

Immunostimulation to reduce recurrence after surgery for anal condyloma acuminata: a prospective randomized controlled trial

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Abstract

Aim Human papillomavirus is the most common cause of sexually transmitted disease. It is associated with immunosuppression and shows a marked tendency to recur. We investigated a natural immunostimulant aimed to reduce recurrence.

Method A randomized controlled study was carried out including 261 patients allocated to surgical excision alone (control group; $n = 122$) and surgical excision plus postoperative immunostimulation for 30 days with a natural product (STET; study group; $n = 139$). Patients with HIV positivity were excluded. All patients gave fully informed consent.

Results The patients were followed for 6 months after surgery. Recurrence occurred in 7.2% (10/139) in the

study group and in 27.1% (33/122) in the control group ($P < 0.0001$). There were no significant differences in the sex, sexual orientation, number of lesions, time to diagnosis and treatment or localization of lesions in the two groups.

Conclusions Immunostimulation using a natural product significantly reduced the incidence of recurrence of anal condylomata in patients undergoing surgical excision.

Keywords Anogenital warts, condylomata acuminata, recurrences, natural therapies, immunomodulation, current therapies

Introduction

Human papillomavirus (HPV) is the most common form of sexually transmitted infection. Its incidence has increased over the past 30 years [1]. In the USA, the prevalence of anogenital HPV is estimated to be 15%, equal to 24 million individuals. In addition, 500 000 to one million new cases of genital warts are believed to occur annually, resulting in 600 000 healthcare provider visits per year [2]. Currently, more than 100 different HPV types have been sequenced and officially classified, about one-third of which have been found to infect the ano-genital epithelium.

Guidelines for management have been published by the Medical Society of Venereal Diseases of the United

Kingdom and the European Course on Human Papillomavirus Associated Pathology Group, but optimal treatment is still undecided.

Recurrence rates vary from 20% to 50% after any form of treatment. Surgery with the local application of imiquimod, an imidazoquinolinamine derivative, seems to be the most effective [3]. One reason postulated for recurrence is the lack of antiviral activity with most of the recommended therapies. Thus, only the wart itself is destroyed, whereas the latent HPV in the surrounding tissue remains. Imiquimod and interferon are the only medical treatments that exhibit antiviral activity. Both have demonstrated lower recurrence rates compared with other therapies [4].

The immune status of patients is an important factor leading to recurrence. Anal condylomata in immunosuppressed patients treated by surgery appear to recur significantly more often and quicker than in patients with a competent immune system. In HIV-seropositive patients, CD4 counts should be maximized to prevent

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early recurrence [5]. In one study, recurrence after treatment was more frequent in HIV-positive (66.4%) than in HIV-negative patients (26.8%) [6].

Many treatments have been used to reduce recurrence, including interferon [7], lentinan [8], imiquimod [4,9,10], autogenous vaccine [11], 5 fluorouracil [12] and squaric acid dibutylester [13]. Some studies have not been generally randomized or have contained small numbers of patients.

The immune system appears to play a role in regression of genital HPV disease through both cellular and humoral immunity. In patients with spontaneous regression, significant differences have been shown in the epidermal and dermal concentration of CD4 + -activated memory lymphocytes compared with those without regression. While there is an association of serum antibodies to HPV proteins with HPV-related diseases, their role is uncertain because their presence does not correlate with wart regression. There is evidence to suggest that T cells in male and the female genital epithelium secrete protective antibodies against many HPV infections, but the significance of this is unclear [14].

Many natural products have been shown to induce an immune response, including *Echinacea* [15–17,19], *Uncaria* [18], *Astragalus* [19], *Glycyrrhiza* [19] and others. In this study, we have used a natural product developed by Erbita (Chiesanuova, San Marino) in a prospective randomized study to determine any effect on recurrence after surgical excision of anal condylomata.

Method

From November 2003 to October 2006, 262 patients (176 male subjects) with anal condylomata were entered in the study. Exclusion criteria included HIV positivity, treatment with immunosuppressants, involvement of the internal part of the vagina or of the cervix and age less than 18 years. One patient entered was subsequently excluded as he became positive for HIV.

After taking a history, a dermatological and anorectal examination was performed in all patients. Female subjects with an involvement of genital tract were studied by a gynaecologist and treated in one or more sessions. These patients were excluded from the study. Informed signed consent was obtained from all patients.

Patients were randomized to surgical removal alone ($n = 122$, male subjects 88; the control group) and to surgical removal followed by the natural product composed of *Echinacea*, *Uncaria*, *Tabebuja*, papaya, grapefruit and *Andrographis* (*Andrographis paniculata*) in a dose of three tablets per day for 1 month postoperatively ($n = 139$, male subjects 87; the study group; Table 1).

