ANALYSIS

24(105), September - October, 2020

ISSN
2321–7359
EISSN
2321–7367

Medical Science

Intra-lesional Injection of Tuberculin Skin Test Antigens as an Immunotherapy for Warts: Open Label Placebo Controlled Clinical Trial

Abdulmajeed Alajlan✉

Dermatology Department, King Saud University, King Saud University Medical City, P.O Box 7805, Riyadh 11472, Saudi Arabia

✉**Corresponding author:**
Abdulmajeed Alajlan, MD
Associate Professor Dermatology Department,
King Saud University,
King Saud University Medical City, P.O Box 7805,
Riyadh 11472, Saudi Arabia
E mail: amajlan@ksu.edu.sa

Article History
Received: 16 August 2020
Reviewed: 16/August/2020 *to* 13/September/2020
Accepted: 14 September 2020
E-publication: 20 September 2020
P-Publication: September - October 2020

Citation
Abdulmajeed Alajlan. Intra-lesional Injection of Tuberculin Skin Test Antigens as an Immunotherapy for Warts: Open Label Placebo Controlled Clinical Trial. *Medical Science*, 2020, 24(105), 3513-3522

ABSTRACT

Background: Warts are typically skin growths caused by Human Papilloma virus affecting all age groups. Resistance to conventional therapy is a common characteristic of the lesion. Immunotherapy with variant antigens is effectively used in wart therapy. Purified

protein derivative (PPD) of tuberculin is tested. *Objective:* To determine the efficacy and adverse effects of intra-lesional injection of PPD antigens in treating common warts. *Methods:* 82 Patients with multiple warts who have not received any treatment for the last 12 weeks have been enrolled in an open labelled, placebo-controlled clinical trial in outpatient dermatology clinics in a tertiary care center. The research has acquired Ethical approval from the IRB in King Saud Medical City and informed consent was signed voluntarily by all patients. Patients were assigned for two groups. Interventional study group for intra-lesional injection of tuberculin skin test antigen, which subdivided in to two different groups: positive PPD skin test (n=40) and negative PPD skin test (n=22). Control group were intra-lesional normal saline was injected (n=20). All patients who lost to follow up 3 months post last injection were excluded from the analysis. We defined success of the injection by complete resolution of one or more warts at injected or none injected sites. *Result:* Seventy-six percent (31/40) of the positive PPD patients in the active group showed clearance of one or more warts compared to 10% (2/20) of normal saline group ($p<0.005$) While 82 % (18/22) of negative PPD patients showed clearance of one or more warts. Out of the responders in the positive PPD patients, 38% percent (12/31) cleared anatomically distant warts as well. However, 78% (14/18) of the responders in the negative PPD group cleared anatomically distant wart. Analysis of the two groups (positive and negative PPD test). in the active arm showed significantly that multiple injections at single session is more efficacious for distant wart than single injection ($p<0.005$). *Conclusion:* Immunotherapy with intradermal injection of PPD of tuberculin skin antigen is an effective and safe treatment modality for multiple and resistant warts. Multiple PPD injections at one session are more effective than single injection. Positive PPD skin test is not mandatory for its efficacy in our study population.

Keywords: Intra-lesional Injection; Tuberculin Skin Test; Antigens; Immunotherapy; Warts; Purified Protein derivative.

1. INTRODUCTION

Verruca Vulgaris (common warts) are frequent skin conditions affecting all human age groups (Al Aboud, 2019). Although, they are self-limiting, many patients want them to resolve sooner for the associated discomfort and cosmetic appearance (Kumar & Zawar, 2007). Topically applied treatment modalities are the standard approach for most of the patients including cryotherapy, salicylic acid, electrocautery, cantheridine, etc., (Tuggy & Garcia, 2011). The first instance of immunotherapy for wart was introduced by Japanese researcher in 1979 (Harada, 1979).

In early 2001, clinical studies of immunotherapies with candida antigen, measles, mumps and rubella were introduced successively with significant benefits (Scott et al., 2001, Johnson et al., 2001, Phillips et al., 2000). At that time, these antigens were not available at our hospital urging us to look to similar available antigen that enhances cell mediated immunity against human papilloma virus. Purified protein derivative (PPD) of tuberculin skin test have been chosen by the author. It had been tested by the author for the first time in pilot patients and found effective before this study to be held. Part of the outcome of this study had been then presented in American Academy of Dermatology in San Francisco in 2006 and published as scientific new (Finn, 2018). Also it has been presented at several dermatological meetings thereafter. Nine years later, group of researchers published the same idea (Amirnia et al., 2015, Daulatabad, 2016, Nofal et al., 2013, Jaisinghani, 2019, Abdelazeim, 2014). However, in addition that our study is the first work to test PPD as in this reference. It has a new significant result namely that negative PPD skin test patients were treated and compared with positive PPD patients with important outcome. The peculiar of immunotherapies is to its efficacy for the recalcitrant warts as well distant none injected warts. The non-clinically apparent warts are potentially at the target of immunotherapy. The possibility of prophylaxis effect of these modalities will be discussed.

