Journal of Pakistan Association of Dermatologists. 2024;**34(4)**:1048-1061.

Review Article

Combination therapy for the treatment of cutaneous and genital warts

Maryam Khalili, Rezvan Amiri, Behzad Iranmanesh, Fateme Ghaysuri*, Mahin Aflatoonian

Department of Dermatology, Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran.
* Kerman University of Medical Sciences, Shiraz, Iran.

Abstract Evidence-based studies demonstrated that combination therapy can optimize treatment efficacy along with faster response rates and lower recurrence rates which are essential factors in increasing patients' adherence to treatment. In this review, we discuss effectiveness, safety and recurrence rates of various combination therapies in the treatment of cutaneous and genital warts in detail. We searched literature with related keywords. Inclusion criteria were randomized clinical trials (RCTs) that investigated efficacy of combination of two or more treatments on various types of cutaneous and genital warts since January 2000 up to August 2023. Thirty articles were enrolled to final investigation. Adjuvant of destructive methods to other therapeutic options was the most common combination therapy in the treatment of warts. Cryo-immuno-therapy is an effective therapeutic method in the treatment of wart that can boost immune system response against human papilloma virus (HPV). This combination therapy demonstrated rapid remission of the both treated and distant untreated lesions, and reduces risk of recurrence. Application of topical destructive methods prior to laser leads to rapid remission and reduces treatment cost as well. Combination of laser with immunologic therapies can be especially effective in recalcitrant warts (i.e. periungual lesions). Combination therapy can particularly be recommended in immunocompromised patients and those with refractory warts that demonstrated poor response to other treatment options.

Key words
Cutaneous warts; Genital warts; Condyloma acuminate; Adjuvant therapy; Combination therapy.

Introduction

Wart is a benign proliferation of mucocutaneous tissue that is caused by infection of basal keratinocytes by HPV. Nowadays, over 150 subtypes have been identified that can cause cutaneous or genital warts.[1,2]

Choice of treatment depends upon various factors such as characteristics of patients (age and immunologic status), clinical features of the lesions (site, size, number, subtype and response to prior treatments) and characteristics of therapeutic modalities (efficiency, recurrence rate, availability, affordability, duration of treatment course and safety profile) and patients' tendency to treatment.[3] Current treatment modalities, especially traditional ones are time consuming, and have high recurrence rates, which can be frustrating, and lead to lower rates of patient's adherence to treatment. Therefore, searching for the best treatment approaches that can enhance efficacy, hasten response rates, have acceptable safety profile and reduce recurrence is always armament. Evidence-based studies demonstrated that combination therapy can result in superior outcomes along with faster response rates and lower recurrence rates that are essential factors in increasing patients'

Manuscript: Received on: January 14, 2024
Accepted: April 19, 2024
Address for correspondence
Dr. Mahin Aflatoonian, Associate Professor, Department of Dermatology, Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran.
Email: maaflatoonian@gmail.com

adherence to treatment.[1-5] In this review we discuss effectiveness, safety and recurrence rates of various combination therapies in the treatment of cutaneous and genital warts in detail.

Methods

We searched Pubmed, Scopus, Web of Science, Medline, Cochrane library and Google scholar with keywords of "wart" OR "verruca" OR "genital wart" OR "condylomata acuminata" OR "cutaneous wart" AND 'combination" OR "adjuvant" OR "treatment". Inclusion criteria were randomized clinical trials (RCTs) that investigated efficacy of combination of two or more treatments on various types of cutaneous and genital warts since January 2000 up to August 2023. Exclusion criteria were non-RCTs, case reports, case series, and articles that were in languages other than English or on non-human subjects or those which evaluated efficacy of treatment on internal genital warts. After initial literature search, one-hundred and fifty-six articles were achieved. Firstly, the abstracts of articles were evaluated by two authors for eligibility. Fifty-eight articles were selected and evaluated based on manuscript. After exclusion of duplicated articles or those that were not met by inclusion criteria, thirty articles were enrolled to final investigation. The results of search strategy are demonstrated in **Figure 1**. Detail of articles including author's name, study design, demographic features of patients, therapeutic methods, final outcome, possible side effects, follow up period and recurrence rates were extracted from articles and illustrated in **Table 1**.

Results

Existing literature shows that combination of destructive methods with other treatments were the most common combination therapies in the treatment of warts. Destructive therapies include physical (cryotherapy, lasers, microneedling and electroporation) and chemical [cantharidin and trichloroacetic acid (TCA)] options. Adjuvant treatment options include intralesional (IL) *candida* antigen, IL Purified Protein Derivative (PPD), zinc (systemic and topical), topical 5-fluorouacil (5-FU), podophyllin/ podophyllotoxin, topical cantharidin and oral isotretinoin.[4-25]

Cryotherapy is the first-line of treatment that has cytodestructive effects and leads to necrosis of keratinocytes, recruits natural killer cells to the treated site, and implies immunomodulatory effects. It also induces inflammation that stimulates immune system to clear distant wart lesions. Four articles were evaluated to demonstrate the efficacy of combined cryotherapy and IL immunotherapy (cryo-immunotherapy) in the treatment of cutaneous and genital warts.[4-7] Cryotherapy was performed with liquid nitrogen every other week up to 4-5 sessions as 10-30 second freeze-thaw cycles (1-3 cycles) until white ice crystal formed in the whole lesion and 1-5mm of margin of the lesion. The volume of injection in immunotherapy with *candida* varied from 0.1 cc to maximum of 0.3 cc. The injection volume in PPD group has inverse in association with the size of induration in skin pretest; the injected volumes were 0.1, 0.2 and 0.3 cc in patients with induration sizes of more than 15mm, 10-15 mm and 5-9 mm, respectively.[4-7] While, two studies demonstrated significantly superior outcomes and faster response rates with immuno-cryotherapy (cryotherapy combined with IL *candida*/PPD) compared to each of these treatments alone;[4,7] other studies showed no significant difference between cryo-immunotherapy (cryotherapy combined with IL PPD) compared to cryotherapy or immunotherapy alone.[5,6] However, combination therapy group demonstrated lower rates of recurrence compared to cryotherapy group.[4,5] Side effects including blister formation and

