



The Effect of Cryotherapy and Local Pharmacological Treatment on Eradication of Highly Oncogenic HPV and Lesions on the Cervix

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Abstract

Persistent infection with highly oncogenic HPV (*Human papillomavirus*) is a risk factor for the development of cervical intraepithelial neoplasia and cervical cancer. The aim of the study was to assess the effect of a double cryotherapy procedure performed on the cervix accompanied by complementary pharmacotherapy with intravaginally administered drugs in cases of highly oncogenic HPV infection. Abnormal cytological examination results were found in 214 patients, of these 138 were diagnosed with ASC-US (atypical squamous cells of undetermined significance), 76 with LSIL (low-grade squamous intraepithelial neoplasia). The presence of HPV in all women was determined by RT-PCR. The presence of DNA HPV was confirmed in a total of 192 women: 116 with ASC-US (84%) and all 76 cases with LSIL.

Sixty-one patients had only one type of HPV-HPV 16 was most common. In 51 women, two types of HPV were detected-most frequent were HPV 16 and 18. 80 women (over 40%) were infected with multiple types of HPV. The patients underwent the cryotherapy procedure twice and, in addition, all the women were given intravaginal therapy; Betadine (Povidone-Iodine) was applied in 158 patients and Papilocare gel in the remaining 34. A HPV eradication level of 95.5% was recorded in the Betadine group and 85% in the Papilocare gel group.

Keywords: HPV infection; Cryotherapy; Betadine; Papilocare

Introduction

Though primary and secondary prevention in cervical cancer is becoming more commonplace, the disease remains a global women's health issue. According to world epidemiological data, over 569,000 women are diagnosed with cervical cancer each year. The data also shows that 311,000 women with cervical cancer die per annum [1].

Over 90% of cervical cancers are associated with persistent infection with highly oncogenic types of HPV (*Human papillomavirus*), including the following 15 types: 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 39, 51, 73, 68 and 82. Some researchers also consider HPV types 26, 53 and 66 as carcinogenic [2-4].

Most women with HPV infection eradicate this common sexually transmitted infection spontaneously but 5% to 10% of those infected with highly oncogenic HPV types have persistent infection. Persistent infection is defined as the presence of the same type of HPV 6 to 12 months after the original infection. It poses the risk of subsequent development of intraepithelial neoplasia and cervical cancer [3,5,6]. Thus, eliminating high-risk HPV infections is a path to avoiding the development of pathological changes in the cervix.

Kim et al. [7] applied zinc citrate (CIZAR) intravaginally in 194 women diagnosed with a highly oncogenic HPV infection, resulting in HPV eradication in 64.47% of patients. Fu et al. [8] used a topical photodynamic therapy with 5-aminolevulinic acid, curing persistent high-risk HPV infection in over 64% of women. Cryotherapy in combination with vaginal treatment with iodine-

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containing preparation (Betadine; Povidone-iodine) also resulted in the eradication of carcinogenic HPV infections [9].

This year, the preliminary results of a randomized clinical trial in Zambia were presented (750 women with HPV infection and acetic acid lesions in colposcopy). Pre-cancerous condition (CIN) was diagnosed in some women. Cryotherapy, thermal ablation or electrosurgical removals of the transition zone using the LEEP system were applied. In more than 60% of patients, similar results were obtained from these three methods at the end point of elimination of HPV and cervical lesions [10]. Treatment of lesions on the cervix, especially CIN, with accompanying HPV infection, is an effective strategy of secondary prevention against the development of cervical cancer [11].

The aim of the study was to evaluate the effect of cryotherapy in combination with local pharmacological treatment (Betadin or Papilocare gel) in the eradication of highly oncogenic HPV in patients with ASC-US (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial neoplasia) on the cervix with concomitant HPV infection.

Materials and Methods

The study included 214 women aged 25 to 68 (mean age 38.7) with abnormal ASC-US (Atypical Squamous Cells of Undetermined Significance) or LSIL (Low-grade Squamous Intraepithelial neoplasia). Cytology was determined via the Bethesda system.

HPV genotyping was performed in the Laboratory of Molecular Genetics using the RT-PCR (Reverse Transcription Polymerase Chain - Reaction) method with the patients' written informed consent. 18 types of highly oncogenic and 12 types of low-oncogenic HPV were determined. The presence of highly oncogenic DNA HPV was the criteria for inclusion into the study.

The cytological examination in 214 patients provided the following results:

- ASC-US was observed in the case of 138 patients.
- LSIL was detected in 76 patients.

Out of 138 patients with ASC-US, HPV DNA results confirmed the presence of HPV infection in 116 women (84.06%). In all 76 women with LSIL, HPV infection was confirmed.

- in 61 (31.7%) patients, only one HPV type was demonstrated, most frequently including:
 1. HPV 16 in 29 (47.5%) patients;
 2. HPV 31 in 8 (13.1%) patients;
 3. HPV 58 in 11 (18%) patients;
- In 51 (26.5%) patients, two types of the virus were detected, one of which was either type 16 or type 18.
- In the remaining 80 (41.6%) patients, multiple HPV types

were detected, including types 68, 73, 56, and 66 (Table 1).

Two or more types of HPV were found more frequently in patients with LSIL compared to ASC-US patients: LSIL 84.2% (64 patients out of 76) vs. ASC-US 57.7% (67 patients out of 116). This result is statistically significant according to Fisher test ($p=0.0001$).