2. MATERIAL AND METHODS

A total of forty consecutive patients who were diagnosed to have one or more warts were enrolled in this study. Inclusion criteria were; 18 years and older, immunocompetent and not receiving any wart therapies (including herbal remedies) for the last 12 weeks. All patients had warts of duration more than 12 weeks. Pregnant and lactating ladies were excluded. In the PPD group, a positive skin test with tuberculin antigen was required, figure 1. All patients provided informed consent and were randomized to two groups: (1). intra-lesional injection with tuberculin antigen skin test or (2). intra-lesional injection with normal saline. At the end of the trial there was clear efficacy in the active group thereby extra 20 patients were enrolled to PPD group to ensure the positive outcome.

All subjects were pre tested according to the randomization, where group 1 injected with 5 units in 0.1 mL of PPD and group 2 with 0.1 mL intradermal normal saline into the skin of the forearm. Reading tests were done 48 hours post injection and the resulted induration of any amount were documented. Reaction to PPD considered positive if the redness is 5 mm or more. Even patients with no reaction to PPD still received active treatment.

One or two warts were treated in each patient. All subjects received injections every 3 weeks into the same wart until complete clearing of the treated wart was achieved or for a maximum of 3 treatments. Patients were examined at study initiation and at each episode of treatment. Complete resolution is only considered positive.

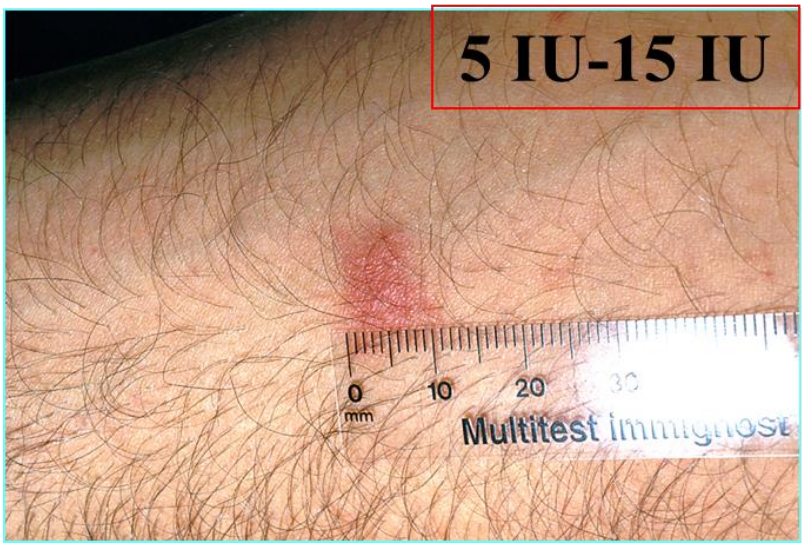

Figure 1: tuberculin skin test Purified protein derivative

For the active arm group; positive and negative PPD skin test patients where received either a single or multiple intradermal injection of PPD at warty lesions each treatment session, respectively. If no response, injections were repeated every 3 weeks for a maximum 3 sessions. For the control group, each patient received a single intradermal injection of normal saline at one wart every three weeks for a total of 3 sessions. Adverse effects were recorded. The research has acquired Ethical approval from the IRB in King Saud Medical City. The trial was submitted on 23/4/2018 to the IRB council in King Saud Medical City as a proposal, following Helsinki guidelines of clinical trials, and we had trial registry number of 79/3SFD/847J acquired on 30/3/2019. We followed and fulfilled all the points in CONSORT 2010 checklist of information to include when reporting a randomized trial. We included the filled checklist form as a flow chart per those guidelines in supplementary-figure 1.

We used the SPSS software version 20.0 (SPSS 16.0; SPSS Inc., Chicago, IL) for statistical data analysis. Statistical significance was considered at a p-value of <0.05. Data was normally distributed and checked by Kolmogorov–Smirnov test. One-way ANOVA, and chi-square tests were used where indicated.

3. RESULTS

The clinical characteristics and demographic data of the two study groups are described in Table 1. The two groups were statistically congruent regarding age, gender, duration of the wart, and resistance to treatment.

Table 1: Over all differences between active and normal saline groups

Variables		**Active PPD group n=62**	**Normal saline group N=20**	**Comparison: p value**
Age	Mean (Range)	23 (18-41)	21(18-31)	NS*
Gender				
	Males	23	6	NS
	Females	39	14	NS
Clearance of warts	N (%)	49 (79%)	2 (10%)	p<0.005
Adverse effects	N (%)	0(0)	0 (0)	NS

*NS: Not Significant

Among 62 patients in the active group, we had 23 males and 39 females. In the control group we had among 20 patients, six males and 14 females. Age range in the active group between 18-41 years with average of 23 years, and in the control group age range between 18-31 years with average of 21 years.

