

artículo original

Ozone therapy in the treatment of recurrent vulvo-vaginitis by *Candida albicans*

Ozonoterapia en el tratamiento de la vulvo-vaginitis recurrente por *Candida albicans*

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Keywords

vulvo-vaginitis,
ozone-therapy,
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Candidiasis,
insufflation,
Candida albicans,
Oleosan^R

Abstract

Vaginitis caused by *Candida albicans* is the most common gynaecological disease found in the primary health care and makes a health issue of great importance because of its high frequency. The objective of the study was to assess the effectiveness of the ozone and diet therapy treatment of recurrent vulvo vaginal by *Candida albicans* infections. Patients (150) were selected with ages ranging from 30 to 50 years, with vulvo-vaginitis for at least 6 months of evolution, refractory to usual drug treatment and positive cultures for candidiasis.

It was performed one hydrocolontherapy with ozonized water. It was given: diet low in carbohydrates of high glucemic index, daily intravaginal instillations with ozonized water (10 sessions), daily intravaginal ozone insufflations at concentration of 20 µg/mL at speed of 0.2 L/min during 10 min (10 sessions), application of ozonized oil (*Oleosan*^R, Lacoser, Italy) with peroxide number of 600 and 400, during 10 days, 4 major autohemotherapy at 2.0 µg once a week. At the end of treatment were repopulated the vaginal flora with *Lactobacillus* vaginal tablets for 7 days and repopulated the intestinal flora with lactobacillus using oral route, during 1 month. Results: 85% of patients favourably responded to treatment, 10% remained asymptomatic for a period of less than one year and 5% of patients did not respond to treatment. Intravaginal ozone therapy, offers an effective alternative to conventional treatment with usual fungicides, not only achieved a remission of symptoms and negative cultures of vaginal exudates in patients with vulvo-vaginitis, but also increased Ig A and *Lactobacillus* in the vaginal epithelium. Also serves to restore the body's own defence abilities by stimulating the normalization of vaginal mucosa local immunity. Without producing a disturbing effect on the saprophytes.

Palabras clave

vulvo-vaginitis,
ozonoterapia,
aceite
ozonizado,
Candidiasis,
insuflación
vaginal, *Candida*
albicans,
Oleosan^R.

Resumen

La vaginitis causada por *Candida albicans* es la enfermedad ginecológica más común encontrada en la atención médica primaria. La vulvo-vaginitis por candida la hace un problema sanitario de indudable importancia por su alta frecuencia, y su diagnóstico en ocasiones resulta difícil, porque puede tener manifestaciones simples o combinaciones de síntomas de diferentes etiologías. El objetivo del estudio fue valorar la eficacia de la ozonoterapia y la dieta en el tratamiento de la vulvovaginitis de repetición refractaria al tratamiento farmacológico habitual. Se seleccionaron 150 pacientes de edades que fluctuaban entre 30 a 50 años, que presentaban vulvovaginitis de repetición de cómo mínimo 6 meses de evolución, refractarias al tratamiento farmacológico habitual y con cultivos positivos a candidiasis. A todas las pacientes se les realizó una hidrocolonterapia con agua ozonizada, se les instauró una dieta baja en carbohidratos de alto índice glucémico, se les administró duchas intravaginales diarias con agua ozonizada (10 sesiones), insuflaciones de ozono intravaginales diarias a una concentración de 20 µg/mL a una velocidad de 0,1 L/min durante 10 min (10 sesiones), seguidas de la aplicación de 1 mL de Fluvix (Gel con Factor de Crecimiento Epidérmico) aceite ozonizado (*Oleosan*^R, Lacoser, Italia) con índice de peróxido de 600 y 400 IP durante 10 días, 4 autohemoterapia mayor a 2.,0 µg una vez a la semana. Al final del tratamiento fue repoblada la flora vaginal con tabletas vaginales de *Lactobacillus* durante 7 días y repoblada la flora intestinal con *Lactobacillus* usando la vía oral, durante 1 mes. El 85% de las pacientes respondieron favorablemente al tratamiento, el 10% permanecieron asintomáticas durante un período de menos de un año y un 5% de las pacientes no respondieron al tratamiento. La terapia de ozono intravaginal, ofrece una alternativa eficaz al tratamiento convencional con fungicidas habituales. Se logró una remisión de los síntomas y cultivos negativos de exudados vaginales..

