

METHYLENE BLUE PHOTODYNAMIC THERAPY VERSUS TUBERCULIN PURIFIED PROTEIN DERIVATIVE (PPD) IMMUNOTHERAPY IN PLANE WARTS IN CHILDREN

By

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ABSTRACT

Background: Plane warts are caused by human papilloma virus (HPV) (most often HPV-2, HPV-3 or HPV-10) and generally multiple, slightly elevated, smooth papules occurring most often on the face, hands, neck, and legs of children and young adults.

Objective: To compare the efficacy and safety daylight photodynamic therapy using methylene blue versus tuberculin purified protein derivative (PPD) immunotherapy intradermal injection in plane warts in children.

Patients and methods: Forty patients (Children) complaining of plane warts were enrolled in this study. They were randomly selected from the attendants of out-patient clinic Dermatology and Venereology Department, Al Hussein University Hospital, Faculty of Medicine, Al-Azhar University from April 2019 to November 2019. Patients were divided randomly into two groups, to undergo either methylene blue day light photodynamic therapy (MB-DL PDT) or tuberculin PPD intradermally.

Results: Tuberculin PPD-treated group showed significantly higher rates of complete response compared with MB- DL- PDT-treated group, complete response (60% versus 30% respectively); partial response (10% versus 30%), and no response (30% versus 40%).

Conclusion: PPD intradermally and MB-DL PDT has significant advantages over other treatments. Most treatment modalities were painful, needing multiple visits (time and money consuming), and were directed to each individual wart. This study confirmed that immunotherapy tuberculin PPD and MB-DL-PDT can be used to treat cutaneous Plane warts in children.

Keywords: Plane warts, HPV, MB-DL PDT, PPD

INTRODUCTION

Multiple plane warts a contagious viral disease that might be a disturbing cosmetic problem. Patients often request treatment because of social stigma or

discomfort. Ideally, the treatment should be simple with a low risk of adverse effects. Treatment failure, recurrence or relapse are common because the traditional therapeutic approaches are sometimes less successful in eradicating

the total HPV infection especially the subclinical and latent infections (*Moreira et al., 2012*).

Photodynamic therapy (PDT) is a technique that utilizes reactive oxygen species produced by a non-toxic dye or photosensitizer molecule in the presence of low intensity visible light to kill mammalian or microbial cells (*Fathy et al., 2017*).

The advantages of PDT are numerous. It is safe and non-invasive. Photodynamic therapy is not free of adverse effects. The most common one is pain during illumination. With the use of portable light sources to optimize the procedure and facilitate outpatient treatment, the concept of ambulatory PDT has emerged. The most important methodological simplification the therapeutic use of daylight (*See et al., 2016*).

Daylight-PDT is a simplified treatment procedure that improves tolerability by altering two key aspects of conventional PDT. The absence of occlusion and the use of daylight instead of an artificial light source make the daylight-PDT a more simple procedure where patients need to spend less time in the clinic. In addition, pain intensity during daylight-PDT is significantly lower than with conventional PDT, probably due to continuous production and photoactivation of small amounts of protoporphyrin IX (*Wiegell et al., 2014*).

Methylene blue (MB) is a widely known histological dye that has been in use to stain living organisms for many years. It belongs to the phenothiazinium class of compounds. It is well known to be photo dynamically active. The characteristic color of MB is caused by

the strong absorption band in the 550-700 nm regions. Methylene blue has shown in vivo activity against several types of tumors when locally injected and illuminated with red laser light (*Fathy et al., 2017*).

Immune mechanisms may explain the spontaneous resolution of warts. The immune response elicited by human papilloma virus (HPV) includes the production of specific antibodies and cell-mediated immunity with activation of T-helper-1 (Th-1) lymphocytes. T-helper-2 (Th-2) secreted interleukin. Interleukin-4 (IL-4) helps in antibody secretion, while Interleukin-12 (IL-12) is one of the proinflammatory cytokines characteristic of Th-1-based immune response (*Shaheen et al., 2015*).

Immunotherapeutic modalities like PPD were used for treatment of recalcitrant warts. PPD is an extract of *Mycobacterium tuberculosis*, used for testing exposure to tuberculin protein, either from a previous vaccination or from the environment (*Eassa et al., 2011*).

Immunotherapy has the advantage over traditional treatments in that it presumably enhances recognition of the virus by the immune system. This allows clearing of the treated wart, and frequently warts at distant anatomic sites. Acquisition of human papillomavirus-directed immunity may also prevent future clinical infection (*Thappa and Chiramel, 2016*).

The aim of this study was to compare the efficacy and safety daylight photodynamic therapy using methylene blue versus Tuberculin Purified Protein Derivative (PPD) immunotherapy intradermal in plane warts in children.