Cryotherapy and pharmacological treatment were applied according to the following scheme:

- 3 min of cryotherapy, covering the lesion on the cervical shield (if present), external opening of the uterus, the transition zone and part of the cervical canal;
- This was then followed by:
 1. In 158 women (52 LSIL, 106 AS-CUS)- Betadine-applied vaginally for 14 days, once per night,
 2. In 34 women (24 LSIL, 10 AS-CUS)- Papilocare gel (Hyaluronic acid niosomes, beta-glucan niosomes, Asiatic Pennywort Phytosomes (*Centella asiatica*), BioEcolia, Aloe Vera, Aloe Vera, *Coriulus versicolor*, Indian Honey Extract (Neem))- applied vaginally for 21 days, once per night, deeply so that the drug reached the cervix.
- A second cryotherapy procedure was performed after 2 months, followed by:
 1. Repeat administration of Betadine in the same 158 women (14 globules vaginally) every other day,
 2. Papilocare gel in 34 women, applied vaginally at night, every other day (14 globules),
- HPV DNA marking after 2 months following the second cryotherapy procedure.

A statistical analysis was performed comparing the two types of treatment (2x cryotherapy + Betadine vs. 2x cryotherapy + Papilocare) using the Fisher exact test.

Results

The post-therapeutic presence of HPV DNA was tested, and the results were as follows: among the 158 women treated with Betadine, 151 (95.5%) had no HPV DNA presence.

Among the 34 women who received Papilocare, HPV eradication was confirmed in 29 (85.2%), 5 were positive (HPV 16 and 33 were found). The performed LEEP procedure did not show any CIN features in the histological picture.

2x cryotherapy + Betadine are more effective than 2x cryotherapy + Papilocare in the eradication of highly oncogenic HPV in patients with ASC-US and LSIL. Fisher test result: 0.041 ($p<0.05$).

Discussion

Over 90% of precancerous conditions and cervical cancer develop as a result of persistent infection with highly oncogenic HPV [3-5].

The results of global studies, including the randomized PATRICIA

Table 1: Number of patients with one or more type of HPV infection according to cytological results of ASC-US and LSIL tests.

	Number of patients with HPV	Number of patients with one type of HPV	Number of patients with two types of HPV	Number of patients with multiple types of HPV
ASC-US	116	49	25	42
LSIL	76	12	26	38
(Total number of patients)	192	61	51	80

study, indicate geographical differences regarding the occurrence of HR-HPV in precancerous conditions and cervical cancers. HPV 16 and HPV 33 seem to be the greatest risk factor for the development of pathological changes of the cervix [3,9,10].

It has been established that screening tests in women: cytology, colposcopy and tests detecting HPV contribute to the elimination of cervical cancers [11].

The elimination of a persistent HPV infection is an important factor in the prevention of the development of cervical pathology. Randomized studies with vaginal infusions of zinc citrate (CIZAR) in women with HPV infection but without precancerous conditions showed - as already mentioned - effective elimination of highly oncogenic HPV as compared to the untreated control group (64.44% vs. 15.25% respectively).

In another study, local photodynamic therapy with 5-aminolevulinic acid, which was administered to 76 patients, was also effective in eradicating highly oncogenic HPV (61.10% vs. 24.32% in the control group).

The conversion rate of abnormal results (cytology + HPV DNA) after the 9-month follow-up was 90.9% in the treatment group and 25% in the observation group. However, 5 out of 6 patients with CIN I did not respond to treatment [8]. In the randomized PATRICIA study involving 4,825 women, the elimination of HPV 16 and 31 was lower compared to cases of other types of HPV [3]. In our study, the most common type of HPV was type 16 (47.5%) occurring as a single infection and also among infections with two types of HPV. Other researchers obtained similar results [12,13]. The PALOMA study showed the efficacy of *Coriolus versicolor*-based vaginal gel in the elimination of HPV in women with cervical lesions [14].

Our results show that in the group of 158 patients treated with cryotherapy and Betadine, the HPV elimination rate is 95.5%, while in the group with cryotherapy and Papilocare- 85.2%. These good results are higher than in the cited CIZAR study. Perhaps such high HPV eradication rates are due to an additional factor: the restoration of the microbial balance in the vagina by using a Papilocare gel with a pH of 5. A meta-analysis of electronic databases also suggests a relationship between the composition of the vaginal microflora and HPV infection [15].

In the presented study, it is possible that the additional pharmacological treatment resulted in such a high percentage of HPV elimination as compared to the above-mentioned treatment in the group of women from Zambia (the destructive method only). Cryotherapy used by this group eliminated HPV in 60% of women, comparable to thermal ablation (64%) [10]. The use of additional local pharmacological treatment in our study increased the effectiveness of HR-HPV eradication to 95.5% of women in the case of Betadine and 85.2% in the group of patients using Papilocare.

Conclusion

2x cryotherapy + Betadine are more effective than 2x cryotherapy + Papilocare in the eradication of highly oncogenic HPV in patients with ASC-US and LSIL.

Authors' Contribution

JM, RH, AG: study design. JM, AM: gynecological examination

of patients, performance of cryotherapy procedures, prescription of medicines. RJ, AG: reference collection. MM: statistical analysis.

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