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Role of intralesional antigen immunotherapy in the treatment of warts

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Abstract

Background:Warts are the most common clinical manifestation of human papillomavirus (HPV) infection in the skin and mucous membranes, mostly found on the hands, feet, face, and genitalia. These benign lesions have different clinical forms. There are over 200 types of this virus and some of them have contributed in the pathophysiology of wart. Each of its types is different in at least 10% of the sequences encoded by major capsid gene (L1). Although these viruses have a tendency to infect some specific body parts, this disease may be manifested in approximately all regions of the skin and mucosa. In some previous studies, it has been shown that mumps-measles-rubella (MMR) vaccine results in regression of warts via immunomodulation and induction of delayed (cellular) hypersensitivity reactions at the wart tissue. This method can be used in larger populations because of vaccine availability and safety. The varicella zoster (VZV) vaccine, which contains live attenuated virus derived from the OKA strain.

Keywords:Warts, Viral Vaccines, Human papillomavirus (HPV).

Human Papilloma Virus:

The last 30 years have witnessed the discovery of many different types of human papillomaviruses (HPVs) as well as the demonstration of their role in human cancer and their significance as targets for diagnosis and therapy **(1).**

Like many viruses, HPV is a unique organism. It is extremely difficult to grow in vitro; but once an individual becomes infected with HPV, it can be difficult or even impossible to eradicate. HPV is associated with mild to moderate disease that even in the absence of therapy may spontaneously regress. On the other hand, some HPV infections progress to cancer, which can be fatal if treatment is delayed **(2).**

HPVs infect stratified epithelium and establish infections that can persist for decades. For these infections to persist, papillomaviruses have evolved strategies to evade the effects of the innate immune system during the initial phases of infection as well as long-term surveillance by the adaptive immune system **(3).**

Type of HPV

At least 15 HPV types associated with malignancy of both genital tract and non-genital tract have been categorized as High Risk (HR) types (HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 59, 66, 68, 69, 73 and 82) whereas those associated with benign lesions such as genital and skin warts, are categorized as Low Risk (LR) types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108). More

than 100 different HPV types have been described till date (table 1) **(4).**
The majority of high-risk HPV types are associated with the development of cancer of uterine cervix, vagina, vulva, adenocarcinoma in women and anus, oro-pharynx and esophagus in both men and women. HPV infection is also reported in skin cancer, lung cancer and in retinoblastoma

Table (1): Clinical manifestations and associated HPV types. (5).

Skin lesions	**Often detectable**	**Rarely detectable**
Verrucae vulgares, verrucae palmares et plantares	1, 2, 4	26, 27, 29, 41, 57, 60, 63, 65
Verrucae planae	3, 1	28, 29
Butcher's wart	7	1, 2, 3, 4, 10, 2
Squamous cell carcinoma of the finger, Bowen's diseas	16	31, 33–35, 5
Epidermodysplasia verruciformis (EV	3, 5, **8**	9, 12, 14, 15, 17, 19–25, 36–38, 46, 47, 49, 50, etc
EV-squamous cell carcinoma	5, **8**	14, 17, 20, 4
Mucosal lesions		
Condylomata acuminate		
High-grade squamous intraepithelial neoplasias and invasive carcinomas of the anogenital trac	**16**	18, 26*, 31, 33, 35, 39, 45, 51, 52, 53*, 56, 58, 59, 62, 66*, 68, 73, 82
Bowenoid papulosis, erythroplasia of Queyra	**16**	
Buschke-Löwenstein tumo	**6,11**	
Laryngeal papillomatosi	**6,11**	
Heck's diseas	**13,32**	

Prevalence of HPV infection
Generally, papilloma virus-associated infections are very frequent and occur not only in patients with ages ranging from 20 to 40 years, but also in children and teenagers **(6).**

The World Health Organization (WHO) estimated an annual increase of 3 hundred million in the number of HPV carriers in the world. Overall HPV prevalence in 157,879 women with normal cervical cytology was estimated to be 10.4 percent **(7).**
An International Agency for Research in Cancer (IARC)- initiated study (Globocan 2002) to determine the global cancer incidence demonstrated that about 5.17 per cent of all cancers can be attributed to HPV **(7).**

