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ORIGINAL ARTICLE

Does Immunotherapy of Viral Warts Provide Beneficial Effects When It Is Combined with Conventional Therapy?

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Background: Cryotherapy has been accepted as the mainstay in treating periunugal and palmoplantar warts. The major drawback of cryotherapy is the requirement of several unbearably painful treatment sessions. **Objective:** This study aims to assess the efficacy of immunotherapy in viral wart treatment, as an adjunctive method to cryotherapy. **Methods:** Retrospective chart review was performed on 124 patients visiting the hospital from January to December 2009 for the treatment of periungual and plantar warts. We analyzed the number of cryotherapy sessions necessary for treating warts and assessed the clinical benefits from the addition of other treatment modalities, by adjusting the various confounding factors. **Results:** Of the 124 investigated patients, immunotherapy with diphenylcyclopropenone (DPCP) was performed in 14 patients (11%), together with cryotherapy. After adjusting the factors related to the therapeutic difficulties of wart, the average number of cryotherapy sessions for the immunotherapy-combined group was significantly lower (3.58 ± 1.25) than that for the cryotherapy only group (5.10 ± 0.44) ($p = 0.026$). However, there were no differences in the number of treatment sessions of cryotherapy when topical 5-FU/salicylic acid agents were added to the treatment. **Conclusion:** Immunotherapy may be a successful adjuvant to cryotherapy in reducing the number of agonizing cryotherapy sessions. **(Ann Dermatol 23(3) 282～287, 2011)**

-Keywords-
Cryotherapy, Immunotherapy, Warts

INTRODUCTION

Warts are common dermatologic disorders which are mediated by the human papilloma virus (HPV). They usually affect the children and adolescents, and some studies report that up to 10% of the young population has warts. Some warts may spontaneously disappear, while others persist and can spread on other body sites, provoking physical and emotional distress to the patients[1]. The current therapeutic options for warts include cryotherapy, eletrocauterization, surgical excision, laser ablation, bleomycin intralesional injection, topical agents, such as 5FU/salicylic acid, and immunotherapy. They work for the treatment of warts by direct destruction and/or induction of immunologic responses to virus-affected-keratinocytes. Some agents have intrinsic antiviral activity[2]. Clinicians should select, from this large array of therapeutic options, the best treatment option suitable for individual cases, considering the patients' age, sex, previous medical history and the clinical characteristics of the warts.

Cryotherapy using liquid nitrogen plays a crucial role in treating warts, especially those located on the periungual and palmopalantar areas[3-5]. In clinical everyday practices, the treatment of these warts usually begins with cryotherapy. However, it can induce pain and even damage the patient's skin by frostbite lesions around the treated area, albeit infrequently.

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Some pain sensitive patients may even give up the therapy before the end, therefore pain limits the success of cryotherapy[4].

Many wart patients have been reported with defective cell-mediated immune mechanisms, especially those showing multiple warts. Diverse immunomodulators, such as dinitrochlorobenzene (DNCB), squaric acid dibutylester (SADBE), and diphencyclopropenone (DPCP), have been used for treating warts[6]. Among them, DPCP is the most widely used for its outstanding safety[7]. Several topical agents containing 5FU, which interferes with the metabolic process of the nucleic acid in HPV are also widely used[8]. Some agents enhance their antiviral efficacy by mixing keratolytic materials, such as formic acid or salicylic acid. Immunotherapy and topical agents have their own clinical advantage over other destructive treatment modalities. Several clinical studies focused on comparing various treatment modalities[9,10]. However, little was verified about the combined therapy, especially cryotherapy combined with the ancillary methods.

This study was designed to evaluate the strength of commonly-used treatment modalities, and aimed at reducing the required number of painful cryotherapy sessions.

MATERIALS AND METHODS

Participants

Charts were reviewed retrospectively in 124 patients who visited our dermatologic clinic from January to December 2009 for the treatment of periungual and plantar warts. This study included the patients who presented with warts on the periungual and/or palmoplantar areas, and received at least one session of cryotherapy. The types and sessions of treatment for every patient, as well as information about age, sex, number and location of the wart lesions, were identified.

Treatment design

Cryotherapy with liquid nitrogen spray was generally applied every 3 or 4 weeks. Cryogen was applied perpendicularly to the wart at a distance of about 2 cm. The wart was sprayed until the ice-ball forming from the lesion center spread to a 2 mm margin of the lesion. The spraying time depended on the treated area. The regimen of 3 complete cycles of freezing, followed by 10 seconds thawing, was used. If the lesion was covered with heavy scales, they were gently peeled off with sterile blade by the clinician, before cryogen application.

