

Evolving role of immunotherapy in the treatment of refractory warts

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ABSTRACT

Cutaneous and genital warts are common dermatological conditions caused by the human papilloma virus (HPV). Although it is a benign condition, it causes disfigurement, has a tendency to koebnerize, and can be transmitted to others. This makes adequate and timely treatment important. There are several conventional treatments available with variable response. Topical and systemic immunotherapy has now found a significant place in the treatment of warts because of its nondestructive action, ease of use, and promising results. Through this review, we would like to present a brief overview of the various immunotherapeutic agents used. These include more established agents such as imiquimod, *Mycobacterium w* vaccine, bacillus Calmette-Guérin vaccine, measles, mumps, and rubella vaccine, Candida antigen, trichophyton antigen, tuberculin, zinc, cimetidine, levamisole, HPV vaccine, and autoimplantation therapy. Other agents such as contact immunotherapy which is sparsely used now than before and newer agents such as *Corynebacterium parvum*, sinecatechins, echinacea, propolis, glycyrrizinic acid, and Vitamin D have also been discussed. The mechanism of action of these agents, along with their dosage, mode of administration, duration of use, expected outcomes and comparative efficacy, evidence for their use, and expected side effects, if any, are reviewed.

Keywords: Cutaneous, genital, immunotherapy, verruca, wart

INTRODUCTION

Immunotherapy is defined as a type of biological therapy that uses substances to stimulate or suppress the immune system to help the body fight cancer, infection, and other diseases. Some types of immunotherapy only target certain cells of the immune system. Others affect the immune system in general. Types of immunotherapy include cytokines, vaccines, and some monoclonal antibodies. It may either be an activation immunotherapy, where immunity is induced or enhanced (used in infections, cancers), or a suppression immunotherapy, where immunity is suppressed (used in autoimmune diseases). The use of immunotherapy is well-established in malignancies and has now found a role in infections as well.

Human papilloma virus (HPV) is a small DNA virus, which is implicated in benign and malignant neoplasia.^[1] Cutaneous and genital warts constitute the benign end of the spectrum, and their management still continues to annoy dermatologists. Although

the spontaneous resolution rate for warts is 65–78%, the cosmetic disfigurement, tendency to spread, and associated poor quality of life warrants quick intervention.^[2] The conventional modalities in treatment of warts include destructive therapies such as salicylic acid, trichloroacetic acid, cryotherapy, silver nitrate, phenol, cantharidin, surgical interventions and lasers; antiproliferative agents such as bleomycin, vitamin D analogs, podophyllin, podophyllotoxin, and 5-fluoro uracil; antiviral agents such as cidofovir and retinoids.^[3] Because of the cumbersome nature of these procedures and a high risk of recurrence, immunotherapy is becoming more and more popular, especially in the treatment of refractory cutaneous and

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Cite this article as: Thappa DM, Chiramel MJ. Evolving role of immunotherapy in the treatment of refractory warts. Indian Dermatol Online J 2016;7:364-70.

Access this article online

Website: www.idoj.in

DOI: 10.4103/2229-5178.190487

Quick Response Code:



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genital warts. These include various topical, intralesional, and systemic agents.

When to consider immunotherapy

There are no well-defined criteria or consensus on when immunotherapy should be tried in a patient with warts. Current indications include

1. Recalcitrant warts
2. Recurrent warts
3. Extensive warts
4. Difficult to treat areas—periungual and palmoplantar sites

The various agents used with the indications, dosage and route of administration are mentioned in Table 1. Individual agents are discussed below.

Imiquimod

Imiquimod is a non-nucleoside heterocyclic amine which acts as an immune response modifier. It increases the cellular levels of interferon alpha (IFN- α), tumour necrosis factor alpha (TNF- α), and interleukin-6 (IL-6), which leads to strong antiviral and antitumor effects.^[4] Arany *et al.* showed that high constitutive levels of signal transducer and activator of transcription 1 (STAT1) and interferon response factor 1 (IRF1) but low levels of IRF2 and protein inhibitor of activated STAT1 (PIAS1) are essential for a complete response to

imiquimod therapy.^[5] In a multicentric, double-blind, randomized controlled trial by Beutner *et al.*, 5% imiquimod cream was more effective than 1% in treatment of genital warts when applied at home by the patient as a thrice-a-week application for 16 weeks.^[4,6] A systematic review by Moore *et al.* in 2001 concluded that imiquimod is an effective home-based treatment modality for genital warts. There was complete resolution of warts in up to 76% of the patients.^[7] The efficacy of imiquimod has been studied in cutaneous warts as well, with 27–89% in immunocompetent and 33–50% in immunocompromised showing complete response.^[8] The commonly reported side effects include burning sensation, pain, erythema, and vitiligo-like depigmentation. It is found to be effective and safe in children and there are reports of safe use in pregnancy.^[9,10]

