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# Impact of a new carrageenan-based vaginal microbicide in a female population with genital HPV-infection: first experimental results

A. PERINO[1], P. CONSIGLIO[1], M. MARANTO[1], P. DE FRANCISCIS[2], R. MARCI[3], V. RESTIVO[4], M. MANZONE[5], G. CAPRA[4], G. CUCINELLA[1], G. CALAGNA[1]

[1]Gynecology and Obstetrics, "Villa Sofia Cervello" Hospital, University of Palermo, Palermo, Italy
[2]Department of Woman, Child and General and Specialized Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy
[3]Department of Morphology Surgery and Experimental Medicine, University Hospital of Ferrara, Ferrara, Italy
[4]Department of Science for Health Promotion and Mother Child Care "G. D'Alessandro", University of Palermo, Palermo, Italy
[5]Department of Obstetrics and Gynecology, IRCCS "Sacro Cuore – Don Calabria" Hospital, Negrar, Verona, Italy

**Abstract. – OBJECTIVE: The objective of this study was to assess safety, satisfaction, and anti-viral effect of a new carrageenan-based vaginal microbicide in a population of fertile female patients with genital human *papillomavirus* (HPV) infection.**

**PATIENTS AND METHODS: Forty healthy and sexually active women aged 18-45 years with genital HPV infection were enrolled. Each subject was treated with a gel formulated with 0.02% carrageenan and Propionibacterium extract (CGP) (Carvir, Depofarma SpA, Mogliano Veneto, Treviso, Italy). The subjects were evaluated at baseline, after the I cycle of therapy and after the II cycle. At final status, treatment acceptability and satisfaction were evaluated using a 5-point Likert scale. Furthermore, the rate of HPV genital infection clearance at final follow-up was evaluated. These data were compared with the HPV genital infection clearance rate in a control group of patients not subjected to any therapy.**

**RESULTS: Overall, 68 HPV infections were detected at baseline, among 40 subjects enrolled. The HPV 16 genotype was the most frequent (12%) followed by HPV 18 (10%), and HPV 53 (9%). At the end of the study, 22 (55%) patients were very satisfied, 14 (35%) were satisfied, 3 (7.5%) were uncertain, and only 1 (2.5%) was dissatisfied, with 0 very dissatisfied. Only 2 patients complained of a local adverse event. Analysing infection clearance at the end of the study, 60% of patients became HPV negative. Among these, 13 cases were high-risk HPV infection. There were 16 patients with persistent infection ("non-responders"). No patient developed a "de novo" genital lesion. After controlling for age, the intervention had an adjusted OR of 4.9 (95% CI 1.6-15.1) to clear HPV.**

**CONCLUSIONS: The results of this work suggest that Carvir vulvovaginal microbicide gel is safe and well-tolerated. Furthermore, this experience supports the hypothesis that CG has a role in accelerating the normal clearance of genital HPV infection in women with a positive HPV-DNA test.**

*Key Words:*
Carrageenan, Papillomavirus, Microbicide, Female population, HPV.

## Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide; it is defined as a necessary condition for the development of cervical cancer, as well as several other anogenital cancers (vulva, vagina, penis, anus)[1,2]. HPV infections are usually transient, subclinical, and self-limiting[3]. Persistent infection with "high risk" HPV (HR-HPV), including genotypes 16, 18, 31, 33, 45, 52, and 58, classified as carcinogens by the World Health Organization (WHO), is the foremost cause of high grade cervical intraepithelial neoplasia (CIN), cervical cancer, and postoperative recurrence[4-6]. Moreover, this evolution is generally favoured by the presence of risk factors, such as tobacco exposure, sexual promiscuity, and immunosuppression[7].


*Corresponding Author:* Gloria Calagna, MD, Ph.D; e-mail: gloria.calagna83@gmail.com

In the last few years, the prevention of HPV infections has been a primary goal of worldwide research, and the availability of vaccines against human papillomaviruses has permitted a huge step forward in this direction. Reports[8-11] from countries with established HPV vaccination programs indicate relevant, beneficial effects as early as 3 years after the introduction of the vaccination program, including decreases in the incidence of high-grade cervical abnormalities and the prevalence of various types of HPV vaccines. Despite the undeniable benefits of HPV vaccination programs, today, the achieved results in terms of cancer prevention and mortality are still sub-optimal[12]. Furthermore, vaccines do not protect against 36 other HPV types associated with anogenital infections, and the availability of the HPV vaccination in developing countries is still inadequate due to limited access to the vaccine[13].

