Effect of combined therapy polyhexamethylene biguanide and Coriolus-MRL on human papilloma virus (HPV) cervical and vulvar-related lesions

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ABSTRACT — OBJECTIVE: Human papillomavirus (HPV) persistent infections of genital tract are strongly related to the development of genital warts and cellular transformation. These infections lead to different type of anogenital cancers, including cervical, vulvar and vaginal. Current therapeutic treatments of genital warts include surgical removal, physician-applied and patient-applied treatments. Most of these treatments aim to remove the wart, with low effect on virus infectivity. Therefore, recurrence rate represents a significant issue that has not been yet addressed. Moreover, adverse events and patient discomfort hamper the potential of these treatments. The aim of our study was to present a protocol proposal for the treatment of primary and recurrent genital warts based on Coriolus-MRL and polyhexamethylene biguanide application (PHMB).

PATIENTS AND METHODS: 42 Patients with primary (15 patients) and recurrent (27 patients) genital warts were treated with the immunostimulator Coriolus-MRL and PHMB solution. Coriolus-MRL (Mycology Research Laboratories Ltd, Luton, Bedfordshire, United Kingdom) was administered as 2 tablets, 3 times per day, for six months while PHMB-gynecological solution, a mono-dose gynecological liquid-gel, was prepared and self-administered by patients before bedtime, every four days for twenty days and, afterward, every twen-ty days for the following six months. The control group was treated only with Coriolus-MRL, 2 tablets, 3 times per day, for six months. All patients with recurrent genital warts were previously treated with pod-ophyllin local therapy.

RESULTS: We have found that the combined treatment of PHMB solution and Coriolus-MRL induced an immediate disappearance and clearance of HPV infection lesions, speeding up the warts healing in comparison to the control, with a total regression of these within the first month for treated group while the control group has reached the same effect after 6 months.

CONCLUSIONS: These results have demonstrated that combined therapy with Coriolus-MRL and PHMB solution provide positive outcomes in cases of primary or recurrent genital warts.

KEYWORDS

HPV infection, LSIL, HSIL, Condyloma, Genital warts, PHMB, Coriolus-MRL.

INTRODUCTION

Viral infections are related to the onset of about 15% of human cancers¹. Particularly, human papillomavirus (HPV) infections are responsible for more than 90% of anal and cervical cancers, of about 70% of vaginal and vulvar cancers and induce the develop-

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ment of genital warts^{1,2}. This transformation process takes around 7-10 years and starts as a consequence of a persistent HPV infection. HPV presence leads to cellular transformation (low to high-grade squamous intraepithelial lesion, LSIL to HSIL) and then to precancer and invasive cancer^{3,4}. Interestingly, cervical epithelial cells are more susceptible to cell transformation than other cells of female genital tract, such as vaginal cells⁵.

Among HPV-induced cancers there is vulvar squamous cell carcinoma (VSCC) that can arise following at least 2 different pathways, similarly to what occurs in head and neck carcinomas⁶⁻⁸. The first pathway is related to persistent infections of specific HPV subtypes in the anogenital or muco-sal tract, and it mainly occurs in young women. The second pathway is HPV-independent; it occurs in el-derly women and is more common in the western countries⁶⁻⁸. Around 43% of VSCC are HPV relat-ed^{9,10}. Some HPV subtypes, such as HPV-16 and 18 are more oncogenic than others, and they are classified as high-risk HPV. Low-risk HPV subtypes are related to the development of genital warts^{11,12}.

Condylomata acuminate (koilocytic atypia) represent 90% of genital warts and are caused by HPV-6 and HPV-11 infections¹³⁻¹⁵. The infectivity of genital warts is extremely high with a rapid onset, within 2/3 months^{16,17}. The highest rate of genital warts occurs among 15 and 24 years old females¹⁸, although an increase in the age-specific prevalence has been found in younger birth cohorts¹⁹. Morphologically, genital warts may be flat, dome-shaped, cauliflower-shaped, or pedunculated. They can start as small colored papules on the skin, either individual, in cluster or they can grow in diameters and cause pain during normal intercourse and childbirth²⁰. The warty shape may also vary in color and in appearance²¹.

The first clinical manifestation of genital warts is between three and eight months from the infection²². By then, genital warts may either increase in number and size or undergo to a spontaneous regression. Although about 30% of warts regress within the first four months of infection, most of them reappear, even after proper treatments, making long-term remission rates uncertain. Host immunosuppression, infection with high-risk HPV subtypes, and patient age represent some of the risk factors related to longterm wart persistence^{23,24}. HPV-related genital warts may progress to grade 2/3 cervical intraepithelial neoplasm (HSIL) and, if not treated, can eventually develop invasive cervix cancer²⁵.

