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Clinical Efficacy of Combination Therapy with Podophyllotoxin and Liquid Nitrogen Cryotherapy in the Treatment of Genital Warts in Men

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ABSTRACT High prevalence, peculiar etiopathogenesis, and ineffective therapies have contributed to the fact that genital warts are one of the most challenging issues in modern medicine. This prospective study was aimed at determining the clinical efficacy of combination therapy with 0.5% podophyllotoxin solution and liquid nitrogen cryotherapy in the local treatment of genital warts in men. One hundred and ten consecutive male patients with genital warts were randomly assigned to two groups. The control group consisted of two subgroups: 30 patients treated with podophyllotoxin and 30 patients treated with cryotherapy. The experimental group included 50 patients treated with combination therapy. The therapy continued until complete regression, but not longer than six weeks. Analysis of the average increase in the number of cleared warts compared to week zero found a significant clinical improvement in the group treated with a combination therapy in relation to the group treated with podophyllotoxin at the end of each of the six weeks and in comparison with the group treated with cryotherapy at the end of each of the first three weeks. After discontinuation of therapy, a significantly lower recurrence rate and appearance of new condylomas was observed at the end of the third month in the group treated with a combination therapy compared with each group treated with monotherapy, and at the end of the sixth month compared with patients treated with cryotherapy. The combination of podophyllotoxin and cryotherapy showed a significantly higher efficacy in the treatment of genital warts in comparison with monotherapy with podophyllotoxin after 6 weeks of treatment ($P<0.001$), with considerably lower recurrence and appearance of new warts compared with cryotherapy during the 6 months after therapy ($P<0.005$).

KEY WORDS: condylomata acuminata, genital diseases, cryotherapy, podophyllotoxin, treatment outcome

INTRODUCTION

External genital warts (EGWs) are visible manifestations of human papillomavirus (HPV) infection and one of the most common sexually transmitted infections (STIs) in the developed world. The incidence of 1% in sexually active adults and 3% in sexually active adolescents, peculiar etiopathogenesis, and still-ineffective therapies have contributed to the fact that EGWs are one of the most challenging issues in

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modern medicine (1). Being prone to recurrence and associated with malignancies, this entity is equally frustrating for both patients and physicians. The lack of effective treatment modalities is the most discouraging problem for clinicians. No specific antiviral treatment is available. Due to the lack of a specific antiviral agent, the treatment is mainly based on the removal of the visible skin lesions. The treatment is a long-term process that involves multiple procedures, and the results are sometimes very modest. Recurrences are frequent and an absolute therapy of choice does not exist yet.

Today, topical chemicals are most commonly used for treatment and include podophyllotoxin, imiquimod, sinecatechins green tea extract, liquid nitrogen cryotherapy, and surgical and electrosurgical methods. In order to achieve better treatment results and reduce the recurrence rate, combined treatment methods (2-6) have also been introduced.

Objective

The aim of this study was to determine the clinical efficacy of the combined application of 0.5% podophyllotoxin solution and liquid nitrogen cryotherapy in the topical treatment of EGWs in men.

PATIENTS AND METHODS

This prospective randomized controlled study included 110 patients treated at the Clinic of Dermatology and Venereology Diseases in Novi Sad, Serbia. The sample included consecutive patients with EGWs who sought medical care.

The inclusion criteria were male sex, age 16 and over, good general condition, EGWs only in the genital region, compromised area under 4 cm^2, and signed informed consent for each patient.

The exclusion criteria were immunocompromised patients, human immunodeficiency virus (HIV) infection, previous cryotherapy, podophyllotoxin and/or podophyllin in the treatment of EGWs, a total compromised area over 4 cm^2, individual lesions over 1 cm^2, other STIs in the genital region, hypersensitivity to podophyllotoxin and/or podophyllin, immunosuppressive therapy in the past six months, and topical corticosteroid therapy in the last four weeks.

All procedures were conducted in accordance with the ethical standards of the Human Rights Expert Committee. The consent of the ethics committee was provided.