In this scenario, the need for identifying other options for preventing HPV infections is an important issue. Several experiences on the use of carrageen-based microbicides against sexually transmitted infections, especially HIV, have been published, with encouraging results[14,15]. Carrageenan (CG), a sulfated polysaccharide compound extracted from red algae, would appear to have high efficacy in inhibiting HPV infection *in vitro* and *in vivo*; thus, it may potentially be used as an adjuvant to enhance the potency of HPV vaccination[1,16-19]. Marais et al[19], for the first time in 2011, reported a negative association of HPV infection with a vaginal 3% CG-based microbicide, based on a phase III trial, comparing compliant *Carraguard* (FMC, Philadelphia, PA, USA) users and compliant placebo users. These preliminary results have supported further clinical testing in the prevention of HPV infection, and new formulations[20]. Recently, a new CG/Proprionibacterium extract (CGP) based vaginal microbicide, *Carvir* gel (Depofarma SpA, Mogliano Veneto, Treviso, Italy) has been proposed as a possible option to reduce the risk of viral transmission, particularly focusing on the HPV infection.

The aim of this study was to assess safety and satisfaction in the use of *Carvir* vulvovaginal gel in a population of fertile female patients with genital HPV-infection. We also recorded the rate of clearance of HPV genital infection after the treatment protocol period, comparing these data with the HPV genital infection clearance rate in a control group of patients not subjected to any therapy.

## Patients and Methods

### Patients

This prospective observational study on female patients with genital HPV infection was conducted in the Department of Obstetrics and Gynecology of the University of Palermo, which is a referral centre for the diagnosis and management of lower genital tract diseases. Forty healthy and sexually active women aged 18-45 years, with documented HPV genital infection (vulvar, vaginal and/or cervical sample), were enrolled in the research, between January 2014 and December 2015. Exclusion criteria included a history of surgical treatment for CIN, previous HPV vaccination, history of malignancy, HIV infection or other immunosuppressive diseases, pregnancy, ongoing immunosuppressive therapy, use of psychotropic drugs, and more than 6-lifetime sexual partners. Patients who were using vaginal therapy (any type of local preparations) at enrolment were asked to suspend these treatments and were evaluated for inclusion in the study after 30 days of suspension.

The presence of HPV-related genital lesion (vulvar, vaginal, cervical, and/or perianal) was not considered an exclusion criterion in term of warts and low-grade squamous intraepithelial lesions (LSIL), the latter defined by histological samples. Based on the presence or absence of genital lesions at enrolment, the study population was divided into 2 groups: group A (patients with HPV-related genital lesions), and group B (patients without HPV-related genital lesions).

Finally, a control group consisted of 35 patients with documented HPV genital infection, with or without warts and/or LSIL, and not subjected to any anti-viral therapy, was also enrolled. The investigation was approved by the Hospital Research Committee. All samples and data were collected with written consent from patients.

### Intervention

Each patient of the study groups was treated with Carvir vaginal microbicide gel, a new CGP based gel developed by Depofarma SpA, (Mogliano Veneto, Treviso, Italy). It is a non-contraceptive water-based, clear, odourless, tasteless gel made from different types of carrageenans naturally derived from red seaweed. The gel is formulated with 0.02% CG and Propionibacterium extract, packaged in a dedicated squeeze tube with a specific applicator: the applicator is designed to dispense approximately 5 ml of

gel per application. Our study protocol provided two phases of gel therapy application (Figure 1). During the first phase, gel therapy (vulvar and vaginal application) was applied once daily for thirty days continuously (I cycle CGP). During this period the patients could have sexual intercourse with condom use. Then, after a colposcopic/vulvoscopic check-up and after eventual physical treatment (laser $CO_2$ vaporization) (if needed), the second phase of gel therapy (II cycle CGP) was started, with an application on alternate days for a total of 45 applications. During this second phase, the patients could have sexual intercourse also without condom.