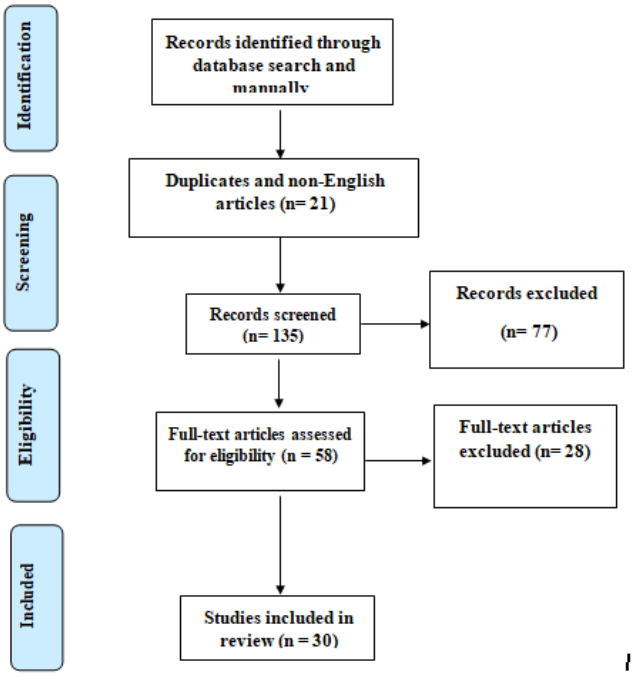

Figure 1 Schematic of the search strategy.

hypopigmentation were significantly more common in combination group compared to monotherapy group; however they were temporary and resolved without any treatment.[5]

Four articles were evaluated the efficacy of combined cryotherapy with zinc sulfate (systemic or topical) in the treatment of warts.[8-11] Two studies revealed significantly faster response with combined 2% topical niosomal zinc sulfate and cryotherapy compared to combined cryotherapy with either placebo or conventional zinc sulfate.[8,9] Whilst combined cryotherapy and oral zinc sulfate (10 mg/kg/day, up to maximum 600 mg/day) showed no significant difference compared to cryotherapy as monotherapy regarding efficacy and number of treatment sessions.[10,11] In addition, gastrointestinal (GI) side effects were reported to be significantly higher in patients who were treated with oral zinc sulfate compared to control group (68.9% vs. 17.7%, respectively).[10] Nonetheless, significantly lower recurrence rates of genital warts were reported with combination of cryotherapy and oral zinc sulfate than cryotherapy alone.[11]

Three studies were evaluated the efficacy of combined cryotherapy and topical podophyllin in genital warts.[12-14] Podophyllin is a plant-based ingredient that attaches to microtubules inside the cells and interrupts cell proliferation and induces apoptosis. Combination therapy of cryotherapy and topical 25% podophyllin demonstrated significantly higher and faster remission of genital warts during only two treatment sessions in comparison to combined TCA and podophyllin, or monotherapy with either of them alone (complete clearance of 72.5%, 62.3%, 37.5%, and 44.3%, respectively).[12] In contrast, two other studies showed equal efficacy of combination of cryotherapy and topical podophyllin/ podophyllotoxin cream with either combined IL bleomycin with placentrex gelor combined cryotherapy with placebo.[13,14]

Oral retinoid is an antiproliferative drug and powerful immunomodulator that decreases HPV replication via increasing epidermal cells differentiation and reducing viral transcription. Reyna-rodriguez revealed faster recovery and lower relapse rate in the treatment of condyloma acuminata lesions with adding low dose of oral isotretinoin (20 mg/day) to cryotherapy compared to cryotherapy alone; however the result was not statistically significant. Percentages of complete clearance after only two weeks of treatment were 53.4% and 20% in combination therapy and monotherapy groups, respectively.[15]

5-FU is an anti-proliferative drug that prevents HPV replication via suppression of synthesis of pyrimidine and thymidine. Luke *et al*. reported no additional efficacy with adding topical 5% 5-FU (twice a day) to cryotherapy (two cycles, over 5 sessions) compared to cryotherapy as monotherapy.[16]

Cantharidin induces keratolysis and apoptosis of epidermal cells by releasing serine proteases. It also has anti-proliferative effects by halting the cell cycle. Meymandi *et al.* compared efficacy of combined cryotherapy with topical cantharidin versus combined cryotherapy with placebo. Although there was no significant difference regarding complete regression of warts between the two groups at the end of treatment; however, faster improvement was reported in cantharidin group compared to placebo group. Complete clearance after three sessions of treatment was 58.1% and 14.5% in combination therapy and monotherapy groups, respectively.[17]

Lasers were the second most common destructive methods, including neodymium-yttrium-aluminum- garnet (Nd: YAG), erbium-doped yttrium-aluminum-garnet (Er: YAG), diode, pulsed dye laser (PDL) and carbon dioxide (CO_2) laser.[18-23] In-situ photo-immunotherapy (ISPI) is a unique therapeutic method that recently has been used in the treatment of various types of cancers and infections. It is a novel combination therapeutic strategy that boosts cell mediated immune system via synergistic effects of immunomodulation and photothermia on immune system. This combination therapy can augment antigen presentation to cytotoxic CD8 T cell to eradicate infected keratinocytes.[18] Shi *et al.* evaluated efficacy of combined local phototherapy (using diode laser) and topical 5% imiquimod compared to topical imiquimod alone. Significantly higher response rates and reduction in size of the lesions were reported in combination therapy group compared to monotherapy group. Complete remissions were reported 61.1% and 32.4% in combination therapy and monotherapy groups, respectively.[18] PDL eliminates infected epithelia cells by destruction of the feeding blood vasculature in papillary dermis and stimulation of immune system. Nd: YAG laser employs both photothermal and photomechanical effects that leads to coagulation of feeding vasculature in papillary dermis and explosion of infected keratinocytes. This leads to more exposure of HPV antigen to immune system that induces keratinocytes apoptosis.[19,20] Two studies showed no significant difference regarding final outcome between combination of laser (either PDL or Nd: YAG) with topical medications (30%SA or 10%KOH) and laser alone (1-5 monthly sessions); however, significantly faster response was achieved with combination therapy compared to monotherapy groups. Mean number of treatment sessions in combination therapy was significantly lower (2.2) in comparison to monotherapy group (3.1).[19,20] In addition, there was no significant difference between two groups regarding adverse effects.[20] Another study compared the efficacy of combined PDL and IL interferon alpha-2b (IFN-α 2b) compared to either of these treatments alone.Significantly superior outcome and lower recurrence rates were reported with combination therapy compared to monotherapy groups.[22] CO_2 laser and Er: YAG laser lead to physical destruction of infected keratinocytes. Both focused and defocused modes can be utilized for cutting of warts as well as vaporization and maintaining hemostasis, respectively. Results of one study indicate superior outcomes with combined long-pulsed Nd: YAG and Er: YAG compared to Er: YAG alone with only one treatment session in recalcitrant warts. Complete remission was reported in 48% and 29% of lesions in combination therapy and monotherapy groups, respectively (P=008). Side effects were not statistically different between two groups, except, bullae that were significantly more common in combination therapy compared to monotherapy group (P=029).[21] One study shows association of higher CD4 T cells, CD4/CD8 ratio and IL-2peripheral blood levels as well as lower levels of CD8 T cells with clearance of wart lesions. Moreover, this study found that

