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Topical Indocyanine Green Nanosystem For Photodynamic Therapy of Cutaneous Warts

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Abstract

Background: Photodynamic therapy was proven very promising for the treatment of dermatological diseases, especially the resistant ones such as recalcitrant cutaneous warts.

Objective: to evaluate the efficacy and safety of photodynamic treatment of cutaneous warts using ICG carried in a Nano system drug carrier and prepared in a topical form (gel) as a photosensitizer in combination with diode laser as a source of radiation.

Methods: the efficiency of the nano-vesicular system (transethosomes) as a potential topical drug delivery system for the photosensitizer indocyanine green (ICG) was investigated followed by clinical photodynamic therapy (PDT) in patients suffering from cutaneous warts.

Results: Results revealed that ICG transethosomal gel displayed curative effects which were excellent in 19 cases, good in 9 cases and poor in 2 cases only. It also shows that recurrence was in only one case out of the total 30 cases with highly significant difference of dermatology life quality index DLQI before and after total treatment sessions and the mean of degree of improvement was (4.90 ± 1.09).

Conclusion: PDT using transethosomal ICG is an effective and safe therapeutic modality for the treatment of cutaneous warts.

Keywords: Topical delivery, Indocyanine green, Transethosomes, Photodynamic therapy, cutaneous warts, Clinical.

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Introduction

Cutaneous warts are a typical human papillomavirus (HPV)-induced dermatological disease. To date, about 100 genotypes of HPV have been identified. Cutaneous warts vary widely, with an average prevalence of up to 22 % in the general population, depending on various age groups and populations [1].

HPV-associated warts, including common warts (verruca vulgaris), plantar warts (verruca plantaris), flat warts (verruca plana), periungual warts, and genital warts (condyloma accuminata), can be diagnosed based on anatomical position or morphology. Cutaneous warts cause not only cosmetic disfigurement but also functional and social concerns that require treatment [2].

Photodynamic therapy (PDT) is clinically safe, effective and less invasive therapeutic option for treatment of

various dermatologic conditions with good cosmetic results. Several researches have shown that PDT produces endogenous molecular oxygen which kills microbes and viruses [3].

Many published studies on the use of PDT in cutaneous warts have found an efficacy of 42- 90%. The early researches used ALA and found that white light was more effective than blue or red light. In order to achieve greater penetration, most studies use ALA as the photosensitizer with long wavelengths light sources and red surroundings [4]. One of the researches used a diode laser in treatment of flat warts and showed a very good response of 94% while another study used a pulsed dye laser in treatment of verruca vulgaris showed a more moderate response [4].

Pain with topical PDT using ALA or Methyl aminolevulinate (MAL) was significantly documented, ranging from stinging or burning sensations to extreme discomfort. The mechanism behind the occurrence of pain is not yet confirmed, and no standard pain relief protocol yet established [5].

Indocyanine Green (ICG) is a biocompatible dye that is commonly used in many medical fields. ICG is commonly used as a photosensitizer in photodynamic therapy and as a laser energy absorber in photothermal tumor therapy, as well as in laser welding and tissue repair due to its high absorption of powerful laser diodes wavelengths [6].

The therapeutic effectiveness of topical PDT depends significantly on the successful delivery of photosensitizers into skin. This is considered to be very difficult because of the stratum cornium, which act as a barrier against the penetration of the photosensitizers, mainly the hydrophilic ones [7].

The usage of ultradeformable lipid vesicles, such as transfersomes, niosomes, and ethosomes, has been emerged as an efficient technique to promote photosensitizer permeation and penetration throughout the skin [7].

Transethosomes are one type of ultradeformable lipid vesicles whose membrane consists of phosphatidylcholines, edge activator and ethanol. The presence of both edge activator and ethanol causes lipid membrane destabilization and fluidization. Transethosomes are thus characterized by high elasticity and high penetration ability through the stratum cornium without being aggregated or cracked [8].

ICG was previously loaded into transfersomes and used for topical PDT in basal cell carcinoma [5]. In this work, ICG was loaded in transethosomes, as an attempt to potentiate its effectiveness and stability in the PDT of cutaneous warts.

The present study was conducted to evaluate the efficacy and the safety of photodynamic therapy of cutaneous warts using ICG loaded in a Nano system drug carrier and prepared in a topical form (gel) as a photosensitizer with use of diode laser as a source of irradiation.

Materials and methods

Materials

ICG was purchased from (Acros, Belgium), while Soybean phosphatidylcholine (PC), Sodium deoxycholate (SDC), and chloroform were purchased from Sigma Aldrich. Carboxymethyl cellulose Na (Na-CMC) was purchased from Normest Company for scientific development, Egypt. Absolute ethanol (99%) and methanol were purchased from El Nasr Pharmaceutical Chemicals Co., Adwic, Egypt.