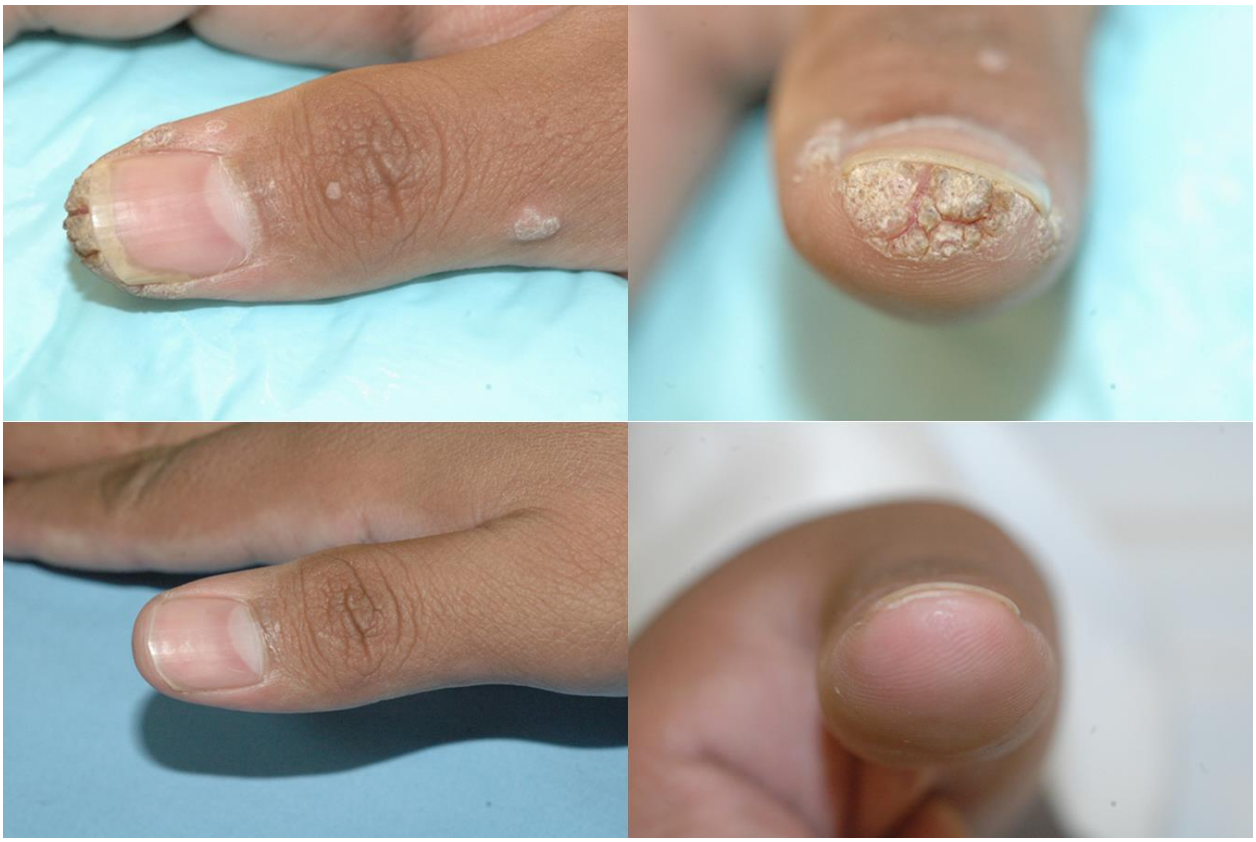

Figure 2: effect of PPD on wart of thumb nail

Seventy-nine percent (49/62) of the active group showed clearance of one or more warts (figure 2-6) compared to 10% (2/20) of normal saline group (p<0.005). Out of the responders in the active group, thirty-eight percent (12/31) cleared the injected and anatomically distant warts. Forty-five percent (14/31) of the responders in the positive PPD required only one single injection, while only 11% of negative PPD responded from single injection. Of interest no single recurrence of any cleared warts of both groups in follow up period. Analysis of the two groups in the active arm showed that multiple injections at single session is statistically (p<0.05) more efficacious for distant wart than single injection (Table 2). Post study completion, further PPD injection beyond 3 sessions resulted in higher response rate for the injected warts in 85% and more obviously in distant warts in 72%. There were no significant adverse effects of either group. One patient only in the interventional group showed a very slight improvement after PPD repeated injections.

Table 2: Comparison between positive and negative PPD patients in the active arm

Variables	Positive PPD patients N=40	Negative PPD patients N=22	Comparison: *p* value
Over all Clearance of warts	31 (76%)	18 (82%)	NS
Clearance after first injection	14/31 (45%)	2/18 (11%)	p<0.005
Clearance of distant warts	12/31 (38%)	14/18 (77%)	p<0.005
Average number of injection sessions per patient*	1.8	2.6	p<0.05

* This analysis based on study protocol to treat till the injected wart resolved or maximum of 3 treatment sessions.