adding traditional Chinese medicine to fractional CO_2 laser was associated with higher percentage of factors related to eradication of warts compared to laser alone.[23]

Ghonemy *et al.* showed no difference in clearance of warts with combined microneedling and topical 5-FU solution, and either IL 5-FU (50 mg/cc) or microneedling as monotherapy. The combination group demonstrated significantly higher reduction in wart's size compared to other groups. In addition, IL injection group required significantly higher numbers of treatment sessions than other groups. Also, higher percentage of patients in injection group complained of pain during treatment compared to other treatment groups.[24]

Bleomycin binds to DNA and interferes with cell proliferation and induces apoptosis and necrosis of infected epithelial cells. Di Chiacchio *et al.* compared efficacy of electrochemotherapy [combination of electroporation and IL bleomycin (3 mg/cc)] in the treatment of ungula warts during one treatment session. Whilst, IL bleomycin group showed significantly superior outcomes with lower response rates; significantly higher percentage of side effects(including haemorrhagic necrosis and pain) were observed in combination therapy compared to monotherapy therapy group (91% and 59%, respectively).[25]

Isik *et al.* compared the efficacy of combined topical 0.5% 5-FU and 10% SA to topical 5% potassium hydroxide (KOH). They demonstrated no significant difference between two groups regarding outcome, safety profile and recurrence rates.[2]

Immunotherapy is a popular noninvasive therapeutic option that can be effective particularly in patients with multiple recalcitrant warts, and can clear distant warts. The treatment with it is simple, non-expensive and has a high safety profile.[26-28] Three studies evaluated combination of different types of immunotherapy in the treatment of warts. Horn *et al.* compared the efficacy of combined IL immunotherapy (using Mumps, *Candida*, and *Trichophyton*) plus IFN-α-2b, to IL immunotherapy plus IL normal saline or each of these treatments alone. Significantly superior outcomes were demonstrated with combination therapy regarding clearance of treated warts and distant warts, compared to monotherapy with IFN-α-2b or placebo. However, there was no significant difference between combined immunotherapy and IFN-α 2b with immunotherapy alone. Side effects were fever, myalgia, edema and erythema that were resolved after 24 hours with antipyretics and cold compress. There was no significant difference between groups regarding side effects.[26] Nofal *et al.* demonstrated superior efficacy along with lower recurrence rates with triple IL antigen immunotherapy [including PPD, *candida* antigen and measles–mumps–rubella (MMR) vaccine] compared to either of them as monotherapy; however the results were not statistically significant.[27] Fawzy *et al.* demonstrated no additional benefits with adding human papilloma virus (HPV) vaccine (Cervarix/ Gardasil) to *candida* antigen in patients with condyloma acuminata compared to *candida* antigen alone.[28]

Imiquimod acts through effects on toll-like receptors and boosts the immune system response via up-regulation of inflammatory cytokines related to TH-1 (IFN-α, TNF-α, IL-12). Stefanaki *et al.* showed no significant difference between combined topical 5% imiquimod and 15% SA solution withcryotherapy. However, plantar warts showed significantly higher response rates with combination therapy compared to cryotherapy (83.3% vs. 30%, respectively).[29]

Table 1 Results of the reviewed literature.

First author (year)	*Design*	*Age*	*M/F*	*Clinical type*	*Study*	*Control*	*Result (%)*	*S/E (%)*	*F/UP (RR, %)*
Attwa (2020)[4]	Prospective RCT	18-68 (32.85 ± 10.79)	25/35	VV	Cryotherapy + IL *candida* antigen (every 2 W, maximum, 5 sessions)	C1: Cryotherapy C2: IL *Candida* antigen	CR: S: 40 C1: 25 C2: 20	S: Erythema: 90, Hypopigmentation: 40, Peeling: 55, Bullae: 95 Fever/ myalgias: 75, C1: Erythema/ edema: 25, Pain: 95, Hypopigmentation: 15, Peeling: 20, Bullae: 70, C2: Erythema: 45, Edema: 85, Pain: 100, ypopigmentation: 5, Peeling: 30, Fever/ myalgias: 65, Headache: 80	3 mo (S: 10 C1: 20 C2: 10)
Awad (2023)[5]	Prospective RCT	31.50 ± 12.2	336/14	VV	Cryotherapy + IL PPD (every 2 W until maximum 4 sessions)	IL PPD	CR: S:44 C: 48	Pain, Erythema, Edema, Blister, Hypopigmentation	2 mo (S: 0 C: 20)
Moubasher (2021)[6]	Prospective RCT	NS	45 (NS)	CA	Cryotherapy + IL PPD	C1: IL PPD C2: cryotherapy (every other W for 4 session)	CR: S:46.7 C1:13.3 C2: 26.7	NS	No
Ibrahim (2021)[7]	Prospective RCT	S: 23.68 ± 11.1 C1:24.00 ± 2.5 C2: 23.36 ± 9.9	37/38	VV	Cryotherapy + IL PPD	C: Cryotherapy (cryogen, every 2 W until maximum 4 sessions) C2: IL PPD (0.1 cc, every 2 W until 4 sessions)	CR: S: 84 C1: 28 C2: 48	S: Pain (100), Edema (8), Swelling (8), Hypopigmentation (4), Flu like (4) C1: Pain (100), Edema (32), Hypopigmentation (32), Swelling (8), C2: Pain (100), Erythema (20), Edema (8), Flu like manifestations (4)	No
Mahmoudi (2018)[8]	Prospective RCT Double-blind Placebo-controlled	S: 45 ±6.60 C:39 ±7.47	40/43	VV	Cryotherapy (every 3 W)+ oral zinc sulfate (10 mg/kg/d, maximum: 600 mg/d)	Cryotherapy + placebo	CR S:68.4 C:63.9	S: Nausea (68.9), Vomiting (17.7), Epigasteric pain (17.7) C: GI adverse effects (5.1)	6 mo (S:7.9, C:16.6)
Farajzadeh (2018)[9]	Prospective RCT Double-blind	S:26.70 ±9.77 C: 28.8±8.84	31/29	VV	Cryotherapy (every 2 W) + 2% niosomal zinc sulfate cream (twice a d)	Placebo with cryotherapy (every 2 W)	CR: S: 60 C: 43.3	S: Pain (52), Blister (46.9), Pruritus (71.4), Hypopigmentation (57.1), PIH (46.2), C: Pain (48), Blister (53.1), Pruritus (28.6), Hypopigmentation (42.9), PIH (53.8)	No

