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Original paper

# Ozone Therapy in Papillomavirus Infection- HPV

# Ozonoterapia en la Infección por Virus del Papiloma Humano (HPV)

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## Keywords

Intravaginal ozone, ozonized oil, Human Papilloma Virus, Ozonated Saline Solution, genotype 16, 18, Condiloma Acuminata, ozone therapy, womb cancer.

## Abstract

We analyze a study of 30 patients infected with human papillomavirus (HPV) without having spontaneous remission after one year as of the first diagnosis. The samples were collected between October 2014 and February 2017. Cervical swabs were analyzed both HPV genotyping by PCR and Reverse Line Blot, and cervical cytology by Pap testing.

The study was carried out with the objective of verify the efficacy of O2 / O3 alone and in combination with other complementary measures. The patients were divided into two groups.

**a) Group O3 + (treated with ozone plus integrative therapy):** It was treated with intravaginal ozone, ozonated saline solution, micro-immunotherapy and administration IV of GSH, Se, Zinc and VitC.

**b) Ozone Group (treated exclusively with ozone):** It was administered with intravaginal ozone therapy alone. Although the number of patients does not allow a safe conclusion, the group of patients treated with ozone plus integrative therapy presented a great advantages ($p<0,05$) compared to the group of patients treated exclusively with ozone.

**Positive (negative cytology after 3 months of finishing the therapy)**

- Group O3 + (patients treated with ozone plus integrative therapy):16 de 20 => 80.0 %
- Ozone group (patients treated exclusively with ozone): 6 de 10 => 60.0 %

It was shown that the synergy of the ozone therapy with the other treatments was more effective, permanent and harmless than the application of intravaginal ozone alone..

Palabras clave

*ozono intravaginal, aceite ozonizado, Virus del Papiloma Humano, Solución Salina Ozonizada, genotipo 16, 18, Condiloma Acuminata, ozonoterapia, cáncer de útero.*

## Resumen

*Se realizó un estudio sobre 30 pacientes infectadas con virus de papiloma humano (HPV por su sigla en inglés), que no habían tenido remisión espontánea al transcurrir un año desde el primer diagnóstico. Las muestras fueron recogidas entre octubre 2014 y febrero 2017. Se analizaron los frotis cervicales tanto por genotipificación del HPV por PCR y Reverse Line Blot, y citología cervical por la prueba de Papanicolaou. El estudio se realizó con el objetivo de comprobar la eficacia del O2/O3 solo y en combinación con otras medidas complementarias. Las pacientes fueron divididas en dos grupos*

*a) **Grupo O3 + (tratado con ozono más terapia integradora):** Fue tratado con ozonoterapia intravaginal, solución salina ozonizada (SSO3) IV, microinmunoterapia oral, Glutatión, VitC, Se y Zinc por vía I.V*

*b**) Grupo ozono (tratado solo con ozono):** Fue tratado únicamente con ozonoterapia intravaginal. Si bien el número de pacientes no permite unas conclusiones seguras, las pacientes del grupo tratado con ozono más terapia integradora presentaron grandes ventajas comparadas (p<0,05) con el grupo tratado solo con ozono*

***Positivos (citología negativa después de 3 meses de concluir la terapia)***

- ***Grupo O3** + (tratado con ozono más terapia integradora): 16 de 20 => 80.0 %*
- ***Grupo ozono** (tratado solo con ozono): 6 de 10 => 60.0 %*

*Se demostró que la sinergia de la terapia del ozono con los otros tratamientos fue más efectiva, permanente e inocua que la aplicación de ozono intravaginal en solitario*

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## Introduction

Human papillomavirus infection (HPV for short) is a global concern. And rightly so. Cervical cancer is caused by infection of the human papillomavirus. Persistent infection with a high-risk virus (especially type 16) can cause cancer of the cervix, vulva, vagina, anus, penis, and oropharynx.[10]

One of the reasons, among others, for the increase in the infection lies in the rise of sexual initiation at very early ages and to have an important and promiscuous sexual activity. The most recent Spanish figures (April 2017) indicate that the percentage of women aged 15 who have had sex is 19%; and the average age range at the time of first intercourse is between 16.5-22.7 years of age.[11]

On the basis of a meta-analysis of 1 million women with normal cervical cytology, around 291 million women worldwide are estimated to have human papillomavirus infection of the cervix at a given point, corresponding to an average prevalence of 10•4%, though prevalence is higher in women younger than 25 years (16•9%). It should be added that human papillomavirus infections detected in women aged older than 30 years persist for longer than those in younger women. The meta-analysis keeps saying: "Human papillomavirus is one of the most powerful human carcinogens and has been implicated in cancers at several sites. Roughly 610000 new cancers per year (5% of all cancers) have been attributed to human papillomavirus infection, of which more than 80% occurred in developing countries."[10]