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Introduction

Vulvo-vaginitis is the most common disease found in the primary health care and usually is not easily solved. Its diagnosis is difficult in some cases, because it may have simple manifestations or combinations of symptoms of different etiologies². In many cases, the patient carries out self-medication without medical consultation^{2,3} creating resistance to usual drugs. The use and extensive abuse of fungicides, antibiotics and vaginal antiseptics, far from solving the problem rather complicates the same, which leads to the search for alternative or complementary methods to solve the problem⁴. A vulvo-vaginitis is considered recurrent when the event repeats four or more times per year. In that case, to carry out an adequate diagnosis and the ensuing treatment it is necessary to perfectly individualise each case, as non-complicated candidiasis perfectly responds to common treatments, including those using single doses, while the complicated cases do not generally respond to single doses and require lengthy treatment periods or prophylactics (prophylaxis of the recurring infection). Vulvo-vaginitis manifests with plentiful thick, whitish cheese type secretions with a characteristic odour, erythema, pain, heat, stinging and irritation. Infectious vulvovaginitis is responsible for 90% of the remaining types of vaginitis, the main types being Bacterial Vaginosis, Candidiasis and Trichomoniasis⁵.

It is thought that 75% of women have suffered from candidiasis once in their life and 40% have shown recurring episodes. *Candida albicans* has shown to be the etiologic agent in 85% of cases followed by *Candida glabrata* for 10% and *Candida tropicalis* for 5%⁶. Under the influence of estrogens, the epithelium produces glycogen which degrades under the action of *Lactobacillus spp* passing to glucose and finally to lactic acid, this latter maintaining a vaginal pH of less than 4.5, [...].

Objective

It has to be taken into account that one of the biologic actions of ozone is the activation of the Nrf2 system through a moderate and controlled stimulus of oxidative stress provoking an inhibition of the inflammation mediated by cytokines by means of the induction of reductase of leukotriene B4. Therefore, the Nrf2 system contributes to the protection against various pathologies, including carcinogenesis, hepatic toxicity, respiratory tract and chronic inflammatory diseases, neuronal ischemia and kidney problems^{10,12}. Furthermore, it is known that ozone has other effects, such as:

1) Direct disinfecting and trophic effects, when applied locally. 2) Systemic antibacterial and antiviral effects because of a discrete peroxide formation. 3) Increases the deformity of red blood cells with a relative improvement of the blood circulation. 4) Improves the delivery of oxygen to tissues. 5) Improves the erythrocytary metabolism enhancing the efficiency of glucose metabolism and 6) Improves the metabolism of fatty acids by the activation of antioxidant enzymes active in the removal of peroxides and free radicals. It has been considered as an objective of this study to demonstrate the effectiveness of the application of ozone therapy in its topical and systemic application forms in recurrent vulvo-vaginitis by *Candida albicans spp*.

Materials and Methods

The research was carried out in Clínica Fiorela Madrid, for two and a half years (2010-2013), under the direction of Dr. Adriana Schwartz, under a retrospective monocentric observational study. The selected patients who entered the program signed a detailed informed declaration before the beginning of the program.

The research protocol was submitted to discussion by the researchers taking part in the program, and it was submitted as well for its revision and approval to the ethics and institutional review committee and (Clínica Fiorela, Madrid). The protocol was executed only after its approval and it fulfilled the ethical procedures for medical research in human subjects established by the Helsinki Statement issued by the World Health Assembly¹³.

150 subjects who had been diagnosed recurrent vulvo-vaginitis were included in the program with at least 6 months of refractory evolution to common pharmacologic treatment and positive cultures of candidiasis, with ages comprised between 30 and 50 years, showing their willingness by means of their informed consent orally and in writing to participate in the program. Subjects were excluded showing: 1) Pregnancy and lactation, 2) Presence of other vaginal infections such as gonorrhea, ureoplasmosis, chlamydia, papilloma virus, 3) Use of fungicides or other locally applied medicines during the one month prior to treatment, 4) Participation in other clinical studies or utilization of drugs in experimentation stage, 5) non-willingness of the subject to participate in the program. The exclusion criteria were: Refusal of the subject to continue the program, decease of the subject or manifestation of a psychiatric illness.

The clinical and microbiological criteria for effectiveness were: 1) Complete clinical and microbiological healing, 2) Absence of subjective clinical symptoms, 3) Absence of inflammatory disorders of the vaginal mucous membrane, and negative vaginal cultures taken after ending the treatment.

It was considered that the patient had improved when a substantial reduction of the objective and subjective symptoms could be evidenced. The infection was considered recurrent when the recurrent presence of subjective and / or objective symptoms of candidiasis was detected with a positive vaginal culture or exudate after 2-4 weeks of having ended the treatment.

General clinical protocol: Vaginal cultures and exudates were taken before and at the end of treatment, being sent to the clinical laboratory for their analysis by conventional methods (culture in Sabouraud dextrose broth, SDB and direct observation or in a solution of 10% potassium hydroxide (KOH)). A low glycemic index, low carbohydrate diet was prescribed. The food sensitivity test (by measuring of IgG antibodies, lactose intolerance test by lactase deficiency and gluten intolerance test by antibodies in antiendomysium blood, antigliadine and antitransglutaminase) was carried out to determine the foods to which they were intolerant or sensitive, these foods being removed from the diet. At the beginning of the treatment a colon hydrotherapy (HC-1 Classic, Transcom SL, Gipuzkoa-Spain) was carried out on the subjects with ozonated water to stop the intestinal migration of microorganisms.