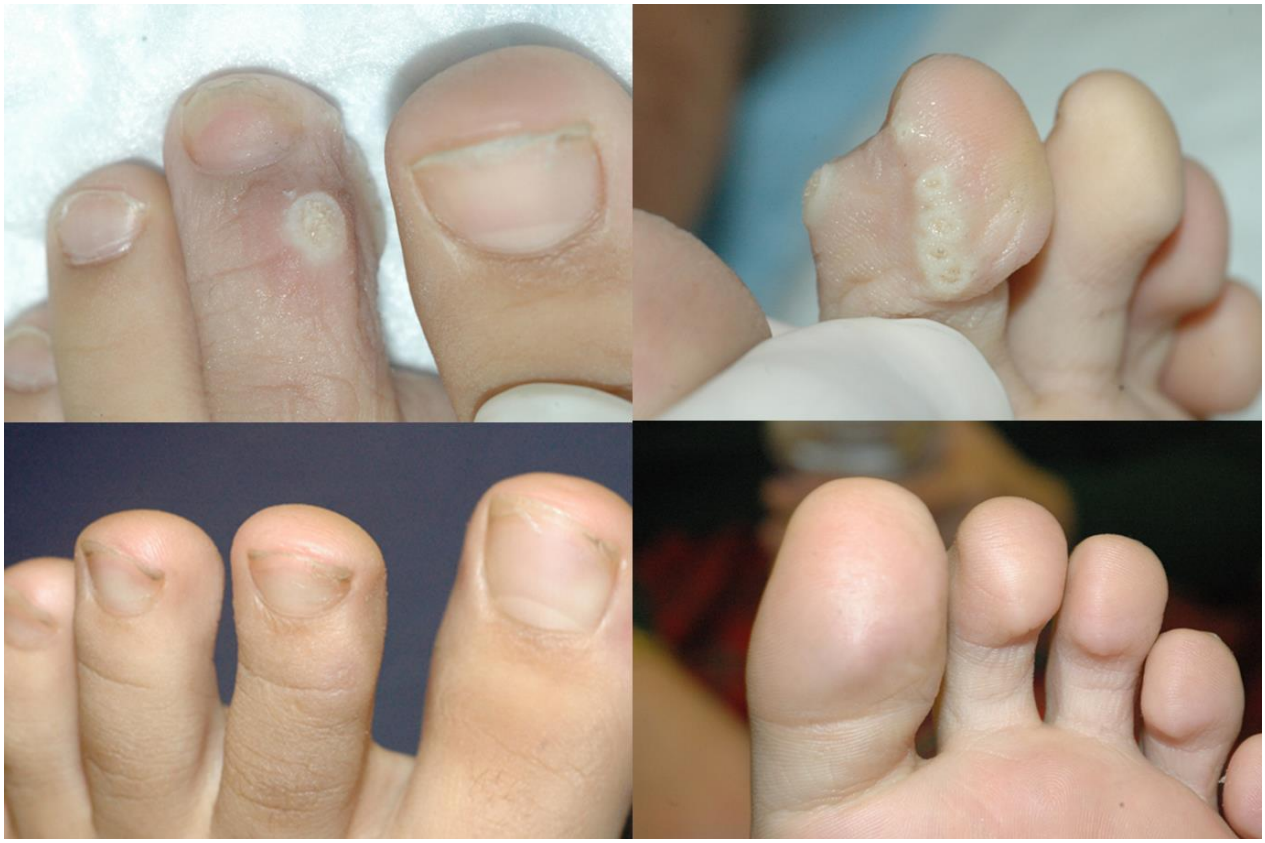

Figure 3: Effect of PPD on wart of big toe

Figure 4: Effect of PPD on wart in middle finger

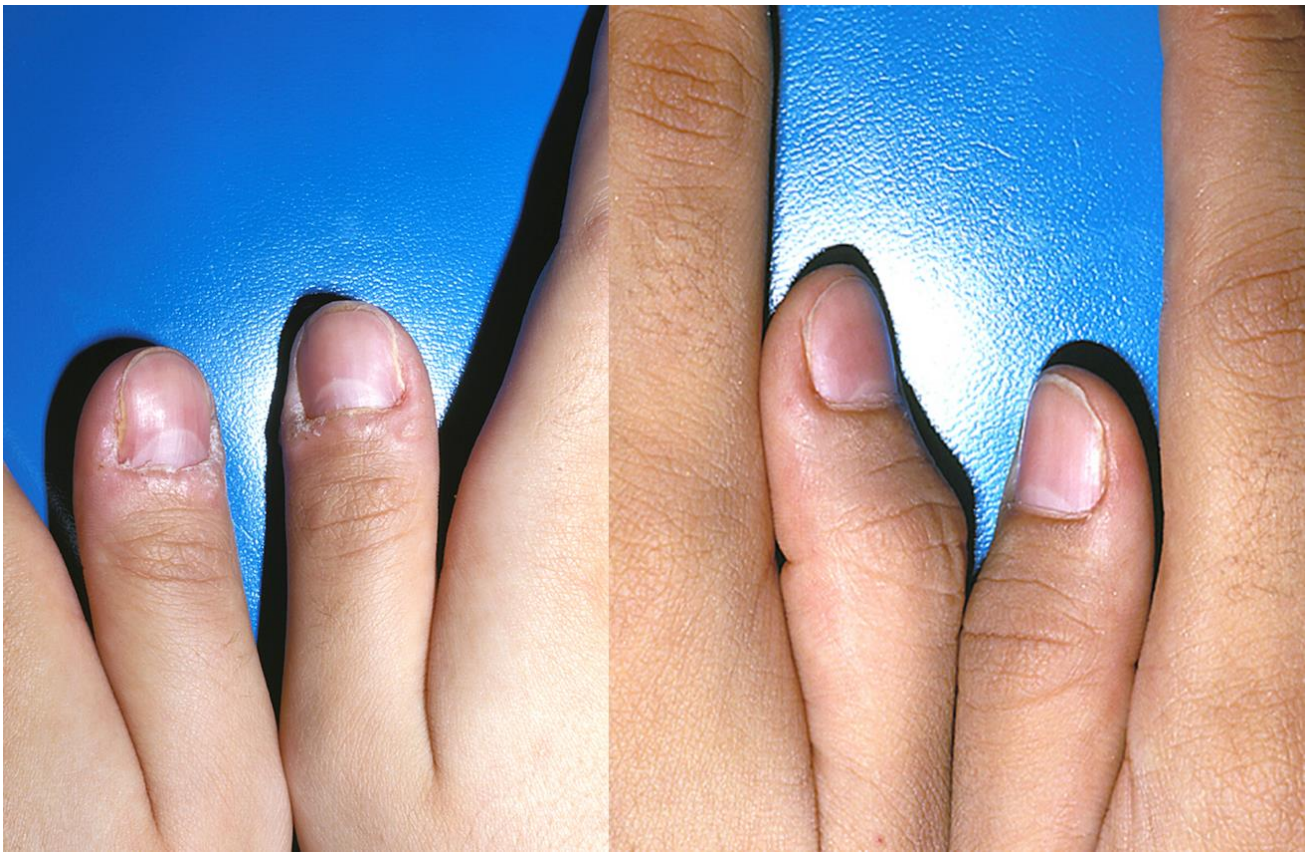

Figure 5: Effect of PPD on wart in thumb of right hand

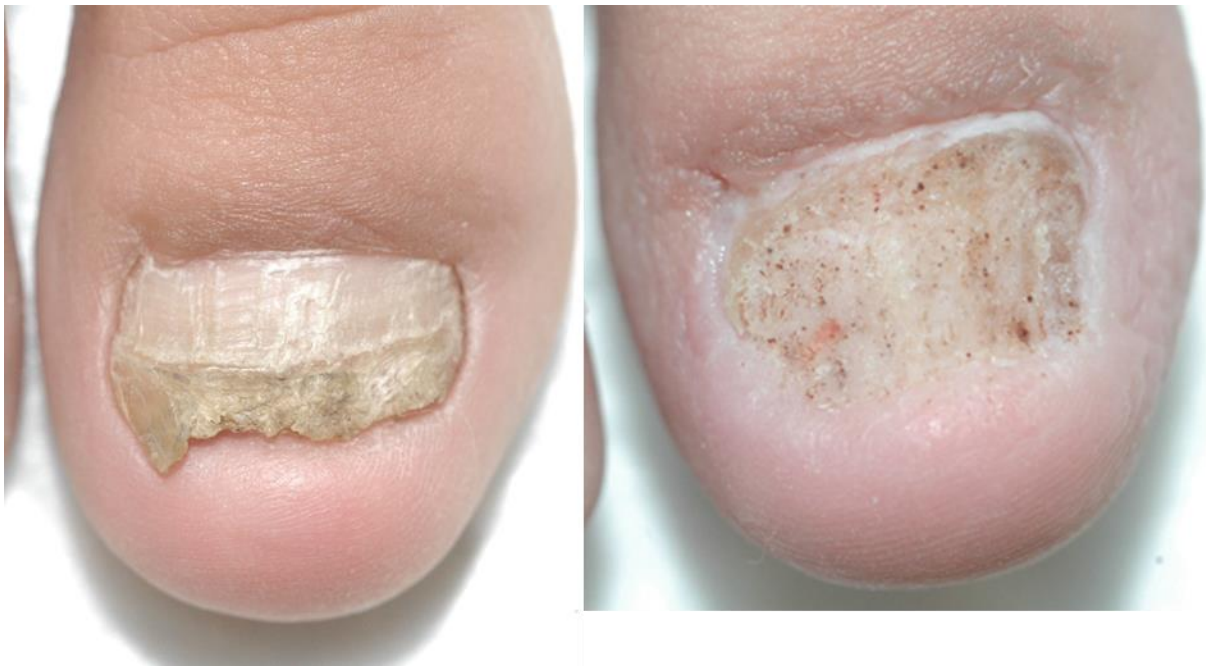

Figure 6: Effect of PPD on wart not so much improvement

The treatment with 5 units of 0.1ml PPD, resolution of the treated warts was significantly more pronounced in the study group than in the normal saline control group (study group: 79%, control group: 10%) with no obvious adverse events in both groups apart of pain at injection sites. Among 40 patients who have positive tuberculin skin test, 31 (76%) showed complete clearance of warts in

comparison to 18 (82%) in patients with negative tuberculin skin test. However, resolution of the treated warts was non-significantly more pronounced between the two groups.

When we segregated the patients with single injection only, we found that 14 (45%) of patients with positive skin test showed complete resolution of warts in comparison to 2 (11%) patients only in negative skin test group ($P<0.005$) when we examined the effect of intra-lesional injection of PPD on distant warts. It was found that 12 (38%) of patients with positive skin test showed complete clearance of distant warts, in comparison to 14 (77%) of patients with negative skin test ($P<0.005$).

4. DISCUSSION

The cellular immune system has an important role in defending the body against viral infections (Meyer et al., 2014). Warts are caused by human papilloma virus which is prevalent all over the world and in certain instances it may lead to cervical cancers and squamous cell carcinama (Pinto et al., 2003). The cellular mediated immunity may abolish the disease or at least participate in the restriction of its proliferation and sequelae. Intra-lesional immune medication has been developed as a promising treatment modality for warts (Meyer et al., 2014).