### Study Data

Relevant demographic characteristics (age, previous pregnancy, region, tobacco exposure, partner status, contraceptive use) and inclusion/exclusion criteria were recorded at baseline (T0), in both the study and control groups. At T0, all candidates underwent the HPV-DNA test (liquid-based monolayer Pap testing with HPV typing using two vulvar and vaginal brushes) with eventual HPV genotyping according to Muñoz et al[21], while only patients with current HPV infection were retrieved. At the same time, all patients were submitted to colposcopy and vulvoscopy to identify possible HPV-related lesions. All suspected lesions were biopsied and sent to histological evaluation for a definitive diagnosis. As previously reported, only patients with no malignant lesions were considered in the work.

All enrolled patients of the study population performed the first follow-up visit (T1) after the I cycle CGP therapy. At T1, patients with persistent HPV-related genital lesion (already identified at T0) had physical treatment. On the other hand, women with a "*de novo*" genital lesion were excluded from the study. After the II cycle CGP therapy, all included patients underwent a second follow-up visit (T2), during which a colposcopy and/or vulvoscopy and the HPV-DNA test were performed. All adverse events (local or systemic) which occurred or were referred by patients (during, immediately after I cycle and until the end of study) were recorded. Any disorder, discomfort, or injury, both local and general, arising in relation to the application of the vaginal gel was considered as an adverse event. Finally, at T2, treatment acceptability and satisfaction were evaluated using a 5-point Likert scale (very satisfied, satisfied, uncertain, dissatisfied, and very dissatisfied). Treatment was considered satisfactory when patient answers were "very satisfied" or "satisfied".

### Outcome Measures

The main outcomes of the report were to evaluate the safety and satisfaction of the enrolled patients through assessment of endpoints: the absence of genital findings with epithelial disruption (abrasions, ulcers, fissures, erythema) and the absence of patient discomfort, respectively. Next, the rate of HPV genital infection clearance at T2 follow-up in the study population was evaluated, and these data were compared with spontaneous genital HPV clearance in control group, with the same follow-up period.

### Statistical Analysis

Normality of the distribution of quantitative variables was evaluated with the Skewness and Kurtosis tests. Normally distributed quantitative variables were summarized as mean (standard deviation) and those not normally distributed, as median (interquartile range). For qualitative variables, absolute and relative frequencies were calculated. The association of normally distributed quantitative variables (age) with a clearance of HPV infection was evaluated with the Student's *t*-test. Bivariate and multivariate logistic analysis model was used to analyse crude and adjusted drug efficacy. All collected data were analysed using Stata MP 14.2 statistical software.

## Results

A total of 40 patients with HPV genital infection and 35 controls were enrolled and concluded the study protocol. Demographic data of intervention group are described in Table I. Women had a mean age of 30 (SD=5) years, 74% (n=31) of them were married/living with a partner, 40% (n=17) used contraceptives and all were Caucasian (European). Control group had a mean age

**Table I.** Demographic data of study population of intervention group.

| Characteristics | Enrolled women N=40 |
|---|---|
| Age, years (SD) | 30.3 (5.4) |
| Previous pregnancy, n (%) | 9 (21.4%) |
| Tabacco exposure n (%) | 16 (38.1%) |
| Married/living with partner n (%) | 31 (73.8%) |
| Contraceptive use n (%) | 17 (40.5%) |

**Table II.** Characteristics of the lesions at baseline of group A patients among intervention group patients.

| Lesion | N (%) |
|---|---|
| L-SIL | 20; (77%) |
| Vulvar warts | 2; 7.7% |
| Vaginal warts | 1; 3.8% |
| L-SIL + warts (vulvar, vaginal or anal) | 3; 11.5% |

of 35 (SD=6) years. At baseline (T0), 26 (65%) patients of intervention group (with HPV-related genital lesion) were included in group A and 14 (35%) patients (without HPV-related genital lesion) in group B; all genital lesions diagnosed at enrolment of patients of group A are listed in Table II. Among controls, 20 patients (57%) were allocated to group A and 15 (43%) to group B.