Preparation of transethosomes loaded by ICG

ICG-loaded transethosomes were prepared by the thin film hydratiom process, which consists of two major steps: thin film formation and hydration of the formed film. As stated in previous studies [9, 10], the thin film formation process was carried out.

In brief, with ratio of 10:1:1, phosphatidylcholines (PC), edge activator (SDC) and ICG is dissolved in a mixture of 2:1 chloroform to methanol. Following the evaporation of organic solvents, under vacuum at 45 ° C using a rotary evaporator (Heidolph-Elektro GmbH+Co KG, Germany), a thin film was obtained. Then the thin film was hydrated by phosphate buffered saline containing 40% absolute ethanol to provide the vesicles with flexibility and elasticity [8].

The obtained dispersion of the transethosomes loaded by ICG (Transetho-ICG) was sonicated to decrease the size of the vesicles, and stored for further use in the refrigerator.

Characterization of the prepared Transethosomes

The shape of Transetho-ICG was examined after negative staining under the transmission electron microscope (TEM, Jeol, Ltd., Tokyo, Japan). The particle size and surface charge (zeta potential) of the Transetho-ICG were measured using a zetasizer (Malvern Instruments Ltd., UK) laser diffraction technique after dilution of 1:100 using distilled water.

To calculate the encapsulation efficiency (E.E%), the unloaded ICG was separated from the Transetho-ICG dispersion by centrifugation at 7000 rpm for 30 min using cooling centrifuge (Centrikon T-42 K, Kontron Instruments, UK). The precipitated vesicles were re-dissolved using absolute ethanol.

The concentration of the ICG in the dispersion was determined using a spectrophotometer (Ray Leigh UV-2601, Beijing, China) to measure the absorbance at 788 nm, and the ICG concentration was derived from a previously constructed standard curve. Finally, the E.E was measured as a percent from the initial ICG concentration.

Under sink conditions, the in vitro release behavior of ICG from the prepared vesicles was tested using a piece of dialysis membrane (molecular weight cut off from 12,000 to 14,000). 1 ml of the Transetho-ICG was placed in the dialysis membrane and immersed in 100 ml of 10% ethanol-containing phosphate buffered saline and kept under continuous stirring at 37°C.

At determined time intervals, aliquots of 1 ml (n=3) were withdrawn and replaced by the fresh medium. The withdrawn aliquots were centrifugated and the supernatants were examined spectrophotometrically at 788 nm to determine the concentration of ICG.

Formulation of Transetho-ICG gel

For clinical applications, the prepared Transetho-ICG dispersion was incorporated into a hydrogel using Na-CMC (5%). Adequate volume of Transetho-ICG was diluted by 50 ml distilled water containing mixture of preservatives (0.1% Methyl paraben and 0.2%propylparaben). The total volume was completed to 100 ml distilled water, then 5gm of Na-CMC was added portion wise under continuous agitation till complete dispersion. The gel was left for 24 h for complete swelling and removal of air bubbles. The final concentration of the ICG in the gel was 0.05%.

Clinical study

Patients

According to the Declaration of Helsinki, this prospective, descriptive observational study was subjected to approval by the Ethics Committee of national institute of laser enhanced sciences – Cairo University with registration number (CU – NILES – 41/19), 30 patients complaining of multiple cutaneous warts of different types on different parts of their bodies were enrolled in the study. Patients were of Fitzpatrick skin types III and IV, with lesions that are resistant to other treatment modalities. Patients which received any treatment modality within the last 6 months and those with immunodeficiency (related to cancer chemotherapy, systemic corticosteroid therapy, genetic immunodeficiency, transplant status) were excluded. All subjects were given a written informed consent to be signed before their enrollment. Patients were treated and followed up in the outpatient dermatology clinic in the National Institute of Laser Enhanced Sciences.

Methods

All patients were subjected to full history recording including patient name, age, gender, address, telephone number, medical history, previous treatments, history of chronic diseases, medications history, and family history. Clinical examination and dermatological examination were performed for every patient including the lesions (warts) types, number, site and any associated signs, also dermoscopical examination was performed using (DermLite II hybrid m; 3Gen, LLC, San Jaun Capistran, USA).