A systematic review determined the absence of benefit in using immunotherapy in treatment of warts due to the scarcity of evidence from randomized controlled trials (Chen et al., 2014). PPD is a frequently used antigen (Froeschle, 2014). The use of this protein derivative for wart immunotherapy is essential in two main aspects. First because the mandatory immunization schedule in many countries requires BCG vaccine, where warts are highly prevalent. The PPD gives a good screening tool for tuberculosis as well may help to indicate active disease in some patients.

Second, while immunotherapy is typically an economical modality of treatment for wart cases, PPD is the cheapest and traditionally used antigens. It has been reported that the price of PPD is less than one dollar (Daulatabad, 2016).

Treatment of resistant warts was always a major challenge for both the dermatologist and the patient (Lipke, 2006). Some studies documented that the rate of success of intra-lesional immunotherapy with specific antigens varies from 22% to 93% in different contexts, varying from one to 32 settings depending on the individual immune response, and normal saline has been documented to cause wart lesions to vanish in about 22% of cases (Gibbs, 2003, Sterling, 2003). Originally, Lahti and Hannuksela used topical tuberculin gel in traditional wart immunotherapy in 1982 at a cure rate 60% (Lahti & Hannuksela, 1982). Nevertheless, the long duration limited its potential use in the future as a standard method of wart immunotherapy.