First author (year)	*Design*	*Age*	*M/F*	*Clinical type*	*Study*	*Control*	*Result (%)*	*S/E (%)*	*F/UP (RR, %)*
Khalili (2022)[10]	Prospective RCT Double-blind	S:26.53±8.25 C: 28.0±9.52	25/35	VV	Cryotherapy (every 2 W)+ 2% niosomal zinc sulfate suspension (twice a d)	Conventional 2% zinc sulfate + cryotherapy	CR: S: 93.3 C: 93.3	S: Pain (83.3), Blister (43.3) Pruritus (40), Hypopigmentation(16.7), PIH (13.3) C: Pain (80), Blister (46.7) Pruritus (20), Hypopigmentation (16.7), PIH (16.7)	3 mo (S:10, C: 13.3)
Akhavan (2014)[11]	Prospective RCT Single-blind	20-50 y	0/228	CA	Cryotherapy + oral zinc sulfate	20% Podophyllin solution (once a w for 8 W), Imiquimod (3/W, for 8 W), Cryotherapy	No significant difference in response	NS	6 mo (significantly lower RR in combination groups compared to monotherapy groups)
Sherrad (2007)[12]	Prospective RCT	S: 25.7 C1: 25.4 C2: 25.4 C3: 26.8 C4: 26	409 (NS)	CA	Cryotherapy + topical 25% podophyllin	C1:Topical podophyllin 25%, C2: TCA C3: Cryotherapy C4: TCA + podophyllin 25%	CR: S: 100, C1: 82.1, C2: 84.5 C3: 92.4 C4: 94	C1: irritation (1.3) C3: intolerable pain (1.2)	No
Mahajan (2013)[13]	Prospective RCT	S: 27.6 ± 9.64 C: 29.56 ± 9.06	43/17	CA	Cryotherapy + topical 20% podophyllin	IL bleomycin + topical 5% placentrex gel	CR: S: 80 C: 83.3	S: Burning/stinging (46.6) Pain (23.3), Bleeding (16.6) Irritation (6.6), Edema (36.6) Infection (16.6), Desquamation (33.3), Hypopigmentation (63.3), Scar (6.6); C: Burning (66.6), Pain (70), Bleeding (56.6), Irritation (16.6), Edema (43.3), Hypopigmentation (10)	3 mo S: 6.6 C: 0
Gilson (2023)[14]	RCT double-blind placebo-controlled	18-58 (26)	91/49	CA	Cryotherapy, (I/W, 12 W)+ Podophyllotoxin 0.15% cream (BID, III/W, 4 W)	Cryotherapy+ placebo cream	CR: S: 60 C: 45.7	S:, Pain: 34.3 C: Pain: 18.6	3 mo (S: 16.7 C: 18.8)
Reyna-Rodriguez (2020)[15]	Prospective RCT Single-blind	21-52	41/5	CA	Cryotherapy + isotretinoin (20 mg/d for 45 d) +	Cryotherapy (every 2 w until 3 sessions)	CR: S:65.2 C:65.2	S: Pain: 47.8, Blister: 26.1 Ulcer: 4.3, PIH: 100 LFT abnormalities: 4.3 C:, Pain: 30.4, Blister: 13, Ulcer: 21.7, PIH: 100, LFT abnormalities: 4.3	22 w (S: 20, C: 42.9)
Luke (2006)[16]	RCT Double-blind	15-75 (S: 39.3, C: 36)	33/47	VV	Cryotherapy+ Topical 5% 5-FU (II/d)	Cryotherapy, (2-cycle, every 3 W, maximum 5 sessions)	CR: S: 30 C: 42.5	S: Blister: 52.5, Pain: 47.5 C: Blister: 35, Pain: 27.5	No