The experimental group of 50 patients was treated with a combination therapy. The control group included 30 patients treated with podophyllotoxin and 30 patients treated with cryotherapy. The examinees were randomly assigned to three groups, one experimental and two control groups. In each patient, the area affected by EGWs was measured at the first examination using two perpendicular diameters, the largest and the smallest (p1 – up to 0.5 cm^2; p2 – from 0.6 to 1 cm^2; p3 – from 1.1 to 2 cm^2; p4 – from 2.1 to 3 cm^2; p5 – from 3.1 to 4 cm^2), as well as the number of EGWs (b1 – 1; b2 – from 2 to 5; b3 – from 6 to 10; b4 – more than 10). Localization in anatomical sites was mapped using a schematic diagram for each individual genital wart: A – penis, B – other body parts (pubic area, scrotum, and thighs). Each genital wart was marked according to the above characteristics, and a schematic diagram was made for each patient.

The experimental group underwent liquid nitrogen cryotherapy on the first day: 5 to 10 seconds for each lesion until the wart and the surrounding healthy skin in a diameter of 1-2 mm were frozen, using a Cry-AcR device (Brymill Cryogenic System, Brymill Company, Ellington, USA); the therapy lasted up to 6 weeks (7). Simultaneously, on the third, fourth, and fifth day the patients applied podophyllotoxin on previously cleaned wart surfaces twice a day using wooden applicator sticks; the therapy was followed by 2 days of rest; the therapy also lasted up to 6 weeks.

In the podophyllotoxin control group, patients were treated for three consecutive days with a 0.5% podophyllotoxin solution twice a day using wooden applicator sticks on previously cleaned surface, and the treatment lasted up to 6 weeks. In the cryotherapy control group the therapy also lasted up to 6 weeks. Liquid nitrogen was applied once a week, 5 to 10 seconds on every lesion until the wart and the surrounding healthy skin in a diameter of 1-2 mm was frozen.

In order to evaluate the treatment effects, check-ups were performed every seven days. After discontinuation of therapy, the therapeutic effects were evaluated after the first, second, third, and sixth month. The treatment effectiveness was assessed for each lesion in terms of: 1) clearance, 2) regression, and 3) recurrence (a new lesion on the previously treated site).

All assessments were performed by the same dermatologist. During each follow-up, local reactions were quantitatively assessed: pain, erythema, edema, and erosions.

Determination of differences in treatment effects in each group included evaluation of average reduction in the skin/mucous membrane areas affected by EGWs and the percentage of patients with complete EGWs clearance. Six months after the end of therapy,

the percentage of recurrent and new EGWs and patients with recurrent and new EGWs was determined.

Statistical analysis

Statistical data processing was performed using the IBM Statistical Package for the Social Sciences (SPSS) software 21.0. Numerical characteristics were obtained using the average values (arithmetic mean) and measure of variability (standard deviation – SD), while descriptive statistics associated with nominal data were represented as *frequencies and percentages*. Comparison of numerical values between three or more groups of data was performed using the a*nalysis* of variance (*ANOVA*) test. Testing the differences of *non-normally* distributed trait *values* was performed using the Kruskal-Wallis test (three or more data sets) and the Mann-Whitney test (two groups). Comparison between the observed counts with the expected counts was done *using the χ2 test*. A *P* value of *<0.05* was *considered statistically significant*.

RESULTS

The age of patients ranged from 18 to 56 years (average age 27.85 years). There was no statistically significant difference in the mean age of the examined groups (*P*=0.718).

The number of sexual partners of patients with EGWs ranged from 1 to 100. There was no significant difference in the number of sexual partners between the examinees (Kruskal-Wallis H =5.049, *P*=0.080).

Sex without a condom was reported by half of the patients treated with cryotherapy, every sixth patient (16.7%) treated with podophyllotoxin, and 40% of patients treated with combination therapy. Condoms were regularly used by every seventh patient treated with podophyllotoxin, one in ten patients treated with cryotherapy, and one in six patients treated with combination therapy. A statistically significant difference in distribution of condom use was found in regard to the applied therapy as well as among the examined groups (χ2=7.726, *P*=0.021).

No significant difference was found in the analyzed data on previous sexually transmitted diseases (STD) in the studied groups (χ2=4.502, *P*=0.105).

More than half of the patients treated with cryotherapy (56.7%) and combination therapy (58.0%), as well as two thirds of patients treated with podophyllotoxin (66.7%) were smokers. The difference in the distribution of smoking status of the examinees was not statistically significant (χ2=0.778, *P*=0.678).