During the research, only 2 patients complained of a local adverse event related to the CGP treatment (1 case of mild vulvar erythema and 1 case of vulvar itch), both resolved spontaneously without the need of therapy. However, these patients did not drop out of the study. At the end of the work (T2), the 5-point Likert scale patient satisfaction was as follows: 22 (55%) patients were very satisfied, 14 (35%) were satisfied, 3 (7.5%) were uncertain, and only 1 (2.5%) was dissatisfied, with 0 very dissatisfied.

At baseline (T0), both in intervention and control groups, high-risk HPV types (56% and 54% respectively) were more frequent than low-risk HPV types (41% and 44%), and intermediate-risk (3% and 3%).

In intervention group, the HPV 16 genotype was the more frequent (12%) followed by HPV 18 (10%), and HPV 53 (9%) (Figure 2A). Among control group, HPV 16 and 31 (11.5% each one) were the more prevalent followed by HPV 6 (9.5%) (Figure 2B). Furthermore, 23 (57.5%) intervention patients and 17 (48.6%) control patients had only one identified HPV-genotype; 12 (30%) intervention and 11 (31.4%) control patients had two HPV-genotypes and 5 (12.5%) intervention, and 7 (20%) control patients had more than two genotypes.

Considering intervention group, at T1, 12 patients from group A (46.2%) became lesion-negative, 4 patients (15.4%) had lesion improvement (defined as a colposcopic dimension reduction of