Ozone treatment: Vaginal instillations with ozonated water were carried out for 10 days. 1.2L sterilized double distilled water was ozonized with 5 mg/L for 20 min with a flow rate of 1 L/h. 10 intravaginal washings (400 mL) were carried out with the ozonated water by means of a 100 mL syringe, followed by insufflation of the oxygen-ozone gas mixture with a concentration of 20 µg/mL for 10 min, with a continuous flow of 0.2 L/min (2 L total volume). An intravaginal device specially designed for this purpose (Figure 1) VIN-100 (Econika SPE, Odessa-Ukraine) was used, allowing optimal intravaginal ozonation, ensuring a greater and more uniform contact of ozone on the vaginal epithelium, allowing the medical worker to operate safely, as no leak of ozone took place.

4 major autohemotherapy (AHTMayor) were applied with a dose of 2.0 mg O₂-O₃ at a rate of one per week. Frequently, urethra and bladder were involved in this inflammatory process, therefore, both organs were instilled with ozonated water in three sessions with a volume of 150 mL.

The ozone generator Bozon-N (Econika SPE, Odessa-Ukraine) was used.

In cases of excoriation and vulvar ulcers, ozonated pads enriched with Epidermal Growth Factor Senitul® and Oxiderm® (Lab. Heberfarma, Spain) were applied.

Given the fungicidal and bactericidal effect of ozonized oil^{14,19}, 1 mL of ozonated oil 600IP Oleosan® (Lacoser, Italy) was applied (intravaginal) daily during the acute infection period, upon controlling the symptoms, the concentration of oil was lowered to 400IP Oleosan® (Lacoser, Italy), applications (intravaginal) of 1 mL Fluvix®, a gel containing recombinant growth factor (Lab. Heberfarma, Spain), to protect the vaginal mucosa from the dryness provoked by the direct action of the ozone gas in mucosae.

At the end of the treatment, the vaginal flora was repopulated with *lactobacillus* (Isadin-to-barcilus-lactobacillus-plantarum-p-17630-isdin) for 10 days through the vaginal route and for 1 month through the oral route (Lactibiane Tolerance. Lab. Pileje).

Prophylactic measures: the application of O₃ 400IP oil (Lacoser, Italy) was continued for 5 post-menstruation days for a period of 3 months as a preventive measure. The male partner of the treatment subject was also treated with applications in the glans penis of Oleosan® of 600IP oil (Lacoser, Italy) for 10 days. Calendar of the Prophylactic treatment of Isadin-to-barcilus through vaginal route: upon finishing the menstrual period, 1 vaginal capsule, for 6 days overnight, for three months. Fig. 2

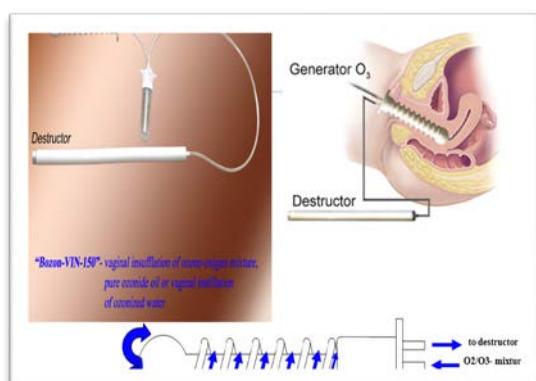


Figure 1. Device used for the vaginal administration of ozone through vaginal route.

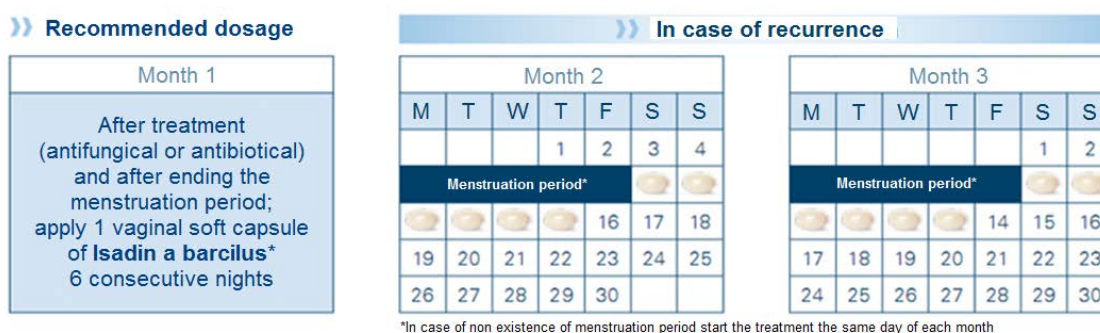


Figure 2 Calendar of the prophylactic treatment of Isadin-a-barcilus through the vaginal route.