Before the initiation of therapy (week zero), there was no significant difference in average number of EGWs (χ2=4.464, *P*=0.614) among the examined groups. Before the beginning of treatment, the average number of condylomas and SD in the group treated with podophyllotoxin was 3.9 (SD=2.07), 4.11 (SD=2.58) with cryotherapy, and 4.48 (SD = 2.67)with combined therapy. Most patients presented with 2 to 5 EGWs: 21 (70.0%) patients treated with podophyllotoxin, 23 (76.7%) patients treated with cryotherapy,

Table 1. Distribution of the total reduction of skin surface affected by condylomas expressed in percentages during the treatment period

	Treatment period⁰					
Group	1	2	3	4	5	6
P	11.11 (17.28)	24.72 (26.76)	24.86 (33.91)	9.44 (17.05)	11.81 (17.06)	5.56 (13.50)
C	21.67 (32.43)	25.97 (27.63)	18.61 (24.63)	14.17 (25.16)	6.75 (12.52)	1.87 (5.84)
PC	32.17 (36.92)	35.83 (33.89)	14.67 (24.66)	4.50 (14.40)	0.33 (2.36)	1.67 (8.41)
Test*	H=6.015	H=2.280	H=2.090	H=5.485	H=19.444	H=5.041
	***P*=0.049**	*P*=0.320	*P*=0.352	*P*=0.064	***P*<0.001**	*P*=0.080
Test**	Z=1.021	Z=0.244	Z=0.480	Z=0.476	Z=1.145	Z=1.067
	P=0.307	*P*=0.807	*P*=0.631	*P*=0.634	*P*=0.252	*P*=0.286
Test***	Z=2.401	Z=1.366	Z=1.441	Z=1.819	Z=4.465	Z=2.234
	***P*=0.016**	*P*=0.172	*P*=0.150	*P*=0.069	***P*<0.001**	*P*=0.026
Test****	Z=1.278	Z=1.089	Z=0.808	Z=2.238	Z=3.458	Z=1.036
	P=0.201	*P*=0.276	*P*=0.419	*P*=0.025	*P*=0.001	*P*=0.300

Legend: ⁰ – time from 1 to 6 weeks; P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – difference between P, C, PC (Kruskal-Wallis); ** – difference between P and C (Mann-Whitney); *** – difference between P and PC (Mann-Whitney); **** – difference between C and PC (Mann-Whitney); data in parentheses – SD

and 30 (60.0%) patients treated with combination therapy. Before the initiation of therapy (week zero), no significant difference was found in the skin surface affected by EGWs among the studied groups of patients (Kruskal-Wallis H = 1.449, *P*=0.485).

Statistical significance of the differences in the reduction of the EGW-affected areas in the examined groups

The test results of the distribution and the average reduction of EGW-affected areas as well as remission of EGWs in the examined groups during the treatment are presented in Tables 1-4.

Table 1 shows the distribution of average reduction of areas affected by condylomas (expressed in percentages) in the examined groups during the treatment period. Data are presented as average values (SD), whereas Kruskal-Wallis test was used for the comparison of the obtained test results and statistical significance of the differences between all three groups, and the Mann-Whitney test was used for ranking the compared items in all three groups. A statistically significant difference was observed between the tested groups at the end of the first and fifth weeks of treatment (Table 1). A significantly higher reduction in the area affected by condylomas was found in the group of patients treated with combination therapy compared with the group of patients treated with podophyllotoxin at the end of the first week of treatment; at the end of the fifth week of treatment it was significantly lower than in the groups treated with podophyllotoxin and cryotherapy (Table 1).

When comparing the total average reduction in the surface area affected by condylomas at week zero (100%) and at the end of each week during the treatment period, the difference between all three groups was statistically significant at the end of the first, second, third, fourth, and fifth weeks (Table 2). A significantly higher total reduction in the surface affected by condylomas was found in the group of patients treated with combination therapy compared with the group treated with podophyllotoxin at the end of the first, second, third, fourth, and fifth weeks of treatment and compared with the group treated with cryotherapy at the end of the second, third, and fourth weeks of treatment. Although at the end of the sixth week the differences between the rates within the three groups using podophyllotoxin, cryotherapy, and combined therapy (87.50, 88.75, 89.17, respectively) were not statistically significantly different, the time of clearance was statistically significantly different (Table 2).