The high cost of medication and difficulty in obtaining the medication in India poses problems in the widespread use of imiquimod. There were also some concerns raised in a Cochrane review in 2014, which showed a high number of industry sponsored studies regarding the efficacy of imiquimod.^[11]

Mycobacterium w

Mycobacterium indicus pranii or *Mycobacterium w* is a rapid growing nontubercular mycobacteria, which has been found to induce a strong proinflammatory response while injected

Table 1: Various agents used in immunotherapy of warts

Agents	Indication, dosage and administration
Topical agents	
Imiquimod	For genital and cutaneous warts, 5% cream, 3 times a week, for 16 week
Sinecatechins	For cutaneous warts, 10% ointment 3 times a day for maximum 16 weeks
BCG	For cutaneous and genital warts, applied topically on the warts in normal saline or salicylic acid, washed after 2 hours, weekly treatment for 6 to 12 weeks
Intralesional (IL) agents	
Mw vaccine	For cutaneous warts, 0.1 ml intradermal into 3-5 warts or all warts, followed by 0.1 ml intralesional, 2-4 weekly, maximum 10 sittings
BCG vaccine	For cutaneous and genital warts, 0.1-0.5 ml intralesional injection in largest wart, in 2 weeks interval in 5 sittings.
PPD	For genital warts, 0.1 ml weekly intradermal injection in the forearm for 12 weeks
MMR vaccine	For cutaneous warts, 0.3-0.5 ml into single largest wart fortnightly for up to 5 sittings
Candidial extract	For cutaneous warts, 0.1-0.3 ml injected into the largest wart at first sitting, then 3 weekly intralesional injections
Trichophyton antigen	For cutaneous and genital warts, 0.3 ml injected into largest wart every 3 weeks, maximum of 5 sittings
Tuberculin	For cutaneous warts, 2.5 units into few warts every 2 weekly
Vitamin D3	For cutaneous warts, 0.2 ml of 7.5 mg/ml, Vitamin D intralesional, 2 sittings 4 week apart
Interferon alpha 2B	For genital warts, 1-2 million units 3 days/week (Monday-Wednesday-Friday) for 3 weeks
Systemic	
Zinc	For cutaneous warts, 10mg/kg/day (2.5 mg/kg/day elemental zinc) for 2 months
Cimetidine	For cutaneous warts, 20-40 mg/kg/day for 3-4 months
Levamisole	For cutaneous warts, 2.5-5 mg/kg/day, 2-3 consecutive days every 2 weeks for 4-5 months.
Echinacea	For cutaneous warts, 600 mg single oral dose (single study)
Propolis	For cutaneous warts, 500 mg single oral dose (single study)
HPV vaccines	For cutaneous warts, 0.5 ml intramuscularly, at 0, 2 and 6 months (2 dose or 3 dose regimen) may be followed

intralesionally. There is a prominent delayed hypersensitivity response with an increase in T helper 1 cytokines such as IL2, IL4, IL6, and IFN gamma and activation of natural killer cells and cytotoxic T cells. The HPV laden cells are caught in the crossfire leading to clearance of warts both at the site of injection and distally.^[12] It is administered in two ways—either with an intradermal sensitizing dose or without it. In the first method, a sensitizing dose of 0.1 ml is administered intradermally in the deltoid region followed by 2–4 weekly intralesional injections in few warts (maximum 0.1 ml in each sitting) for up to 10 sittings. In the latter, the sensitization dose is missed and direct intralesional injections are started.^[2,12] The response varied by 54–93% in cutaneous warts and 89% in genital warts.^[2,12-14] In an open label comparative study involving 66 patients with extragenital warts by Dhakar *et al.*, complete clearance of treated warts was seen in 66.7% (20/30) and 65.5% (19/29) of the patients in the Mycobacterium w (Mw) and cryotherapy groups, respectively. Clearance of distant warts was significantly high ($P = 0.004$) high in the Mw group. Improvement in the Dermatology Life Quality Index was greater in the Mw group.^[15] In a double-blind randomized control trial by Kumar *et al.* involving 89 patients of genital warts comparing Mw vaccine with 5% imiquimod cream, 67% of patients in the Mw group and 59% in the imiquimod group showed complete resolution. ($P = 0.52$, nonsignificant difference).^[16] In another open label study in multiple extragenital warts by Meena *et al.* in 40 patients, 83% showed complete resolution.^[14] The reported side effects include pain, nodularity, ulceration, scarring at the site of injection, flu-like symptoms, fever, and lymphadenopathy.^[14] Paraesthesia on the limb distal to the site of injection have also been reported.^[17]

