Abstract. – OBJECTIVE: To explore the potential correlation between three-dimensional color power Doppler ultrasound (3D-CPA) parameters and high-grade cervical lesions and early cervical cancer microvessel density (MVD) and investigate the role of transvaginal three-dimensional power Doppler ultrasonography in the detection of cervical intraepithelial neoplasia.

PATIENTS AND METHODS: Totally 90 subjects were randomly divided into three groups: the control group (n = 30, including patients with chronic cervicitis), the high-grade cervical intraepithelial neoplasia (CIN) group (n = 30, mainly CIN II-III), and the early cervical cancer group (stage Ia-IIa) (n = 30). All patients received preoperative 3D-CPA, and the cervical blood flow was graded. The cervical and intra-mass parameters including vascularization index (VI), flow index (FI), and vascularization-flow index (VFI) were measured. The immunohistochemistry of the anti-CD34 monoclonal antibody was performed for the post-operative specimens obtained from each group. The MVD of the tumors was calculated. The difference of each parameter was compared among these three groups, and the correlations between the ultrasound vascular parameters and MVD were analyzed. The high-grade CIN group was followed up for 6 months after the loop electrosurgical excision procedure (LEEP) conization surgery with 3D-CPA.

RESULTS: Compared with the other two groups, the early cervical cancer group had significantly higher VI, FI, and VFI parameters (p < 0.01). Compared with the control group, all of the three parameters of the high-grade CIN group were significantly higher (p < 0.01). The MVD values increased from the control group to the high-grade CIN group, and in turn to the cervical cancer group, with significant differences between each pair (p < 0.05). MVD was positively correlated with the ultrasound parameters VI and VFI (r = 0.723, r = 0.692). There were significant differences among the three groups in terms of vascular morphology and type. However, the ultrasound parameters and vascular types were not significantly different between the postoperative CIN group and the control group.

CONCLUSIONS: 3D-CPA can be used to assess blood flow in the cervix. It is particularly useful for the early diagnosis of cervical cancer and CIN and for the postoperative follow-up of CIN.

Key words: Color Doppler, Microvessel density, Cervical cancer, Cervical intraepithelial neoplasia.

Introduction

Currently, with the application of the “three-step” cervical cancer screening technique and in-depth understanding of the pathogenesis of human papilloma virus (HPV), the diagnosis rates of high-grade cervical intraepithelial neoplasia (hereinafter referred to as “high-grade CIN”) and early invasive cervical carcinoma have gradually increased every year, particularly in younger groups. However, due to the diversity and complexity of the pathogenesis, misdiagnosis and overtreatment are still common. In recent years, the relationship between angiogenesis and tumor has been a concern among many investigators. It has been confirmed that angiogenesis is involved from the occurrence of CIN to the development of cervical...
cancer. Microvessel density (MVD) is believed to be the gold standard to reflect tumor angiogenesis. The three-dimensional color power ultrasonography (3D-CPA), increasingly applied in recent years, can reveal the spatial relationship of tumor vascular perfusion and the three-dimensional structure in an intuitive manner, and quantitative analysis is also possible using the virtual organ computer-aid analysis (VOCAL) software. Hence, it has become a noninvasive method for the evaluation of pelvic perfusion. In this study, we applied three-dimensional transvaginal power ultrasound combined with MVD measurement to evaluate the correlation between 3D-CPA ultrasound parameters and high-grade CIN and early cervical cancer, and explore the value of the 3D-CPA technology in the diagnosis and follow-up of high-grade CIN.

**Patients and Methods**

**Clinical data**

This study enrolled 30 patients who received colposcopy and confirmed as early cervical cancer (Ia-IIa) by biopsy in our clinic from January 2011 to December 2012, aged 35 to 60 years, with a mean age of 48.19 ± 8.61 years. The high-grade CIN group included 30 patients, aged 22 to 45 years, with a mean age of 36.32 ± 6.40 years; the control group included 30 patients, aged 20 to 56 years, with a mean age of 33.76 ± 10.47 years. All patients received preoperative three-dimensional power Doppler sonography; the cervicitis and CIN groups underwent loop electrosurgical excision procedure (LEEP) conization surgery; and the cervical cancer group underwent radical hysterectomy. Postoperative biopsies of all specimens and immunohistochemical MVD measurements were performed. The CIN group was followed up for 6 months after surgery with 3D-CPA. Informed consent was obtained from all patients.