Table 1 Patients' sex and median age (interquartile range).

	Group	
	Control	Study
Sex		
M	88; 34 (18) years	87; 32 (9) years
F	34; 33 (21) years	52; 30 (14.5) years

M, male subject; F, female subject.

Surgery included diathermy destruction or excision (or both) of each lesion.

Patients were subdivided into three groups: heterosexuals, homosexuals and bisexuals. All female subjects were heterosexual. In the control group, 45 men were heterosexual, 39 homosexuals and 4 bisexual. In the study group, 48 men were heterosexual, 34 homosexual and 5 bisexual.

In the study group, 34 (24.4%) patients had extensive disease affecting the anal canal and in the control group this aspect involved 29 (23.7%) patients.

Surgical treatment was necessary in multiple sessions to avoid anal stenosis in 13 (9.3%) patients in the study group and in 14 (11.4%) patients in the control group.

The study was not sponsored by the manufacturer. Patients were followed in the outpatient department weekly for 1 month and then at 3 and 6 months after surgery when an anoscopy was performed. All patients were contacted by telephone when they did not attend an outpatient visit. A further visit at 1 year was recommended but many patients defaulted.

Statistical analysis

Homogeneity of the groups was tested against age, sex, sexual orientation, time to diagnosis and localization using Fisher's exact test or Wilcoxon's test where appropriate. A probability value of less than 0.05 was taken to be statistically significant. Any difference in the incidence of recurrence was analysed using Fisher's exact test.

Results

All 261 patients completed the study with no patient lost to follow up to 6 months. One (0.7%) patient in the study group and two (1.6%) in the control group did not attend the booked control visit at 1 month but were called by telephone and as a result they attended. Three (2.2%) patients in the study group and four (3.3%) in the control group did not attend the 6-month appointment. All did so, however, after a telephone call.

Only 56 (46%) patients in the control group and 57 (41%) in the study group attended the 1-year appointment.

One patient (in the study group) was excluded from the study owing to the subsequent development of HIV positivity.

No side-effects were observed in the study group. Surgical complications occurred in three patients including postoperative bleeding ($n = 1$) and mild anal stenosis treated conservatively ($n = 2$).

At 1 month, 22 patients in the control group had developed recurrence compared with five in the study group (18% vs 3.6%, $P < 0.001$; Table 2). Recurrence rates at 6 months were 27.05% and 7.19% ($P < 0.001$; Table 3).

There was no statistical difference between the two groups with regard to age, sex, sexual orientation, perianal lesions, genital lesions and time to diagnosis of recurrence ($P = 0.11, 0.11, 0.11, 0.11, 0.32$ and 0.11 , respectively). The only difference was the presence of associated peri and endoanal condyloma, which were more frequent in the study group ($P < 0.0001$; Table 4).

Table 2 Recurrence at 1 month.

Frequency	Group	
	Control	Treatment
No. of patients	122	139
Recurrence (%)	22 (18.0)	5 (3.6)

Table 3 Recurrence at 6 months.

	Group	
	Control	Treatment
No. of patients	122	139
Recurrence		
No (%)	89 (73)	129 (93)
Yes (%)	33 (27)	10 (7)

Table 4 Differences between the two groups.

	Group	
	Control	Treatment
No. of Patients	122	139
Endoanal warts		
No (%)	51 (42)	26 (19)
Yes (%)	71 (58)	113 (81)

Discussion

Various therapies have been used to treat anal condylomata [20]. Some are based on long-standing practice, small trials and case-series data, whereas recently developed treatment modalities have been supported by more rigorous methodology [21]. No consensus has been reached on the appropriate treatment. No data are available to indicate whether treatment eliminates infectivity, the primary aim of treatment being to remove the lesions. There is a paucity of published randomized trials [10]. Despite the introduction of antiviral treatments such as interferon or imiquimod, surgical destruction or removal remains the treatment of choice for some clinicians.

The present study has shown that recurrence of anal condylomata after surgical treatment is reduced by the addition of the natural product of a mixture of *Echinacea*, *Uncaria*, *Tabebuja*, papaya, grapefruit and *Andrographis*. There was a significant lower incidence of recurrence or persistence of lesions at 1 and 6 months when compared with patients treated by surgery alone. The high rate of follow-up obtained in the study was guaranteed by a telephone call to all patients who failed to attend the booked appointment.

We inform patients that the recurrence rates are high, and there is also the risk of neoplastic transformation. We suggest an anoscopy every year but only 40–50% of patients attended.