Risk factors for HPV infection
Infection with high-risk HPV combined with other risk factors such as immunosuppression,

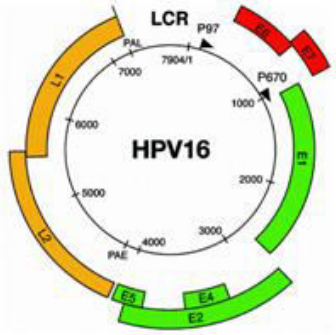
cigarette smoking and coinfection with human immunodeficiency virus can lead to the development of cervical cancer. Also, high sexual activity has been reported to increase the risk of HPV infection in some women **(8).**

Authors looking at changes in eating habits indicated that regular intake of papaya, a fruit rich in carotenoids (lutein, zeaxanthin, and β-cryptoxanthin) and citric fruits (vitamin C) leads to a reduction in the persistence of infection by HPV. Evidence also indicate that increased levels of folic acid reduce the risk of infection for high-risk HPV strains **(8).**

Virus structure and genome

This virus has a double DNA strand divided into early (E) and late (L) proteins, and regulating regions. The early region (E1-E8) codes genes for viral replication and host cell transformation. Proteins E6 and E7 are coded for oncogenic HPV types and have been shown to have a role in negatively affecting the cell cycle by binding and deactivating tumor suppressing genes p53 and pRB. The late regions code capsidproteins L1 and L2. L1 is the largest capsid protein and it is highly preserved among the HPV types. The long control region (LCR) is a regulating region that contains the origin of viral replication that occurs exclusively in the nucleus of the host cell **(9).** Depending on the composition of these segments, various types of HPV are identified with different pathogenic characteristics **(10).**

Papillomavirus Genes and Gene Functions are shown in table 1



PROTEIN	FUNCTIONS
E6	Destruction of p53 tumor supressor protein (Accumulation of mutation and Apoptosis inhibition)
E7	Inactivation of pRb tumor supressor protein (Cell cycle progression and accumulation of p16INK4a)
E1	Viral DNA replication
E2	Viral DNA replication; repression of E6/E7 genes
E4	Assembly and release of the viral particle
E5	Interaction with the Epidermal Growth factor (EGF)
L1	Major capsid protein in the viral particle
L2	Minor capsid protein in the viral particle

Figure (1): Genes Functions **(11).**

Immunology of HPV and virus life cycle

The papillomaviruses are very successful infectious agents. They induce chronic infections that have no apparent systemic sequelae and rarely kill the host, but periodically shed large amounts of infectious virus for transmission to naïve individuals. To achieve this successful lifestyle, HPVs must either avoid or negotiate with the powerful immune defense systems of the host. Host defence is a partnership between innate immunity (phagocytes, soluble proteins e.g., cytokines, complement and epithelial barriers) together with adaptive immunity (antibody, cytotoxic effector cells). In simple terms, the innate immune system detects the pathogen and acts as first line defence clearing up to 90 per cent of microbial assaults alone **(12).**

Both the antigen-specific and innate arms of the immune system are involved in the control of virus infections. Indeed, natural killer cell (NK) activity is associated with the regression of HPV-induced cervical lesions **(13)**.

However, it is the antigen-specific immunity that can specifically target responses and

immunological memory. Mechanisms used to eliminate virus infections include viral neutralization where antibodies specific for virus surface antigen contain the spread of acute infection and protect against re-infection. Serum IgG and secretory IgA present in mucus secretions may play an important part in host defense by blocking viral attachment to mucosal epithelial cells. Indeed, secretory IgA has been considered to be the first line of defense at the mucosa **(14).**

Circulating antibody or complement may also agglutinate and opsonize virus particles facilitating Fc or C3b receptor-mediated phagocytosis. However, once an infection has occurred, antibodies are not usually able to eliminate a virus, particularly if the virus is capable of entering a latent state in which its DNA is integrated into host chromosomal DNA. Cluster of differentiation antigen 8 (CD8+) positive CTLs (Cutaneous T-lymphocyte) and CD4+ T-helper type 1 cells (TH1) are the main components of a CMI (Cell mediated immunity) response. Activated TH1 cells produce cytokines, including interferon-γ (IFN-γ) and interleukin-2 (IL-2). IFN-γ acts directly to eliminate virus by inducing an antiviral state in cells while IL-2 acts indirectly by assisting the activation of CTL precursors into an effector population. Both IFN-γ and IL- 2 activate NK cells which are important in the first few days of infection until a specific CTL response develops **(Fig. 1). (15).**

