

Intralesional and subcutaneous application of *Viscum album* L. (European mistletoe) extract in cervical carcinoma in situ

A CARE compliant case report

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Abstract

Rationale: Carcinoma in situ (CIS) of the uterine cervix is a premalignant condition of squamous epithelium. The standard treatments are excision and ablation procedures; for women with positive margins, hysterectomy is recommended.

Patient concerns: A 47-year-old Peruvian woman with recurrent candidal vaginitis had been diagnosed with colpocervicitis and squamous metaplasia 8 years ago, which were not treated.

Diagnoses: The patient was diagnosed with CIS after cervical conization procedure. She refused radical hysterectomy and opted for integrative medicine treatment.

Interventions: She was treated with intralesional and subcutaneous *Viscum album* L. extract (VAE) injections. VAE is a widely used herbal cancer treatment with cytotoxic, apoptogenic, and immunological effects, but it has not been investigated in cervical CIS.

Outcomes: Ending month 5 of treatment, complete remission of cervical CIS was observed. The patient is still in remission after 30 months (until publication).

Lessons: This is the first report on complete remission of cervical CIS after intralesional and subcutaneous injection with VAE. Prospective studies should evaluate to what degree the treatment effect is reproducible.

Abbreviations: CIN = cervical intraepithelial neoplasia, CIS = carcinoma in situ, HPV = human papillomavirus, IL = interleukin, ML = mistletoe lectins, Pap = Papanicolaou, VAE = *Viscum album* L. extract.

Keywords: case report, cervical carcinoma in situ, cervical intraepithelial neoplasia, complete remission, european mistletoe, fraxini, *Viscum album* L

1. Introduction

Carcinoma in situ (CIS), the last grade of dysplasia observed before invasive cancer, is defined as cervical intraepithelial neoplasia grade 3 (CIN 3).^[1] CIN is a premalignant condition of the squamous epithelium of uterine cervix and could be low-grade (grade 1 [CIN 1]) or high-grade (grade 2 [CIN 2] and grade

3 [CIN 3]).^[2] CIN 3 refers to severely atypical cellular changes encompassing more than two-thirds of the epithelial thickness and includes full-thickness lesions but with no signs of invasion into the stroma.

More than 83,000 women were diagnosed with cervical cancer, and almost 36,000 died from this disease in the Region of the Americas in 2012; according to the Pan American Health Organization (PAHO), the number of deaths in the Americas is projected to increase 45% by 2030.^[3] The National Institute of Neoplastic Diseases (INEN) of Peru reported 1485 new cases of cervical cancer registered in 2014, ranking it highest on the list of cancer incidence in the country.^[4] The main 2 factors associated with the development of high-grade CIN and cervical cancer are the subtypes 16 and 18 of human papillomavirus (HPV) and the persistence of the virus. Essential cofactors are immunosuppression, cigarette smoking, and long-term use of oral contraceptives.^[5]

The spontaneous remission rate for CIN 3 (severe dysplasia and CIS) is 32% to 47% and has a slow evolution over several years.^[6] The standard treatment for high-grade CIN includes excision (e.g., cervical conization using loop electrosurgical excision procedure [LEEP]) and ablation (e.g., cryotherapy, laser) of the transformation zone of the cervix (an anatomic area that contains the transition from the squamous epithelium of the ectocervix to the glandular epithelium of the endocervix). Hysterectomy is an option for women for whom other treatment has been unsuccessful or who have recurrent CIN.^[1] Cervical

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Ethics approval was not necessary for this type of study. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

The authors report no conflicts of interest.

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treatments can reduce the risk of invasive cancer of the cervix by 95% in the first 8 years after therapy, so CIN has a high rate of cure when the entire lesion has been excised (negative margins). On the other hand, studies have consistently shown that patients with a positive margin after an excisional procedure of the cervix are at significantly higher risk for residual disease—as determined at subsequent hysterectomy or with repeat excision—than patients with clear margins.^[7,8]

Viscum album L. extract (VAE) is an aqueous whole-plant extract made from European mistletoe, a hemi-parasitic plant that grows on different host trees (e.g., ash, birch, apple, oak).^[9] It contains cytotoxic and immunoactive compounds including mistletoe lectins (ML), viscotoxins, and other low-molecular-weight proteins, oligosaccharides and polysaccharides, flavonoids, and triterpene acids. The antitumor activity of ML, including prophylactic effects, has been linked to their strong apoptosis-inducing and immune-stimulatory effects. These include in vivo and in vitro activation of monocytes/macrophages, granulocytes, natural killer cells, T cells, dendritic cells, and the induction of a variety of cytokines.^[10–12] Clinical trials show improvement of the quality of life and potential beneficial effects on survival.^[13,14] Clinical trials are currently being conducted in Sweden (NCT02948309), United States (NCT03051477), and Germany–Egypt (NCT02106572).