In Spain women of 15 years and older (20.17 millions) are at risk of developing cervical cancer. "Every year 2511 women are diagnosed with cervical cancer and 848 die from the disease. Cervical cancer ranks as the 10th most frequent cancer among women in Spain and the 2nd most frequent cancer among women between 15 and 44 years of age. About 2.7% of women in the general population are estimated to host cervical HPV-16/18 infection at a given time, and 63.1% of invasive cervical cancers are attributed to HPVs 16 or 18." [11]

US figures show that each year 12.966 women are diagnosed with cervical cancer and 6.605 die from the disease. "Cervical cancer ranks as the 13th most frequent cancer among women in USA and the 4th most frequent cancer among women between 15 and 44 years of age. About 3.9% of women in the general population are estimated to host cervical HPV-16/18 infection at a given time, and 71.2% of invasive cervical cancers are attributed to HPVs 16 or 18."[12]

These chilling figures are on the rise and merit a stronger and integrative treatment such as the one we are proposing here.

High-risk genotypes are found in 90% of cervical cancers, and HPV16 in particular appears to be the most virulent. In men, more than 200 genotypes are known and about 40 of them infect the epithelial cells of the mucous membranes, in particular the genital apparatus, the oral cavity and the skin. Among these, 14 genotypes are considered to be carcinogenic (HPV-RH), namely HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.[1]

The best known are genotypes 16 and 18, since they are linked to the development of cervical cancer, is the second most common cancer in women after breast cancer. All of them are transmitted sexually or cutaneous and are responsible for both benign and malignant lesions. Recently, these genotypes have also been found in certain prostate and bladder cancers.[1, 2] Two types of HPV (6 and 11) are the cause of most cases of genital warts, but this types are low-risk virus, since they do not progress into cancer or other serious health problems.

Papilloma is a circular double-stranded uncoated DNA virus containing approximately 8000 base pairs and 8 genes. The target cell of HPV is the keratinocyte, a stratified growth cell, since this virus needs such stratification to mature and reproduce itself.

This stratified squamous epithelium is found in the skin, vaginal epithelium and junction areas (cervix). The cell receptor for HPV is an α6β4 type integrin present on the surface of basal layer of keratinocytes. When the virus enters into the cell, seeks integrating into the DNA of the cells to survive and replicate itself. Being an uncoated virus, makes him especially virulent, but also has the ability to immortalize the infected cells, which can cause cancer. In fact, it has been demonstrated that there is a link between the high prevalence of cervical cancers and infections with this virus.[3, 6]

When the virus enters into the cells puts in motion a complete machinery to produce different viral proteins. On one hand, early proteins are produced to help the virus to integrate and "deceive" the cell with the interest of continuing cell division, manipulating cellular proteins, such as p53, p21 or pRb. Also late proteins are produced that will form the viral capsid. In addition, virus recognition by the Toll-like receptor (TLRs) initiates an inflammatory environment that hinders the apoptosis of the infected cell.

On the other hand, HPV causes a decrease in the maturation of antigen presenting cells such as Langerhans cells, which results in a poor immune response to viral infection.[5]
Viral RNAs will activate the Toll-like receptors, causing an inflammatory cascade through NF-kB, a nuclear transcription factor capable of increasing the expression of anti-apoptotic genes.

Therefore, the goals of ozone therapy are to:
Prevent the virus from multiplying and infecting new cells.
Decrease the activation of Toll-like receptors and avoid excessive activation of NF-kB.[7]
Favor the immune response of the T cells dependent, as well as the humoral response B dependent, thanks to a better presentation of antigens.
Rebalance the immune response by controlling persistent infection and related diseases.
Neutralize the production of viral oncoproteins, involved in the origin and progression of the neoplastic process.

Keratinocytes (non-professional antigen presenting cells) usually do not have the class II histocompatibility complex on their surface.[2, 5] However under certain conditions (possibly exaggerated expression of proinflammatory mediators) HLA-DR molecules (typically found in antigen presenting cells) are expressed on the surface of keratinocytes. In the case of cervical neoplasia associated with HPV, this is associated with the progression of the disease.[4] The objective of ozone therapy in this case is to hinder the appearance of HLA-DR molecules on the surface of keratinocytes.[4]

The interest in using ozone in the present study was to verify the efficacy of O2 / O3 alone and in combination with other complementary measures in the treatment of HPV infections in the cervix of patients who had not had spontaneous remission within one year from the first diagnosis.