Common warts dermoscopically display multiple densely packed papillae, each containing a central red dot or loop, which is surrounded by a whitish halo. Hemorrhages represent a possible additional feature, appearing as irregularly distributed small red to black tiny dots or streaks [11]. Dermoscopy of plantar warts typically reveals multiple prominent hemorrhages within a well-defined, yellowish papilliform surface in which skin lines are interrupted [12]. Dermoscopy of filiform warts shows the same features as common warts, with more prevalent papillae [13]. Cure is detected by disappearance of the previous features and appearance of normal skin markings.

Wart lesions were subjected to repeated weekly photodynamic therapy sessions using previously prepared ICG gel under occlusion for 45 minutes prior to exposure to 810 nm gallium-aluminum-arsenide diode laser therapy with power output of 200 mW for 15 minutes till clearance or for a maximum of 6 sessions and were followed up for any recurrence for 3 months after the last session of treatment.

The degree of improvement of cutaneous warts was evaluated clinically and dermoscopically at base line and 1 week after the last treatment session. The curative effect was graded as excellent (complete clearance), good (≥50% clearance), poor (< 50% clearance) and invalid [3]. Number of sessions and healing time were recorded for each lesion. Patient satisfaction was reported, dermatology life quality index (DLQI) was recorder before and after the end of treatment protocol. Any side effect was recorded including erythema and pain which assessed using pain analogue scale.

Clinical response and cosmetic outcome were evaluated with each session, after end of total treatment sessions and 3 months after end of treatment sessions and this assessment was performed by 2 independent doctors. DLQI score was used to indicate improvement of the patients' quality of life.

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric.

P-value > 0.05: Nonsignificant (NS); P-value < 0.05: Significant (S); P-value < 0.01: highly significant (HS).

Results

Characterization of of indocyanine green transethosomes for PDT

ICG was incorporated in the transethosomes membrane with high encapsulation efficiency of 60% ± 1.5. Transetho-ICG exhibited spherical shape as illustrated by TEM photo [Figure 1] with average particle size of 135 ±7 nm. Moreover, the prepared Transetho-ICG exhibited Zeta potential of -25, indicating high stability and low tendency for aggregation. The ICG was released from the Transetho-ICG in a controlled manner with initial rapid release of 20% after 2 h, then the release was sustained for 24h with % cumulative release of 70%.

Demographic and descriptive clinical data of the study sample:

This study included 30 patients with multiple cutaneous warts, they were 19 males (63.3%) and 11 females (36.7%). Their age range was from (5 – 55) years with a median of (28.50) as shown in **table (1)**.

All the selected patients presented with multiple cutaneous warts of different number (2-6) with mean (3.33 ± 1.52) and different types (common, filiform and plantar) located in different sites including scalp, ear, hand, foot, neck and face.

The duration of the disease ranged from 2 months to 24 months with a mean of (9.17 ± 4.65) as shown in **table (1).**

Clinical response:

The range of number of sessions for each lesion was from (3-6) sessions with mean of (4.90 ± 1.09).

Curative effects of treatment were excellent in 19 cases, good in 9 cases and poor in 2 cases only as shown in **table (2)**. [Figures 2-5].

Table (2) also shows that recurrence was in only one case out of the total 30 cases and it was a newly formed lesion in a different site.

This study showed that there is a highly significant difference

of dermatology life quality index DLQI before and after total treatment sessions and the mean of degree of improvement which was (4.90 ± 1.09) as shown in **table (3)** and that degree of improvement of dermatology life quality index was highly related to the curative effects of treatment as shown in table (4).

One Way ANOVA test

Among the total 100 lesions that were treated using the photodynamic therapy, 67 lesions were common warts, 22 were filiform warts and 11 were of plantar type as shown in **table (5)**

There was a significant difference between the curative effects of the therapy among the different types of the warts as the filiform type shows more response followed by plantar and common types.

Discussion

This study was conducted to evaluate whether ICG-PDT could be beneficial therapy for different types of cutaneous warts without causing severe side-effects.

ICG, is a nontoxic tricarbocyanine dye, that show little phototoxicity and has been widely used in fluorescence angiography and both liver and heart function tests [14]. ICG is easily absorbed and rapidly distributed throughout and excreted from the body [15].

The maximal absorption of ICG is around 800 nm, similar to the wavelength of a near infrared (NIR) diode laser, which is a U.S. Federal Drug Administration approved light source for PDT. Additionally, there is no need for photoprotection with ICG due to its NIR range [16].

In present study, transethosomes have been used to enhance the penetration of the photosensitizer across the skin, transethosomes were successfully prepared by thin film hydration method. The membranes of the formed vesicles were essentially made of lipid (PC). Edge activator (SDC) and ethanol were added to impart elasticity and flexibility to the vesicles, thus they can squeeze themselves and penetrate through the skin layers without losing their shape [8, 10]

Regarding the effect of indocyanie green photodynamic therapy on different types of cutaneous warts that have been studied in our study, curative effects of treatment were excellent in 19 cases, good in 9 cases and poor in 2 cases only. This was proven by the clinical assessment. Furthermore, the disappearance of black dots together with total restoration of the normal skin markings confirmed the cure of the lesions by dermoscope.