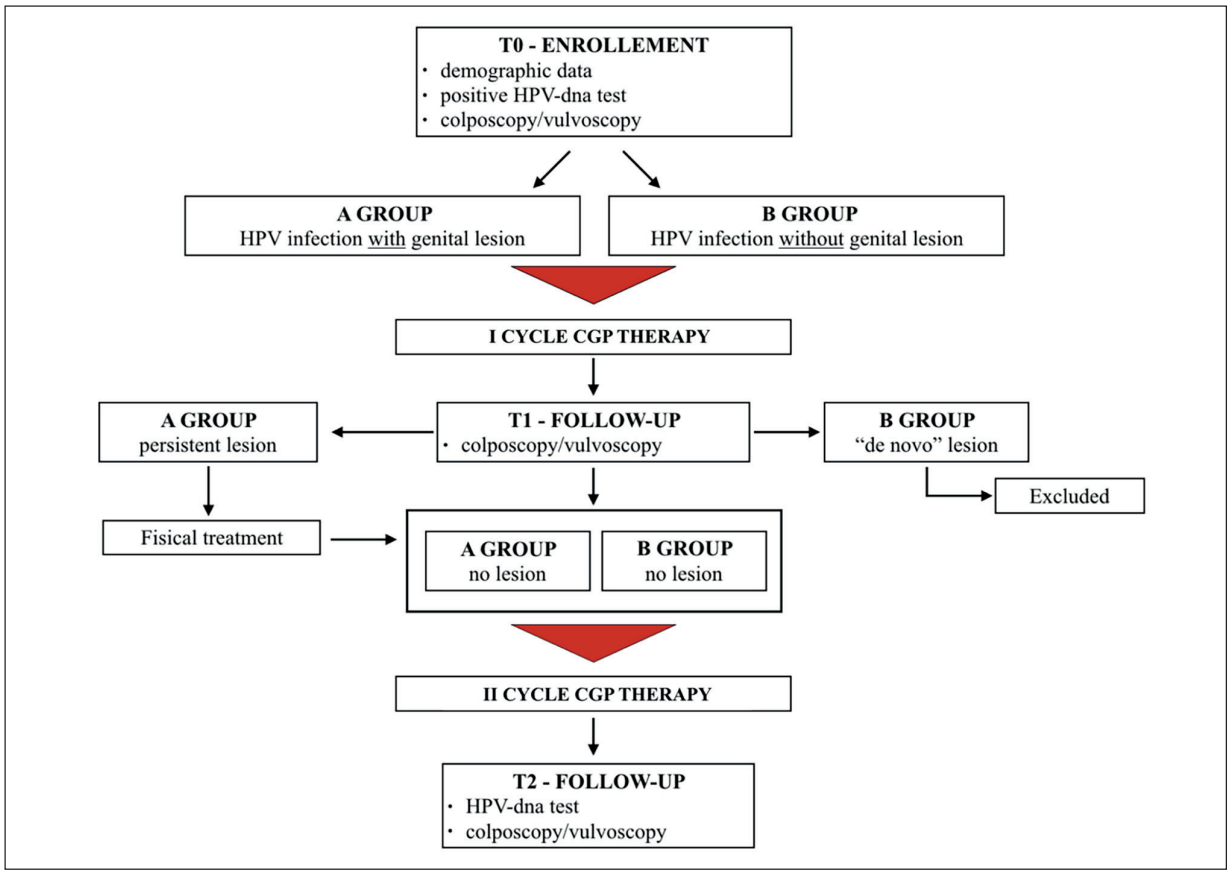


**Figure 1.** Study Flow Chart.

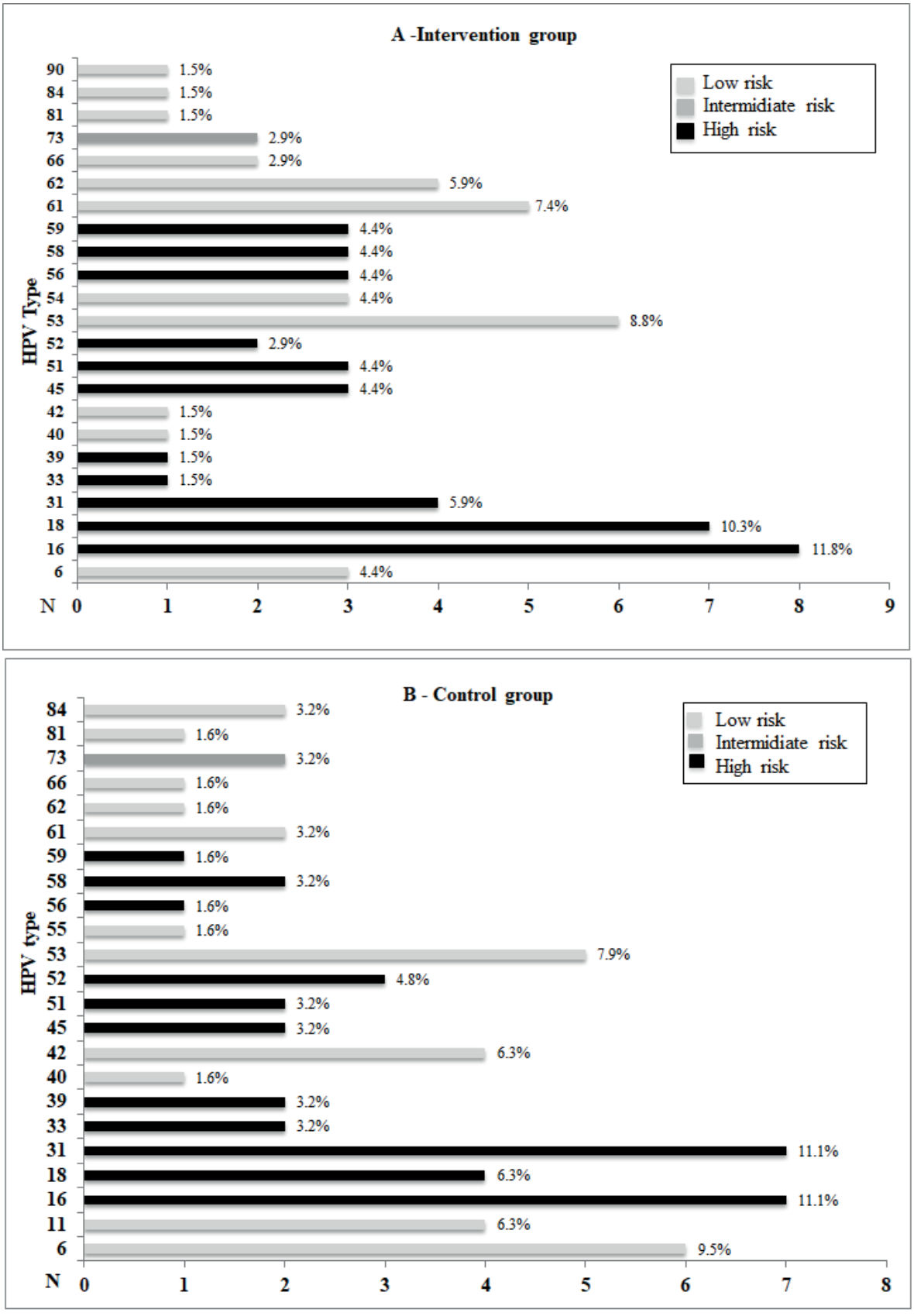


**Figure 2.** ***A-B,*** HPV type infection at baseline in intervention and control group.

the lesion >50%), and only 10 (38.5%) had an unchanged persistent genital lesion, requiring a physical treatment. Among controls, only 5 patients of group A became lesion-negative (25%), and 15 had persistent genital lesion with successive physical treatment. On the contrary, regarding patients of group B, no patient developed a "*de novo*" genital lesion either in case or control group.

At T2, a total of 24 patients (60%) were HPV negative (15/26, 57.7% of group A; 9/14, 64.3% of group B) (Figure 3A). In detail, analysing infection clearance considering the HPV genotype, 13 patients (62%) with high-risk infection were negative at T2 (Figure 4A). In control group, 9 patients (26%) were HPV negative (5/15, 25% of group A; 4/11 26.7% of group B (Figure 3B). In particular, 3 patients (23.1%) with high-risk infection were negative at T2 (Figure 4B).

Finally, considering intervention patients, among 16 with persistent infection at T2 ("non-responders"), 10 (62.5%) had only one HPV genotype, 4 (25.0%) had two HPV genotypes, and 2 (12.5%) had more than two genotypes (Figure 5A); in control group, regarding 26 patients without HPV clearance, 14 patients (53.8%) had only 1 HPV genotype, 10 (38.5%) had 2 HPV genotypes, and 2 (7.7%) 3 genotypes (Figure 5B).

Logistic regression analysis showed that intervention had a crude OR of 4.3 (95% CI 1.6-11.6) to clear HPV infection and an adjusted OR of 4.9 (95% CI 1.6-15.1) after controlling for age. In particular, the adjusted OR was 3.5 (95% CI 0.8-15.0) among patients with lesions and 7.9 (95% CI 1.20-52.7) among patients without lesions.