In a prospective non-controlled study, subcutaneous VAE injection was used as a neoadjuvant treatment in HPV-related CIN 1-3, resulting in 41% complete and 27% partial remissions (determined by biopsy in case of complete response).^[15] Moreover, authors of another study investigating VAE and human recombinant interferon alpha in CIN 1-2 reported an increase in regression and a significant decrease in progression rates.^[16] However, to our knowledge, no studies have been

published on the use of intralesional VAE injection in treating cervical CIS.^[17] Therefore, we present the following case, reported in accordance with the CARE (CAse REporting) guidelines.^[18]

2. Case presentation

A 47-year-old Peruvian teacher and mother who had a history of recurrent candidal vaginitis were diagnosed with a high-grade CIN 2-3 and chronic cervicitis by biopsy. Eight years before, she had been diagnosed with colposcervicitis and squamous metaplasia, which were not treated. Nine months after CIN 2-3 diagnosis, she underwent a cervical conization that indicated CIS with exocervical extension lesion; according to the medical report, the lesion could not be excised entirely (positive margins). Three months later, a biopsy again indicated CIS with endocervical extension lesion (Fig. 1 A–B). Radical hysterectomy was recommended by her gynecologist; however, the patient refused this intervention.

The patient came to Centro Médico Antroposófico (CMA) in Lima-Peru, where she was evaluated, and intralesional and subcutaneous VAE injections therapy was recommended. Treatment with VAE was suggested because of the reported long-term remission of malignant and premalignant lesions and the promising results from studies using high-dose VAE applications (intralesional, intravenous, and intratumoral).^[9,19–22]

The treatment began with stepped-dose, thrice weekly subcutaneous VAE applications from the host tree ash (AbnobaVISCUM Fraxini 0.2mg–2mg; 2mg contains about 2 µg mistletoe lectin/mL; the ML-concentrations in the lower dose are respective). The injections were applied in the