Further to taking into consideration the medical history of the patients, a structured interview was conducted with them, making it possible to obtain general information to complete the data collecting form. The parameters collected on the form were: registration number, medical history number, full name, gender, ethnic group, age, weight, height, blood pressure, education level, toxic habits, physical activity, pathologic background, use of drugs or supplements and food habits and data to evaluate the index of quality of life according to a questionnaire of the Spanish Association of Candidiasis (www.candidiasiscronica.info) Table 1

Table 1. Indicators of quality of life before and after treatment.

Quality of life indicators	Before treatment	Data at the end of 3 months after treatment
		Treated group
Sleep disturbances	3.4 = 0.2	1.8 = 0.2*
Dietary restriction	2.9 = 0.4	2.1 = 0.3
Anxiety	4.2 = 0.3	1.4 = 0.3*
Depression	2.3 = 0.3	0.7 = 0.3
Insecurity	3.1 = 0.4	1.2 = 0.4*
Attention and memory deficit	3.7 = 0.3	1.8 = 0.4*
Irritability	4.1 = 0.3	1.8 = 0.4*
Fear that the disease will worsen	3.4 = 0.4	1.5 = 0.3*
Sexual dysfunction	3.9 = 0.3	1.2 = 0.3*
Lack of interest for life	1.1 = 0.1	0.9 = 0.3
Family conflicts	4.0 = 0.5	1.1 = 0.3*

Legend: * meaningful differences ($p < 0.05$).

Statistical processing

Exploratory analysis of data was performed through an analysis to find aberrant points (outliers). Subsequently, the experimental data were subjected to descriptive analysis. To determine statistical differences between the proportions of patients before and after treatment the McNemar test was used.

Results

The initial microbiological study evidenced that the vaginal cultures showed the following non specific microorganisms:

- In 150 patients with vulvo-vaginitis *Candida albicans* was present in 100%;
- *Streptococci* group B, was present in 12 patients (8%) with 10^3 - 10^8 cells/mL;
- *Streptococci* D in 9 patients (6%) in an amount of 10^5 cells/mL; *Epidermal staphylococcus* in 3 patients (2%) in an amount of 10^5 - 10^3 cells/mL;
- *Bacteroides* in 10 patients (6.6%) in an amount of 10^3 - 10^4 cells/mL;
- *Proteus spp* in 5 patients (3.3%) in an amount of 10^4 cells/mL;
- *Escherichia coli* in 30 patients (20%) in an amount of 10^5 cells/mL.

Furthermore, in the group under study it was determined that:

- In 90% (135) of the patients with vulvo-vaginitis the vaginal microflora index was very low; the number of *Lactobacillus* did not reach 10^2 cells/mL.
- 30% (45) of the patients were affected by atopic dermatitis.
- 100% (150) of the patients systematically self-administered antiseptics, oral antibiotics and topics.

85% of the patients responded favorably to treatment. 10% had a recurrence before one year and 5% of patients did not respond to the treatment.

Discussion

After finishing the treatment (application of the clinical protocol and ozone treatment), it was determined that:

- o 100 patients (66.7 %) observed the disappearance or a meaningful reduction of the symptoms to the third day of treatment.
- o 50 patients (33.3%) recorded a slight itching around the 7th day of treatment with a moderate vaginal discharge.
- o In 10 days, only 15 patients (10%) complained about moderate vaginal pruritus, 5 additional insufflation were applied to them for the complete resolution of the symptoms
- o In 8 subjects (5.3 %) by abandonment or for not correctly following the specifications, it was not possible to evidence improvements.

The germicidal action of ozone is based on: the oxidative transitory stress, which is deadly for the microorganism due to the weakness of its antioxidant defense system^{20,24}. Microorganisms lack enzymes such as dismutase superoxide, catalase and glutathione peroxidase which conform the cellular defense system capable to challenge and neutralize the oxidative action of ozone. Ozone is capable of stimulating a certain number of cells of the immune system^{25,27}. As a result, these cells may release small amounts of immunostimulating and immunosuppressing cytokines^{28,29}.

Ozone in its forms of ozonated oil, ozonated water and gas acts as an excellent fungicide and germicide due to its high oxidating power^{30,33}. Ozone is capable of alerting the immune system, usually without provoking an imbalance between activation and suppression of the immune system, acting as an immunomodulator.

During the performance of this study the following was evidenced: 1) A synergistic action of ozone treatment in its different application forms: ozonated water, gas and ozonated oil with the adopted nourishment measures. 2) Reduction of symptoms and negativation of cultures and vaginal exudates.

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