First author (year)	*Design*	*Age*	*M/F*	*Clinical type*	*Study*	*Control*	*Result (%)*	*S/E (%)*	*F/UP (RR, %)*
Meymandi (2017)[17]	Prospective RCT Double-blind Placebo-controlled	5-52	69/41	PP, VV	Cryotherapy (every 2W) + topical cantharidin solution (I/d, 6 W)	Cryotherapy+ placebo	CR: S:100 C:100	S:, Hypopigmentation (29.1) PIH (41.8), Pain(61.8), Blister (50.9), Atrophic scar (9.1), Itching (12.7), Hypertrophic scar (7.3) C: Hypopigmentation (10.9), PIH (45.5), Pain (78.2), Blister (32.7), Atrophic scar (29.1), Itching (9.1), Hypertrophic scar (6.3)	3 mo (S:32.7, C:36.4)
Shi (2023)[18]	RCT	12-61 (33.8 ± 12.9)	78 (NS)	VV, FW, PW	Diode laser (I/w, 5w, WL, 808 nm; spot, 2 cm^2; 900 J/cm^2; time, 600 s) + topical 5% imiquimod	Imiquimod 5% (I/d, 5d/w, 42d)	S: 61.1 C: 32.4	S: Erythema: 66.7, Erosion: 52.8 C: Erythema: 38.2, Erosion: 23.5	6 mo (0)
Akarsu (2005)[19]	Prospective RCT Single-blind	9-54 (22.05)	14/5	VV	PDL (WL: 585 nm, PD: 350µs, spot: 5mm, F: 6-9 J/CM2, margin: 5 mm) + 30% SA (BID for 5 d)	PDL (1-5 monthly sessions)	CR: S: 69.7 C:66.6	S: Erythema: 100, Blister:6.06, PIH:9.09, Hypertrophic scar: 3.03, Crust: 27.27, Scar: 9.09 C: Erythema: 100, Blister:9.09, PIH:6.06, Crust:18.18, Scar:6.06	No
Khattab (2019)[20]	Prospective RCT Comparative	9-54 (22.05)	28/10	VV, PP	Long-pulsed Nd:YAG + 10% potassium hydroxide (nightly)	Paring + Long-pulsed Nd:YAG (5 sessions, monthly intervals, spot: 5mm, PD: 10 mm, F: 120 J/cm2 margin: 1 mm)	CR: S:78.7 C: 69.7	S: Blister (6.06), Crust (27.27), Scar (9.09 VS), Hyperpigmentation (9.09), Hypertrophic scar (3.03), Blister (9.09), Crust (18.18), Scar (6.06), Hyperpigmentation, (6.06), Hypertrophic scar (0)	6 mo (0)
Jiryis (2023)[21]	Prospective RCT	12-72 (34.67)	12/12	PP PU VV	Long pulsed Nd:YAG (spot size of 2 or 6 mm, PD, 15–45, ms; F, 70–350 J/cm^2, one session) + Er:YAG	Er:YAG (spot size of 1–4 mm, 2 Hz, 5.5–11.1 J/ cm2, one session)	CR: S:48 C:29	S: Crust: 100, Blister: 5, PIH: 2.5, Hypopigmentation: 1.7, Hypertrophic scar: 3.3 C: Crust: 100, PIH: 5, Hypopigmentation: 3.3, Hypertrophic scar: 3.3	5 w
Choi (2002)[22]	Prospective RCT Comparative	5-62	23	PU	PDL + IL IFN-α 2b (3 weekly sessions)	C1: IL IFN-α 2b C2: Paring + PDL (WL: 585, spot: 5 to 7 mm, F: 8 J/cm^2, PD: 450	CR: S:92.3 C1: 50 C2: 0	NS	6 mo (S: 8.3 C1:20 C3:NS)

First author (year)	*Design*	*Age*	*M/F*	*Clinical type*	*Study*	*Control*	*Result (%)*	*S/E (%)*	*F/UP (RR, %)*
						µs, pulse No: 2, overlap: 1 to 2 mm, margin: 2 mm)			
Xu (2020)[23]	Prospective RCT	23-70 (38.7 ±11) y	84/57	CA	CO_2 laser + Oral traditional Chinese medicine)	CO_2 laser	Significantly higher RR in monotherapy compared to combined therapy	NS	6 mo (S:25.7, C: 40.8)
Ghonemy (2020)[24]	Prospective RCT	18-60	52/38	PP	Microneedling+ 5-FU solution (under occlusion for 3 h) Every 2 weeks Maximum: 6 sessions	C1: Microneedling (dermapen, depth: 2 mm) C2: IL 5-FU (500 mg/10 cc, 0.1 cc, 4 mg/each lesion, maximum: 2cc/session+ superficial paring with blade	CR: S: 86.7 C1:70 C2:76.7	S: Pain (20) C1: Pain (23) C2: Pain: (43.4), Hemorrhagic Eschar (56.7)	6 mo (0)
Di Chiacchio (2019)[25]	Prospective RCT Double-blind	19-57 (36)	14/30	PP, PU	Electroporation (8 pulses, 1000 v,/cm^2, 100 µs) + IL bleomycin	IL belomycin, Reconstituted with 2% lidocaine, 3 mg/cc, Volume: 0.1 cc, One session	CR: S: 50 C: 85.7	Pain, Necrosis, Onycholysis, Inflammation, Infection, RSD	9 mo (S:30.4, C: 4.7)
Isik (2014)[2]	Prospective RCT Open-label	S: 33.7±9.59 y C: 37.0± 9.51 y	41/19	CA	10% SA (daily for 12 w)+ 0.5% 5-FU	5% KOH solution (daily for 12 w)	CR: S: 76.7 C: 70	Burning sensation, Erosion	4 weeks (S: 10, C: 6.6)
Horn (2005)[26]	Prospective RCT Single-blind	Average age: 34-40	84/117	NS	Immunotherapy (mumps, *Candida*, or *Trichophyton)* (0.3 cc) +IL IFNα-2b (10^6 IU, 0.08 cc)	C1: Immunotherapy C2: IFNα-2b C3: Normal saline	S: 68 C1: 54 C2: 26 C3: 22	Fever and myalgia S: 73.2, C1: 12.9, C2: 19.6, C3: 1.6, Edema/erythema: 4.5	No
Nofal (2022)[27]	Prospective RCT	12-67 (27.15)	71/89	VV, PP, PW, PU	Combination of IL PPD, candida antigen, and MMR(0.1 ml each, every 2 w, up to 5 sessions)	C1: IL PPD (0.3 cc) C2: IL candida antigen (0.3 cc, 1/100) C3: IL MMR (0.3 cc)	S: CR: 77.5 C1:57.5 C2:72.5 C3:62.5	Pain, Erythema, Blister, Edema, Hypopigmentation, Flu-like syndrome	6 mo (S:6.45, C1:8.7, C2:13.8, C3: 12)
Fawzy (2023)[28]	Prospective RCT Single-blind	20-66 (36.2 ± 11.31)	18/62	CA	S1: IL bivalent HPV vaccine (Cervarix, 0.2 cc, 1-w interval, 2 sessions)+ *Candida* S2: IL quadrivalent HPV vaccine (Gardasil, 0.2 cc, 1-w interval, 2 sessions) +	C1: IL Candida antigen (0.2 cc, 1/1000 units, 2-w interval, up to 3 sessions) C2: normal saline	CR: S1: 20 S2: 60 C1: 40	S1: Pain (100), Itching (30), Anaphylaxis (5) S2:Pain (100), Itching (30), Anaphylaxis (5) C1: Pain (100), Itching (25) C2: Pain (100)	3 mo (0)