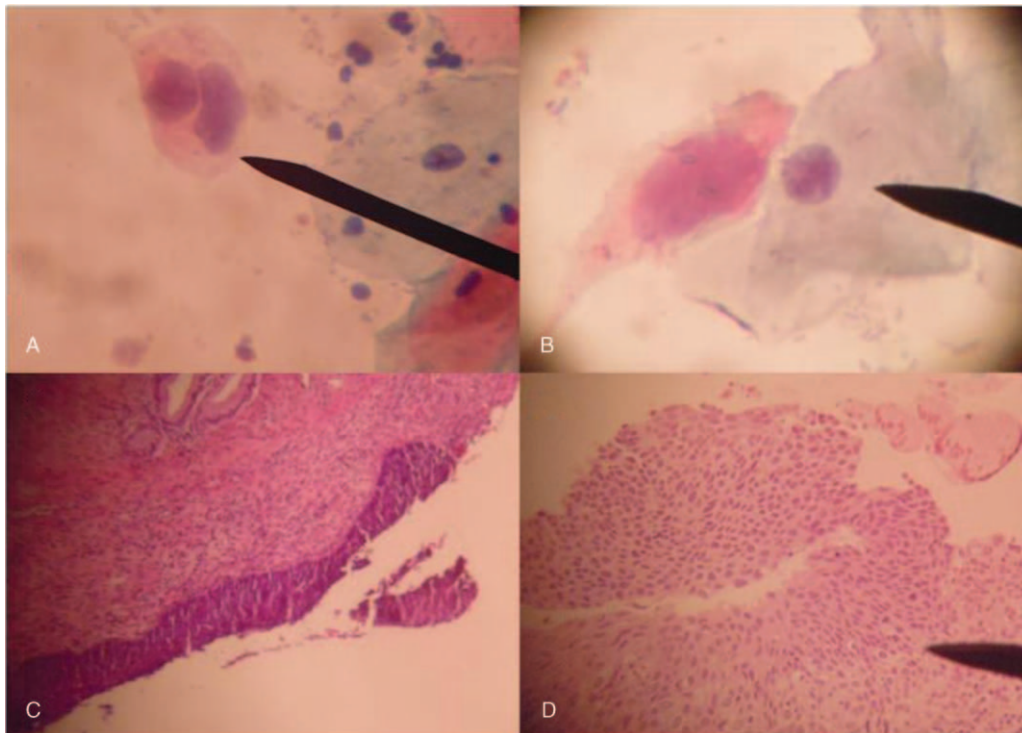


Figure 1. A-B: Cervical biopsy of the patient 2 weeks before VAE treatment: Cervical carcinoma in situ. C-D: Cytologic test from Pap after 9 weeks of VAE treatment: Cervical intraepithelial neoplasia grade 1. VAE = *Viscum album* extract.

Table 1
Viscum album L. extract therapy: Vials per month.

Month	0.2 mg*	2 mg*	20 mg*	20 mg*	Colposcopy and Papanicolaou test results course
	SC†	SC	SC	IL‡	
1	7			2 (week 1,2)	
2	7	3			CIN 1 (week 9)
3		10	1	5 (week 14, 15)‡	
4			9	4 (week 16)	
5			8		CIN 0 (week 22)
6			8		
7			9		CIN 0 (week 31)
8			12		
9			8		
10			12		
11			9		
12			6		
13–36			8		CIN 0 (week 62,97,115,143)

CIN = cervical intraepithelial neoplasia.
 * Vials of 1 mL; of these, only 0.25–1.0 mL were used for each injection depending on the infiltration dose tolerated by the patient.
 † SC: subcutaneous, 1 vial maximum used per application; IL: intralesional, 4 vials maximum used per application.
 ‡ Week 14: 2 vials, week 15: 3 vials used.

periumbilical area, the first time by the nurse and then by the patient herself. Intralesional VAE injections (AbnobaVISCUM Fraxini 20mg, 1 mL per vial) were applied by the physician with the help of a speculum, starting in the first month with 1 vial in week 1 and the same in week 2 (Table 1). In the week of intralesional application, just 2 subcutaneous applications were administered. Due to logistical challenges, the intralesional intervention was paused for 3 months; the subcutaneous injections, however, were continued through this period.

In month 2 of treatment, the patient’s Papanicolaou (Pap) test indicated CIN 1 (Fig. 1 C–D). The dose of subcutaneous injections had been continuously increased, and by month 3 the patient started to use ampoules of 20 mg. Between months 3 and 4, she received the last intralesional injections (specifically, in weeks 14, 15, and 16 of 2, 3, and 4 VAE vials of 20mg, respectively). Ending month 5, her Pap test was negative for CIN, and colposcopy evaluation showed a normal cervix (no cervicitis was reported). It continued in this manner until this case was reported (36 months after the start of the VAE treatment). From

month 13, the subcutaneous applications occurred twice a week. For VAE treatment and course of disease Figure 2.

Side effects of treatment were swelling and occasional itching of the skin at the subcutaneous injection site. When intralesional VAE injections were applied, the patient felt mild uterine contractions beginning about 6 hours after application and lasting about 1 hour; occasionally, mild headache was reported. The patient consistently reported having a very good quality of life, as indicated in her responses to the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) that was conducted after complete remission and until the final Pap test (Fig. 3).

2.1. Antecedent and concomitant therapies

The patient reported experiencing a lot of pressure and stress at work. Otherwise, she had been healthy (weight: 58 Kg, height: 1.67 m) and without any particular risk factors for CIN/CIS. She drank 1 to 2 glasses of wine per week and ate a balanced diet.