Bacillus Calmette-Guérin vaccine

The principle behind using Bacillus Calmette-Guérin (BCG) vaccine is the same as that of the Mw vaccine. The delayed hypersensitivity response against the antigen is the key to clinical response against warts. It increases the serum levels of IL-12 and decreases the level of IL-4.^[18] One to three doses are administered 1 month apart. In a single-blind placebo-controlled trial by Sharique *et al.* involving 154 patients of cutaneous warts (common, plantar and plane warts), there was 39.7% resolution with BCG vs. 13.7% with placebo.^[19] In another placebo controlled trial ($n = 80$), topically applied BCG paste (weekly for 6 weeks) has also been found to be effective in children with common warts and plane warts with 65% and 45% resolution, respectively.^[20] No side effects were reported in these two studies. However, another report by Daulatabad *et al.* in 7 patients from India showed a high incidence of flu-like symptoms precluding further doses in 57% patients, making one question its safety in tuberculosis endemic countries like India.^[21]

Measles, mumps, rubella vaccine

Measles, mumps, rubella (MMR) viral vaccine accelerates the clearance of virus and viral infected cells by stimulation of

cell-mediated and humoral immunity.^[22] It has been used in a dose of 0.5 ml injected into each cutaneous wart once in two weeks for up to 5 sittings to produce 63% complete resolution by Nofal *et al.* Pain, itching, erythema, and flu-like symptoms were the side effects noted.^[23] Another study by Na *et al.* involving 136 patients of cutaneous warts showed more than half reduction in the size of wart in 51% patients whereas only 5.6% had complete resolution.^[24] Pain at the site of injection was the only adverse effect noted. Eighty seven percent complete resolution was noted in 40 patients with multiple plantar warts within 3 sittings by Gamil *et al.*^[25] In a study by Shaheen *et al.*, comparing MMR vaccine with intralesional purified protein derivative and saline in 10 patients each, the rate of lesional and distal resolution were 60% each with purified protein derivative (PPD) and 80% and 40% with MMR and 0% with saline.^[26]

Candida antigen

Candida albicans extract is injected intralesionally to provoke a cellular response and clearance of warts. 0.3 ml candida extract is injected into the largest wart at first visit and consequently every 3 weeks. Distal clearance was seen in 82% of patients in a phase 1 trial in 18 patients suffering from cutaneous warts by Kim *et al.* Immune response to HPV-57 L1-peptide (380-412) was most commonly seen.^[27] In 220 children treated by Munoz Garza *et al.*, a 71% complete resolution of the injected wart was noticed with an average of 2.7 sittings of intralesional candida. Distant response was seen in 21% patients with only half showing a complete response.^[28] Other studies showed a 39–87% complete response with or without adjuvant treatment.^[29-31] Three out of 7 human immunodeficiency virus (HIV) positive patients showed clearance of warts when treated with this modality.^[32] The most common side effect was pain and discomfort during injection, however, serious side effects such as vitiligo-like depigmentation and painful purple digit have also been reported.^[33] Another rare side effect described by Signore *et al.* is post-immunotherapy revealed cicatrix (PIRC). This refers to the scar of previous destructive procedures tried on a wart that becomes visible once immunotherapy starts working and the wart starts healing. The scar should not be wrongly attributed to the immunotherapy *per se*.