For patients who received immunotherapy concurrently with cryotherpy, 0.1% DPCP was applied by the physician with the tape occlusion to the inner side of the upper arm, for 48 hours. After the desirable sensitization reaction of visible erythema was obtained on the site, patients were instructed to apply $1\times10^{-3}\%\sim1\times10^{-2}\%$ of DPCP onto warts daily to potentiate more specific local immune reaction against the HPV affected keratinocytes.

5FU/salicylic acid is the primary topical agent prescribed for warts in our clinic. When the crust after cryotherapy came off naturally with the topical antibiotics, the patients were recommended to use 5FU/salicylic acid on the warts on a daily basis.

At every visit, the clinical improvement was checked and marked. When no single wart lesion was found by the clinical inspection with the help of magnifying lens, the patients were considered cured. The total treatment period was the time from the first visit to the endpoint of treatment. Every treatment modality was summarized, including the number of cryotherapy and bleomycin injection sessions, as well as the information whether the immunotherapy or topical agents were applied to patients. Patients who, in addition to cryotherapy, received DPCP application and experienced successful sensitization were included in the immunotherapy group, whereas those who had ever used 5FU/salicylic acid were included in the topical agent group.

Statistical analysis

The t-test was performed to compare the number of cryotherapy sessions required for clearing out every single wart, between the patients treated with cryotherapy and additional modalities (i.e. the combined therapy group) and those treated only with cryotherapy (i.e. the cryotherapy only group). Cryotherapy was the mainstay of treatment in this study; the combined therapy group included the immunotherapy group (i.e. DPCP sensitization was successfully achieved), and the topical agent group (i.e. 5FU/salicylic acid was recommended). This was a retrospective study using the chart review. Therefore, several confounders could bias the results. To minimize the unwanted bias, we chose the analysis of covariant (ANCOVA) for controlling the confounders. Statistical analyses were done with the appropriate procedures, as applicable. The statistical significance was set at the 0.05 level and the confidence interval at 95%. Data were analyzed with the SPSS software package Version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic analysis

Of 124 investigated patients, 60 were male and 64 were

female. Immunotherapy using DPCP (14 cases), and treatment with 5FU/salicylic acid topical agent (49 cases) was conducted concurrently with cryotherapy, while the rest of the patients reveived cryotherapy alone. Among 14 patients in the immunotherapy group, 9 patients were male and 5 patients were female. The topical agent group included almost the same number of male and female patients (i.e. 25 male and 24 female). The average age was 21.2±14.3 years for total patients, 18.3±10.0 for the immunotherapy group, and 20.9±11.9 for the topical agent group. No statistically significant differences were found in gender ratio and average age between the investigated groups (Table 1).

Immunotherapy combined with cryotherapy (The immunotherapy group)

To achieve clinical resolution, the patients were treated on average 4.84±3.75 times with cryotherapy and cryotherapy was applied 5.64±3.62 times in the immunotherapy group. The number of treatment sessions did not show any statistically significant differences ($p=0.4$) (Table 2). The mean number of warts was 2.78±2.22 in the cryotherapy alone group and 4.14±2.41 in the immunotherapy group. The mean period of treatment was 4.98±3.56 months in the cryotherapy alone group, and 7.93±5.22 months in the immunotherapy group. Both number of warts and total treatment period were significantly higher in the immunotherapy group than in the cryotherapy alone group ($p=0.03$ and 0.05 respectively) (Table 2). Generally speaking, more wart lesions and longer treatment periods imply higher therapeutic difficulties. Thus, it can be suggested that the addition of immunotherapy to cryotherapy was more likely in severe wart cases. To eliminate the bias produced by these confounders, the ANCOVA test was conducted, with the number of lesions and the treatment period as covariants. After adjustment, the number of cryotherapy sessions required for cure was 5.10±0.44 for the cryotherapy alone group, and 3.58±1.25 for the immuno-

Table 1. Demographic characteristics in the treatment groups

	Total	+Immunotherapy	+5FU/ Salicylic acid
Age (years)	21.2±14.3	18.3±10.0	20.9±11.9
p-value		0.415	0.864
Sex (M/F)	60/64	9/5	25/24
p-value		0.326	0.914

Age and sex distribution between different therapeutic groups is shown here. Each *p*-value stands for the statistical significance level of the difference between the group that utilized the specific modality and the rest of the sample. For instance, the mean age of the group that received the combined therapy of cryotherapy and immunotherapy is 18.3 years old, and shows no statistically significant deviation from that of the patients who did not receive immunotherapy. Statistical evaluation was performed with the Student's t-test (p-value<0.05). M: male, F: female. 5FU: 5-Fluorouracil.