PATIENTS AND METHODS

Forty patients (Children) complaining of plane warts were enrolled in this study. They were randomly selected from the attendants of out-patient clinics of Dermatology and Venereology Department, El Hussein University Hospital, Faculty of Medicine, Al-Azhar University. From April 2019 to November 2019. Patients were divided randomly into two equal groups.

• **Group I:** Patients were treated with daylight photodynamic therapy using methylene blue (MB-DL PDT).

• **Group II:** Patients were treated with tuberculin purified protein derivative (PPD) immunotherapy (intra-dermal).

Cases included in this study were subjected to the following:

Parents gave informed consent to participate in this work. Participants or their parents had complete explanation about the nature, risks and purpose of the study.

1. Complete history taking included: Personal history, Present history and Past history.
2. General examination.
3. Complete dermatological examination: To determine the type, number, size, sites of warts and the presence or absence of distant lesions. The diagnosis of warts was made by clinical examination and patients were advised not to use any other wart treatment during the study period. Proper written consent was obtained after counseling.
4. Each patient was photographed. Photos were taken before starting treatment

and every 1 or 2 weeks according to the group for 6 sessions.

5. Follow up of the patient for 3 months to detect recurrence or appearance of new lesions.

Preparation of Methylene blue:

Methylene blue hydrogel concentration was 0.1 % was prepared by laboratory of Alezaby pharmacy:

- Methylene blue (0.1 g).
- Carboxymethyl cellulose (2g).
- Phenoxyethanolamine (0.5g).
- Potassium sorbate (0.5 g).
- Distilled water to (100g).

Storage of Tuberculin PPD:

Tuberculin PPD was available at Vacsera Company. Tuberculin PPD solutions can be adversely affected by exposure to light. So we stored it in dark except when doses being actually withdrawn from the vial. Store between 2 and 8°C also the product which has been exposed to freezing was not used, avail of tuberculin PPD which had been in use for more than one month was discarded because oxidation and degradation may have reduced the potency.

Post procedure care for all patients in both groups:

1. Topical, systemic antibiotics and analgesic anti-inflammatory were prescribed to the patient (in needed cases) to guard against infection and relieve pain.
2. The patients were asked to return after one week to Group 1 and two weeks to Group 2 for assessment of healing, the

need for another session and complications.

3. Photographs were taken before and after response with the same camera settings and illumination.

Patients were following up at least for three months after the last treatment as regards observation of recurrence or appearance of new lesions.

Statistical analysis:

Data were analyzed using Statistical package for Social Science (SPSS) version 24. Quantitative data were expressed as mean \pm standard deviation

(SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance: was used when comparing between two means.
- Chi-square test: was used when comparing between non-parametric data.
- P-value < 0.05 was considered significant.

RESULTS

There was no statistical significant difference between studied groups as regard age, sex, site and sessions of complete improvement.

The mean age in MB-DL PDT group was 9.8 ± 2.04 years with minimum age of 6 years and maximum age of 13 years while the mean age in PPD group was 9.6 ± 2.5 years with minimum age of 5 years and maximum age of 13 years.

Were 3 males (15%) and 17 females (85%) in MB-DL PDT group while there were 8 males (40%) and 12 females (60%) in PPD group.

There were 13 patients (65%) affected in the face and 7 patients (35%) affected in other sites in MB-DL PDT group also there were 13 patients (65%) affected in the face and 7 patients (35%) affected in other sites in PPD group.

There were 8 patients (40%) showed no response, 6 patients (30%) showed partial response and 6 patients (30%) showed complete response in MB-DL PDT group while there were 6 patients (30%) showed no response, 2 patients (10%) showed partial response and 12 patients (60%) showed complete response in PPD group.

For complete improvement in MB-DL PDT group, 1 patient (16.7%) required 3 session, 1 patient (16.7%) required 4 sessions, 1 patient (16.7%) required 5 sessions and 3 patient (50%) required 6 sessions while for complete improvement in PPD group, 1 patient (8.3%) required 3 session, 3 patients (25%) required 4 sessions, 3 patients (25%) required 5 sessions and 5 patients (41.7%) required 6 sessions (**Table 1**).

