Clinical response to glycyrrhizinic acid in genital infection due to human papillomavirus and low-grade squamous intraepithelial lesion

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Abstract

Human papilloma virus (HPV) can infect any of the mucosal areas of the body and cause cervical cancer. Until recently, no specific treatments were available for this condition; therefore, any damaged tissue had to be removed or destroyed, which may have presented obstetrical repercussions for some women. Recently, new drugs have been developed that have shown to be effective for the cure of HPV infection. Glycyrrhizinic acid (GA) has shown fewer side effects and its systemic use makes it possible to reach difficult-to-treat lesions. The purpose of this study was to evaluate the clinical outcome of GA to eliminate the epithelial lesion and HPV. We carried out a longitudinal, descriptive study that included women of reproductive age who were diagnosed with HPV associated with low-grade squamous intraepithelial lesion (LSIL). Subjects began treatment based on GA using two routes of administration - systemic (oral) and topical (spray) - with assessments every month to determine the clinical changes of the lesions through colposcopy and Papanicolaou (Pap) smear. Simple statistics were used along with two-tailed Student's t-test; $P<0.05$ was considered statistically significant before and after treatment. There were 70 eligible patients, of whom 62 fulfilled the inclusion criteria. Age of subjects was 27.8±9.5 years. At the time of the study, 100% of the patients had HPV infection, 40% were associated with LSIL, and only 16% used a barrier contraceptive (condom) method. Resolution was achieved in all patients from 4 weeks of treatment initiation and improvement was achieved in the majority of patients at 12 weeks (74%) ($P<0.001$). However, there was persistence of LSIL in 27.7% of patients and only one patient progressed to cervical intraepithelial neoplasia (CIN) II. The use of GA proved to be effective in resolving clinical HPV lesions. For cervical lesions with epithelial changes (LSIL), treatment may be required for a longer period as with other drugs used for this infection, as well as monitoring for at least 1 year according to the natural evolution of the disease.

Introduction

In recent years, human papilloma virus (HPV) has become one of the most frequent sexually transmitted infections, producing initial lesions known as genital warts. These may appear as small individual pimples or as small, flat or raised groups and can appear weeks or months after sexual intercourse.[1,2] Estimates of the prevalence of HPV range from 14% to 45%, and >100 viral genotypes have been reported, of which 40 can infect any mucosal body areas and are classified as high and low risk.

The high-risk group is constituted by genotypes 16 and 18 and the low-risk group by genotypes 6 and 11. The latter are rarely found in neoplastic lesions but cause condyloma acuminata.[3,4] However, it has been established beyond dispute that oncogenic HPV infection represents a risk factor for the single most important development of squamous intraepithelial lesion.[5,6] The high incidence and mortality due to preventable tumors has been linked to disparities in access to care and treatment, placing cervical cancer as the second most common cause of death in women as well as causing enormous social unrest in those women with HPV infection.[7,8] Primary prevention with vaccination against HPV is often inaccessible due to its high cost.[9,10]

In addition, if left untreated, these lesions can have implications in regard to reproduction, recurrent opportunistic genital infections and, in extreme cases, anatomic changes. Until recently, there were no specific treatments for this condition. Damaged tissue had to be destroyed or removed which, in young women, could have reproductive consequences.[11,12] Recently, however, new drugs have shown good effectiveness to cure HPV infection, among which is GA[13,14] that has shown fewer adverse effects regarding irritation, burns, complexity in application and control of dosage, as well as the possibility of systemic use that allows treating those lesions not visible or difficult to reach anatomically. The purpose of this study was to evaluate the clinical outcome of GA on the elimination of intraepithelial lesions and HPV.

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Materials and Methods

The study design was longitudinal and descriptive. Women of reproductive age who were sexually active were included. Women were recruited from the outpatient gynecology clinic and were diagnosed with HPV on colposcopy with low-grade squamous epithelial lesions (LSIL). Subjects had a history of vaginal discomfort and recurrent transvaginal discharge, without previous treatment. Treatment based on GA was initiated using two routes of administration - systemic (oral) and topical (spray) - carried out for a minimum period of 8 weeks. After being explained the reason for the study and treatment with GA, all patients were asked to sign informed consent for their participation. During the initial evaluation a survey was conducted and demographic data were recorded in regard to sexual behavior and reproductive health. After initiation of therapy, all patients had colposcopy and Pap test repeated each month to determine any clinical and lesion changes that were recorded graphically. Disposable materials were used for sampling and periodic reviews. Disposal of contaminated materials was in accordance with safety standards for the elimination of biological and toxic waste products.[15] Treatment and follow-up were carried out without interruption until cure was demonstrated (no HPV lesion established by colposcopy and Pap smear). Persistence of the disease was considered when there was no improvement in the lesion and there was advancement to other stages of the LSIL.

For statistical analysis, simple statistics were used with measures of central tendency and dispersion, and two-tailed Student's t-test

to compare between-group differences of the changes; $P < 0.05$ was considered statistically significant before and after treatment.

Results

There were 70 eligible patients, of whom 62 patients fulfilled the inclusion criteria. Patients were aged 27.8±9.5 years. Obstetric characteristics are described in Table 1. At the time of the study, 100% of the patients presented with HPV infection, and 40% were associated with LSIL. Only 16% were using barrier method contraception such as a condom, which offers some protection for HPV; 50% did not use any form of contraception and the remainder were distributed between mechanical methods and hormonal action (Table 2). Recovery was achieved in all patients with the use of GA, which was observed from 4 weeks of treatment initiation but without significant difference. Improvement in most patients (74%) was demonstrated at 12 weeks ($P<0.05$), and in all patients after 13 weeks ($P<0.001$) (Table 3). However, there was persistence of LSIL in 27.7% of patients and only one patient progressed to CIN II; therefore, the usual treatment for the lesion had to be complemented.