The skin area affected by condylomas was analyzed every week in comparison with the previous week (the area affected by condylomas in the previous week was always 100%), and the difference between all three investigated groups was statistically significant at the end of the first, second, and fifth weeks of treatment. Compared with the previous week, a significantly higher reduction in the area affected by condylomas was found in the group treated with combined therapy compared with the group treated with podophyllotoxin at the end of the first and second weeks and compared with the group

Table 2. Reduction of skin surface affected by condylomas expressed in percentages during the treatment period compared with week zero

	Treatment period⁰					
Group	1	2	3	4	5	6
P	11.11 (17.28)	35.83 (25.72)	60.69 (29.39)	70.14 (27.90)	81.94(20.60)	87.50 (19.63)
C	21.67 (32.43)	47.64 (33.58)	66.25 (28.96)	80.42 (24.64)	86.49 (22.51)	88.75 (22.45)
PC	32.17 (36.92)	68.00 (37.05)	82.67 (31.13)	87.17 (28.29)	87.50 (28.33)	89.17 (27.73)
Test*	H=6.015	H=17.570	H=16.224	H=14.011	H=6.218	H=2.213
	***P*=0.049**	***P*<0.001**	***P*<0.001**	***P*=0.001**	***P*=0.045**	*P*=0.331
Test**	Z=1.021	Z=1.535	Z=0.728	Z=1.536	Z=1.159	Z=0.530
	P=0.307	*P*=0.125	*P*=0.467	*P*=0.125	*P*=0.246	*P*=0.596
Test***	Z=2.401	Z=2.401	Z=3.728	Z=3.698	Z=2.462	Z=1.459
	***P*=0.016**	***P*=0.016**	***P*<0.001**	***P*<0.001**	***P*=0.014**	*P*=0.145
Test****	Z=1.278	Z=2.584	Z=2.999	Z=2.097	Z=1.263	Z=0.893
	P=0.201	***P*=0.010**	***P*=0.003**	***P*=0.036**	*P*=0.207	*P*=0.372

Legend: ⁰ – time from 1 to 6 weeks; P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – difference between P, C, PC (Kruskal-Wallis); ** – difference between P and C (Mann-Whitney); *** – difference between P and PC (Mann-Whitney); **** – difference between C and PC (Mann-Whitney); data in parentheses – SD

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Table 3. Statistically significant reduction of the skin surface affected by condylomas expressed in percentages compared with the previous week of treatment within different groups

	Treatment period⁰		
Group	1	2	5
P	11.11	26.11	39.29
C	21.67	33.02	36.67
PC	32.17	57.15	7.69
Test*	H=6.015	H=14.190	H=7.233
	***P*=0.049**	***P*=0.001**	***P*=0.027**
Test**			
	P=0.307	*P*=0.352	*P*=0.823
Test***	Z=2.401	Z=3.504	Z=2.593
	***P*=0.016**	***P*<0.001**	***P*=0.010**
Test****		Z=2.572	Z=2.347
	P=0.201	***P*=0.010**	***P*=0.019**

Legend: ⁰ – time from 1, 2 and 5 weeks; P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – difference between P, C, PC (Kruskal-Wallis); ** – difference between P and C (Mann-Whitney); *** – difference between P and PC (Mann-Whitney); **** – difference between C and PC (Mann-Whitney)

treated with cryotherapy at the end of the second week of treatment, whereas at the end of the fifth week of treatment area reduction was significantly lower than in the groups treated with podophyllotoxin and cryotherapy (Table 3).

The number of cleared condylomas was compared to week zero (week zero is always 100%) at the end of each week during the treatment period, and there was a statistically significant difference between all three groups at the end of each week. A significantly higher percentage of cleared condylomas was found in the group of patients treated with combination therapy compared with the group treated with podophyllotoxin at the end of each of the six weeks of treatment and compared with the group treated with cryotherapy at the end of the first, second, and third weeks of treatment (Table 4).

Comparison between three groups of patients based on the percentage of clinically cured patients with regard to the applied therapy during the treatment period

The percentage of clinically cured patients was analyzed at the end of each week of the treatment period, and the difference between all three groups was statistically significant at the end of each of the first five weeks of treatment. After six weeks of treatment, the distribution of clinically cured patients in the investigated groups showed no statistically significant difference. A significantly higher percentage of clinically cured patients were found in the group treated with combination therapy in relation to the group treated with podophyllotoxin at the end of each of the first five weeks and at the end of the second, third, and fourth weeks of treatment with regard to the group treated with cryotherapy (Table 5).