First author (year)	*Design*	*Age*	*M/F*	*Clinical type*	*Study*	*Control*	*Result (%)*	*S/E (%)*	*F/UP (RR, %)*
					Candida				
Stefanaki (2015)[29]	RCT	2-12 (8.06 ± 8)	51/35	VV, PP, FW, PW	Imiquimod 5% + 15% SAsolution (5/W, 3 mo)	Cryotherapy (every 2W, 3 mo)	CR: S: 81.1 C: Pain	S: Erythema: 2.7 C: Pain	No
Akhavan (2014)[11]	Prospective RCT Single-blind	20-50 y	0/228	CA	Oral zinc sulfate (400 mg/d for 8 w) + podophyllin, Oral zinc sulfate + imiquimod	20% Podophyllin solution (once a W for 8 W), Imiquimod (3/W, for 8 W), Cryotherapy	No significant difference in response	NS	6 mo, (significantly lower RR in combination groups compared to monotherapy groups)
Parsad (2001)[3]	Prospective RCT	5-16 (12.2)	10/12	VV, PW, PP	Oral levamisole (2.5 mg/kg/W)+ Cimetidine (30 mg/kg/d) for 12W	Oral cimetidine (30 mg/kg/d)	CR: S: 65 C: 31.6	S: Metallic taste:5% Nausea:10%	No
Fathy (2020)[30]	Prospective RCT Single-blind	S:18-43 (28.6±8.6) C:19-45 (31±8.6)	20/20	PP	IL combined digoxin+ Furosemide (every week, maximum: 5 sessions, 0.1 cc)	IL normal saline	CR: S: 50 C: 0	Pain (100)	5 W
Lofty (2022)[31]	Prospective RCT Double-blind	18-50	38/42	VV, PP	IL furosemide + IL digoxin, Weekly interval, Volume: 0.2 cc (150 µl digoxin and 50 µl furosemide) Maximum: 5 sessions	IL normal saline	CR: S: 92.5 C: 10	S: Pain (95), Edema(5), Hypo (5%) C: Pain (45)	6 mo (S: 0, C: 50)
Rijsbergen (2019)[32]	Prospective, RCT Double-blind, Placebo-controlled Parallel-group	25.8 ± 10.6	31/49	VV. PP	Topical digoxin+ furosemide gel (0.125%, 5-30 mg/lesion/d, 42 d)	C1: Topical digoxin C2: Topical furosemide C3: Placebo	CR: S: 16 C1: 15 C2: 15 C3: 0	Nasopharyngitis, Headache, Influenza-like illness	No

Abbreviations: M, male; F, female; S/E, side effect; F/UP, follow up; RR,relapse rate; RCT, randomized controlled trial;VV, verruca vulgaris;w, week;IL, intralesional; CR, complete remission; S, study group; C, control group; mo, month; PPD, purified protein derivative;NS, not stated;CA, condyloma acuminata;d, day, PIH, post inflammatory hyperpigmentation; TCA, trichloroacetic acid ; 5-FU, 5-fluorouracil; LFT, liver function test;SA, salicylic acid; PDL, pulsed dye laser; IFN-α, interferon-α; MMR, measles–mumps–rubella ; HPV, human papilloma virus;WL, wavelength; F, fluence; PD, pulse duration; PP, palmoplantar wart; Nd:YAG, neodymium-yttrium-aluminum- garnet; Er: YAG,erbium-doped yttrium-aluminum-garnet; FW, flat wart;PW, plane wart;CO2 laser, carbon dioxide laser; PU, periungual; RSD, Reflex sympathetic dystrophy ;

Cimetidine shows immunomodulatory effects via interaction with histamine (H)-2 receptors, and suppression of T inhibitory cells. Levamisole also has immunomodulatory effects through stimulation of cytokines associated to TH1 and increased serum levels of interferon (IFN) and interleukin (IL)-2. Parsad *et al.* demonstrated significantly higher clearance rates and faster response rates with combined oral cimetidine (30 mg/kg/day) and levamisole (2.5 mg/kg/week) compared to oral cimetidine alone.[30]

Zinc sulfate can up regulate cytokines related to TH-1 (such as IL-1, IL-2, IL-6, IL-8 and TNF-α), and augments the immune system via balancing TH-1 to TH-2 ratio, and recruitment of T cytotoxic cells, neutrophils, NK-T cells, and macrophages to defend against viral infections. One study showed that although adding oral zinc sulfate (400 mg/day) to topical 20% podophyllin solution (once a week for 8 weeks) or imiquimod (thrice a week for 8 weeks) shows no significant additional efficacy; however; lower recurrence rates were observed with combination therapy compared to monotherapy groups.[11]

Three studies investigated efficacy of combined digoxin and furosemide (topical/IL injection, containing 150 µl digoxin and 50 µl furosemide in each session) compared to either of these treatments alone or placebo.[30-32] While, two studies showed significantly higher percentage of complete clearance of treated warts in combination therapy group compared to placebo (92.5-50% vs. 10-0%, respectively);[30,31] the other study demonstrated no significant difference in any active treatment groups compared to placebo group regarding complete clearance rates. Nevertheless, significant reduction in size of warts was only observed in combination therapy group compared to either of these drugs as monotherapy or placebo group.[32]

Discussion

Nowadays, there are various treatment options including traditional destructive methods, antiproliferative treatments and immunomodulators for the treatment of warts. Combination therapy might have synergistic effect that augments immune system response to eradicate HPV. Combination therapy can balance TH-1 and TH-2 immune response, and activate various pathways in immune system.[4] Combined cryotherapy with topical medical therapy leads to enhancement of penetration of topical medication by inducing local edema. Moreover, performing cryotherapy prior to IL injections can lead to less resistance to injections and a more convenient treatment due to swelling and softening of warts after cryotherapy.[4-7] Existing evidence shows that efficacy of cryotherapy as monotherapy is relatively low.[33] Therefore, adjuvant cryotherapy with other treatment modalities can lead to superior outcomes, shorter treatment courses, and reduction of recurrence rates, and as a result augments patient's adherence to treatment as well. Cryo-immuno-therapy is an efficient therapeutic strategy in the treatment of warts. Evidence reveals that applying destructive methods such as cryotherapy before immunotherapy can expose viral antigens to immune system, and attracts immune cells to the site of treatment and boosts immune system response. It can be recommended to those with multiple recalcitrant warts, especially immunocompromised individuals.[4-11]