In the only accessible placebo-clinical controlled trial, Abd-Elazeim et al. examined intra-lesional immunotherapy with PPD antigen in 20 patients with resistant multiple clinical warts (Abd-Elazeim, 2014). Compared to our findings, a full improvement was recorded in 70% of patients after 5 therapy sessions (Wananukul et al., 2009). Another open-label analysis by Kus et al showed total recovery of palmoplantar and periungual warts was reported in 93% of cases after 6 weeks of intra-lesional PPD injection (Kus et al., 2005). It was proposed to be an effective and safe approach for immunotherapy in these cases.

In this study, no complications were observed during PPD injections or later on, and the recurrence was nil during the period of follow-up. Our finding came in concordance with other studies (Na Ch et al., 2014). Traditional therapy may also be effective against warts with 70% response (Goncalves, 1975). However, it is associated with recurrence and some side effects favoring immunotherapy in selected cases (Mulhem & Pinelis, 2011).

Traditional modalities like cryotherapy are associated with recurrence rate up to 30% (Youn et al., 2011). Most probably due to existence of warts in the avascular epidermis which has low immune response (Resnick et al., 1990). Studies have also shown adverse reactions to these modalities such as inflammation, wounds ulcers, scarring and hypo-or hyperpigmentation (Focht et al., 2002). These complications rarely occur when intra-lesional PPD immunotherapy is used. This may allow us to propose the intra-lesional immunotherapy among the first line solution for treating patients with multiple warts.