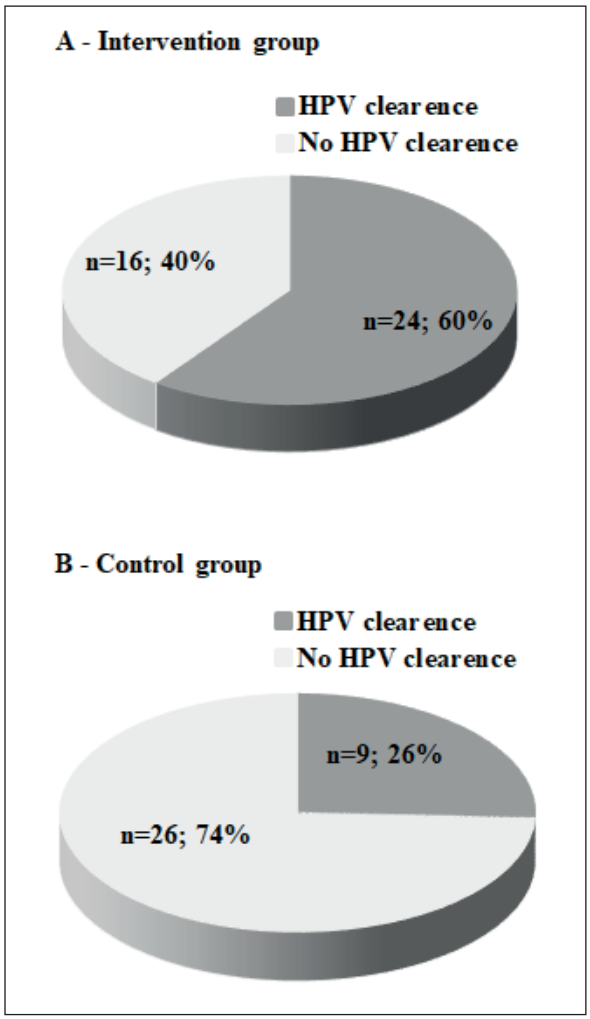


**Figure 3.** ***A-B,*** HPV infection clearance after treatment in intervention and control group.

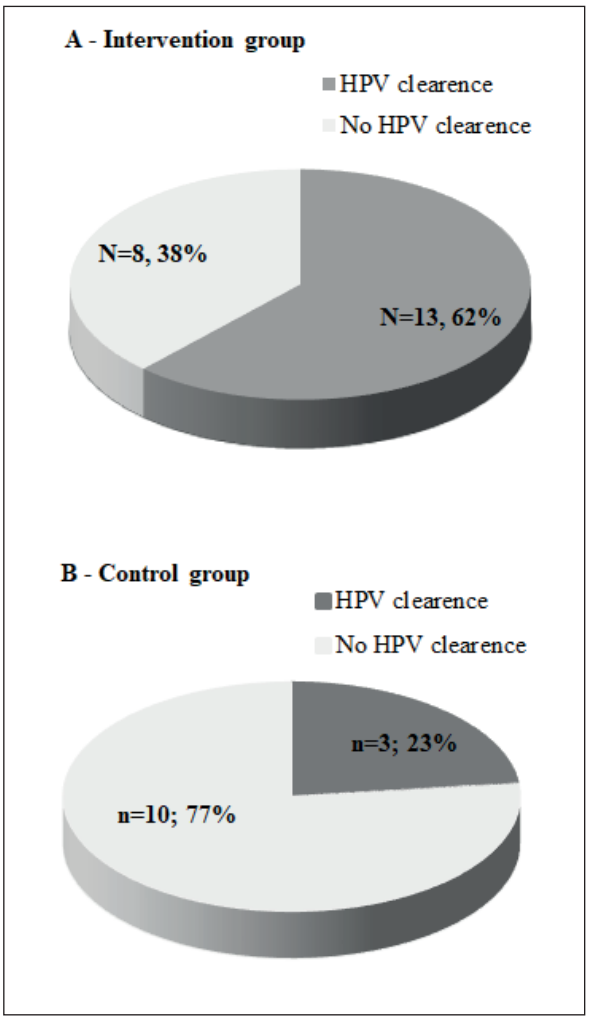


**Figure 4.** ***A-B,*** HPV infection clearance among women with high grade HPV types after treatment, in intervention and control group.

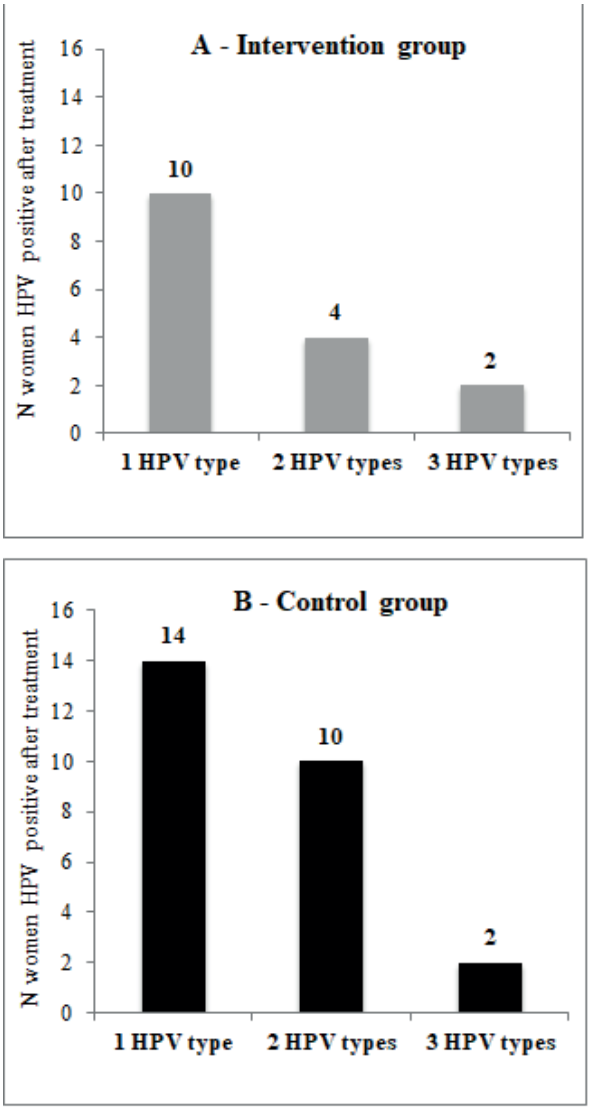


**Figure 5.** ***A-B,*** Number of HPV coinfection among women positive after treatment, in intervention and control group.