HPV infections resemble those of non-lytic viruses as they do not cause cell death but are released from infected cells by desquamation. The ideal defense against these infections would therefore be a combination of neutralizing antibodies and CTL-mediated cell lysis of infected cells and prevention of subsequent re-infection by released virions **(16).**

The target cell for CTL-mediated lysis would be the keratinocyte present in the intermediate layers of the squamous epithelium where viral transcription and replication take place and the early proteins (E1, E2, E4, E5, E6 and E7) are abundantly expressed. The late proteins L1 and L2 would be unsuitable CTL targets as they are expressed in the superficial layers where cells are already shedding. However, neutralizing antibodies would have to be directed at these proteins to prevent de novo infections **(17).**

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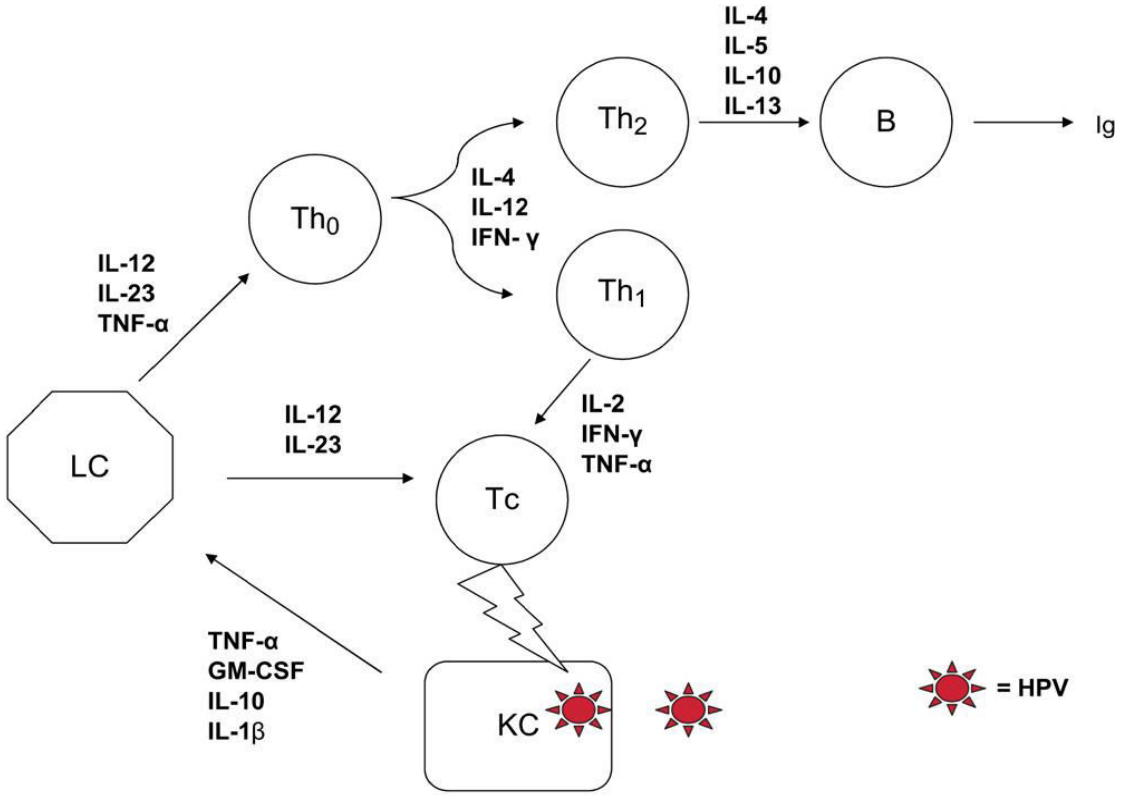

