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ORIGINAL ARTICLE

Efficacy of carboxymethyl beta-glucan in cervical intraepithelial neoplasia: a retrospective, case-control study

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ABSTRACT

BACKGROUND: Persistent human papillomavirus (HPV) infection constitutes the principal risk factor for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. For this reason, new drugs have been studied to support the host immune system against the HPV infection. The aim of this retrospective, case-control study was to detect the efficacy and safety of carboxymethyl β-glucan (Colpofix®) gel as adjuvant therapy in HPV infection.
METHODS: The medical records of patients attending the Colposcopy Service of four hospitals in Rome from 2011 to 2013 were collected. Case arm consisted of patients submitted to local therapy with Colpofix®. Control arm comprised patients who did not receive this therapy. A total of 999 patients were included, divided into four groups, according to their cytological and histological specimens, colposcopy and subsequent management.
RESULTS: Local therapy with Colpofix® gel resulted effective with respect to no therapy for the regression of low-grade CIN (CIN1) in patients submitted to follow up (P=0.0204), while it was no effective for the regression of CIN1 submitted to ablative therapy and high-grade CIN (CIN 2+) (P value not significant).
CONCLUSIONS: In conclusion, Colpofix® gel represents a valid alternative to "wait and see" strategy in patients affected by CIN1. Further prospective studies are warranted to confirm these results.

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Key words: Papillomaviridae - Cervical intraepithelial neoplasia - Beta-glucans.

Human papillomavirus (HPV) represents a significant source of morbidity and mortality worldwide. High-risk, oncogenic HPV types are associated with 99.7% of all cervical cancer.[1]

HPV16 is the most common type and, combined with HPV18, accounts for over 70% of all cases of cervical cancer.[2] The majority of HPV infections are transient and only persistent oncogenic HPV infections constitute a risk factor for the development of cervical intraepithelial neoplasia (CIN) and cervical invasive cancer.[3]

Immunity plays a key role in the clearance of HPV infection. Innate immune response represents the first line of defense against the infection during the early stages of HPV infection, promoting a cytokine-mediated pro-

inflammatory process, which links the innate with the adaptive immune response. Evasion of the immune response by HPV, on the other side, is critical for the persistence of the infection and leads to a higher risk of progression to cervical cancer precursor lesions (CIN3).[3]

Thus, stimulation of the innate immune response through adjuvant medical devices may represent a promising therapeutic strategy for disrupting the evasion mechanisms of HPV. Between these medical devices, one of the most promising is the β-glucan. β-glucans consist of polysaccharides of β-D-glucose that, through immune response modulation, may take part in the clearance of HPV infection by supporting the host immune system. In the present study, the efficacy and safety of carboxymethyl β-glucan (Colpofix®) gel are described for the treatment of women performing colposcopy for abnormal Pap test.

Materials and methods

A multicenter, retrospective, case-control study was conducted reviewing medical records of patients attending Colposcopy service for abnormal Pap test in four hospitals in Rome (Umberto I Polyclinic, Sant'Eugenio Hospital, Cristo Re Hospital, and San Carlo di Nancy Hospital) from May 2011 to May 2013. Case arm consisted of all the patients to whom therapy with carboxymethyl β-glucan (Colpofix®) gel was administered. Conversely, the control arm comprised patients who did not receive this therapy. The study protocol was approved by the Institutional Review Board of the Umberto I Polyclinic. This retrospective cohort study did not require patient informed consent, in accordance with the current rules of our hospital.

For all the patients, both in the case that in the control arm, a colposcopy-guided biopsy was performed if the examination yielded positive results. According to histological results, patients were submitted to therapy (destructive or excisional) or follow-up.

Follow up provided to perform colposcopy and Pap test every six months in two years. Patients submitted to therapy were also checked every six months with colposcopy and cytology in two years. When colposcopy resulted positive by follow-up examinations, another biopsy was carried out. All participants received standard of care treatment, according to the guidelines released by the Italian Society of Colposcopy and Cervico-vaginal Diseases.[4]

Exclusion criteria for study population selection were: prior cervical treatment within 18 months, immunodeficiency, state of pregnancy, inappropriate colposcopy (non-visualized squamocolumnar junction, endocervical lesion) and incomplete follow-up (less than 12 months).

Selected patients of both case and control arms were divided into four groups according to their cytological and histological specimens, colposcopic appearance, and their subsequent management:

— Group 1: atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL) and negative colposcopy;

— Group 2: ASCUS/LSIL with positive colposcopy and histologic cervical intraepithelial neoplasia grade 1 (CIN1); management: follow up;

— Group 3: ASCUS/LSIL with positive colposcopy and histologic CIN1; management: ablative therapy;

— Group 4: high-grade squamous intraepithelial lesions (HSIL) or LSIL with positive colposcopy and histologic cervical intraepithelial neoplasia grade 2+ (CIN2+); management: loop electrosurgical excision procedure (LEEP).