Table (1): Comparison between studied groups as regard age, sex, site, response and sessions of improvement

	MB – DL PDT	PPD
Age	N =20	N =20
Range	6 – 13	5 – 13

Mean ± SD	9.80 ± 2.04		9.60 ± 2.50	
P. value	0.783			
Sex		MB – DL PDT	PPD	Total
Male	N	3	8	11
	%	15.0%	40.0%	27.5%
Female	N	17	12	29
	%	85.0%	60.0%	72.5%
Total	N	20	20	40
	%	100.0%	100.0%	100.0%
P-value	0.077			
Site		MB – DL PDT	PPD	Total
Face	N	13	13	26
	%	65.0%	65.0%	65.0%
Other	N	7	7	14
	%	35.0%	35.0%	35.0%
Total	N	20	20	40
	%	100.0%	100.0%	100.0%
P-value	1.0			
Response		MB – DL PDT	PPD	Total
No	N	8	6	14
	%	40.0%	30.0%	35.0%
Partial	N	6	2	8
	%	30.0%	10.0%	20.0%
Complete	N	6	12	18
	%	30.0%	60.0%	45.0%
Total	N	20	20	40
	%	100.0%	100.0%	100.0%
P-value	0.117			
Sess. of Improvement		MB – DL PDT	PPD	Total
3	N	1	1	2
	%	16.7%	8.3%	11.1%
4	N	1	3	4
	%	16.7%	25.0%	22.2%
5	N	1	3	4
	%	16.7%	25.0%	22.2%
6	N	3	5	8
	%	50.0%	41.7%	44.4%
Total	N	6	12	18
	%	100.0%	100.0%	100.0%
P-value	0.905			

There were no complications except for hypopigmentation which was occurred in 1 patient (5%) in MB-DL PDT group

and local pain which was occurred in 6 patients (30%) in PPD groups (Table 2)

Table (2): Complications in MB-DL PDT and PPD groups

MB – DL PDT (n=20)	N	%
Burning pain	0	0%
Mild itching	0	0%
Mild irritation	0	0%
Hypopigmentation	1	5%
Hyperpigmentation	0	0%
PPD (n=20)	N	%

Systemic side effect	0	0%
Local pain	6	30%
Imm. Hypersensitivity	0	0%
Abcess	0	0%



Photo (1): Female patient 6 y. with plane warts, complete improvement after four sessions of MB-DL PDT



Photo (2): Male patient 13 y. with plane warts, complete improvement after five sessions of intradermal tuberculin PPD injection

DISCUSSION

The results of the present study revealed there were 40% showed no response, 30% showed partial response. This partial response mean decrease in size or number of plane warts only

without clearance of wart, and 30% showed complete response in MB-DL PDT group.

Our results relatively matching with the results of using aminolevulinic acid as PDT in treatment of plane wart with plane

wart (20%), that results make the methylene blue has the priority in treatment of plane and common wart on aminolevulinic acid as it is more effective, cheap and more available (*Yin et al., 2013*).

Immune mechanisms have been suggested to explain the spontaneous resolution of warts. If this immunity could be enhanced, wart resolution could be long lasting. The stimulated immune system would destroy all warts in the body, saving the patients from local treatment for each individual wart (*Mohamad et al., 2013*).

It has been reported that untreated warts resolve after injection of immunotherapy that induces HPV-directed immunity. Antigens used for immunotherapy include tuberculin; BCG (*Bacillus Calmette–Guérin*); mumps, candida and trichophyton and MMR (vaccine against measles, mumps, and rubella) (*Nofal & Nofal, 2010* and *Garg & Baveja, 2014*).

The exact mechanism of the clearance of warts with tuberculin PPD is not known. Its injection into the HPV infected Patient probably generates strong pro-inflammatory signals and attracts antigen presenting cells, which also recognize and process low profile HPV particles in the infected tissue. This leads to a strong adaptive immune response not only against mycobacterium tuberculosis but also against HPV. A similar mechanism has been proposed for the resolution of warts with skin test antigens such as mumps, candida and trichophyton antigens both at the injected as well as distant sites (*Moubashera et al., 2016*).

The mechanism of action of immunotherapy is still unclear. It may act through induction of strong nonspecific inflammatory response against the HPV-infected cells. It has also been suggested that the trauma itself may cause wart clearance in previously sensitized individuals (*Mohamad et al., 2013*).

Release of cytokines by immune system such as IL-2, IL-4, IL-5, IL-8, IFN- γ and TNF- α stimulate a strong immune response against HPV may be another possible mechanism of action (*Medrano et al., 2017*).

The response to antigen injection was associated with proliferation of peripheral blood mononuclear cells that promotes Th1 cytokines, including interferon gamma and interleukin 2, which further activate cytotoxic T cells and natural killer cells that eradicate HPV-infected cells. The clearance of warts strongly indicates the development of a widespread HPV-targeted immunity as a response of antigen injection and represents a major advantage of the immunotherapy (*Mohamad et al., 2013*).

Our results with tuberculin PPD-treated group showed different response rate to those previously reported by the study which reported complete clearance in 93% of the target wart and 87% complete clearance of distant wart; (Palmoplantar and periungual warts) this may be explained as that study was based on intralesional injection (Palmoplantar and periungual warts) while in our study intralesional in children is so difficult and painful (*Moubashera et al., 2016*).