Figure 2: Host response to viral infection **(18).**

Human Papilloma Virus (HPV) Vaccines

HPV vaccines are vaccines that protect against infection with human papillomaviruses (HPV). HPV is a group of more than 200 related viruses, of which more than 40 are spread through direct sexual contact. Among these, two HPV types cause genital warts, and about a dozen HPV types can cause certain types of cancer—cervical, anal, oropharyngeal, penile, vulvar, and vaginal.

three vaccines that prevent infection with disease-causing HPV types are licensed for use in the United States: Gardasil®, Gardasil® 9, and Cervarix®. All three vaccines prevent infection with HPV types 16 and 18, two high-risk HPVs that cause about 70% of cervical cancers and an even higher percentage of some of the other HPV-caused cancers (1, 2). Gardasil also prevents infection with HPV types 6 and 11, which cause 90% of genital warts (3). Gardasil 9 prevents infection with the same four HPV types plus five additional cancer-causing types (31, 33, 45, 52, and 58) that together account for 10 to 20% of cervical cancers.

Gardasil 9 is now the only HPV vaccine available for use in the United States. Cervarix and Gardasil are still used in other countries.

Given the worldwide burden of HPV infection (anogenital warts and neoplasia of several sites), prevention of infection could provide relief from an important public health threat. With the introduction of cervical screening in developed countries, the number of deaths from cervical cancer has declined dramatically **(15).**

Warts

Warts are the cutaneous manifestations of HPV infection. Warts may exist in different forms according to the epithelial surface and HPV type responsible for the infection. Common warts (Verruca vulgaris), plantar warts (Verruca plantaris), flat or plane warts (Verruca plana) and genital warts (Condyloma acuminata) are some of the clinical manifestations of HPV infection **(19).**

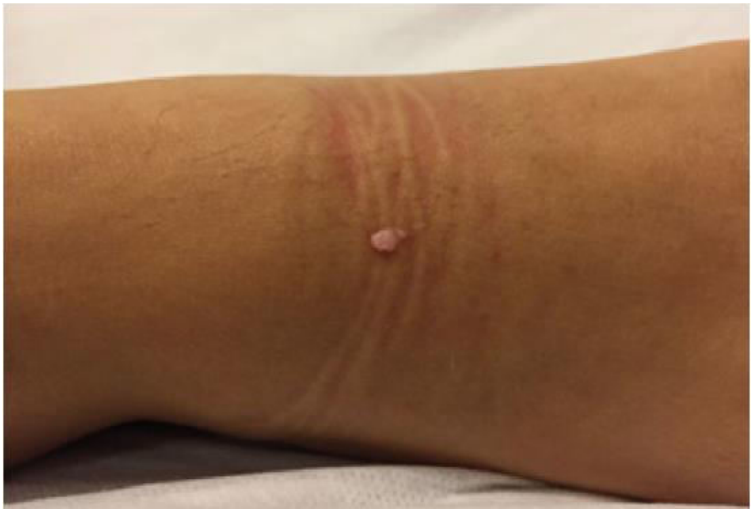

Figure 3: Common wart **(20).**

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. 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) **(21).**

Antigen

Topical Bacillus Calmette-Guerin (BCG)

BCG is one of the agents used for immunotherapy in warts. Its precise mechanism of action is not known, but it is speculated to upregulate the Th1 type of cytokine response IFN-α, IL-2, 12, 18, IFN-γ, and TNF-α as well as induce a nonspecific inflammatory response against the virus. Most of the researchers used intralesional injections of the BCG vaccine to treat recalcitrant warts, and a few of them used the intradermal route **(22).**

Intralesion antigen immunotherapy

The common incidence of warts in addition to their clinical significance highlights the need for immune protection against HPV infection. This is particularly true in view of the absence of specific antiviral therapy against HPV, the variable efficacy of the available therapeutic modalities (**22**).

Although the mechanism is not entirely understood, these injections are thought to work by inducing a systemic T-cell mediated response. Cytokines released from Th1 cells such as interleukin-2 and interferon-gamma are predominantly increased in response to injection. Injecting intralesionally might also play a role in concentrating the local immune response; however, some argue that the trauma of injection alone may be enough to induce a sufficient immune response in immunocompetent patients **(23)**.