## Materials and methods

A mono-centric, prospective, non-randomized interventional study with ozone and adjuvant therapies was conducted. Thirty female patients with a diagnosis of HPV were treated in a range of ages between 35 and 75 years who attended the consultation on Fiorela Clinic between October 2014 and February 2017.

The research protocol was discussed by the participating researchers and reviewed and approved by the Ethics and Institutional Review Committee (Fiorela Clinic). Once the committee determined that the research complied with the ethical procedures for medical research in humans set up by the Helsinki Declaration issued by the World Medical Assembly[13] the protocol started to be applied. The patients received a detailed explanation of the research and were taken their consent in both verbal and written form.

### Inclusion Criteria

- CIN I to III confirmed with old and recent cytology
- Minimum of 8 months of evolution, maximum 5 years and 3 months.
- Do not perform any other treatment.

### Exclusion Criteria

- Perform other treatments.
- Do not present the Pap smears.
- Do not follow the guidelines.

We used Ozonette generator (Sedecal) classified as medical device IIb and with CE marking.[14] Vaginal devise [7] with continuous circuit of continuous ozone administration (specially designed for this purpose which guaranteed the homogenous and hermetic distribution of ozone in the vaginal epithelium), ozonated oil (Oxonid® 600 IP), Fluvix®, ozonized bidistilled water, 2LPAPI.[5]

Two groups of patients were formed:

a) **O3 Group + (treated with ozone plus integrative therapy)** (n = 20): It was administered with ozone, ozonated saline solution, micro-immunotherapy and administration IV of GSH, Se, Zinc and VitC.

**Ozone Group (treated exclusively with ozone)** (n = 10): It was administered with intravaginal ozone therapy alone during 10 days.

**O3 group + I (treated with ozone plus integrative therapy** (n = 20): It was performed 10 daily vaginal insufflations of ozone, after washing the vagina with ozonated water. 6 sessions of SSO3 [7,8] twice a week, dose: 0.4-0.8 mg; administration IV: GSH 600 mg, VitC 1 g, Zinc 5 µg/mL, IV infusion of Selenium 100 µg/mL twice weekly; Micro-immune-therapy treatment, 2LPAPI (LaboLife)[5,2] for 3-6 months. Daily intravaginal application of ozonized 600 IP oil (Oxonid®)[9]. Daily application of Fluvix® (gel with epidermal growth factor, Lab. Heberfarma).

**Ozone group (patients treated exclusively with ozone)** (n = 10): It received only 10 ozone vaginal insufflations with the same concentrations used in the previous group and that are specified below.

Vaginal insufflation through a vaginal device at concentration of 20 µg/mL O2 / O3, at continuous flow of 200 mL/min during 10 min. The concentration was gradually increased up to 50 µg/mL, at this concentration the flow was maintained for 3-5 min.

Ozonization of bi-distilled water: 400 mL of bi-distilled water at ozone concentration of 50 µg/mL during 10 min at continuous flow rate of 500 mL/min.

The evaluation of the efficacy of the treatment was concluded with vaginal cytology three months after the last therapy.

An exploratory analysis of the data was performed to detect aberrant points (outliers). The experimental data were subjected to a descriptive analysis where the proportions of the main variable (success or failure) were estimated. Contingency tables were developed. A ji-Square test (X) was used to determine statistical differences between proportions of success or failure according to the treatment. Data processing was performed using SPSS software (version 2015).

## Results

**Table 1. Characterization of the studied patients**

Group O3 + (treated with ozone plus integrative therapy)

- Age range: 29 to 75 years
- Average age: 47.6
- CIN I: 9 CIN II: 10 CIN III: 1

Ozone Control Group (treated exclusively with ozone)

- Average range: 27 to 41
- Average age: 35.2
- CIN I: 4 CIN II: 5 CIN III: 1

**Table 2. Classification by degrees of CIN**

- CIN I: 13 patients
- CIN II: 15 patients
- CINIII: 2 patients

**Table 3. Evolution of the patients according to the criteria of therapeutic success**

Positive (negative cytology after 3 months of therapy completion)

- Group O3 + (treated with ozone plus integrative therapy: 16 of 20 => 80.0%
- Ozone group (treated exclusively with ozone): 6 of 10 => 60.0%

