irregular echo (about 5×4×3 cm)

Table 2. Correlations of transvaginal color Doppler ultrasound features with p53 protein expression

| Ultrasound features (n=78) | n | p53 protein expression | | χ^2 | p value |
|---------------------------------|----|------------------------|-----------------|----------|---------|
| | | Positive (n=48) | Negative (n=30) | | |
| Cervical space-occupying lesion | | | | 6.206 | 0.011 |
| Yes | 53 | 38 | 15 | | |
| No | 25 | 10 | 15 | | |
| Tumor diameter (cm) | | | | 3.411 | 0.036 |
| >2 | 57 | 40 | 17 | | |
| ≤2 | 21 | 8 | 13 | | |
| Pelvic lymph node metastasis | | | | 1.427 | 0.084 |
| Yes | 32 | 22 | 10 | | |
| No | 46 | 26 | 20 | | |

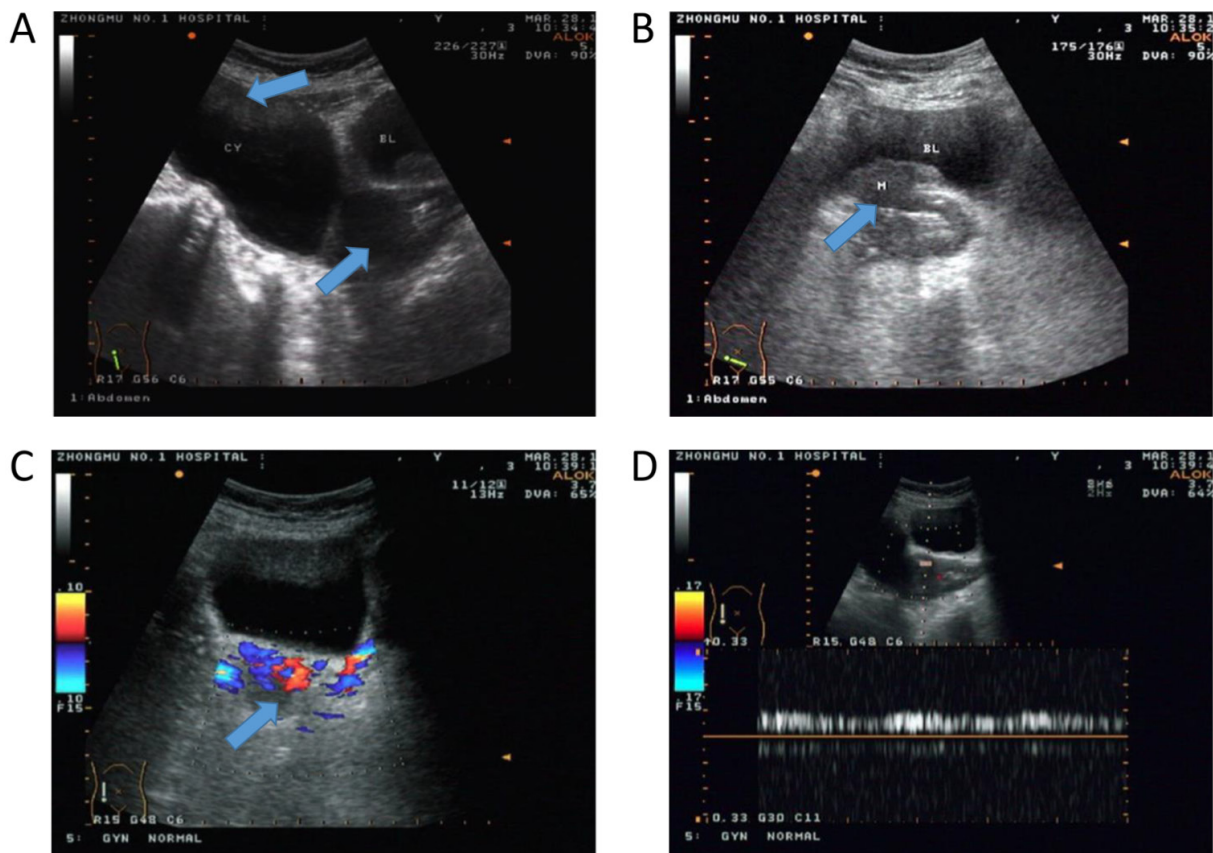


Figure 3. Transvaginal color Doppler ultrasound features of cervical cancer. **A:** Ultrasonography revealed cervical hypertrophy, muscle displayed null echoes with high-echoes, and the cervical structure was unclear (arrows); **B:** An about 5×4×3cm echoless space inside the uterine cavity (the arrow shows the tumor); **C and D:** CDFI: rich blood flow signals of internal cervical mouth, which were arteriovenous spectrum (the arrow shows the tumor); (RI: 0.37; BL: bladder; CY: cyst; m: mass).

Table 3. Correlations of p53 with CDFI grading and RI

| Ultrasound features (n=78) | n | p53 protein expression | | χ^2 | p value |
|----------------------------|----|------------------------|-----------------|----------|---------|
| | | Positive (n=48) | Negative (n=30) | | |
| CDFI grading | | | | 7.821 | 0.008 |
| 0-I | 26 | 8 | 18 | | |
| II-III | 52 | 40 | 12 | | |
| RI | | | | 2.614 | 0.026 |
| >0.4 | 33 | 15 | 18 | | |
| ≤0.4 | 45 | 33 | 12 | | |

with poor internal acoustic transmission in the uterine cavity. CDFI: abundant blood flow signals could be seen in the inner opening of uterus, showing arterial and venous frequency spectrum (RI: 0.37; Figure 3).

Correlations of transvaginal color Doppler ultrasound features with p53 protein expression

A total of 53 cases were diagnosed with cervical space-occupying lesions via transvaginal color Doppler ultrasound, the mass diameter was larger than 2 cm in 57 cases, and 32 cases had pelvic lymph node metastasis (Table 2). Results of chi-square test showed that the positive rate of p53 protein was significantly correlated with cervical space-occupying lesion and mass diameter shown in transvaginal color Doppler ultrasound ($p < 0.05$), but it had no significant correlation with pelvic lymph node metastasis ($p > 0.05$).

