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Evaluation of the relationship between Doppler predictors with human papillomavirus types, smear and cervical biopsy results

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ABSTRACT

Objectives: The study aims to investigate whether there is any difference between human papillomavirus (HPV) types, smear results, and colposcopic biopsy results in terms of iliac, uterine, and cervical artery pulsatility and resistive index values in high-risk HPV positive patients.

Methods: Iliac, uterine, and cervical artery pulsatility and resistive index values were determined by pelvic Doppler ultrasonography in patients who applied for high-risk HPV positivity and underwent colposcopy-guided cervical biopsy.

Results: There was no difference between HPV types and Pap-smear results and the pulsatility and resistive indices of the iliac artery, uterine artery, and cervical artery. It was observed that the mean cervical artery pulsatility index of the patients whose colposcopic cervical biopsy result was cervical intraepithelial neoplasia (CIN) 1 was 1.61 ± 0.43 and the cervical artery pulsatility index of the patients with CIN 2-3 was 1.15 ± 0.28, and a statistically significant difference was found between them ($p = 0.038$). There was no difference between other Doppler indices and colposcopic cervical biopsy results.

Conclusions: Doppler indices such as cervical artery pulsatility index may be helpful in the evaluation of cervical cancer precursor lesions.

Keywords: Doppler ultrasonography, human papilloma virus, cervical intraepithelial neoplasia, Pap smear

Cervical cancer is the 4th most common cancer among women worldwide [1]. Almost all cervical cancers are associated with human papillomavirus (HPV). HPV 16 50%, HPV 18 20% and other high-risk types such as HPV 31, 33, 45, 52 and 58 are also responsible for 19% [2, 3]. The HPV test, cervical cytology (PAP-test), or a combination of the two tests can be used in cervical cancer screening. The colposcopic examination is the most valuable method in managing HPV positivity and/or abnormal pap-smear test results.

Angiogenesis is the production of new vessels in a specific area and is required for tumor growth and progression [4]. It has been shown that the progression of the lesion from cervical intraepithelial neoplasia (CIN) to cervical cancer is accompanied by angiogenesis [5, 6]. It has been reported that angiogenesis in cervical cancer is an independent prognostic factor and can predict recurrence [7-9].

Color Doppler ultrasonography (US) is a sonogra-

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phy technique used to evaluate blood flow in the area of interest semi-quantitatively. Pulsatility Index (PI) and Resistive Index (RI) are flow parameters used in doppler ultrasound, mainly used to assess resistive in the vascular system. It is an inexpensive, easily available, and non-invasive examination. Doppler US is an effective method to evaluate cervical carcinoma vascularization, and is associated with specific tumor characteristics and is effective in predicting therapeutic response to treatment [10].

In our study, we aimed to determine whether there is any difference between HPV types, smear results, and colposcopic biopsy results in terms of iliac, uterine, and cervical artery PI and RI values detected by Doppler US in high-risk HPV positive patients.

METHODS

Patients aged between 30-65 years, who applied to the gynecological oncology outpatient clinic due to high-risk HPV positivity and underwent colposcopy-guided cervical biopsy between June 2020 and August 2020 at Zonguldak Maternity and Child Health Hospital, were included in this prospective study. The smear and HPV information of the patients were obtained from the results of the national cervical cancer screening program that the patients had at the time of application. According to the American Society for Colposcopy and Cervical Pathology (ASCCP) guideline, a colposcopy and accompanied cervical biopsy were performed by a gynecologist oncologist (A.T.Ç.). Patients who underwent hysterectomy for any reason, patients with a history of using birth control pills or vaginal drugs, patients with a history of cervical precancerous lesions or cervical cancer, patients who underwent uterine artery embolization, or who received chemoradiotherapy were excluded from the study.

Before colposcopy, all of the patients underwent sonography. Pelvic ultrasound was routinely performed using an Acuson S3000 Ultrasound System (Siemens Healthineers, Erlangen, Germany) equipped with an 8C3 HD convex transducer probe (Siemens Healthineers, Erlangen, Germany), which has color and pulsed Doppler capabilities in the supine position. The procedure was performed after ideal bladder filling. The same radiologist (6 years of experience in genitourinary sonography) evaluated all scans and quantitative measurements and was blinded to the patient's clinical information. Additionally, Doppler parameters are standardized. Morphological evaluation of both ovaries and uterus was performed in systematic gray-scale 3D US examination; to rule out fibroids, endometriosis, adenomyosis, or tumors that may affect vascularization. Color flow Doppler was activated in all cases after morphological evaluation. First, the iliac, uterine, and cervical arteries were identified by color Doppler US, then spectral Doppler parameters were calculated automatically as resistive index (RI) and pulsatility index (PI), and the lowest parameters were used for analysis.