Colpofix® Gel was applied in vagina by single-dose applicators of 1 mL once a day for 20 days per month, in two months, in patients of Groups 1 and 2. In patients of Groups 3 and 4, Colpofix® was administered one month after treatment for 20 days per month in two months.

For group 1 and 2, patients were considered positive during follow-up when positive cytology and/or colposcopy were found at 6 and/or 12 month's control. Group 3 patients were considered positive when histologic diagnosis of CIN1+ was obtained after an abnormal colposcopy at 6 and/or at 12 months' follow-up. Group 4 patients were considered positive when histologic diagnosis of CIN2+ was ob-

TABLE I.—*Baseline characteristics of the study population.*

Characteristics	Group 1	Group 2	Group 3	Group 4	Total
Median age	35.3±10	32.5±9.8	35.2±8.9	41.3±10.1	36.1±10.0
Median age at first intercourse	17.6±2.5	16.6±2.1	17.4±2.9	17.3±2.0	17.3±2.5
Median N. sexual partners	3.4±1.3	3.9±2.2	4.0±3.0	3.8±2.5	3.7±2.3
Parity	0.9±1.1	0.7±0.9	0.9±1.0	1.1±1.2	0.9±1.0
Smokers	145 (46.6%)	125 (40.0%)	108 (54.9%)	45 (25.0%)	425 (42.6%)

tained after an abnormal colposcopy at 6 and/or 12 month's follow-up control.

Apart from Colpofix® Gel administration, no other treatment was administered, prescribed, or modified as part of this study. Furthermore, no diagnostic test was done for the sake of this study.

Statistical analysis

The effect size of each study arm was estimated using the R statistical software package. Data analysis was performed using an unpaired *t*-test.

Results

A total of 999 patients were selected for the study. Characteristics of the patients are described in Table I. Median age was 36.1±10.0 years (range 18-65); median age of the first sexual intercourse was 17.3±2.5 years (range 13-30); number of sexual partners was 3.7±2.3 (range 1-20), parity was 0.9±1.0 (range 0-4). Smokers were 42.6% of the total.

There was no significant difference in patient characteristics between the case and the control arms.

Group 1

At six months follow-up, negative cytology and colposcopy were obtained in 181 of 187 patients (96.8%) from the Colpofix® arm, and in 96 of 124 patients (78%) from the control arm (P=0.02). At 12 months' follow-up, 183 of 187 (98%) patients from the Colpofix® arm and 108/124 (87%) from the control arm had negative cytology and colposcopy (P=0.0204) (Table II).

Group 2

Negative cytology and colposcopy were observed at 6 months' follow-up in 95.7% (161/168) of patients from the Colpofix® arm and in 71% (102/144) from the control arm. At 12 months' follow-up, negative cytology and colposcopy were observed in 95.7% (161/168) of patients from the Colpofix® arm, and in 79% (114/144) from the control arm (P=0.0204) (Table II).

Group 3

At six months' follow-up, 91/99 (91.9 %) patients in the Colpofix® arm and 88/98 patients (90%) in the control arm resulted negative (P=NS). At the 12-month visit, 94/99 (95%) patients from the Colpofix® arm and 94/98 (96%) patients from the control arm resulted negative (P=NS) (Table III).

TABLE II.—*Negative cytology and/or colposcopy at 6 and 12 months' follow-up in groups 1 and 2.*

Group	6-month follow-up	12-month follow-up	P value
Group 1			
Colpofix®	181 (96.8%)	183 (98%)	0.02
Control	96 (78%)	108 (87%)	
Group 2			
Colpofix®	161 (95.7%)	161 (95.7%)	0.02
Control	102 (71%)	114 (79%)	

TABLE III.—*Negative histology (<CIN2+) at 6 and 12 months' follow-up in groups 3 and 4.*

Group	6-month follow-up	12-month follow-up	P value
Group 1			
Colpofix®	91 (92%)	94 (95%)	NS
Control	88 (90%)	94 (96%)	
Group 2			
Colpofix®	81 (100%)	78 (96.4%)	NS
Control	96 (98%)	94 (96%)	

Group 4

At 6 months' follow-up, 81/81 patients (100%) from the Colpofix® arm and 96/98 (98%) from the control arm were negative (P=NS). At the second visit, at 12 months, 78/81 (96.4%) patients from the Colpofix® arm and 94/98 (96%) patients from the control arm resulted negative (P=NS) (Table III).

No adverse event related to administration of Colpofix® was observed during this study. No cases of invasive cervical cancer were observed during this study.

Discussion

The principal risk factor for development of cervical carcinoma is the persistence of HPV infection. Anyway, about 90% of HPV infections are transient and these subjects do not develop clinical signs and symptoms, because the host immune system resolves most of infections.[5]

Innate, humoral and cellular-mediated immunity are involved in HPV infection resolution.