Correlations of p53 with CDFI grading and blood flow RI in transvaginal color Doppler ultrasound

The maximum RI of cervical tumor in patients was 0.612, the minimum RI was 0.273, and the average RI was 0.45. The p53 protein expression level was significantly correlated with CDFI grading and RI ($p < 0.05$; Table 3).

Discussion

Two major factors determine the kinetics of cell turnover in cancer. The first factor is cell proliferation, and the second is apoptosis or "programmed cell death". The imbalance of their cell signaling pathways may lead to cell transformation and cancer development [8,9]. The wild-type p53 protein is a transcription factor, which will temporarily stop cell division and repair the damaged DNA upon activation in the G1/G2 phase, and induce apoptosis when DNA damage cannot be re-

paired [10]. Some studies have shown that overexpression of p53 protein is significantly correlated with the survival time of patients with cervical cancer [11], suggesting that p53 plays an important role in RT of cervical cancer.

Since cervical cancer is mostly associated with human papilloma virus (HPV) infection, it has been reported in a significant number of studies that during the occurrence of cervical cancer, p53 is inactivated through forming a complex with HPV-E6 oncoprotein, accelerating the tumor progression [12,13]. Recent studies have shown that after cervical cancer cells are exposed to RT rays, the p53 protein level is increased significantly, leading to cell cycle arrest in the G1 phase and apoptosis [14]. A study by Kumar et al. showed that the p53 index is significantly increased in patients with cervical cancer after radiation with 27 Gy [15]. In addition, Su et al. [16] found that the mean p53 index is 6.6% in patients with cervical cancer before treatment, but it is significantly increased to 13.9% after RT, and the p53 expression is more common in cervical squamous cell carcinoma than adenocarcinoma. Moreover, Yildirim et al. [17] studied and found that no case had p53 mutation before RT, whereas 4 out of 5 cases showed p53 mutation after RT. In view of these results, they thought that the wild-type p53 protein is overexpressed before RT, the mutant-type p53 is overexpressed after RT, and the transformation process of p53 gene from wild type to mutant type is due to the effect of RT. Each p53 exon (5-8) contains frequently-mutated sites and RT can directly induce the generation of p53 mutations. However, Sultana et al. argued that RT can increase the wild-type p53 [18].