Recently, immunotherapy with intralesional antigens (autogenous vaccine, Candida antigen, mumps antigen, Trichophyton skin test antigen, and tuberculin) or vaccines (BCG vaccine, measles, mumps, rubella virus (MMR) vaccine, Mycobacterium w vaccine) has been tried for the treatment of common warts with encouraging results. Intralesional immunotherapy perhaps employs the ability of the immune system to recognize viral antigens that induces a delayed-type

hypersensitivity reaction not only to the antigen but also against the HPV. Consequent to this, the stimulated immune response clears all lesions on other body sites with prominent decrease in the recurrence rates **(24).**

Mechanism of Action:

In a competent body immune system, Th1 cells (CD4+) secrete many different types of cytokines, the most important of which are IFN-γ, IL-2, and IL-12. IL-2 stimulates the maturation of the killer T cell and enhances the cytotoxicity of natural killer cells. The critical function of the killer T cell is cytotoxicity, which means recognizing and destroying cells infected with viruses. Some observations have demonstrated that a CD4-dominant immune reaction in an HPV-infected tissue is associated with a high chance of clearing the HPV infection **(25).**

In summary, the mode of action of intralesional immunotherapy is basically related to its ability to induce a strong cell-mediated immune reaction to alter the balance between Th1 and Th2 responses in favor of the former, leading finally to eradication HPV **(Figure 4).**