Genital warts diagnosis follows a careful clinical history and physical examination. Biopsy is not frequently used, although it is often recommended for lesions suspected, potentially malignant or resistant to treatments²². Although genital wart lesions are not considered painful, they often cause severe discomfort, burning, itching and, in some cases, subjected to bleeding and irritation upon contact with clothing or during sexual intercourse.

Therapeutic options for treatment of genital warts are mainly surgical, interrupting warty growth. Surgical methods include the application of trichloroacetic acid 80-90% solution (TCA), podophyllin, cryotherapy and surgery/electrosurgery²². However, these approaches do not eradicate the underlying viral infection. Other approaches, mostly patient-administered, have demonstrated contrasting results on infection eradication in the long term. Patient-applied local treatments include podophyllotoxin, imiquimod and sinecatechins. However, adverse events and variable recurrence rates may hinder their effectiveness²⁶.

Modern antiseptics are currently used as alternative therapies to antibiotic treatment for wounds and bacterial vaginosis^{27,28}. Among all antiseptics, polyhexamethylene biguanide (PHMB)-based compounds have shown a strong selectivity against pathogens²⁹. PHMB's efficacy has also been demonstrated against viral infections^{30,31}, and in particular against HPV genital infections³².

Coriolus-MRL is a food supplement which contains biomass of the fungus *Coriolus versicolor*. It possesses immunomodulatory and anti-tumor activities whose effects have been evaluated in preclinical and clinical studies^{33,34}. Recently, studies have demonstrated the benefits of this non-specific immunomodulator for treatment of LSIL-HPV-patients^{35,36}.

Given the scarcity of clinical data and the variable efficacy of current therapeutic options, we aimed to investigate the Coriolus MRL immunostimulatory effect combined with the antiviral activity of PHMB antiseptics, for the treatment of primary and recurrent genital warts.

PATIENTS AND METHODS

Patients with primary (23 patients) and recurrent (43 patients) genital warts (classified with pathological Pap smear test, a positive "high risk" HPV DNA test and a positive colposcopic find) were enrolled at the Clinical Institute for Reproductive Medicine (Pleven, Bulgaria), between September 2015 and December 2017. All patients gave their oral consent to participate at the study. This study was conducted following the Ethical principles of the Declaration of Helsinki and national laws.

The patients' age was between 16 and 34 years (Table 1). Twenty-four of the women were nulliparas, 28 had a child and 14 women were multiparas. The genital wart diagnosis was performed a year before the beginning of the treatment for the diagnosis, gynecological examination, Pap smear test, vulvoscopy, colposcopy, HPV DNA test of cervical mucus and biopsy were performed.

Age	Study group	Control group	
mean age (years) (SD, range)	25.33 (5.1, 16-34)	25.96 (4.9, 17- 33)	
HPV subtype	n (%)	n (%)	
6	7/42 (16.6)	2/24 (8.3)	
11	15/42 (35.7)	8/24 (33.3)	
16	13/42 (31.0)	9/24 (35.5)	
18	7/42 (16.6)	5/24 (20.8)	

Table I. Summary of the characteristics of the studied groups.

Abbreviations: SD- standard deviation; HPV- human papilloma virus.

HPV DNA test revealed: high-risk HPV subtype 16 and subtype 18 infections in 22 and 12 patients respectively, HPV-11 infection in 23 women, and HPV-6 infection in 9 women (Table 1). All smear tests revealed grade 2 lesions (low grade squamous intraepithelial lesion, LSIL), which came out as negative at the end of the treatment.

During the vulvoscopy and colposcopy atypical changes were identified. Patients in the study were young women in active reproductive age. Surgery and hospitalization were not necessary, and anatomy of the external genitalia was preserved. Patient's self-esteem and daily activities were not impaired.

Treatment scheme

Women enrolled in this work were divided in two groups, the study group, consisting of 42 patients (15 patients with a primary infection and 27 patients with a recurrent infection – average age 25.33), and the control group, consisting of 24 patients (8 patients with a primary infection and 16 patients with a recurrent infection – average age 25.96). In the first-one, women were treated with Coriolus-MRL and PHMB for 6 months, while, in the control group, women have received only Coriolus-MRL treatment for 6 months.

PHMB-gynecological solution (Biguanelle/ Monogin, Lo.Li. Pharma, Rome, Italy) is a mono-dose gynecological liquid-gel composed by PHMB, EDTA, glycerol, hydroxymethyl cellulose, potassium chloride, lactic acid, and water. Vaginal application was prepared by patients and self-administered before bedtime, following the information reported in the leaflet. PHMB-gynecological solution was administered every four days for twenty days and, afterward, every twenty days for the following 6 months. Coriolus-MRL (Mycology Research Laboratories Ltd, Luton, Bedfordshire, United Kingdom) was administered as 2 tablets, 3 times per day, for 6 months.