## Discussion

Although HPV infections are extremely common, so that it has been estimated that most sexually active women and men are infected at least once in their life, the majority of these infections are temporary[22]. HPV is generally erased by the local immune system and about 60-90% of infections resolve spontaneously within one or two years[23,24]. In particular, the monthly viral clearance rate is globally higher for non-oncogenic types than for oncogenic HPV infections: it has been estimated that the mean infection duration is 8.2 and 13.5 months for non-oncogenic and oncogenic types, respectively[25]. Recent data[3] obtained from the large control arm of the double-blind, randomized controlled *PATRICIA* trial, showed a natural clearance of 53% among all HPV infections at 12 months. Thus, fewer than 10% of HPV infections are persistent, and only a few persistent infections progress to different grades of cervical intraepithelial neoplasia (CIN) and, eventually, to cancer. The entire process of carcinogenic transformation from HPV infection to invasive cancer takes many years in most cases, thus providing a wide window of opportunity to detect the disease at the pre-invasive stage when treatment is highly effective[26,27].

For this reason, recently, researchers have focused attention on CG, a sulfated polysaccharide compound extracted from red algae, used as a thickening agent for a variety of foods and cosmetic products[28]. Its structure is similar to heparan sulfate, which is a HPV cell-attachment factor, and has been shown to prevent the binding of HPV virions to cells *in vitro*; it also exerts a second heparan sulfate-independent inhibitory effect, possibly due to the action of CG as an obstacle between virion surfaces and cellular proteins involved in the infectious process. These CG characteristics could be associated with the negativization of the HPV genital infection[1,16,17,29].

Considering the potential role of CG in HPV infection, in the last few years, clinicians have focused their attention on evaluating how tolerable and safe CG-based gels are. It seems easy to apply, is pleasant or neutral in feel and smell, and non-irritating. Furthermore, most women considered the extra lubrication it gives an advantage[18,30].

Literature data demonstrate how CG-based gel is well tolerated and safe after vaginal application[15]. The results of our study supported this evidence, indicating that the gel formulated with CGP is not associated with severe adverse reaction nor significant genital irritation when administered once daily or on alternate days. In particular, the 5-point Likert scale data showed evident satisfaction in 90% of patients.

Regarding the secondary objective of the report, the results on HPV clearance at T2 follow-up, after 4 months of treatment, seem to be very interesting. Indeed, 60% of patients of the intervention group (15/26, 57.7% of group A; 9/14, 64.3% of group B) became HPV negative. Among these, 13 cases were high-risk HPV infection. These results appear to be notable, mainly due to the short time frame considered and the evidence of literature on the topic. Comparing our research data, patients treated with CG gel showed a HPV

clearance five-time higher ($p$=0.005) in comparison to control group: despite the small number of patients in the study, the effectiveness of intervention seems to be even higher among patients without lesion ($p$=0.032) suggesting more clearance when the infection was recent.

Besides, the fact that no patient of intervention group (both group B and group A patients, after physical treatment) developed a "*de novo*" genital lesion, is worth highlighting, despite the small study sample.

However, our analysis had some limitations. It was a single-centred study and patients were not randomized. We could not value how effective CG based gel is in the clearance of a specific *papillomavirus* genotype and we did not have data on two extreme categories of women (under 20 and over 45 years old), who are in any case sexually active. Moreover, the short study observation period does not allow conclusions to be drawn on long-term results.

## Conclusions

We suggest that *Carvir* vulvovaginal microbicide gel is safe and well-tolerated. Our experience also supports the hypothesis that CG has a role in accelerating the normal clearance of HPV infection in women with a positive HPV DNA test, as well as limiting the genital damage, caused by the papillomavirus itself. This fact reflects on the opportunity that the study is expanded with randomization protocols and a prolonged follow-up. Further researches could also clarify whether a single course of treatment is always sufficient or if a further treatment cycle could be necessary, especially in the case of HPV high-risk infection.

### Conflict of Interests

The Authors declared that they have no conflict of interests.

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