**Methods**

**Instruments and reagents**

GE Voluson E8 color Doppler ultrasonic diagnostic device, with a cavity volume probe, frequency at 5-9 mHz. The VOCAL software was provided for automatically measurement of relevant vascular parameters. The pulse repetition frequency (PRF) was selected at 0.9, three-dimensional volume sampling set to high quality, and once section for every 15°, totaling 12 sections for each lesion. The enclosing contour of each lesion area was manually depicted.

**Reagents**

Streptomycin avidin-peroxidase (S-P) immunohisto-chemistry was used, with mouse anti-human CD34 monoclonal antibody as the primary antibody.

**Three-dimensional power Doppler ultrasonography**

In the grayscale mode, routine two-dimensional transvaginal sonography of the cervix was first conducted to identify the characteristics of tumor lesions, if any. The scan was conducted in the region of interest – the lesion area for patients with masses detected in the ultrasound examination or the whole cervix if no mass was detected. Three-dimensional reconstruction of the region of interest was then performed in the color power Doppler mode. While the three-dimensional structures of the cervical vascular course and branches were fully revealed, the cervical vessels were classified into the corresponding types. The classification criteria were: Type I: small, evenly distributed straight vessels; Type II: vessels with circular local thickening; Type III: vessels with visible local disorganized branches; and Type IV: vessels with uneven thickness that were twisted into spherical masses (Figures 1 and 2).

Quantitative analysis of the vessels and blood flow inside the lesions was conducted using the manual contour depicting and three-dimensional power Doppler histogram functions in the VOCAL software. The following parameters were generated: vascularization index (VI), an index of the amount of color signals in the region of interest, indicative of the number of detected blood vessels within the tissue; flow index (FI), the average density of color values or blood flow density of all blood vessels, indicative of the amount of blood cells passing through at the time of the three-dimensional scan; and vascularization-flow index (VF1), weighted color value in the region of interest, providing the combined information of existing vessels and blood flow. All patients were examined by the same ultrasound doctor using the same ultrasonic diagnostic device under the same conditions. The parameters (VI, FI, VF1) derived from the three groups of patients were compared.

**MVD measurement**

The cervical lesions from eligible patients were selected, embedded in paraffin and sliced. The specimens were measured using SP immunohistochemistry in strict accordance with the
instructions. The MVD counting was carried out following the method reported by Weidner et al.\textsuperscript{[3]} to determine the MVD based on CD34 staining counts. Specifically, for vessels with single staining-positive endothelial cells or vascular endothelial cell clusters or staining cavity of a diameter less than eight red blood cells and without base blood vessels within the tumor tissue, the area richest in microvascular tissue was selected to be observed at low magnification and in turn high magnification. Five non-overlapping visions were chosen to count the number of microvessels. Any vessel with relatively thick lumen and a diameter of more than six red blood cells was discarded. The MVD counts of all specimens were averaged as the final result.

**Figure 1.** Three-dimensional (3D) power Doppler ultrasound of a patient with stage Ib cervical cancer.

**Figure 2.** Vascular parameters obtained by VOCAL software in a patient with stage Ib cervical cancer (VI: 18.285; FI: 34.058; and VFI: 6.230).
Statistical analysis

The data were input, sorted, and verified using EpiData 3.2 and analyzed in SPSS 17.0 (SPSS Inc., Chicago, IL, USA). If the measurement data meet their application conditions, they were subject to the t-test or analysis of variance; if the data were skewed and still failed to satisfy the test conditions after parametric conversion, the non-parametric test would be used. The count data were compared using the chi-square test or non-parametric test. Relationships between variables were analyzed using the Pearson correlation analysis or the Spearman correlation analysis. \( p < 0.05 \) was considered statistically significant.