**Table 4. Evolución por grados de CIN**

| Variable | Group O3 + | Group O3 | Total in nº & % |
|---|---|---|---|
| Patients with complete resolution: CIN I, CIN II, CIN III | 16 | 6 | 21=> 70% |
| Patients who evolved to a lesser degree of CIN | 2 | 1 | 4=> 13.3% |
| Patients who evolved from CIN II to CIN III | 1 | 1 | 2=> 6.6%<br>With cauterization of lesion. |
| Patients whose lesion persisted | 1 en CIN II | 1 en CIN III | 2=> 6.6%<br>One underwent conization womb neck. |
| One patient with *Giant Condiloma Acuminata.* Genotype 11 with a complete resolution. | | 1 | 1=> 100%<br>Surgical removal, and subsequently treated with ozonated water rinses and 800IP ozonized oil for 21 days[9] |

**Legend: Group O3 +** (treated with ozone plus integrative therapy). **Group O3** (treated exclusively with O3). **CIN Grades**, Intraepithelial Cervical Neoplasia. **CIN I**, mild dysplasia. **CIN II**, high-grade intraepithelial squamous lesion. **CIN III**, severe displasia-carcinoma in situ.

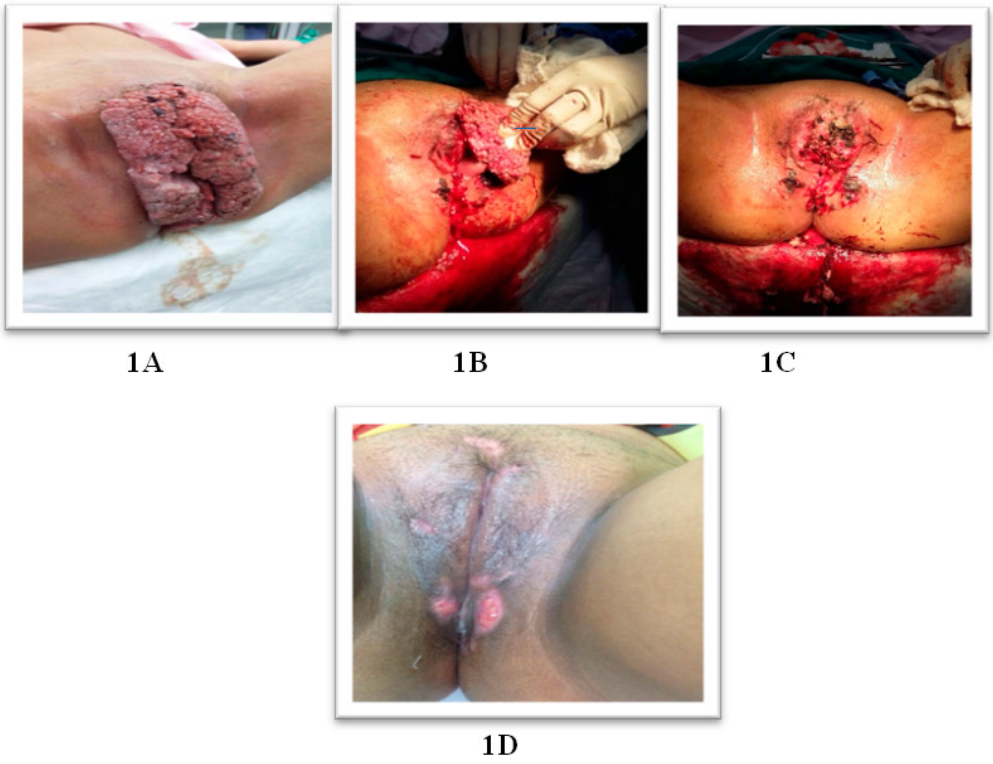


**Figure 1**. Photographs of a giant condyloma acuminate case. 1 A, Global picture of condyloma, 1B Surgical removal, 1C Total removal, 1D Global picture 21 days after removal.

## Conclusions

Intravaginal therapy with O2 / O3 is shown to be effective in a complicated group of patients with no spontaneous remission. The data presented were more favorable to the group receiving the combination therapy ($p<0,05$), apparently because of the synergy of the complementary treatments that were administered.

O2 / O3 vaginal therapy is a simple, economical and effective therapy. It was perfectly accepted by all patients. Apparently, the effects obtained are stable. It can be combined with any other therapy and does not interfere with any therapy.

Although the number of patients does not allow a safe conclusion, patients who received combination therapy with Glutation, VitC, Se, Zinc, Microimmunology and Ozone presented great advantages compared to O2 / O3 exclusively.

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