Table 4. The percentage of cleared external genital warts (EGWs) with regard to the applied therapy during the treatment period

	Treatment period⁰					
Therapy	1.	2.	3.	4.	5.	6.
P	3.4	19.7	43.6	53.8	70.9	80.3
C	19.5	44.7	65.0	80.5	87.0	89.4
PC	29.4	63.6	81.0	87.9	90.0	91.8
χ2 test*	32.476	60.826	49.898	52.432	22.429	10.041
P*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.007**
χ2 test**	15.07	17.162	11.13	19.401	9.367	3.885
(*P*)**	<0.001	<0.001	<0.001	<0.001	0.002	0.049
χ2 test***	32.040	60.115	50.151	50.705	20.693	9.566
(*P*)***	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
χ2 test****	4.111	11.728	10.963	3.499	0.762	0.563
P***	**0.043**	**0.001**	**0.001**	0.061	0.383	0.464

Legend: ⁰ – time from 1 to 6 weeks; P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – testing refers to the differences among all three types of therapy; ** – testing refers to the differences between podophyllotoxin and cryotherapy; *** – testing refers to the differences between podophyllotoxin and combination therapy; **** – testing refers to the differences between cryotherapy and combination therapy

Table 5. Statistical significance of differences expressed in percentages in the distribution of clinically cured patients in the investigated groups during the treatment period

	Treatment period[0]											
	1		2		3		4		5		6	
Patients	n	%	n	%	n	%	n	%	n	%	n	%
P	0	0	1	3.3	6	20.0	9	30.0	13	43.3	20	66.7
C	3	10.0	5	16.7	9	30.0	15	50.0	18	60.0	21	70.0
PC	7	14.0	22	44.0	33	66.0	37	74.0	38	76.0	40	80.0
χ2 test*	4.448		18.02		19.25		15.192		8.689		1.998	
P*	0.106		<0.001		<0.001		<0.001		0.013		0.368	
χ2 test**	3.158		2.963		0.800		2.500		1.669		0.077	
P*	0.076		0.085		0.371		0.114		0.196		0.781	
χ2 test***	4.603		15.138		15.88		14.584		8.658		1.778	
P*	0.032		<0.001		<0.001		<0.001		0.003		0.182	
χ2 test****	0.274		6.265		9.744		4.747		2.286		1.035	
P*	0.601		0.012		0.002		0.029		0.130		0.309	

Legend: [0] – time from 1 to 6 weeks; n – number of patients; P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – testing refers to the differences among all three groups; ** – testing refers to the differences between P and C groups; *** – testing refers to the differences between P and PC groups; **** – testing refers to the differences between C and PC

Percentage of recurrent and new genital warts with regard to treatment

Analyzing the difference between the efficacy of the three applied treatments (%) with regard to recurrent (a new lesion on the previously treated site) and new genital warts during 6 months after discontinuation of therapy found a significantly lower percentage of recurrent and new warts after three months in the group of patients treated with combination therapy compared with patients treated with podophyllotoxin and cryotherapy; after six months, the percentage of recurrent and new warts was significantly lower in the group treated with combination therapy compared with the group treated with cryotherapy (Table 6).

Distribution of patients with recurrent and new warts in the examined groups

Analysis of the percentage distribution of patients with recurrent and new warts after discontinuation of therapy found a lower percentage of patients in the group treated with combination therapy in comparison with other forms of therapy (Figure 1), but the difference was not statistically significant after the first three months following the completion of therapy. Six months after treatment, statistically significantly fewer patients were found with recurrent and new warts in the group treated with combination therapy compared with patients treated with cryotherapy ($\chi2=6.701$, $P=0.010$). The difference was not statistically significant compared with the group treated with podophyllotoxin ($\chi2=2.966$, $P=0.085$).

Adverse local treatment reactions

Pain was present in 33.3% of patients treated with podophyllotoxin, 73.3% of patients treated with cryotherapy, and in 82% of patients treated with combination therapy. A significantly higher incidence of pain was found in the group of patients treated with combined therapy compared with the group treated with podophyllotoxin ($\chi2=20.792$, $P<0.001$).

Edema was equally present in patients treated with podophyllotoxin and cryotherapy (6.7%) and was present in 22% of patients treated with combination therapy, but the difference was not statistically significant ($\chi2=5.445$, $P=0.066$).