The control group was treated only with Coriolus-MRL, 2 tablets, 3 times per day, for 6 months (Table 1). All patients with recurrent genital warts were previously treated with podophyllin local therapy.

HPV infection and genital warts evaluation

Cervical scrapings for HPV-analysis, Pap smears and a colposcopy examination were used to evaluate treatment effectiveness. This procedure was performed at the beginning of the treatment and six months after the initial diagnosis of HPV infection.

RESULTS

As reported in this study, genital warts disappeared in both primary and recurrent cases at the end of PHMB solution and Coriolus-MRL combined treatment. In 38 cases (90.5%), genital warts disappeared on the 20th day after the application of PHMB and in 4 cases (9.5%) genital warts lesions disappeared a month after the beginning of PHMB treatment (Table 2). Immunostimulation with Coriolus-MRL continued for six months. At the end of the treatment, the HPV DNA-test was negative for all treated patients, as well as pap smears and colposcopy examination (Table 2). No patient needed a surgical removal of the lesions.

In the control group, we found a negative HPV DNA-test, six months after the beginning of the treatment with Coriolus-MRL (Table 2).

DISCUSSION

Persistent HPV infections are related to the development of cervical, vulvar and other anogenital cancers, as well as anogenital warts¹. Clinical manifestations of HPV infections may be either asymptomatic or show dysplastic cellular changes, which can range

Table I. Percentage (%) of patients with presence of primary or recurrent genital warts (Condylomata ac. vulvae, LSIL) at the timepoints: T0 (Baseline), T1 (20 days), T2 (30 days) and T3 (180 days) of combined treatment.

	Baseline	T1 (20 days)	T2 (30 days)	T3 (180 days)
Patients with genital warts (%)				
Study group	42 (100)	4 (9.5)	0 (0)	0 (0)
Control group	24 (100)	24 (100)	24 (100)	0 (0)

from minor histological perturbations, such as koiliocytosis, to precancerous and malignant¹⁵.

HPV subtypes are classified in low- and highrisk, depending on their likelihood to develop cancers¹. Generally, HPV-18 and HPV-16 carry a high risk to have oncogenic potential, while HPV-6 and HPV-11 are considered low risk and often linked to the development of genital warts¹⁵. However, studies have shown that oncogenic genotypes 16 and 18 may be also related with condylomatous lesions in nearly 12% of cases³⁷. Moreover, HPV-driven VSCC and high-grade squamous intraepithelial lesions (HSIL) are mainly related to HPV-16, although other HPV subtypes are also frequent^{38,39}. It has been proven that approximately 10-15% of women with vulvar HPV-changes have genital warts on the cervix, and 50% of them show cytological and colposcopic evidences of HPV infections^{1,32,40}. Therefore, inappropriate treatment of both low and high-risk HPV subtypes infections carries a significant risk factor for the development of precursor lesions and neoplasia⁴¹.

Genital warts do not carry a major morbidity or mortality; however, they may cause psychological distress, and significant medical costs^{16,42}. The treatment of genital warts aims to ameliorate symptoms and remove symptomatic warts, but does not eliminate infectivity risk. Crucial factors for choice of therapy are location and spread of the lesion, invasion of the lesion, advantage of the different therapeutic regimens, side effects and contraindications of various medicaments, preference of the patient and of the physician, special cases, such as pregnancy, relapses, immunocompromised patients (HIV-positive)^{16,26}. The most conservative approaches are surgical excision, electrosurgery, cryotherapy and physical ablation²⁶. Physician-applied treatment includes the use of cytotoxic agents such as TCA and podophyllin. However, eradication rate ranges from satisfactory to extremely high, and recurrence occur in 25-40% of cases⁴³. Podophyllin was the first topical treatment of genital warts, but it has been considered less effective than podophyllotoxin, cryotherapy, or electrosurgery, when used as single modality, as a result of a lack of standardized drug preparation, a wide range of adverse skin reactions, and reduced remission rates²². In rare circumstances podophyllin over-application may induce an excessive systemic absorption, with the development of enteritis, bone-marrow suppression, abdominal pain, and neurological compromise⁴⁴.