Clearance of distant warts is a further advantage of the immunotherapy. The exact mechanism is unknown (Gupta et al., 2008). However, in our study 77% and 38% of patients completely cured from distant warts in both negative and positive PPD groups respectively. The physiological mechanism of cure might be due to the induced delayed-systemic type IV hypersensitivity reaction against human papilloma virus antigens as well direct stimulation of cytotoxic T-cells and natural killer (NK) cells against the virus. According to this hypothesis, the usefulness of intra-lesional immunotherapy in the eradication of distant warts and the better result for patients with limited sensitivity to used antigens might be explained (Clifton et al., 2003; Wendling & Patrice, 2008; Horn et al., 2005, Schneffeold & Lehman, 2006).

Some authors have recommended intra-lesional immunotherapy only for positive tuberculin cases with previous PPD antigen immunization. In this study, we propose that intra-lesional immunotherapy to be considered for both tuberculin reactors and non-reactors.

Apart from the limitations of our study such as small number of patients, spontaneous resolution of warts, and open labeled study, our findings came in concordance with other reports. The response of the wart to the immunotherapy depends mainly on the immune response of the patients regardless the site, size, number and duration of the wart (Metawea et al., 2005, Tomson et al., 2011)

More clinical trials should be done on large number of patients prospectively putting in consideration the types, sites, sizes, numbers and duration of warts, as well as number of sessions and combination with other therapies (Bacelieri & Johnson, 2005).

5. CONCLUSION

Apart from late reporting of results of this trial, this study is considered the first experiment tested the effect of PPD on warts. We concluded that Intra-lesional injection of 5 units of 0.1ml PPD antigen for one to six times at 3-weeks interval is highly safe and efficient in treating resistant warts, regardless the site of injection and the immune status of the tuberculin reactor prior to therapy.

Acknowledgement:
We thank the patients who were all participated in and contributed samples to the study. we can also thanks to your guides, professors, lab support, and material support.

Funding:
This study has not received any external funding.

Conflict of Interest:
The author declares that there are no conflicts of interests.

Informed consent:
Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Ethical approval:
The study was approved by the Medical Ethics Committee of king saud University (ethical approval code: #987659).

Capsule summary

- The first instance of immunotherapy for wart was introduced by Japan
- Purified protein derivative (PPD) of tuberculin skin test had been tested by the author for the first time in pilot patients and found effective. Part of the outcome of this study had been then presented in American Academy of Dermatology in San Francisco in 2006 and published as scientific new.

Data and materials availability
All data associated with this study are present in the paper.

Peer-review
External peer-review was done through double-blind method.

REFERENCES AND NOTES

1. Al Aboud AM, Nigam PK. Wart (Plantar, Verruca Vulgaris, Verrucae). [Updated 2019 Sep 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK431047/
2. Kumar R, Zawar V. Threading warts: a beauty parlor dermatosis. Journal of Cosmetic Dermatology, 2007; 6(4), 279–282.
3. Tuggy M, Garcia J. Wart Treatment. Atlas of Essential Procedures, 2011;26–29.
4. Harada S. Clinical application of fungus extracts and its culture filtrate in the treatment of skin diseases; (3). Candida vaccine in the treatment of warts (author's transl). Nihon Hifuka Gakkai Zasshi. 1979; 89:397–402.
5. Scott M, Nakagawa M, Moscicki AB. Cell-mediated immune response to human papillomavirus infection. Clin Diagn Lab Immunol. 2001; 8:209-220.