Erosions were present in 76% of patients treated with combination therapy, 20% of those treated with cryotherapy, and 10% of those treated with podophyllotoxin. A statistically significantly higher erosion rate was observed in the group treated with combination therapy compared with the groups treated with podophyllotoxin ($\chi2=32.690$, $P<0.001$) and cryotherapy ($\chi2=23.758$, $P<0.001$).

Erythema was found in 66% of patients treated with combination therapy, 40% of patients treated with cryotherapy and 26.7% of those treated with podophyllotoxin. A statistically significantly higher

Table 6. Statistical significance of the difference in the percentage of recurrent and new genital warts during six months after therapy in regard to the therapy applied

	Period after treatment			
Recurrent and new EGWs	**1 month**	**2 months**	**3 months**	**6 months**
P	3.4%	7.7%	15.4%	17.1%
C	2.4%	8.9%	19.5%	22.0%
PC	1.7%	4.8%	8.2%	10.4%
χ^2 test*	0.977	2.594	9.888	8.877
P*	0.614	0.273	0.007	0.012
χ^2 test**	0.203	0.123	0.708	0.898
P*	0.652	0.726	0.400	0.343
χ^2 test***	0.984	1.231	4.190	3.161
P*	0.321	0.267	0.041	0.075
χ^2 test****	0.207	2.407	9.582	8.700
P*	0.649	0.212	0.002	0.003

Legend: P – group of patients treated with podophyllotoxin; C – group of patients treated with cryotherapy; PC – group of patients treated with combination therapy; * – testing refers to the difference between all three types of therapy; ** – testing refers to the difference between P and C groups; *** – testing refers to the difference between P and PC groups; **** – testing refers to the difference between C and PC

incidence of erythema was found in the group treated with combination therapy compared with the group treated with podophyllotoxin (χ2=11.952, *P*=0.003).

DISCUSSION

There is currently no specific antiviral treatment for EGWs. It is also impossible to predict relapse in treated patients. Although therapy may accelerate natural regression of EGWs, it is difficult to distinguish it from natural regression in untreated patients. There is no accurate data to confirm that treating EGWs reduces the contagiousness of treated patients, so the primary goal of treatment is improvement of symptoms and/or removal of skin lesions (8,9).

The results published in the global literature on the cure percentage of patients clinically treated with podophyllotoxin according to the protocol used in our study ranged from 56% (10) to 88% (11). The cure percentage of 66.7% of patients treated with podophyllotoxin achieved in our study was within this range. The results of other authors referring to the frequency of recurrence in patients treated with podophyllotoxin are scarce and range from 17% (12) to 91% (13). In our study, recurrent and new warts occurred in 40% of all patients treated with podophyllotoxin three months after cessation of treatment, and the same percentage was maintained even after 6 months after discontinuation of therapy.

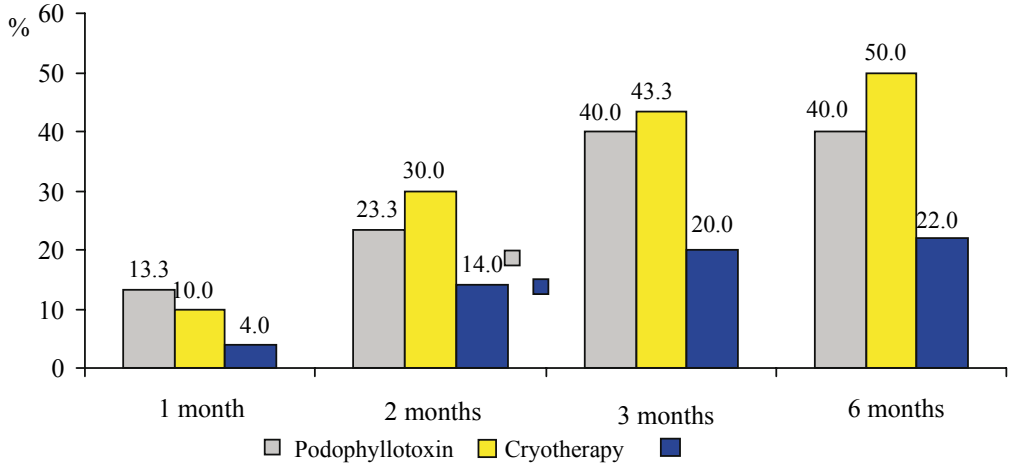

Figure 1. Percentage distribution of patients with recurrent and new warts in examined groups after therapy.