In this experiment, the positive expression of p53 in cervical cancer tissues was summarized, based on which HE staining was used to examine the histological changes after RT, IHC method was used to study the p53 protein expressions in 78 cases of stage II-III cervical cancer tissues,

and the relationship between p53 expression and transvaginal color Doppler ultrasound after RT of cervical cancer. No tissue atypia was found in normal cervical tissues by microscopy after HE staining. After RT, however, most cervical cancer tissues showed nuclear degeneration, karyolysis, cytoplasmic keratosis (vacuolization), and regeneration and fibrosis of cancer tissues. IHC results showed that p53 expression was deleted in normal cervical tissues. In this experiment, there were 48 p53-positive cases (61.54%) and 30 p53-negative cases (38.46%) among 78 patients with cervical cancer and the difference was statistically significant ($p < 0.05$). What was inconsistent with the study of Akbayir et al. [19] was that among 48 p53-positive cases, there were 27 p53-positive cases (56.25%) in squamous cell carcinoma and 21 p53-positive cases (43.75%) in adenocarcinoma, but without statistically significant difference between the two groups ($p > 0.05$).

Transvaginal color Doppler ultrasound can display the internal situation of cervical cancer, uterine cavity, bladder and rectum and its blood flow objectively and comprehensively [20]. In this study, there were no significant changes in cervical morphology in the early stage of cervical cancer, and there were no positive findings in transvaginal color Doppler ultrasound. With the progression of cervical cancer, the cervix was irregularly increased, showing rough and uneven surface. It could be seen from transvaginal color Doppler ultrasound that local cervical edge was irregular, the border was less clear, and sometimes specific nodules showed hyperechoic solid mass or flake plaque. Cervix showed shoe-like hypertrophy, and the internal echo was heterogeneous [21]. Cervical canals were not shown clearly, a little tumor infiltration led to the obstruction of cervical canals, and there might be regular anechoic areas with poor acoustic transmission in the pelvic cavity. CDFI can evaluate the blood flow signals inside the cervical cancer from a macro perspective before operation, which can be assisted by RI. In this study, CDFI showed that there were little blood flow signals in normal cervical tissues, while there were abundant blood flow signals inside the cervical cancer tissues, often showing high-speed low-resistance arterial blood flow spectrum ($RI < 0.40$) [22], as well as high-speed high-resistance blood flow spectrum when complicated with infection. Two-dimensional ultrasound shows different signs

for exophytic cervical cancer, endophytic cervical cancer, invasion of cervical cancer into the uterus and bladder [23]. With the development of ultrasound technique and accumulation of clinical application experience, transvaginal color Doppler ultrasound has become an important tool for auxiliary diagnosis of middle-advanced cervical cancer.

In this experiment, there were 2 cases of no echo, 4 cases of equal echo (the shape was hard to be defined, the tumor echo intensity was close to that of the surrounding tissues, and the internal echo was homogeneous), 36 cases of low echo (the cervical echo was lower than that of uterine body, mostly homogeneous, the edge of lesion was clear, and strong echo could be occasionally seen in cervical solid mass) and 36 cases of high echo (the shape was irregular, light spots were less enhanced, the edge was clear and the distribution was slightly uneven) among 78 patients. Fifty-three cases were diagnosed with cervical space-occupying lesions via transvaginal color Doppler ultrasound, the mass diameter was larger than 2 cm in 57 cases and 32 cases had pelvic lymph node metastasis. Results of chi-square test showed that the positive rate of p53 protein was significantly correlated with cervical space-occupying lesion and mass diameter shown in transvaginal color Doppler ultrasound ($p < 0.05$), but it had no significant correlation with pelvic lymph node metastasis ($p > 0.05$).

Conclusions

In conclusion, the p53 protein expression in cervical cancer after RT is significantly correlated with cervical space-occupying lesions and tumor size shown in transvaginal color Doppler ultrasound, CDFI grading and RI. p53 level and transvaginal color Doppler ultrasound can provide certain valuable clinical data for the treatment and monitoring of cervical cancer.

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Conflict of interests

The authors declare no conflict of interests.

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