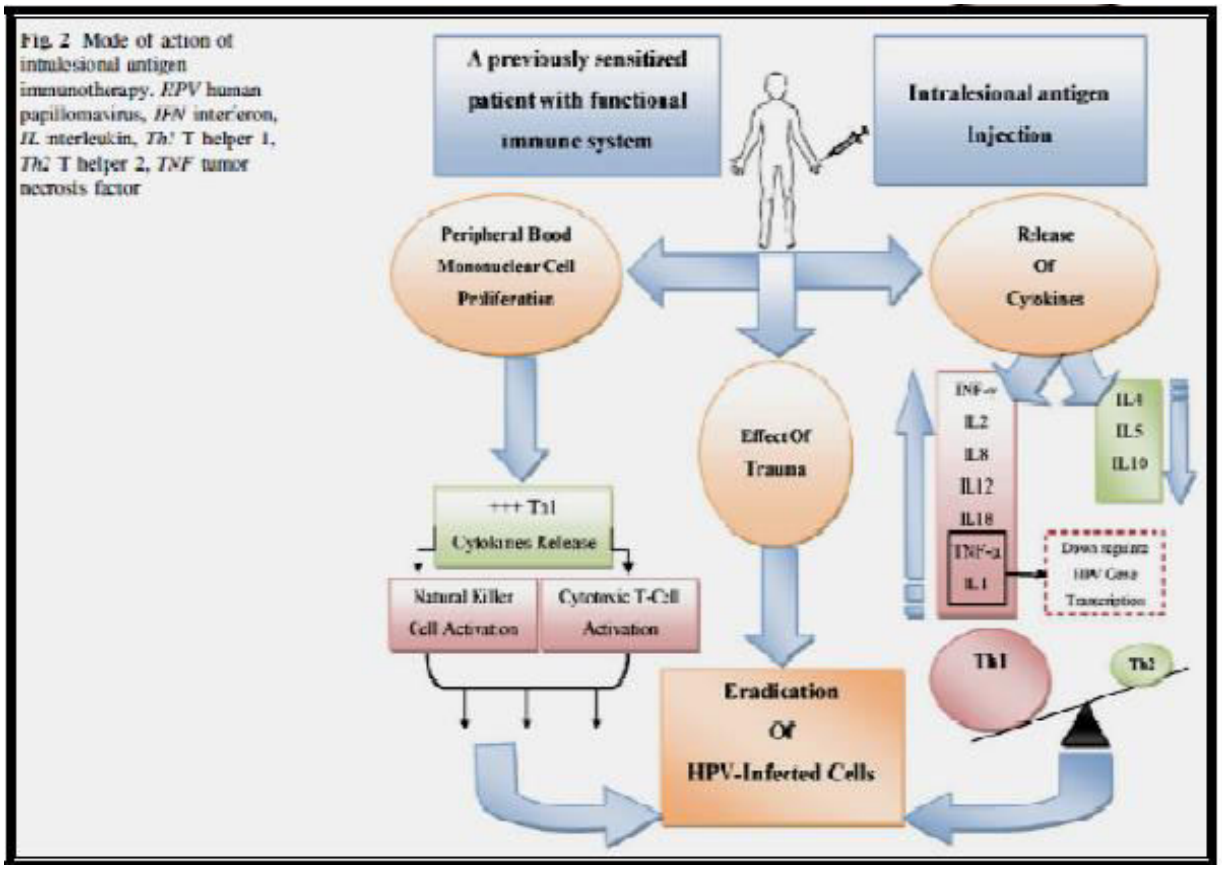

Fig. 2 Mode of action of intralesional antigen immunotherapy. *EPV* human papillomavirus, *IFN* interferon, *IL* interleukin, *Th1* T helper 1, *Th2* T helper 2, *TNF* tumor necrosis factor

Figure 4: Mode of action of intralesional antigen immunotherapy. HPV human papillomavirus, IFN interferon, IL interleukin, Th1 T helper 1, Th2 T helper 2, TNF tumor necrosis factor **(22).**

Success Rates:

Variable success rates have been demonstrated among the studies utilizing this recent modality . No definite explanation for the great variability in the response rates among these studies has been settled; however, factors related to study characteristics, antigen used, treated warts and patient immune response (**22).**

Procedure:

Two different approaches have been used by various studies. In most of the studies, a pre-sensitization test is used before the start of the trial, where 0.1 ml of the antigen to be used is

injected intradermally on the volar aspect of the forearm and a positive reaction required (erythema and induration of at least 5 mm in diameter within 48–72 h). Responders are enrolled in the study, and the non-responders are excluded **(22).**

In the other approach, some authors have injected the antigen directly into the wart without previous sensitization, proposing that this approach would be more practical in terms of time, cost and patient compliance **(26).**

This has been supported by the absence of a significant relationship between the clinical response and the extent of sensitization reaction as shown by many studies **(24).**

Advantages:

The simple easy application into only the 'mother' wart, the promising efficacy, the high safety profile, and the absence of limitation of movement, scarring and pigmentary changes are other advantages of intralesional antigen immunotherapy over traditional therapies **(22).** Several studies have shown that intralesional immunotherapy plays an important role in the reduction or even prevention of recurrences after successful therapy, a finding that represents a promising advantage over traditional therapies **(27).**

This effect might be explained by its ability to induce CMI, which enables the body to recognize HPV, stimulates the production of memory T cells against the virus and intensifies the effector response mechanism **(24)**

Disadvantages:

Trials on intralesional immunotherapy are limited by the absence of standardization in different aspects of intralesional immunotherapy such as the concentration and quantity of injected antigen, number of treatment sessions, intervals between sessions and follow-up period necessary for adequate evaluation of recurrence rates **(28).**

Although pain associated with the injection is relatively well tolerated by most patients, it remains a disadvantage for children who prefer non-painful topical application and for patients with warts in very painful sites, such as those with periungual warts **(22).**

Types of Antigens:

Different types of antigens have been used for the intralesional treatment of different types of warts. These include:

♦ MMR vaccine

Intralesional immunotherapy using MMR vaccine has the potential advantages of clearance of both treated and untreated distant warts without scarring, a presumed low rate of recurrence, and a high safety profile **(29).**

♦ Mycobacterium w (Mw) vaccine

Mycobacterium indicus pranii or Mycobacterium w is rapid growing non-tubercular mycobacteria, which has been found to induce a strong proinflammatory response while injected intralesionally. There is a prominent delayed hypersensitivity response with an increase in T helper 1 cytokines such as IL2, IL4, IL6, and IFN-γ 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 **(21).**

♦ Bacillus Calmette –Guerin (BCG) vaccine

BCG (live-attenuated vaccine derived from Mycobacterium bovis) as an intralesional immunotherapy is a promising, inexpensive, safe, and effective modality for multiple warts as it

has high tolerability, widespread response, and sustained effect with low recurrence rates **(30).**