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The treatment results in our patients treated with cryotherapy are in agreement with the results published in the literature. In our study, 70% of patients were clinically cured at the end of the six-week treatment protocol. However, recurrent or new warts were reported three months after discontinuation of therapy by 43.3% of patients and six months after cessation of therapy by 50% of all patients treated with cryotherapy. The published data of other authors show that after six weeks of cryotherapy, clinical cure varied from 66% (14) to 96% of patients (15). Data on relapses are absent in the majority of studies, and those published range from 21% five months after cessation of therapy (7) to 59% after 12 months (16). In our study, the interval between two cryotherapy sessions was 7 days, since the results reported by other authors in previous trials showed that 7 to 8 days between cryotherapy was more effective than 14 to 21 days apart ($P<0.0001$) (17). Pain was the most common of the adverse effects reported in the literature and was found in 18.6% to 84.4% of subjects, followed by erythema and irritation (16,18,19). In our study, the prevalence of pain in the group treated with cryotherapy was 73.3%.

The high recurrence rate of EGWs associated with monotherapy was the reason to attempt combination therapy. Basically, combination therapy is a simultaneous or sequential application of two or more treatment methods (6). The term "combination therapy" is not clearly defined in the treatment of genital warts, although in most cases it refers to a combination of immunomodulation and ablation therapy. We combined cryotherapy as a form of the ablation therapeutic modality and podophyllotoxins whose lignans, in addition to well-known cytotoxic, antitumor, and antiviral capacities may also have numerous modulatory effects on the immune system, which are still insufficiently investigated [20,21].

Combination treatment with podophyllotoxin and cryotherapy resulted in clinical resolution in 80% of patients (50 patients) after six weeks of therapy. Gilson *et al.* also used the combination of these two methods in treating EGWs, and clinical resolution of lesions was observed in 60% of 70 treated patients after four weeks of therapy (18). The effects of combination therapy were visible earlier than in monotherapy, and 29.4% EGWs were cured after the first week of treatment while only 19.5% of EGWs were cured in a group treated with cryotherapy and 3.4% in the group treated with podophyllotoxin. The difference in favor of combination therapy was noted in the continuation of treatment, and was statistically significant in the first three weeks compared with cryotherapy and in all six weeks compared with the treatment with podophyllotoxin (Table 4).

Six months following the treatment, recurrent and new warts were observed in 22% of all our patients treated with combination therapy. In the study by Gilson *et al.*, recurrent or new EGWs were found in 26.6% of subjects, 4 to 12 weeks after discontinuation of combination therapy (18). In the same study, adverse treatment effects were reported at the site of therapy in 64.3% of subjects, while pain was present in 34.3% of patients (18). In our patients, the incidence of local adverse effects was higher than in monotherapy, and this difference was statistically significant for pain, erythema, and erosions. All adverse effects were transient. Although pain was present in 82% and erosions in 76% of patients treated with combination therapy, these adverse effects did not require therapy discontinuation. Adverse effects on the quality of life will be the subject of further research.

The current principles of combination therapy for EGWs include: immunotherapy after laser ablation by intralesional injection of interferon or by local application of imiquimod cream, which results in lower relapse incidence after cytodestruction (22,23); systemic administration of isotretinoin combined with interferon alfa 2b (24); intralesional administration of interferon alpha 2b and topical administration of podophyllin (25); electrocauterization and administration of 1% cidofovir gel in HIV-positive patients with EGWs, with complete regression achieved in 100% of cases after two weeks of therapy, whereas recurrence occurred in 27% after six months of follow-up (26); podophyllin and cryotherapy; trichloroacetic acid and podophyllin (27). In 27 patients treated with cryotherapy combined with 25% podophyllin and post-ablation topical *15%* sinecatechin ointment during 12 - 16 weeks, clinical cure was achieved in 96.3% of patients and recurrence occurred in 7.4% of patients after six months of monitoring (28).

Further studies should be conducted with a larger patient sample for better treatment results, and efforts should be made to develop an effective therapeutic HPV vaccine (6,27,29,30).

CONCLUSION

The combination of podophyllotoxin and cryotherapy demonstrated a significantly higher efficacy in the treatment of genital warts in comparison with monotherapy with podophyllotoxin after 6 weeks of treatment ($P<0.001$), with considerably lower recurrence and appearance of new warts compared with cryotherapy during 6 months of follow-up ($P<0.005$).

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