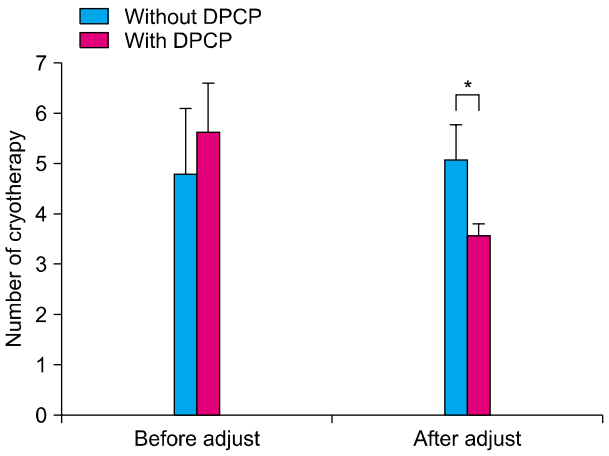

Fig. 1. The required number of cryotherapy sessions for complete remission of warts. Before adjusting the confounders, patients treated with the combined method paradoxically required more sessions, although the difference was not statistically significant. However, after adjustment, the combined therapy significantly decreased the number of cryotherapy sessions. Data are presented as mean±SEM (*$p<0.05$). Statistical significance was determined using the Student's t-test and adjustment was done by ANCOVA. DPCP: diphencyclopropenone.

Table 2. Comparison of the required number of cryotherapy sessions, according to adoption of the concurrent immunotherapy

	Before adjust			After adjust
	Cryotherapy sessions (times)	Warts (lesions)	Period of treatment (months)	Cryotherapy sessions (times)
Without DPCP	4.84±3.75	2.78±2.22	4.98±3.56	5.10±0.44
With DPCP	5.64±3.62	4.14±2.41	7.93±5.22	3.58±1.25
p-value	0.4	0.03*	0.05*	0.02*

Before adjusting for confounders (i.e. the number of wartsand the total treatment period), the patients who were also treated with immunotherapy paradoxically required more sessions of cryotherapy, but the difference was not statistically significant. After adjustment, the combined therapeutic group needed far less cryotherapy sessions (mean 3.58), compared with those without immunotherapy (mean 5.10) The adjustment was done with ANCOVA (*p-value<0.05). DPCP: diphencyclopropenone.

Table 3. Comparison of the required number of cryotherapy sessions, according to adoption of the concurrent topical agent

	Before adjust			After adjust
	Cryotherapy sessions (times)	Warts (lesions)	Period of treatment (months)	Cryotherapy sessions (times)
Without 5FU/Salicylic acid	4.03±2.53	2.61±2.16	4.12±2.84	5.01±0.55
With 5FU/Salicylic acid	6.31±4.75	3.43±2.36	7.14±4.51	4.80±0.69
p-value	0.003*	0.05*	<0.001*	0.6

Before adjustment, the patients who applied 5FU/Salicylic acid with cryotherapy paradoxically required more cryotherapy sessions (mean 6.31) than the rest of the sample (mean 4.03). After adjusting for confounders, there was no statistical difference in the mean number of cryotherapy sessions between the investigated groups (*p-value<0.05). 5FU: 5-Fluorouracil.

therapy group (p=0.026) (Fig. 1).

5FU/Salicylic acid topical treatment combined with cryotherapy (The topical agent group)

The number of warts was higher in the topical agent group (2.61±2.16 vs 3.43±2.36, p=0.05) and the longer treatment period was noticed in the topical agent group, as well (4.12±2.84 months vs 7.14±4.51 months p=0.000). Adjusting the number of lesions and the treatment period, the number of cryotherapy sessions was 5.01±0.55 for the cryotherapy alone group and 4.80±0.69 for the topical agent group. However, the difference was not statistically significant (p=0.6) (Table 3).

DISCUSSION

Cryotherapy using liquid nitrogen is a widely adopted dermatologic treatment for curing warts, and it is usually chosen as the first line therapy in warts[11]. The success rate is variable depending on the reports. However, over 70% of cure rate was guaranteed by several clinical trials[9,12]. Cryotherapy can directly destroy the viral affected keratinocytes[13], and can also trigger the immunologic reaction causing secondary cell damage[14].