Keratinocytes and stem cells on the basal layer of the cervical epithelium represent the target of HPV infection and play an important role in the immune response. These cells express on their surface Toll-like receptors (TLRs), a family of immunological receptors that recognize pathogen-associated molecular patterns (PAMPs). Viral nucleic acids are recognized by TLRs, promoting T-helper 1 (Th1) and T-helper 2 (Th2) cytokines expression and CD4 and CD8 memory T cells.

In particular, activation of TLR-9 in keratinocytes results in production of tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), chemokine ligand 2, chemokine ligand 20 (CCL2, CCL20), chemokine ligand 9 (CXCL9) and type 1 interferon (IFN-1). The relationship between TLRs and HPV infection clearance has been subject of study over the last few years.

A recent study revealed that greater cervical expression of TLR2, -3, -7, -8, and -9 after incident infection with HPV16 is significantly associated with subsequent clearance within 4 months. In women who cleared the infection, expression of TLR1, -3, -7, and -8 was significantly associated with increased secretion of antiviral alpha 2 interferon (IFN-α2).[6] Another study of the same group examined the clearance of HPV16 infection following periods of persistence founding that higher expression of TLR3 or TLR7 was predictive of HPV16 clearance by the following visit.[7]

Another important part of innate immune response was to be given to natural killer cells (NK cells), which respond quickly to stressed cells.[8]

In patients with intraepithelial and invasive cervical HPV lesions Th2 cytokine profile is prevalent: IL2 and TNF-levels (both belong to the Th1 pattern) appear lower in HPV lesion than in healthy women.[9] Overall, in HPV lesions both Th1 and Th2 phenotypes are suppressed, especially Th1.

The β-glucans consist of linear unbranched polysaccharides of β-D-glucose. The basic β-D-glucan is a repeating structure with the β-D-glucose units joined together in linear chains by β-bonds. The sources of glucans are various, including fungi (*e.g.*, mushrooms), yeast and seaweed, as well as barley.[10] These polysaccharides are considered biological response modifiers because they take part in the immune response modulation. In fact, they are recognized as non-self by the immune system and act on specific receptors on host cells: dectin-1, complement receptor 3 (CR3), TLR 2-6.

In particular, linkage between TLR2 and beta-glucan promotes activation of signaling pathways taking to arachidonic acid metabolism and production of cytokines (IL-12, IFN-α), activation of dendritic cells and adaptive immune response with Th-1pattern and consequent less activation of NK cells and Th-2 pattern. Studies of literature revealed that such controlled activation of immune response plays a protective role against pathogen organisms.

Only one study in literature investigated efficacy of Beta-glucan as local therapy for HPV cervical infection. This study demonstrated the effectiveness of the treatment with beta-glucan in women with ASCUS-LSIL lesions and

HPV-CIN1 lesions, with increasing regressions rate after 12 months of the treatment in 15-20% of the subjects.[11] However, no control arm was considered in this study.

The present survey is the first case-control study investigating the efficacy of treatment with Colpofix® on HPV infection related diseases in four groups of patients, including patients treated with either destructive or excisional methods for low- and high-grade cervical intraepithelial neoplasia.

As described in literature, the cumulative probability of cytological regression within 12 months in biopsy-negative LSIL patients is 71.2% [12] and is consistently lower (61%) in LSIL/CIN1 histological-proven patients.[13] In the present study, regression rate in Group I and Group II, evaluated as negative cytology and/or colposcopy after 6 months, was respectively 98% and 95.7% in Colpofix® arm and resulted statistically higher when compared with regression percentage in the control arm: 78% and 71% (P=0.0204).

In the present study, recurrent CIN1 after ablative therapy for low grade intraepithelial neoplasia was about 10% at 6 months and 4-5% at 12 months, both in Colpofix® that in control arm (P=NS). These outcomes are similar to results described in the literature.[14]

No patient in the Colpofix® arm and 2 (2%) patients in the control arm developed recurrent disease 6 months after LEEP; whereas 3-4% of patients experienced relapse after one year follow up, both in the Colpofix® and in the control arm, showing no statistically significant difference between the two arms. Furthermore, the recurrence rate of both the arms observed in our population after excisional therapy for high grade intraepithelial neoplasias resulted much lower than that described in literature: 9-19%.[14-16]

Limitations of the study

The main limitation of this study is that it was conducted retrospectively. However, a large study population was considered and results are statistically significant.

Conclusions

In conclusion, local therapy with application of Colpofix® gel resulted effective with respect to no therapy for the regression of low-grade cervical intraepithelial neoplasias in patients submitted to follow-up for cytologic ASCUS/LSIL. It represents a valid alternative to the "wait-and-see" strategy in this setting of patients. Furthermore, it is a self-managed, low-cost therapy, with a high grade of compliance and no proven adverse event. Further prospective studies are needed to validate the use of this kind of therapy.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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