Trichophyton skin antigen

Trichophyton antigen has been prepared as an allergic extract from *Trichophyton* species by adding extracting solution. Horn *et al.* in a randomized controlled single blind study found that 62% patients with cutaneous warts responded to intralesional trichophyton injections (0.3 ml every 3 weeks, maximum of 5 sittings) and the response was not statistically different from the response to mumps antigen and candida antigen, with or without IFN α added to it.^[34] There is a dearth of studies with trichophyton as a single agent. It has been useful in combination with candida and mumps antigen, and the combination of all

three antigens has been reported to have a 71% response rate which was significantly better than individual agents.^[35,36]

Tuberculin

PPD or tuberculin stimulates the cell-mediated immunity nonspecifically by activating Th1 cells, NK cells, and cytokine production. An increase in IL-12 as a process in boosting the cell-mediated immunity contributes to the mechanism of action.^[37] It has been injected intralesionally into difficult to treat cutaneous warts (palmoplantar warts, periungual warts, facial warts (>10 lesions), verruca vulgaris (>10 lesions) and verruca plana (>10 lesions) at a dose of 2.5 units into few warts every 2 weeks by Saoji *et al.* to produce 76% complete resolution at the end of 4 injections. Over a 6-month follow-up, only one recurrence was noticed. Side effects were mild and included erythema, edema, and pain.^[38]

Interferons

Interferons are natural small proteins that play an *in-vivo* role in viral interference. Therapeutically, IFN α -2B has been used for its immunomodulatory, antiviral, and antiproliferative properties. There are conflicting reports on its efficacy in warts. In a systematic review, 12 randomized controlled trials with 1445 patients showed that there was a significant difference in response rate of genital warts between treatment with topical interferons and placebo (44.4% vs 16.1%). However, there was no significant difference in response between systemic interferons and placebo (27.4% vs 26.4%). The rate of relapse was also less with topical interferons.^[39] Flu-like symptoms have been reported as a possible side effect. Horn *et al.* conducted a single-blind, randomized controlled trial in 233 patients with one or more cutaneous warts and found that there was no added benefit of combining IFN α 2a or IFN α 2b to candid antigen, trichophyton antigen, or mumps antigen.^[34]

Zinc

Zinc is important for immune regulation and stimulates the leucocytes and natural killer cells. Both oral and topical zinc has been found to be useful in the treatment of cutaneous and genital warts. It has been shown that there is a deficiency of zinc in patients with multiple or recurrent warts.^[40,41] Oral zinc sulphate given in a dose of 10 mg/kg/day has been used, with approximately 84–87% patients showing complete resolution of warts in 2 months in two randomized placebo-controlled trials.^[42,43] It has been used as single agent as well as in combination with other modalities such as imiquimod, podophyllin, and cryotherapy.^[44] It has been found to be more effective than cimetidine.^[45] Topical 5% and 10% zinc solution has been used in cutaneous warts, 3 times a day for 4 weeks with only 5% and 11% response, respectively.^[46] Topical zinc oxide 20% ointment showed almost similar efficacy as salicylic acid–lactic acid combination.^[47]

H2 receptor blockers

H2 blockers, such as cimetidine and ranitidine, have been tried in treatment of warts. They block the type 2 histamine receptors on suppressor T cells and augment cell-mediated immunity. It increases mitogen-induced lymphocyte proliferation and inhibits suppressor T cells.^[48] It increases the levels of IFN γ and IL-2 and decreases the levels of IL-18.^[49] It has been used in a dose of 20–40 mg/kg/day for 3–4 months with response ranging from 30–87%.^[50,51] Side effects were mild and included nausea, vomiting, and headache. There are only preliminary studies using ranitidine with doubtful efficacy.^[52] Other double blind studies, however, show conflicting reports of minimal-to-no response especially to low dose cimetidine.^[53,54] Safety and a slightly better efficacy in children than adults is reported.^[55] It was also shown to be more effective when used in combination with levamisole.^[56] A systematic review in 2007 concluded that there is insufficient evidence for efficacy of cimetidine and ranitidine in viral warts.^[57]

Levamisole

Levamisole was introduced as an antihelminthic agent but was soon found to have immunomodulatory effects, making it effective in various dermatological disorders. It has been used in treatment of cutaneous warts at a dose of 2.5–5 mg/kg/day for 3 consecutive days every 2 weeks for 4–5 months.^[58–60] The response to levamisole was approximately 60%. However, in a double blind study by Schou *et al.*, no difference was seen between levamisole 150mg/day for 3 days every alternate week and placebo.^[61] Levamisole can cause rash, nausea, abdominal cramps, taste alteration, alopecia, arthralgia, and a flu-like syndrome but rarely causes myopathy, leucocytoclastic vasculitis, lichenoid eruptions, and leucoencephalopathy.^[56,62]