Patient-applied approaches comprise podophyllotoxin 0.15% cream (Wartec), Imiquimod cream (Aldara) or sinecatechins 15% ointment (Veregen). Podophyllotoxin is a purified extract from the *Podophyllium* sp. plant, with a clearance rates ranging between 45 to 77%²². Adverse events, such as skin irritation, itching or a burning sensation have been reported. Moreover, recurrence rates vary from 38%

to 55%. Imiquimod (1-[2-methylpropyl]-1H-imidazo[4,5-c]quinolin-4-amine) 5% cream is an immunomodulator, that induce immune response against viral antigens²². Possible side effects include skin irritation, a burning sensation, pain or skin ulcers. Two formulations have been commercialized: imiguimod 5% formula and the 3.5% cream. Although the latter has not been associated to any local or systemic side effects, an important efficacy difference has been demonstrated with wart clearance rate of 56% for 5% formulation and 36.6% for 3.5% formulation²². Sinecatechins (Polpyphenon E) derive from a botanical drug isolated from green tea leaves (Camellia sinensis), whose mechanism of action has not been clearly understood, vet⁴⁵. Sinecatechins effectiveness is 54.9%, with a 6.5% recurrence rate. Side effects include redness, irritation or a burning sensation, and they have been reported in 1 out of 3 treated patients. Patient-applied treatment may not be able to destroy all the very small or subclinical lesions in the surrounding skin and this may be the cause of recurrence²⁶.

The significant recurrence rate of current therapeutic approaches generates the need of further clinical studies aimed at finding safe and effective treatment of HPV-lesions.

Here, we present a clinical study on 42 patients, carrying primary and recurrent HPV-warty lesions, treated with PHMB-solution (Biguanelle/Monogin, Lo.Li. Pharma, Rome, Italy) and Coriolus-MRL (Mycology Research Laboratories Ltd, Luton, Bedfordshire, United Kingdom) combined treatment. Patients with recurrent warts were all previously treated with podophyllin. Podophyllin induced genital warts to become darker and flatter than usual (Figure 1) and sometimes may lead to transitional histological changes in warts, which can represent an issue for the diagnosis. These cases are very difficult to be differentiated from vaginal intraepithelial lesions (VIN) or other pre-cancerous lesions. When lesions are hyperpigmented, a differential diagnosis for VIN must be considered. Vulvoscopy and biopsy are required to exclude the presence of multifocal VIN III or invasive disease. Keratinized lesions are large sections with leucoplakia and look like rare form of wart cancer (planocellular cancer) (Figure 1).

Coriolus-MRL and PHMB combined treatment induced the regression of the lesion and elimination of the HPV-infection within six months from the beginning of the treatment. In particular, PHMB solution and Coriolus-MRL induced an immediate disappearance and clearance of HPV infection lesions, speeding up the warts healing in comparison to the control, with a total regression of these within the first month for treated group while the control group has reached the same effect after six months. No adverse events were reported.

PHMB is a cationic antiseptic that has shown to be an effective option for the treatment of bac-



Figure 1. Warty VIN – corrugated papillary surface with expressed surface koilocytosis and remarkable polymorphism with multinuclear cells.

terial vaginosis⁴⁶, as well as to induce regression of HPV-lesions³². It has a broad antimicrobial spectrum, with low toxicity and good applicability²⁷. PHMB is a strong base which interacts with acidic phospholipids leading to an increase of fluidity and permeability of bacterial cellular membrane and consequently to the pathogen death. Neutral phospholipids, typical of human cell membranes, are not affected by PHMB, justifying the high selectivity of this molecule^{27,47}.

Antiviral activity of PHMB and other biguanides has been also reported^{48,49}. Specifically, other studies have demonstrated the antiviral activity of PHMB against herpes simplex viruses, HSV-1 and HSV-2³¹. Coriolus-MRL has also demonstrated to have an important immunomodulatory effect in HPV lesions³³⁻³⁶. Furthermore, it has been demonstrated that Biguanelle/Monogin[®] solution does not carry any mutagenic potential and it can be used safely for treatment of genital tract infections⁵⁰.

Unlike other approaches, combined treatment of PHMB solution and Coriolus MRL did not cause

pain, incisions, or need of anesthesia and surgery, preserving anatomical and aesthetic characteristics of female external genitalia. Hospitalization was not needed, and patient's confidence and social life were well-maintained.

CONCLUSIONS

Although the size of this study is relatively low, we have provided consistent data on the effectiveness of a long-term combined treatment with PHMB solution and Coriolus-MRL for primary and recurrent genital warts, which have demonstrated to be safe and effective in clearing the lesions and controlling HPV infections. This therapy is not an alternative to HPV vaccination. The combination of Coriolus-MRL and PHMB solution-based therapy may be considered as an alternative treatment for patients with HPV-lesions, with no complications or side effects, usually related to conventional surgical excision and other current therapeutic approaches.

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CONFLICTS OF INTEREST:

The authors declare no conflicts of interest.

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