The study protocol was approved by the Ethics Committee of Zonguldak Bülent Ecevit University (Number: 2020/12, Date: 10/06/2020). Written informed consents were obtained from all patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows version 20 software (IBM Corp., Armonk, NY, USA). Descriptive statistics for continuous variables were expressed as mean ± standard deviation or median (minimum-maximum), whereas nominal variables were expressed as number and percentage (%). The variables were investigated using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's tests) to determine whether or not they are normally distributed. ANOVA test was used for variables with normal distribution, and Kruskal-Wallis test was used for those that did not show normal distribution. Tukey's test performed pairwise post-hoc tests when an overall significance was observed. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 45 patients aged 30-65 years who applied for high-risk HPV positivity and underwent colposcopy and cervical biopsy were included in the study. The mean age of the patients was 42.58 ± 7.31. Twenty-three (51%) patients were HPV 16+, 3 (7%) patients were HPV 18+, 19 (42%) patients were high-risk (other than HPV 16/18) HPV+. Smear results of

Table 1. Doppler indices according to HPV types

	HPV-16	HPV-18	Other HR* HPV	*p* value
IA PI	2.34 (1.94-5.16)	2.78 (2.22-2.98)	2.51 (1.62-3.42)	0.704
IA RI	0.90 ± 0.04	0.86 ± 0.09	0.89 ± 0.04	0.274
UA PI	1.97 ± 0.40	2 ± 0.09	2.10 ± 0.50	0.634
UA RI	0.81 ± 0.07	0.81 ± 0.02	0.82 ± 0.08	0.967
CA PI	1.35 ± 0.34	1.32 ± 0.25	1.43 ± 0.43	0.745
CA RI	0.72 ± 0.10	0.73 ± 0.09	0.76 ± 0.10	0.571

HPV = human papillomavirus, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index

Values are presented as median(minimum-maximum) or mean±standard deviation

*high risk

29 (64%) of the patients were normal, 8 (18%) were inflammation, four (9%) were low-grade squamous intraepithelial lesions (LGSIL), three (7%) were atypical squamous cells of undetermined significance (ASCUS), and one (2%) was insufficient. According to the results of cervical biopsy performed with colposcopy, it was chronic cervicitis in 29 (64%) cases, CIN-1 in 7 (16%) cases, and CIN 2-3 in 9 (20%) cases.

There was no difference between HPV types in terms of the iliac artery, uterine artery and cervical artery pulsatility and resistive indices (Table 1). No difference was found between Pap-smear results in terms of the iliac artery, uterine artery, and cervical artery pulsatility and resistive indices (Table 2).

When colposcopic cervical biopsy results and iliac artery, uterine artery, and cervical artery pulsatility and resistive indices were examined; the mean cervical artery pulsatility index of the patients with CIN-1 was 1.61 ± 0.43, and patients with CIN 2-3 was 1.15 ± 0.28, and a significant difference was found between them (p = 0.038). There was no difference between other Doppler indices and colposcopic cervical biopsy results (Table 3).

DISCUSSION

Cervical cancer continues to be a significant cause of cancer morbidity and mortality in countries without screening programs. HPV is the primary etiological agent of cervical intraepithelial lesion and cervical cancer, and it can be detected in 99.7 percent of cervical cancers [1-3]. Cytology and HPV or both (co-test) are used in cervical cancer screening. A direct biopsy performed under colposcopy is accepted as the gold standard method in the diagnosis of cervical intraep-

Table 2. Doppler indices according to Pap-smear results

	Normal	Chronic Cervicitis	LGSIL	ASCUS	Insufficient	*p* value
IA PI	2.61 (1.66-5.16)	2.50 (2.24-3.38)	2.22 (2.13-2.94)	2.05 (1.62-2.37)	2.78	0.246
IA RI	0.90 ± 0.04	0.92 ± 0.04	0.84 ± 0.05	0.89 ± 0.01	0.88	0.055
UA PI	2.07 ± 0.46	1.83 ± 0.30	2.31 ± 0.46	1.82 ± 0.26	1.85	0.335
UA RI	0.80 ± 0.6	0.80 ± 0.9	0.84 ± 0.5	0.92 ± 0.5	0.83	0.065
CA PI	1.40 ± 0.40	1.38 ± 0.32	1.53 ± 0.34	1.06 ± 0.36	1.45	0.588
CA RI	0.74 ± 0.11	0.75 ± 0.07	0.75 ± 0.05	0.70 ± 0.17	0.73	0.981

LGSIL = low-grade squamous intraepithelial lesions, ASCUS = atypical squamous cells of undetermined significance, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index