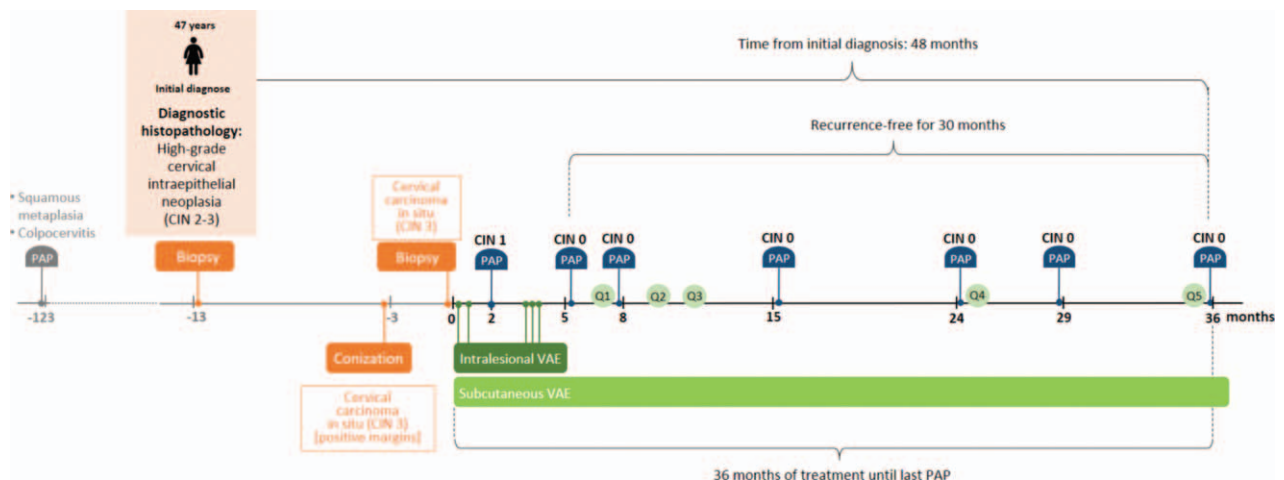


Figure 2. Timeline: VAE treatment and course of disease. PAP = Papanicolaou, Q = EORTC QLQ-C30, VAE = *Viscum album* extract.

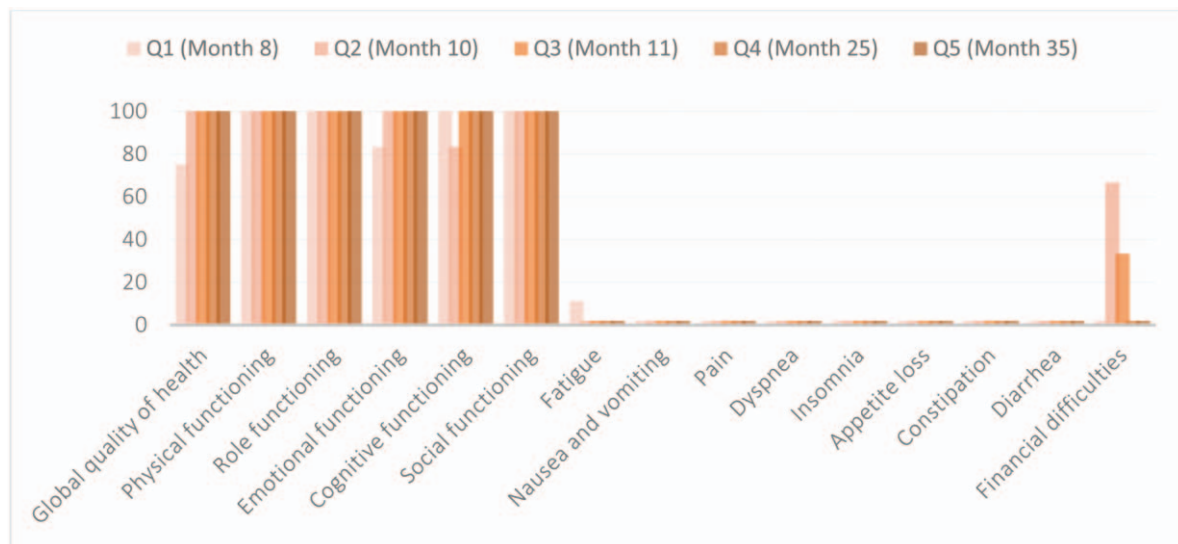


Figure 3. EORTC QLQ-C30 results of Global health and functional status of the patient (score 0: absence—100: full).

Almost a year after starting VAE treatment, the patient was diagnosed with subclinical hypothyroidism (TSH=4,08 UI/mL [0.35–2.5]), which was treated with anthroposophic remedies: Thyroid gland D5. Her symptoms of fatigue and lack of concentration were treated with Levico D3, Absinthium D1/Resina Laricis D3, and Amara-Tropfen. Her laboratory analysis results showed a progressive reduction of TSH (last result: 3.01 UI/mL). The patient currently expresses improved vitality and concentration.

After completion and submission of the article, the patient had an additional colposcopy, 1 year after the end of observation described above. She was still CIN 0 and still continuing the treatment.

2.2. Patient perspective

“I believe the body expresses what a person does not bring to consciousness, then the body makes it evident and one has the task of dissolving it. Mistletoe therapy helped me with this task.”