Recently, lasers have become a popular treatment modality for the treatment warts. Nowadays, various types of lasers have been used in the treatment of cutaneous and genital warts.[1,34] Application of topical destructive methods prior to laser dissolves stratum corneum, and leads to dehiscence of epidermal cells, and consequently increases penetration of

laser. This combination can also induce irritation and therefore recruits immune cells, augments immune system and leads to rapid remission and reduction of treatment cost as well. Combination of laser with immunologic therapies can be especially effective in recalcitrant warts (i.e. periungual lesions).[18-23,34]

Other physical therapies include microneedling and electroporation, which can augment efficacy of topical medications via enhancing the penetration of topical drugs into epidermis through creating pores or increased cell membrane permeability without interference in cell viability. This adjuvant therapy is also more tolerable compared to IL injections, and might result in fewer treatment sessions. Microneedling might also stimulate immune system by recruitment of inflammatory cells and immune cells into the site of infection, and presentation of viral antigens to immune cells.[24,25]

Although existing evidence demonstrates no additional efficacy by adding oral zinc sulfate to other treatment options against wart lesions, it might lead to lower risk of recurrence. Nevertheless, high doses of oral zinc are usually required to be effective, which can lead to GI disturbance and might not be tolerated by patients. In contrast, combined topical zinc sulfate, especially niosomal formulations with other treatment options can be effective on wart lesions due to enhanced absorption.[8-11]

Evidence shows DNA viruses such as HPV are dependent on potassium for replication. Therefore, suppression of entry of potassium into cells might be effective to inhibit replication of virus. Digoxin and furosemide are both inhibitors of potassium entry into cells that recently have been used in clinical trials in the treatment of warts. Moreover, they augment immune system and can lead to clearance of distant warts. Existing evidence shows good efficacy of combined topical/ IL digoxin and furosemide compared to placebo or monotherapy of either of these drugs on various types of warts including palmoplantar lesions.[30-32]

Conclusion

Combination of destructive therapeutic methods with other treatment options can augment treatment efficacy, reduces duration of treatment course and leads to lower recurrence rates. Cryo-immunotherapy is particularly a safe and effective therapeutic strategy that can boost immune system, and also clears distant warts. Adjuvant ablative therapies (such as lasers and microneedling) and topical therapies can enhance penetration of topical drugs and lead to faster treatment responses.

Financial support and sponsorship None.

Conflict of interest Authors declared no conflict of interest.

Author's contribution

MK, RA, BI, FG, MA: Substantial contributions to study design, acquisition of data, manuscript writing, has given final approval of the version to be published.

References

1. Iranmanesh B, Khalili M, Zartab H, Amiri R, Aflatoonian M. Laser therapy in cutaneous and genital warts: A review article. *Dermatol Ther*. 2021;**34(1)**:e14671.
2. Işik S, Koca R, Sarici G, Altinyazar HC. A comparison of a 5% potassium hydroxide solution with a 5-fluorouracil and salicylic acid combination in the treatment of patients with anogenital warts: a randomized, open-label clinical trial. *Int J Dermatol*. 2014;**53(9)**:1145-50.
3. Parsad D, Pandhi R, Juneja A, Negi KS. Cimetidine and levamisole versus cimetidine alone for recalcitrant warts in children. *Pediatr Dermatol*. 2001;**18(4)**:349-52.

4. Attwa E, Elawady R, Salah E. 'Cryo-immuno-therapy' is superior to intralesional Candida antigen monotherapy in the treatment of multiple common warts. *J Dermatol Treat*. 2021;**32(8)**:1018-25.
5. Awad SM, Gomaa AS, Hassan HA, Tawfik YM. Efficacy of cryotherapy combined with intralesional purified protein derivative (PPD) versus intralesional PPD monotherapy in the treatment of multiple common warts. *J Cutan Med Surg*. 2023;**27(2)**:117-25.
6. Moubasher AA, Kolta M. Tuberculin purified protein derivative and cryotherapy in the treatment of genital warts: A randomized controlled trial. *Human Androl*. 2021;**11(11)**:1-6.
7. Ibrahim H, El Taieb M, Nada E, Kamal E, Hegazy E. Combined intralesional injection of tuberculin purified protein derivative plus cryotherapy versus each alone in the treatment of multiple common warts. *Dermatol Ther*. 2022;**35(4)**:e15350.
8. Khalili M, Mehrabadi R, Mohammadi S, Pardakhty A, Amiri R, Aflatonian M. The Efficacy of Immunocryosurgery with Combined 2% Niosomal Zinc Sulfate Suspension and Cryotherapy in the Treatment of Verruca vulgaris. *J Kerman Uni Med Sci*. 2022;**29(2)**:139-45.
9. Farajzadeh S, Pardakhti A, Mohammadi S, Fadaei F, Khalili M, Mohebbi A, Aflatonian M. A randomized clinical trial of using niosomal zinc sulfate plus cryotherapy in comparison with placebo along with cryotherapy in treatment of common wart. *J Kerman Uni Med Sci*. 2018;**25(1)**:1-8.
10. Mahmoudi H, Ghodsi SZ, Tavakolpour S, Daneshpazhooh M. Cryotherapy plus oral zinc sulfate versus cryotherapy plus placebo to treat common warts: A double blind, randomized, placebo-controlled trial. *Int J Women's Dermatol*. 2018;**4(2)**:87-90.
11. Akhavan S, Mohammadi SR, Modarres Gillani M, Mousavi AS, Shirazi M. Efficacy of combination therapy of oral zinc sulfate with imiquimod, podophyllin or cryotherapy in the treatment of vulvar warts. *J Obstet Gynaecol Res*. 2014;**40(10)**:2110-3.
12. Sherrard J, Riddell L. Comparison of the effectiveness of commonly used clinic-based treatments for external genital warts. Int J STD AIDS. 2007;**18(6)**:365-8.
13. Mahajan BB, Tilak Raj RB, Kumar R. A comparative evaluation of therapeutic efficacy and safety of the cryotherapy (liquid nitrogen) with topical 20% podophyllin vs. intralesional bleomycin with topical 5% placentrex gel in the treatment of condyloma acuminata. *Asian J Pharm Clin Res*. 2014;**7(1)**:36-42.
14. Gilson RJ, Ross J, Maw R, Rowen D, Sonnex C, Lacey CJ. A multicentre, randomised, double-blind, placebo controlled study of cryotherapy versus cryotherapy and podophyllotoxin cream as treatment for external anogenital warts. *Sex Transm Infect*. 2009;**85(7)**:514-9.
15. Reyna-Rodríguez IL, Chavez-Alvarez S, Garza-Rodríguez V, Franco-Marquez R, Gonzalez-Martinez G, Ocampo-Candiani J, Villarreal-Martinez A. Cryotherapy plus low-dose oral isotretinoin vs cryotherapy only for the treatment of anogenital warts: a randomized clinical trial. *Arch Dermatol Res*. 2021:1-3.
16. Luk NM, Tang WY, Tang NL, Chan SW, Wong JK, Hon KL, Lo KK. Topical 5-fluorouracil has no additional benefit in treating common warts with cryotherapy: a single-centre, double-blind, randomized, placebo-controlled trial. *Clin Exp Dermatol*. 2006;**31(3)**:394-7.
17. Meymandi SS, Vaseli MB, Aflatoonian M, Abroud F. Efficacy of cryotherapy combined with topical cantharidin application versus cryotherapy and placebo in the treatment of verruca vulgaris: A randomized, controlled clinical trial. *J Pak Assoc Dermatol*. 2017;**27(1)**:42-7.
18. Shi L, Luo M, Zhang F, Zhang L, Wang B, Liu P, Zhang Y, Zhang H, Yang D, Zhang G, Zhou F. Photothermal therapy enhanced the effectiveness of imiquimod against refractory cutaneous warts through boosting immune responses. *J Biophotonics*. 2019;**12(2)**:e201800149.
19. Akarsu SE, Ilknur TU, Demirtaşoğlu M, Özkan Ş. Verruca vulgaris: pulsed dye laser therapy compared with salicylic acid+ pulsed dye laser therapy. *J Eur Acad Dermatol Venereol*. 2006;**20(8)**:936-40.
20. Khattab FM, Khashaba SA. Evaluation of combined treatment with long-pulsed neodymium-doped yttrium aluminum garnet laser and potassium hydroxide for the treatment of recalcitrant wart: a prospective comparative study. *J Dermatol Treat*. 2020;**31(1)**:56-60.