Combination antigen therapy

1. Mumps, Candida, and Trichophyton **(26).**
2. Mumps, Candida, Trichophyton and interferon alpha-2b **(31).**

♦ **Purified protein derivative (PPD)**

Intralesional immunotherapy with PPD stimulates IL-12 cytokine, suggesting activation of a cell-mediated immune response. This ensures control of infection and protection against recurrence. Intralesional PPD injection is an acceptable and safe modality in the treatment of warts (multiple common, palmoplantar, periungual, and genital types) by intralesional route or intradermal route **(32).**

♦ **Candida Albicans Antigen**

Intralesional Candida antigen injection is an immunotherapeutic approach that has recently received increasing attention and has shown promising efficacy in the treatment of warts. This modality is associated with the production of Th1 cytokines such as IFN-γ and TNF-α, which activate cytotoxic and natural killer cells to eradicate HPV infection in both injected and non-injected lesions **(22).**

ConflictofInterest: Noconflictofinterest.

References

1.Moscicki, A. B., Schiffman, M., &Franceschi, S. (2020). The Natural History of Human Papillomavirus Infection in Relation to Cervical Cancer. In Human Papillomavirus (pp. 149-160). Academic Press.

2.Bienkowska-Haba, M., Luszczek, W., Myers, J. E., Keiffer, T. R., DiGiuseppe, S., Polk, P., ... & Sapp, M. (2018). A new cell culture model to genetically dissect the complete human papillomavirus life cycle. PLoS pathogens, 14(3), e1006846.

3.Ferreira, A. R., Ramalho, A. C., Marques, M., & Ribeiro, D. (2020). The Interplay between Antiviral Signalling and Carcinogenesis in Human Papillomavirus Infections. Cancers, 12(3), 646.

4.ZurHausen H. (2009): Papillomaviruses in the causation of human cancers. A brief historical account. Virology; 384: 260-5.

5.Handisurya A, Schellenbacher C and Kirnbauer R (2009): Diseases caused by human papillomaviruses (HPV). Journal der eutschen Dermatologischen Gesellschaft; 7(5):453–466.

6.Singh, S., Ahmad, S., Srivastava, A. N., &Misra, J. S. (2020): A Review on Role of Human Papilloma Virus (HPV) in Health-Related Diseases.

7.Shukla S, Bharti AC, Hussain S, Mahata S, HedauS,Kailash U. (2009): Elimination of high-risk human papillomavirus type HPV16 infection by 'Praneem' polyherbal tablet in women with early cervical intraepithelial lesions. J Cancer Res Clin Oncol; 135: 1701-9.

8.Yang, J., Yang, A., Wang, Z., Wang, W., Wang, Z., Wang, Y., ... & Zhao, W. (2018).

Interactions between serum folate and human papillomavirus with cervical intraepithelial neoplasia risk in a Chinese population-based study. The American journal of clinical nutrition, 108(5), 1034-1042.

9. **Castro TMPPG. (2007):** Frequency of Appearance of Human Papillomavirus (HPV) in Oral Mucosa of Men with Anogenital HPV by a Molecular Technique. Arch. Otorhinolaryngol; 11(1): 36-44.

10. **Weng, S. L., Huang, K. Y., Weng, J. T. Y., Hung, F. Y., Chang, T. H., & Lee, T. Y. (2018).** Genome-wide discovery of viral microRNAs based on phylogenetic analysis and structural evolution of various human papillomavirus subtypes. Briefings in Bioinformatics, 19(6), 1102-1114.

11. **Castillo, A. (2013).** Human papillomavirus and carcinogenesis in the upper aero-digestive tract. Carcinogenesis, 45.

12. **Medzhitov R, Janeway CAJ. (1997):** Innate immunity: the virtues of a non-clonal system of recognition. Cell; 91: 295-8.

13. **Garzetti GG, Ciavattini A, Goteri G, De Nictolis M, MensoS, Muzzioli M, Fabris N. (1995) HPV** DNA positivity and natural killer cell activity in the clinical outcome of mild cervical dysplasia: integration between virus and immune system. GynecolObstet Invest; 39: 130-35.