In the study that used tuberculin (PPD) as topical jelly in treatment of common warts 57% showed complete

disappearance of their warts. The disappearance of warts usually occurred in the 3rd or 4th month. The strength of the tuberculin reactivity was not correlated with the disappearance of the warts. There were no side effects as pain and edema as seen with intralesional injection of PPD. The major disadvantage of topical tuberculin jelly was the long duration of treatment (*Pundir et al., 2019*).

Some studies used immunotherapy with different antigen injection; those antigens either single or multiple antigens. Single antigen using candida antigen with 85% clearance and slightly higher than 74% clearance, 72% clearance and with 51% clearance, Studies using single antigen either mumps or Candida antigens with 47% clearance. Other studies used combination of skin test antigens as mumps, candida and trichopyton with 70.9% clearance and with 53% clearance (*Nofal and Nofal, 2010*).

MMR vaccine was used in treatment of resistant warts either plantar warts as by *Gamil et al. (2010)* with 87% complete clearance, and common warts as by *Nofal and Nofal (2010)* with complete clearance in 80%.

The relatively different response in the present study compared to the other related studies which utilize either a single antigen or a combination of antigens may be attributed to the differences in the number of the studied patients, the duration and the resistance of warts may also explain this difference. The difference may be attributed also to number of sessions.

Immunotherapy with tuberculin PPD is well tolerated. No serious side effects were reported in patients included in the

present study. Only tolerable pain which was occurred in 30% during injection was the main side effect.

That responded to cold compression and topical corticosteroids Furthermore, none of our patients developed new lesions at the site of warts or at any distant site during the next 3 months following the procedure.

CONCLUSION

As regards the benefits to the patients, tuberculin PPD intradermal injection and MB-DL PDT has significant advantages over other treatments. Most treatment modalities are painful, needing multiple visits (time and money consuming), and are directed to each individual wart. The use of topical methylene blue and day light as a photodynamic therapy in the treatment of warts (plane warts) gave some results in plane warts. It was effective, cheap, available, and easy to use with minimal side effects. In immunotherapy treatments patients were able to resume normal daily activities and were free of residual scars which were very appreciated by all patients.

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مقارنة العلاج الضوئي النهاري مع الميثيلين الأزرق بالعلاج المناعي بواسطة لقاح التيوبركلين (بي بي دي) في علاج الثآليل المسطحة في الأطفال

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خلفية البحث: يسبب فيروس الورم الحليمى البشرى (غالباً الورم الحليمى ٢، ٣، ١٠) الثآليل المسطحة. وعادة تكون هذه الثآليل عديدة ومرتفعة قليلاً وملساء، وغالباً توجد على الوجه والأيدى والرقبة والقدمين فى الأطفال والشباب صغار السن.

الهدف من البحث: مقارنة فاعلية وأمان العلاج الضوئى النهاري باستخدام الميثيلين الأزرق بالعلاج المناعى باستخدام مشتقات البروتين النقى (بى بى دي) فى علاج الثآليل المسطحة فى الاطفال

المرضى وطرق البحث: تمت هذه الدراسة بقسم الجلدية والتناسلية بمستشفى الحسين الجامعى بجامعة الأزهر من شهر ابريل ٢٠١٩ حتى نوفمبر ٢٠١٩، وتضمنت ٤٠ طفلاً مصابون بالثآليل المسطحة تم اختيارهم عشوائياً قسموا عشوائياً إلى مجموعتين متساويتين: مجموعة العلاج الضوئى النهاري باستخدام الميثيلين الأزرق، ومجموعة العلاج المناعى باستخدام مشتقات البروتين النقى (بى بى دي).

نتائج البحث: حدث ارتفاع واضح فى مجموعة العلاج المناعى باستخدام مشتقات البروتين النقى (بى بى دي) مقارنة بمجموعة العلاج الضوئى النهاري باستخدام الميثيلين الأزرق، حيث أن معدل الشفاء التام كان ٦٠% مقارنة بـ ٣٠%، وإستجابة جزئية كانت ١٠% مقارنة بـ ٣٠%، وعدم استجابة كانت ٣٠% مقارنة بـ ٤٠%.

الاستنتاج: حقن مشتقات البروتين النقي (بى بى دى) داخل الجلد و ايضا العلاج الضوئى النهارى باستخدام المثيلين الأزرق لهما فوائد بارزة عن طرق العلاج الأخرى لأن غالبية الطرق العلاجية الأخرى مؤلمة وتحتاج إلى عدة جلسات (تكلفة مادية وإستهلاك للوقت)، وكذلك توجه إلى كل ثألول على حده. والعلاج المناعي باستخدام مشتقات البروتين النقى (بى بى دى) والعلاج الضوئى النهارى باستخدام المثيلين الأزرق يمكن استخدامهما فى علاج الثأليل المسطحة فى الأطفال.