21. Jiryis B, Avitan-Hersh E, Mirmovich O, Nevet MJ, Khamaysi Z. Evaluation of combined treatment with Er: YAG laser and long-pulsed Nd: YAG laser for the treatment of recalcitrant warts: a prospective randomized controlled trial. *J Eur Acad Dermatol Venereol*. 2023;**37(12)**:2569-74.
22. Choi GS, Park JH, Kim YK, Choi GS. Combination therapy with Intralesional interferon α-2b and pulsed dye laser for the treatment of periungual warts. *Ann Dermatol*. 2002;**14(2)**:82-7.
23. Xu H, Shen Q, Fan B, Xi C, Wang L. Effectiveness of a combination therapy of traditional Chinese medicine and CO2 laser treatment for condyloma acuminatum. *J Med Virol*. 2020;**92(12)**:3889-94.
24. Ghonemy S, Ibrahim Ali M, Ebrahim HM. The efficacy of microneedling alone vs its combination with 5-fluorouracil solution vs 5-fluorouracil intralesional injection in the treatment of plantar warts. *Dermatol Therapy*. 2020;**33(6)**:e14179.
25. Di Chiacchio NG, Di Chiacchio N, Criado PR, Brunner CH, Suaréz MV, Belda Junior W. Ungual warts: comparison of treatment with intralesional bleomycin and electroporation in terms of efficacy and safety. *J Eur Acad Dermatol Venereol*. 2019;**33(12)**:2349-54.
26. Horn TD, Johnson SM, Helm RM, Roberson PK. Intralesional immunotherapy of warts with mumps, Candida, and Trichophyton skin test antigens: a single-blinded, randomized, and controlled trial. *Arch Dermatol*. 2005;**141(5)**:589-94.
27. Nofal AA, Elkholy BM, Abd-Elmonsef ER, Nofal HO. Triple Intralesional antigen immunotherapy versus Monoantigen in the treatment of multiple recalcitrant warts. *Dermatol Ther*. 2022;**12(5)**:1225-37.
28. Fawzy M, Nofal E, Abdelkhalek N, Ehab R. Intralesional bivalent and quadrivalent human papillomavirus vaccines didn't significantly enhance the response of multiple anogenital warts when co-administered with intralesional Candida antigen immunotherapy. A randomized controlled trial. *Arch Dermatol Res*. 2023;**315(10)**:2813-23.
29. Stefanaki C, Lagogiani I, Kouris A, Kontochristopoulos G, Antoniou C, Katsarou A. Cryotherapy versus imiquimod 5% cream combined with a keratolytic lotion in cutaneous warts in children: A randomized study. *J Dermatol Treat*. 2016;**27(1)**:80-2.
30. Fathy G, Abo-Elmagd WM, Afify AA. Intralesional combined digoxin and furosemide in plantar warts: Does it work? J Cosmet Dermatol. 2021;**20(8)**:2606-11.
31. Lofty AR, Elbakry AM, Omar GA, Hamdino M. Intralesional combined furosemide and digoxin in cutaneous warts treatment: A randomized controlled clinical trial. Dermatol Ther. 2022;**35(12)**:e15935.
32. Rijsbergen M, Niemeyer-van der Kolk T, Hogendoorn G, Kouwenhoven S, Lemoine C, Klaassen ES, de Koning M, Beck S, Bouwes Bavinck JN, Feiss G, Burggraaf J. A randomized controlled proof-of-concept trial of digoxin and furosemide in adults with cutaneous warts. *Br J Dermatol*. 2019;**180(5)**:1058-68.
33. Soenjoyo KR, Chua BW, Wee LW, Koh MJ, Ang SB. Treatment of cutaneous viral warts in children: A review. *Dermatol Ther*. 2020;**33(6)**:e14034.
34. Gheisari M, Iranmanesh B, Nobari NN, Amani M. Comparison of long-pulsed Nd: YAG laser with cryotherapy in treatment of acral warts. *Lasers Med Sci*. 2019;**34**:397-403.