14. **Van Ginkel FW, VanCott JL, Kiyono H, McGhee JR. (1997):** Mucosal immunity to human papillomavirus in the female reproductive tract. Papillomavirus Rep; 8: 1-10.

15. **Vorsters, A., Bosch, F. X., Bonanni, P., Franco, E. L., Baay, M., Simas, C., ... & Muñoz, N. (2020).** Prevention and control of HPV infection and HPV-related cancers in Colombia-a meeting report. In BMC proceedings (Vol. 14, No. 9, pp. 1-13). BioMed Central.

16. **Lambie, M., & Bratman, S. V. (2018).** Fundamental Biology of Human Papillomaviruses. In Recurrent Respiratory Papillomatosis (pp. 1-17). Springer, Cham.

17. **Schiller JT, Roden RB. (1995):** Papillomavirus-like particles. Papillomavirus Rep; 6: 121-8.

18. **Urman CO, Gottlieb AB. (2008):** New viral vaccines fordermatologic disease. J Am AcadDermatol; 58(3): 361- 70.

19. **Tong, Y., Tyring, S. K., &Szalai, Z. Z. (2019).** Human Papillomavirus Infection. Harper's Textbook of Pediatric Dermatology, 588-597.

20. **Cupertino, F., Carvalho, F. N. D. M. P. D., & Caffaro, B. L. (2020).** New recreational category associated with common wart transmission. Revista Brasileira de Medicina do Esporte, 26(4), 354-357.

21. **Thappa DM and Chiramel MJ (2016):** Evolving role of immunotherapy in the treatment of

refractory warts. Indian Dermatology Online Journal; 7(5): 364.

22. **Nofal A, Salah E, Nofal E, et al., (2013A):** Intralesional antigen immunotherapy for the treatment of warts: current concepts and future prospects. American Journal of Clinical Dermatology; 14(4):253-260.

23. **Aldahan AS, Mlacker S, Shah VV, et al., (2016):** Efficacy of intralesional immunotherapy for the treatment of warts: A review of the literature. Dermatologic Therapy; 29(3):197– 207.

24. **Clifton M, Johnson S, Roberson P, et al., (2003):** Immunotherapy for recalcitrant warts in children using intralesional mumps or Candida antigens. Pediatric Dermatology; 20(3): 268-271.

25. **Rezai MS, Ghasempouri H, Marzidareh O, et al., (2019):** Intralesional Injection of the Measles-Mumps-Rubella Vaccine into Resistant Palmoplantar Warts: A Randomized Controlled Trial. Iranian Journal of Medical Sciences; 44(1): 10.

26. **Johnson S and Horn T (2004):** Intralesional immunotherapy for warts using a combination of skin test antigens: a safe and effective therapy. Journal of Drugs in Dermatology; 3(3):263- 265.

27. **Kim K, Horn T, Pharis J, et al., (2010):** Phase 1 clinical trial of intralesional injection of Candida antigen for the treatment of warts. Archives of Dermatology; 146(12):1431–1433.

28. **Chandrashekar L (2011):** Intralesional immunotherapy for the management of warts. Indian Journal of Dermatology, Venereology, and Leprology; 77(3): 261 263.

29. **Na CH, Choi H, Song SH, et al., (2014):** Two-year experience of using the measles, mumps and rubella vaccine as intralesional immunotherapy for warts. Clinical and Experimental Dermatology; 39(5):583-589.

30. **Jaisinghani AK, Dey VK, Suresh MS, et al., (2019):** Bacillus Calmette– Guerin immunotherapy for recurrent multiple warts: An openlabel uncontrolled study. Indian Journal of Dermatology; 64(2): 164.

31. **Horn TD, Johnson SM, Helm RM, Roberson PK. (2005):** Intralesional immunotherapy of warts with mumps, Candida and trichophyton skin test antigens: A single-blinded, randomized and controlled trial. Archives of Dermatology.;141:589-594.

32. **Abd-Elazeim FM, Mohammed GF, Fathy A, et al., (2014):** Evaluation of IL-12 Serum Level in Patients with Recalcitrant Multiple Common Warts, Treated by Intralesional Tuberculin Antigen. Journal of Dermatological Treatment; 25(3): 264- 267.