3. Discussion

The presented case shows a complete remission of cervical CIS (CIN 3) without recurrence in the following 30 months (until publication) after intralesional and subcutaneous injection of VAE. This treatment protocol was decided upon because of the high risk and probability of progression due to the positive margin of conization and high recurrence of vaginitis (as evidenced by the recommendation of the patient’s gynecologist for a hysterectomy).

Diagnosis of cervical CIS was confirmed by histopathology (cervical biopsy). Although the spontaneous regression rate for CIN 3 (severe dysplasia and CIS) is 32% to 47%, studies describe it as a slow process that takes many months (approximately from CIN 1 to normal: 20–72 months, CIN 2 to normal: 24–72 months, no reference from CIN 3 to normal).^[6] In the case of this patient, at month 2 of treatment, she regressed to CIN 1 and ending month 5 to normal. No other specific tumor therapy was used. Therefore, we presume that the remission occurred as a result of intralesional and subcutaneous VAE injections. A Pap

test was performed post-intralesional treatment, and cytologic findings confirmed remission of CIS.

Other tumor remissions have been observed after high dosage and local application of VAE in breast and gynecological cancers (e.g., ovarian, endometrial, cervical, vaginal, vulval, and fallopian cancers), and studies reported a statistically significant benefit in quality of life in cervical cancer patients treated with VAE.^[13,17] Still, to our knowledge, no studies have been published on the use of intralesional VAE injection in treating cervical CIS.

CIN and cervical cancer are immune-sensitive given the known risk of both HPV infection and CIN increases with increasing degrees of immunosuppression.^[23] Additionally, cervical cancer is one of the most common acquired immunodeficiency syndrome-related malignancies in women.^[24] VAE shows strong immune-stimulatory effects on the innate and the adaptive immune system (in vivo and in vitro activation of monocytes/macrophages, granulocytes, natural killer cells, T-cells, dendritic cells, and induction of variety of cytokines such as interleukin-1 [IL-1], IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, granulocyte-macrophage colony-stimulating factor [GM-CSF], tumor necrosis factor α [TNF- α], interferon γ [IFN- γ]).^[17,18,23] Therefore, we presume that VAE contributed to the complete and long-term remission described in this case. This immunostimulating effect also was reflected by a reduction of the patient’s recurrence of candidal vaginitis since the patient started treatment, as it is known candidal infections are more common in immunosuppressed patients.^[25] Immunotherapy of candidal vaginitis for both prevention and treatment is a therapeutic approach that is under investigation. Furthermore, the remission may have resulted from the strong cytotoxic and apoptotic effects of VAE. The cytotoxicity of lectins is inhibited by serum proteins and antibodies when applied systemically; therefore, local application restricted by intralesional injection may also help in the clinical response.^[26] As part of the intended immune reaction, patients usually experience local skin reactions like swelling of the surrounding skin; erythema and itching are well-known side effects of subcutaneous VAE injections. That type of skin reaction in a patient during treatment with epidermal growth factor receptor inhibitor predicts a better clinical outcome than in

patients who did not develop them and may be interpreted as immune stimulation, similar to that provoked by VAE treatment.^[27] Mild uterine contractions (well tolerated by the patient) were a normal response to inflammatory stimulation of the cervix with intralesional VAE injections that caused the release of prostaglandins responsible for the contractions.

HPV is the primary etiologic agent of cervical precancer and cancer, particularly with subtypes 16 and 18, which are strongly associated with high-grade lesions (50% to 60% of cases).^[28] Moreover, HPV infections, particularly persistent infections, are a recognized risk factor for development of precancer lesions (stage of this patient), and invasive cancer.^[29] Recent studies have revealed the molecular bases of HPV-induced tumorigenesis and interaction with the host immune system. At present, available HPV vaccines do not have a therapeutic effect on preexisting HPV infections or related lesions. The focus now is to develop a treatment that can mount a therapeutic immune response against HPV-infected cells. We believe that this case report contributes to the knowledge of VAE in CIN and cervical CIS. It suggests an added benefit to their treatment, which should be further investigated. Until otherwise indicated by clinical trials, standard treatment should be the primary intervention in cases of CIN and cervical CIS, with VAE administered as a promising adjuvant therapy.

4. Conclusions

This is the first report showing complete remission of CIS after intralesional and subcutaneous application of VAE. Prospective studies should evaluate whether this result can be reproduced and become a second-line treatment option for patients who refuse hysterectomy.

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