Discussion

In most patients, partial clinical remission of HPV at 2 weeks of treatment initiation was observed as demonstrated by colposcopy and Pap test, although without statistical difference that, after 4 weeks, become significant. Some cases persisted with LSIL because the drug has no specific action on these types of lesions, similar to the drugs currently used to treat HPV infection.

There were no secondary side effects with the doses used for the required time up until clinical improvement compared with other recently used drugs. This allowed better treatment adherence by patients because once the drug was initiated, treatment was not abandoned until a positive clinical outcome was achieved. In addition, this also allowed application of a topical drug for the same patient quickly and easily without the need for frequent scheduled medical visits as necessary with other drugs used for this same purpose.

Cervical cancer has a high incidence in developing countries. Therefore, in these preventable lesions it has been observed that there are great disparities in access to care and treatment such as inequality in regard to access to health services in low-income women as well as poor quality and poor infrastructure in rural-area preventive programs.[16-18] Understanding the natural history of HPV infection and the principal characteristics related to its evolution are among the most important epidemiological aspects of this disease. Information available regarding the clinical infection of HPV is identical to the evolution of CIN. These consist of developing latent and subclinical infections and the tendency of the virus to remain in a persistent state for an extended period and then reactivate itself or undergo spontaneous resolution.[19]

HPV DNA has been found in 99.7% of cervical cancer cases. The clinical lesion is visible and is characterized by condylomas or genital warts. The subclinical lesion causes no symptoms and is diagnosed by colposcopy or histology. Latent infection is associated with DNA of HPV in tissue that has no clinical or histological abnormalities. Approximately 70% of women with HPV infections become negative for HPV DNA determination in a year and up to 91% at 2 years; 10% of infected women will suffer from persistent infections, which depends on the immune response of the female genital tract as part of an integral systemic response as well as on other factors such as genetic, environmental, nutritional, and even cultural.[20] Adding to this, there is the risk of developing precancerous lesions and even to develop cervical cancer. This process normally takes an average of 15 years, offering many opportunities for detection and treatment.[21]

Along with the immune response, several risk factors have been identified that appear to be associated with the behavior of HPV, the most important being smoking, prolonged use of oral contraceptives, parity, sexually transmitted infections, chronic inflammation and nutritional factors as deficiency of antioxidants such as folate and vitamins A, C and E.

It is know that, in general, simple dietary changes, maintaining an optimal body weight and regular physical activity may prevent up to 30% of cancers and merely changing one's diet with increased fruits and vegetables could prevent 20% of cancers. Identification and modification of cofactors are important because they can have a significant impact on modulating the persistent infection and progression of HPV infection to neoplasia.

At present there are various treatments for squamous intraepithelial lesions, but these are not effective for HPV. These treatments include chemically induced cytotoxicity and ablative methods such as cryotherapy, electrocoagulation diathermy, thermocoagulation, CO_2 laser vaporization, loop diathermy, cold knife conization and hysterectomy that allow the destruction and excision of HPV-infected tissue. There are topical treatments such as organic acids, trichloro- and bicloracetic acid and antimetabolites that include 5-fluorouracil and antifungal agents such as podophyllin and podophyllotoxin. There are also therapeutic vaccines, antivirals (acyclovir, vidarabine) and, finally, immunoregulators such as interferon-alpha and imiquimod.[12,22] In relation to the drug used in the current study, it is known that the beneficial effect is obtained because GA increases the production of viral cyclin, which induces the selective death of virus-infected cells. GA also stimulates production of interleukin-12 in macrophages, which facilitates the development of T-helper lymphocytes in cell-mediated immune response, inhibits viral protein phosphorylation mediated by cellular kinases and modifies the posttranslational signals essential for growth. It also promotes inhibition of prostaglandin E2 in damaged tissue, causing inflammation and increased tis-

Table 1. Obstetrical characteristics of the patients (n = 62).

Variable	Mean ± SD
Gestations	1.7±1.0
Births	1.9±0.3
Abortions	1.7±0.1
Cesareans	1.3±0.2
Rhythm	30.9±8.7×4.2±1.6

Table 2. Contraceptive method used and reason for no pregnancy (n=62).

Method	Frequency (%)
None	50
Condom	16
IUD	10
Oral hormones	10
Tubal ligation	8
Coitus interruptus	8
Injectable hormones	2
Primary sterility	2

Table 3. HPV infection cure (n=62).

Weeks	Frequency (%)	Cumulative (%)	P
4	14.5	14.5	NS
8	27.6	42.1	NS
12	31.9	74.0	<0.05
>13	26.0	100.0	<0.001

NS, non significant.

sue damage as well as inducing interferon production, which promotes the activation of macrophages and, consequently, an increase in their phagocytic properties and destruction of microorganisms. Use of GA demonstrated efficacy in resolution of clinical HPV lesions. For cervical lesions where there may be epithelial changes, it may be required to be used for longer periods of time, as well as to carry out follow-up for at least 1 year according to the natural evolution of the disease. In order to detect an early-stage cervical lesion, it is necessary to have regular pelvic examinations as well as periodic Pap smears. Timely medical care is imminent if there are symptoms of postcoital bleeding, metrorrhagia, and thick or bloody vaginal discharge, as well as ongoing pelvic pain or pain during sexual relations.

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