RESULTS

Cervical ultrasonography and vascular typing (Table I)

There was no significant difference in the sonographic cervical morphology between the control group and the CIN group. Increased volume of the cervix was observed in some early cervical cancer. Normal control group: the cervical blood flow was not rich, signals were rare, and most vessels were Type I, accounting for 90%; high-grade CIN group: rich regional cervical blood was seen, strip vessels or “short stick” vessels were visible, and most were Type II, accounting for 66.67%; early cervical cancer group: the cervical blood flow was rich and messy, vessels were twisted like fire balls, dominated by Type III and Type IV, while the latter accounted for 76.67%.

Postoperative ultrasound parameters and MVD values across the cervical cancer, CIN, and control groups

Compared with the other two groups, the early cervical cancer group had significantly higher VI, FI, and VFI parameters. Compared with the control group, all of the three parameters of the high-grade CIN group were significantly higher (\( p < 0.01 \)). The MVD values increased from the control group to the high-grade CIN group, and in turn to the cervical cancer group, with significant differences between each pair (\( p < 0.05 \)) (Table II).

Correlations between the ultrasound parameters (VI and VFI) and MVD measurement (Table III)

The ultrasound parameters (VI and VFI) were positively correlated with MVD measurement.

Discussion

Correlation between 3D-CPA ultrasound parameters and high-grade cervical lesions and early cervical cancer MVD

Weidner[3] first reported that the MVD that reflected tumor angiogenesis was closely related to the survival of breast cancer patients. It has been found that MVD is significantly indicative of human tumor growth, metastasis and prognosis, and can be used as an important biological predictor of tumor metastasis and prognosis[4-6]. It has been demonstrated that angiogenesis is involved from the development of CIN to the development of cervical cancer; with the progression of lesions, the MVD and a variety of cancer angiogenesis factors are increased significantly, which are closely related to the prognosis[7,8]. The TR3D-CPA technology can fully demonstrate the tumor periphery and internal vascular structure in a dynamic, three-dimensional, more intuitive and complete manner, which provides a relatively non-dependence perspective with high sensitivity and reproducibility, and can detect the course, distribution and morphology of tiny blood vessels with lower blood perfusion, and thus reflect the tumor blood supply more objectively. By using the VOCAL software, quantitative calculation and analysis of the color intensity and values of the region of interest can be achieved to reveal low blood flow changes and the distribution of blood flow in the lesions or tumor regions. The cervical vascular parameters VI, FI, and VFI can

Table I. Results of cervical ultrasonography and vascular typing in three groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer group</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>High CIN group</td>
<td>30</td>
<td>6</td>
<td>20</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>27</td>
<td>3</td>
<td>0</td>
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</table>
be automatically calculated, and images can be stored for future analysis. This study measured three ultrasound parameters of cervical cancer, high-grade CIN (preoperative and postoperative) and chronic cervicitis, and conducted correlation analysis with MVD measurement following the immunohistochemical staining. The results showed that the MVD values increased from the control group to the high-grade CIN group, and in turn to the cervical cancer group, with significant differences between each pair ($p < 0.05$). MVD values were significantly positively correlated with ultrasound parameters VI and VFI ($r = 0.723$, $r = 0.692$), suggesting that the TR3D-CPA technique could be used to evaluate early angiogenesis in high-grade CIN and cervical cancer.

**Value of 3D-CPA in diagnosis of early cervical cancer and severe CIN**

It is reported that three-dimensional power Doppler ultrasound vascular typing is correlated with MVD and vascular growth factor (VEGF) expressions. This suggests that vascular typing and VI may reflect the microscopic blood vessel expressions within a tumor [9]. In this study, semi-quantitative analysis of blood vessels and blood flow in lesions of the early cervical cancer, high-grade CIN, and control groups showed significant differences across the three groups. Rich blood flow was seen in cervical cancer, with vascular thickening, messy courses, and partial fire ball features, dominated by Type III and Type IV vessels, with Type IV accounting for 76.67%; in the chronic cervicitis group, only a peripheral portion of the cervix was associated with blood vessels, without blood flow inside the cervix, or only sparse blood vessels were seen, as Type I vessels accounted for 90%; in the high-grade CIN group, however, the vascular typing was between the other two groups, dominated by Type II, accounting for 66.67%, suggesting that there was neovascularization in the cervix. Therefore, cervical vessels could reflect the degree of cervical lesions, providing the basis for the diagnosis and differential diagnosis.