As cryotherapy is usually done at several weeks intervals, the patients are not bothered by frequent visits to the clinic, as previously shown[14]. It is conveniently performed using a spray, as well as cotton balls, and is only performed by clinicians. Thus, it assures more exact treatment, compared with other modalities, such as topical agents which are applied by patients themselves. However, severe pain around the treated area is often unbearable, especially when the lesion is located in the highly innervated distal ends of the limbs, such as the periungium and palmoplantar areas. This modality definitely needs several sessions to eradicate warts and clinicians should exert every effort to reduce the number of agonizing treatment sessions. Some clinicians have tried to lower the discomfort by applying topical anesthetic agents before shooting the cryogen; however, it requires additional time and does not warrant a complete pain-free procedure[15].

DNCB was initially used as an immunomodulating agent. As its major side effects such as teratogenesis and carcinogenesis were revealed, it is now replaced by a safer topical agent, i.e. DPCP[16]. Immunotherapy is particularly helpful for patients with multiple warts, because it stirs up specific as well as non-specific inflammation in the whole body, eradicating all warts simultaneously[17]. Previously Orecchia et al. reported successful treatment results in multiple recalcitrant warts by weekly application of DPCP for fifteen weeks. Among 44 patients, 20 patients were completely cured and 17 were improved, with reduced number and size of warts[6]. Aghaei also conducted a similar clinical trial to evaluate the efficacy of immunotherapy, and found that four out of six patients achieved complete clearance in twelve weeks, while the remaining two only exhibited slight improvement[18]. According to previous reports, immunotherapy showed clear clinical benefits in the treatment of warts. However, this treatment is generally the second choice method, and is particularly used in refractory warts which are unresponsive to cryotherapy. Immunotherapy might produce minor rash or pruritus at the site of application. However, the discomfort for patients is far less than those of cryotherapy and bleomycin injections. Nevertheless, it requires frequent, weekly visits to the clinic and might therefore take more time to eradicate warts[6].

In many clinics, it is a common practice to combine immunotherapy and/or topical agents with cryotherapy. Unexpectedly, the previous reports on cryotherapy combined with either podophyllin cream or 5FU showed no increment in the curing rate, compared with cryotherapy alone[19,20]. No specific explanations about the disappointing outcomes of topical agents were provided in both previous studies, and the authors just suggested

that the insufficient sample size might be a reason.

In this study, the retrospective chart review revealed no meaningful decrease in the number of cryotherapy sessions in the immunotherapy group, and, at first, there was even a paradoxical increase in the topical agent group, compared with the cryotherapy alone group. Multiple and deep-seated warts were usually treated with combined therapy and the simple chart review did not count for it. In general, the patients with more difficult warts are more likely to receive additional therapy. Multiplicity and the total treatment period were thought to reflect the therapeutic difficulties[21]. To take into account the therapeutic difficulties, such as the number of lesions and the treatment period, covariant analysis was performed in this study. Thus, we found that the number of cryotherapy sessions decreased significantly in the immunotherapy group, while there were no differences in the topical agent group, compared with cryotherapy alone group. The discordant results between immunotherapy and topical agents might be explained by the fact that immunotherapy produces more damage to the viral affected cells by triggering the systemic inflammatory reaction and by the fact that it is generally done much precisely by clinicians, while the topical treatment is carried out by the patients themselves. Daily application of 5FU/Salicylic acid might be irritating or the patients may simply forget to do it. This result supports results from previous studies demonstrating no clinical benefit of topical agents, when associated with cryotherapy[19,20].

Cryotherapy techniques differ between practitioners with regard to freezing/thawing time, mode of application (i.e. cryogen spray versus cotton ball application) and treatment intervals[2]. When the spray gun is used, it is held perpendicular to the wart at a distance of 1～2 cm and liquid nitrogen is sprayed until the ice ball has spread from the center to include the edge of the wart and a 2 mm margin[21]. The lesion is then allowed to thaw. The thawing time depends on complete disappearance of the ice ball. From accumulated experience of cryotherapy conducted in our clinic, the ice ball disappears completely in less than 10 seconds. To minimize variation of the treatment results, we applied this procedure of freezing (i.e. the nitrogen gun) and 10 seconds of thawing.

This retrospective research bears the limitations that every confounder was not completely controlled and that the size of the combined therapy group was not sufficient. However, we propose that the combination of cryotherapy with immunotherapy could be very helpful, as it reduces the number of required sessions of this painful procedure. As the pain hinders the wart treatment and lowers the success rate, the clinicians can achieve desirable treatment outcomes with concurrent DPCP immunotherapy, by reducing the number of painful cryotherapy sessions and also by raising the patients' compliance.

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