Human papilloma virus vaccines

The quadrivalent HPV vaccine that comprises the L1 protein of HPV types 6, 11, 16, and 18 has been in use on a large scale in countries like Denmark with a decline in the prevalence of genital warts. The risk of warts decreased with each dose of HPV vaccine. Patients who received 3 doses had complete protection from genital warts whereas, in those receiving 2 doses, increase in interval between the 2 doses led to a further decline in genital wart incidence.^[63] Similar decline in genital warts has been noticed in the UK and Australia.^[64,65]

There are also reports of HPV vaccine being used in patients with cutaneous warts which has led to resolution of warts, both in immunocompetent and immunocompromised individuals.^[66,67] Now, with the development of nonavalent HPV vaccine against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58, a further decline in HPV-related diseases including warts is expected.^[68]

Autoimplantation therapy

The concept of autoimplantation is that the cell-mediated immunity that is oblivious to the HPV infection is stimulated by

introducing a higher load of the same antigens at a location where a strong immune system activation can occur. Full thickness excision of one wart is made, and after mincing it, the particles are introduced into a dermal pocket. Flexor aspect of the left forearm approximately 2 inches below the antecubital fossa is the usual site of autoimplantation. In a study by Shiva Kumar *et al.* involving 60 patients, 73.3% total clearance of warts was noticed, with a majority of them (91%) within 2 months.^[69] In a modification of this procedure in palmoplantar warts, the pared wart tissue was introduced into the subcutis using a single stab incision and 74.1% resolution was noticed.^[70] Post-inflammatory hypopigmentation and formation of an inflammatory nodule at the site of implantation are the potential side effects.

Others

Various other agents have been tried in immunotherapy of warts. This includes *Corynebacterium parvum*, contact immunotherapy, glycyrrhizic acid, Echinacea, green tea extracts, and intralesional vitamin D with variable results. Among these, contact immunotherapy with dinitrochlorobenzene (DNCB), diphencyprone, and squaric acid dibutyl ester (SADBE) has been used the longest with moderate response.^[71] *C. parvum* has a role in macrophage activation.^[72] In a study involving 10 patients with cutaneous warts (common warts) that compared 0.1 ml immune stimulant *Corynebacterium parvum* vs saline administered intradermally every month for 3–5 months, 9 patients showed complete resolution.^[73] Herbal preparations such as Echinacea and propolis are reported to boost the immunity when administered orally.^[74] Sin catechins are derived from green tea extract (*Camellia sinensis*) and is marketed as a 10% ointment. It contains around 8 catechins, the most abundant and potent of which is epigallocatechin-3-gallate. It works as an antiviral (by inhibiting transcription of activator protein 1 (AP-1), proapoptotic (by upregulation of proapoptotic and downregulation of antiapoptotic genes), and anti-inflammatory (induces IL-12 and decreases IL-10) action. A clearance rate of 46–52% has been noticed in various studies.^[75] Glycyrrhizic acid, obtained from the root of *Glycyrrhiza glabra* has antiviral, anti-inflammatory, and antiulcerative properties. It causes inactivation of extracellular free virus particles, prevention of the intracellular decapsulation of infectious particles, and the deterioration of the assembling capacity of the structural components of the virus. When used with an immunostimulant, it was shown to have a slightly better efficacy than podophyllin (87%).^[76]

In a study involving 20 patients with plantar warts, intralesional injection of 0.2 ml of 7.5 mg/kg Vitamin D3 for a maximum of 2 sittings 4 weeks apart led to 80% complete resolution.^[77]

CONCLUSION

There are many agents used for immunotherapy which show significant results in terms of safety and efficacy. There is always

an issue that the tendency for spontaneous resolution in warts may cause a false increase in treatment response that can be attributed to each agent. When to opt for immunotherapy and which agent to use, still remains unanswered. This issue needs to be addressed on a patient-to-patient basis after considering factors such as disease burden, availability of medication, cost of therapy, potential side effects, and immune status of the patient. Combination of immunotherapy with other destructive modalities such as cryotherapy and radiofrequency ablation or concomitant use of multiple modalities of immunotherapy has shown to enhance the treatment response, however, further studies are needed in this area.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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