Values are presented as median(minimum-maximum) or mean ± standard deviation

Table 3. Doppler indices according to colposcopic cervical biopsy results

	Chronic Cervicitis	CIN-1	CIN-2,3	*p* value
IA PI	2.42 (1.66-5.16)	2.51 (1.94-2.98)	2.22 (1.62-3.45)	0.901
IA RI	0.89 ± 0.04	0.90 ± 0.04	0.89 ± 0.05	0.896
UA PI	2 ± 0.44	2.03 ± 0.23	2.11 ± 0.55	0.804
UA RI	0.8 ± 0.08	0.80 ± 0.03	0.83 ± 0.09	0.785
CA PI	1.40 ± 0.35	1.61 ± 0.43*	1.15 ± 0.28*	**0.038**
CA RI	0.73 ± 0.10	0.81 ± 0.04	0.71 ± 0.12	0.105

CIN = cervical intraepithelial neoplasia, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index
Values are presented as median(minimum-maximum) or mean±standard deviation
*$p = 0.038$ (Tukey Test)

ithelial lesions. However, misdiagnosis and over-treatment may also be mentioned.

Angiogenesis is a rate-limiting step for various pathological conditions, including cancer growth. As the tumor grows and the cells in the center of the tumor become hypoxic, the tumor begins to supply its blood requirement by shifting the angiogenesis stimulator-inhibitor balance in favor of stimulation [11]. Neovascularization is a priority and necessary for tumor progression and metastasis [12]. Angiogenesis is a complex process controlled both negatively and positively by growth factors. The dominant growth factor that regulates angiogenesis is the vascular endothelial growth factor (VEGF) [13].

Transvaginal Doppler ultrasound evaluates the vascularization of the tumor non-invasively. Studies have shown that women with cervical cancer have a lower average PI in the uterine and cervical arteries than healthy women [14, 15]. Hsieh *et al*. [16] reported that intratumoral blood flow evaluated by transvaginal color Doppler ultrasonography correlated with higher proliferation index, higher HPV infection incidence, and pelvic lymph node metastasis in cervical cancer.

Wu *et al*. [17] compared the intratumoral blood flow in the cervical cancer group with the cervical blood flow in the control group. They found that PI and RI values were significantly lower in the cervical cancer group. The authors stated that the evaluation of the intratumoral blood flow of the cervix could be helpful in the early diagnosis and treatment of cervical cancer [17].

Liang *et al*. [18] investigated the role of transvaginal 3D Doppler ultrasonography in the diagnosis of cervical intraepithelial neoplasia. Vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were found to be significantly higher in the early cervical cancer (stage 1a-2a) group than in the control group and high grade CIN group. (CIN 2-3) ($p < 0.01$). Again, when compared with the control group, the VI, FI, and VFI parameters of the high-grade CIN group were found to be significantly higher ($p < 0.01$) [18].

Ping *et al*. [19] investigated the relationship between VI and VEGF expression in three-dimensional color angiography in chronic cervicitis, CIN, and cervical carcinoma. It was observed that VEGF expression gradually increased in chronic cervicitis, CIN, and cervical carcinoma, also VI was correlated with VEGF expression level [19].

In the study conducted by Doğan *et al*. [20], HPV positive patients had higher uterine and cervical artery RI values than the control group. Additionally, they investigated the diagnostic effectiveness of uterine and cervical artery vascularity alone or combined with HPV DNA test and smear. As a result, they observed that combining cervical artery RI with high-risk HPV or smear reduced sensitivity but increased specificity. They also found that combining uterine artery PI with high-risk HPV slightly increased the positive predictivity compared to the high-risk HPV test alone [20].

Although colposcopy-guided cervical biopsy is important for early diagnosis of high-grade CIN and cervical cancer, it scares the patients and is not always accepted because it is an invasive procedure. There is a search for non-invasive examinations to reduce the

colposcopy process. In our study, we examined the difference between HPV types, smear results, and colposcopic biopsy results in terms of the iliac, uterine, and cervical artery PI and RI values detected by pelvic Doppler US, which is a non-invasive and easily tolerated examination by patients. Consistent with the literature, we found a significant difference in cervical artery pulsatility index between patients with cervical biopsy results CIN-1 and CIN 2-3.

Limitations

Our study had some limitations. The main limitation of this study was the smaller sample size (45 cases). Only one radiologist evaluated all scans; interobserver or intraobserver reproducibility could not be evaluated. Sonography was not reproducible since it was an operator-dependent and real-time examination. The data were collected from a single center. In our study, we could not distinguish between premenopausal and postmenopausal, which can affect blood circulation. Our data needs to be supported by prospective, multicenter studies with a large patient population.

CONCLUSION

If our results support multicenter studies, pelvic doppler US can be considered an important step in selecting patients to be referred for colposcopy after cervical cancer screening.

Authors' Contribution

Study Conception: ATÇ; Study Design: ATÇ; Supervision: ATÇ, AA; Funding: ATÇ, AA; Materials: ATÇ, AA; Data Collection and/or Processing: ATÇ, AA; Statistical Analysis and/or Data Interpretation: ATÇ, AA; Literature Review: ATÇ; Manuscript Preparation: ATÇ and Critical Review: ATÇ, AA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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