Topical treatments include podophyllotoxin, imiquimod cream and trichloroacetic acid. In a meta-analysis, the cure rates of imiquimod and podophyllotoxin treatment were 50.3% and 56.4%, respectively [22]. Combined analysis of three randomized controlled studies of imiquimod showed a statistically significant difference to the placebo group, as did a combined analysis of the nine studies on podophyllotoxin. In this meta-analysis, podophyllotoxin gave more serious adverse effects.

Invasive therapies include cryotherapy, argon plasma beam treatment and surgical excision. Surgical excision is highly effective. Repeated sessions are needed with cryotherapy and it is mainly indicated for small lesions [10].

Many treatments may cause complications. Podophyllin can cause serious systemic side-effects if applied in excess, and given the potential oncogenic and teratogenic effects it should be avoided on the cervix and in the anal canal and in pregnancy. Marrow suppression, hepatic dysfunction, neurological effects, hallucinations, psychosis, nausea, vomiting, diarrhoea, abdominal pain and genital burns have been rarely described. Trichloroacetic acid may cause a cutaneous intense burning sensation and ulceration to the dermis and it is therefore not

recommended for large warts. 5 fluorouracil has limited use owing to severe local effects and may be teratogenic. Interferon has limited value owing to its expense, the occurrence of systemic side-effects and a variable response rate. It should only be used on specialist advice. Imiquimod is not recommended in pregnancy or internally. Mild to severe erythema may occur and other side-effects include localized erosions, an impetigo-like reaction, and an itching or burning sensation [14,23]. Horn *et al.* [24] reported the clinical efficacy of intralesional immunotherapy using *Candida*-, mumps- or *Trichophyton*-derived antigens.

Electrosurgery is the most effective treatment and it is suitable for all lesions whether internal or external. It is important to leave skin bridges between wounds to allow epithelization to minimize scarring and avoid anal stenosis. Laser treatment has similar indications to electrosurgery, and the results and complications are similar. Scissor excision does not damage the adjacent skin as diathermy does [25]. Podophyllin is the cheapest medical therapy but surgery is more cost effective [26]. All treatment methods have a recurrence rate of around 25% [27].

It is often unclear whether recurrence is due to recrudescence, reinfection, or other factors for example individual immune response or inadequate treatment [21]. The presence of genital HPV types in plucked pubic and perianal hair suggests that there is an endogenous reservoir for HPV which may play a role in recurrence [28].

Many treatments have been proposed to reduce recurrence. Adjuvant interferon treatment can reduce it after surgical excision [7] and is particularly effective in reducing recurrence in patients with condylomata present for more than 6 months ($P < 0.04$) and with condylomata containing HPV subtype 6/11 ($P < 0.05$).

It has been suggested that imiquimod reduces recurrence after laser therapy; but in one study, 8.5% of patients had to stop treatment owing to skin reactions at the application site [4]. Other forms of chemotherapy such as Lentinan combined with laser therapy reduced recurrence compared with a control group [8] and others studies have confirmed a reduction of recurrences after surgery [10,11,13]. The importance of the immune system in patients with anal condylomata is further supported by the observation of a lower mean monocyte count [29].

There is, therefore, evidence to indicate that immunostimulation may lead to a reduction in the size of lesions and of recurrence after surgery. Many naturally derived products have been suggested to have an immunostimulatory action in humans. *Echinacea* herbal preparations are derived from the root and stem of the cornflower. They are primarily used for their immuno-

stimulatory effect but if continued for more than 6–8 weeks, they may be immunosuppressive [16]. The increased level of the complement component properdin may be an indication of one aspect of immune system stimulation in patients treated with *Echinacea* [17]. It stimulates immune cells as quantified by CD69 expression on CD4 and CD8 T cells. This activation takes place within 24 h of ingestion and continues for at least 7 days [19].

Possible side-effects of *Echinacea* include allergic reactions and its use by patients with autoimmune diseases has to be carefully monitored. Contraindications include HIV positivity, AIDS, collagenoses, tuberculosis, leucocytosis, multiple sclerosis and patients undergoing immunosuppressive treatment.

Uncaria tomentosa is a potent alkaloid that induces the apoptosis pathway. The degree of apoptosis is not reduced in the crm-A-expressing cell line, suggesting that the extrinsic pathway may not be essential for pteropodine-induced apoptosis [18].

When the high cost and side-effects of interferon, imiquimod and lentinan are considered, these appear to be of less benefit than the natural product used in the present study. This agent led to a reduction of recurrence after surgery as tested by a randomized controlled trial containing a large number of patients in each group with no significant side-effects. Long-term follow-up is necessary to determine whether there are any long-term dangers such as malignant transformation.

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