6. Johnson S, Roberson P, Horn T. Intralesional injection of mumps or Candida skin test antigens. Arch Dermatol. 2001; 137:451-455.
7. Phillips R, Ruhl T, Pfenninger J, et al. Treatment of warts with Candida antigen injection. Arch Dermatol. 2000; 136:1274-1275.
8. Finn, R (2018, December 6). Tuberculin Skin Test Promising as Wart Therapy. Retrieved from https://www.mdedge.com/internalmedicine/article/12339/dermatology/tuberculin-skin-test-promising-wart-therapy.
9. Amirnia M, Khodaeiani E, Fouladi DF, Masoudnia S. Intralesional immunotherapy with tuberculin purified protein derivative (PPD). in recalcitrant wart: A randomized, placebo-controlled, double-blind clinical trial including an extra group of candidates for cryotherapy. Journal of Dermatological Treatment, 2015; 27(2), 173–178.
10. Daulatabad D, Pandhi D, Singal A. BCG vaccine for immunotherapy in warts: is it really safe in a tuberculosis endemic area? Dermatologic Therapy, 2016; 29(3), 168–172.
11. Nofal A, Yosef A, Salah E. Treatment of recalcitrant warts with Bacillus Calmette-Guérin: a promising new approach. Dermatologic Therapy, 2013;26(6), 481–485.
12. Jaisinghani A, Dey V, Suresh M, Saxena A. Bacillus Calmette–Guerin immunotherapy for recurrent multiple warts: An open-label uncontrolled study. Indian Journal of Dermatology. 2019; 64(2), 164.
13. Abd-Elazeim FM, Mohammed GF, Fathy A, Mohamed RW. Evaluation of IL-12 serum level in patients with recalcitrant multiple common warts, treated by intralesional tuberculin antigen. J Dermatolog Treat. 2014;25:264–7.
14. Meyer, Sonja Izquierdo, et al. "Cell-Mediated Immune Response: a Clinical Review of the Therapeutic Potential of Human Papillomavirus Vaccination." Acta Obstetricia Et Gynecologica Scandinavica, vol. 93, no. 12, 2014, pp. 1209–1218.
15. Pinto LA, Edwards J, Castle PE, et al. Cellular immune responses to human papillomavirus (HPV)-16 L1 in healthy volunteers immunized with recombinant HPV-16 L1 virus-like particles. J Infect Dis. 2003;188:327-338.
16. Chen, Juping, and Yan Wu. "Local Treatments for Cutaneous Warts." Evidence-Based Dermatology, June 2014, pp. 320–328.
17. Froeschle JE, Ruben FL, Bloh AM. Immediate hypersensitivity reactions after use of tuberculin skin testing. Clin Infect Dis. 2002; 34:E12–13.
18. Lipke MM. An armamentarium of wart treatments. Clin Med Res. 2006;4:273–93.
19. Gibbs S, Harvey I, Sterling JC, Stark R. Local treatments for cutaneous warts. The Cochrane Database Syst Rev. 2003;(3): CD001781.
20. Sterling JC, Handfield-Jones S, Hudson PM. Guidelines for the management of cutaneous warts. Br J Dermatol. 2001; 144:4–11.
21. Lahti A, Hannuksela M. Topical immunotherapy with tuberculin jelly for common warts. Arch Dermatol Res. 1982;273:153–4.
22. Wananukul S, Chatproedprai S, Kittiratsacha P. Intralesional immunotherapy using tuberculin PPD in the treatment of palmoplantar and periungual warts. Asian Biomedicine. 2009; 3:739–43.
23. Kus S, Ergun T, Gun D, Akin O. Intralesional tuberculin for treatment of refractory warts. J Eur Acad Dermatol Venereol. 2005; 19:515–6.
24. Na CH, Choi H, Song SH, Kim MS, et al. Two-year experience of using the measles, mumps and rubella vaccine as intralesional immunotherapy for warts. Clin Exp Dermatol. 2014;39:583–9.
25. Goncalves JC. 5-Fluorouracil in the treatment of common warts of the hands. A double-blind study. Br J Dermatol. 1975;92: 89–91.
26. Mulhem E, Pinelis S. Treatment of nongenital cutaneous warts. Am Fam Physician. 2011;84:288–93.
27. Youn SH, Kwon IH, Park EJ, Kim KH, Kim KJ. A Two-week Interval Is Better Than a Three-week Interval for Reducing the Recurrence Rate of Hand-foot Viral Warts after Cryotherapy: A Retrospective Review of 560 Hand-foot Viral Warts Patients. Ann Dermatol. 2011;23(1):53–60.
28. Resnick RM, Cornelissen MT, Wright DK, et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. J Natl Cancer Inst. 1990;82:1477-1484.
29. Focht III DR, Spicer C, Fairchok MP. The Efficacy of Duct Tape vs Cryotherapy in the Treatment of Verruca Vulgaris (the Common Wart). Arch Pediatr Adolesc Med. 2002;156(10):971–974.
30. Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed Mycobacterium w vaccine for the treatment of ano-genital warts: an open label pilot study. J Eur Acad Dermatol Venereol. 2008; 22:1089–93.
31. Clifton MM, Johnson SM, Roberson PK, et al. Immunotherapy for recalcitrant warts in children using intralesional mumps or Candida antigens. Pediatr Dermatol. 2003;20:268–71.
32. Wendling, Patrice. "Genital Wart Treatment Options Expanding." Ob.Gyn. News, vol. 43, no. 4, 2008, p. 16.
33. 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;141:589–94.

34. Scheinfeld N, Lehman DS. An evidence-based review of medical and surgical treatments of genital warts. Dermatol Online J. 2006; 12(3):5.
35. Metawea B, El-Nashar AR, Kamel I, et al. Application of viable bacille Calmette-Guerin topically as a potential therapeutic modality in condylomata acuminata: a placebo-controlled study. Urology. 2005;65:247–50.
36. Tomson N, Sterling J, Ahmed I, et al. Human papillomavirus typing of warts and response to cryotherapy. J Eur Acad Dermatol Venereol. 2011;25:1108–11.
37. Bacelieri R, Johnson SM. Cutaneous warts: an evidence-based approach to therapy. Am Fam Physician. 2005;72:647–52.

SUPPLEMENTARY

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	

CONSORT 2010 checklist — Page 1

Supplementary Figure 1: CONSORT 2010 checklist of information to include when reporting a randomized trial