With the three-dimensional power Doppler ultrasound, ultrasound parameters VI, VFI, and FI can be measured and statistically analyzed in the VOCAL software. VI (angiogenesis index) was the cervical vascular coefficient per unit volume. As a reflection of the degree of tumor vascular blood supply, the higher it is, the richer newborn blood vessels are there within the tumor. This is consistent with our findings. The VI was significantly increased in the cervical cancer group compared with the high-grade CIN group and the control group ($p < 0.01$), while there were also differences between the high-grade CIN group and the control group. The FI and VFI values were similar to the results with VI, which was not completely consistent with Geng et al.'s

<table>
<thead>
<tr>
<th>Group</th>
<th>VI</th>
<th>FI</th>
<th>VFI</th>
<th>MVD</th>
</tr>
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<tbody>
<tr>
<td>Cervicitis</td>
<td>2.26 ± 1.37</td>
<td>30.13 ± 5.08</td>
<td>0.75 ± 0.50</td>
<td>18.41 ± 8.25</td>
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<tr>
<td>CIN</td>
<td>5.90 ± 3.61</td>
<td>32.58 ± 4.07</td>
<td>2.19 ± 2.14</td>
<td>24.73 ± 6.31</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>14.67 ± 8.17</td>
<td>36.47 ± 4.22</td>
<td>4.57 ± 2.38</td>
<td>16.5 ± 4.33</td>
</tr>
<tr>
<td>After the surgery</td>
<td>2.26 ± 1.32</td>
<td>29.53 ± 7.06</td>
<td>0.92 ± 1.31</td>
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</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.044</td>
<td>0.001</td>
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<td>&lt;0.001</td>
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<td></td>
<td>0.997</td>
<td>0.704</td>
<td>0.517</td>
<td>0.124</td>
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<tr>
<td></td>
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<td>0.001</td>
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<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.045</td>
<td>0.008</td>
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<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Table II. Postoperative ultrasound parameters and MVD values across the cervical cancer, CIN, and control groups.

Note: $P_1$: comparison between the cervicitis group and CIN group; $P_2$: comparison between the cervicitis group and cervical cancer group; $P_3$: comparison between the cervicitis group and “After the surgery for CIN” group; $P_4$: comparison between the CIN group and cervical cancer group; $P_5$: comparison between the CIN group and “After the surgery for CIN” group; $P_6$: comparison between the cervical cancer group and “After the surgery for CIN” group.

MVD

| MVD | VI Correlation coefficient ($r$) 0.723  
|     | $p$-value 0.010 |
|     | VFI Correlation coefficient ($r$) 0.692  
|     | $p$-value 0.035 | 1983
study. He believed that FI was not statistically different across the three groups, which could be a result of deviations due to manual painting of the cervical samples. Thus, further confirmation is needed. However, the results are sufficient to demonstrate that the 3D-CPA technique can be used in combination with Thinprep cytologic test (TCT) and HPV for early prediction of high-grade CIN and early cervical cancer.

Value of 3D-CPA in postoperative follow-up of high-grade CIN

Since cervical lesions are mainly treated by conization, postoperative cervical stenosis and adhesions are likely to occur. The follow-up of such lesions is currently performed using cervical cytology, HPV, colposcopy, and other similar means, with which there can be a high risk of false negative reports due to inward transition of the cervical transformation zone, adhesions at the opening and other factors that compromise the collected specimens. These will undoubtedly result in a delay or missed diagnosis of recurrent lesions. On the contrary, transvaginal three-dimensional power Doppler ultrasound can detect targeted cervical blood flow and predict tumor recurrence through the vascular parameters and vessel typing, thus alarming the clinicians to strengthen screening, and can be used as a supplementary to other measures. Ferrandina et al confirmed that a short treatment with the cyclooxgenase-2 (COX-2) inhibitor celecoxib in human cervical cancer could reduce the expression of MVD, while the two-dimensional and three-dimensional Doppler evaluation showed no significant difference in pretreated tumors versus posttreated tumors.

Conclusions

It needs further study, patients with cervical cancer using surgery or other treatment, whether the three-dimensional power Doppler ultrasound can be applied to the patient’s follow-up.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References