We hypothesize that the reason of this clinical effect is the direct phototoxic effects of ICG PDT on the target tissues as PDT has the ability to destroy infected keratinocytes and inactivate viral replication [17] , and the immune specific responses during PDT which include the production of various cytokines, such as interleukin interleukin-1β (IL-1β), IL-2, and tumor necrosis factor-α; matrix metalloproteinase-1 (MMP-1) and MMP-3 are also secreted by fibroblasts in response to PDT, resulting in immunomodulatory effects in skin disorders, including cutaneous warts [18].

This may be the main reason why ICG PDT can effectively cure cutaneous warts and reduce the recurrence rate, as the study shows that recurrence was in only one case out of the total 30 cases and the new lesion was in different site. Also, our study showed the highly significant difference of dermatology life quality index DLQI that has been achieved after total treatment sessions and the mean of degree of improvement was (4.90 ± 1.09). This improvement of DLQI is highly related to the curative effects of treatment and this relation is highly significant.

Results of the clinical study revealed that the ICG transethosomal gel applied at a therapeutic concentration of 0.05% presented a promising treatment modality and required short incubation period (45 min) compared to other previously used photosensitizers, also treatment program was completely safe for the patients, as manifested by the absence of swelling or erythema in irradiated areas. Furthermore, none of the patients reported significant pain directly after or long after the treatment session.

This was considered beneficial because standard photodynamic treatment protocols based on 5-aminolevulinic acid (ALA-PDT) showed that the patients reported pain leading to the discontinuation of the treatment sessions, in a study done by Guarino et al. [4], whom used methyl aminolevulinate (MAL) as a PS, incubation period was 3 h, and the sessions were painful. Another study conducted by Ohtsuki et al. [19] used topical 20% of 5-aminolevulinic acid (ALA) as a PS with incubation period 5 h and reported side effects of pain and erythema. In 2009, Chong and Kang [20] published a case report of clearance of recalcitrant acral viral wart using methylaminolevulinate-red light PDT with a long incubation period of 3 h, and they reported the presence of pain, erythema and bullae as treatment associated side effects.

To the most of our knowledge, there's neither published data on using ICG PDT for treatment of different cutaneous warts nor it's encapsulation on transethosomes.

This treatment modality proved a positive impact on the patients' quality of life. Patients were impressed by disappearance of social embarrassment, improvement of their social relations and feeling safe to deal with others with no fear about transmission of infection especially to their children.

Conclusion

Based on the present research findings, photodynamic therapy with the indocyanine green nanosystem can be considered as a promising therapeutic option for treatment of patients with multiple recalcitrant cutaneous warts as it is safe, highly effective with good curative effects and a low recurrence rate.

References

1. Leerunyakul K, Thammarucha S, Suchonwanit P, Rutnin S. A comprehensive review of treatment options for recalcitrant nongenital cutaneous warts. Journal of Dermatological Treatment. 2020 Mar 10:1-8.
2. Sterling JC, Gibbs S, Haque Hussain SS, et al. British Association of Dermatologists' guidelines for the management of cutaneous warts 2014. Br J Dermatol. 2014; 171:696-712.
3. Wu L, Chen W, Su J, Li F, Chen M, Zhu W, Chen X, Zhao S. Efficacy of the combination of superficial shaving with photodynamic therapy for recalcitrant periungual warts. Photodiagnosis and photodynamic therapy. 2019 Sep 1; 27:340-4.
4. Guarino MF, Harto A, Jaén P. Treatment of recalcitrant viral warts with photodynamic therapy with mal and red light. J. Cosmet., Dermatol. Sci. Appl. 2013 Mar 7; 3:117-20.
5. Fadel M, Samy N, Nasr M and Alyoussef A. Topical colloidal indocyanine green-mediated photodynamic therapy for treatment of basal cell carcinoma. Pharmaceutical Development and Technology 2016, 22(4): 545-550.
6. Genina EA, Kuzmina MY, Pankov SS, Bashkatov AN, Tuchin VV. In vitro study of indocyanine green solution interaction with skin. InSaratov Fall Meeting 2006: Optical Technologies in Biophysics and Medicine VIII 2007 Apr 26 (Vol. 6535, p. 65351H). International Society for Optics and Photonics.
7. Moolakkadath T, Aqil M, Ahad A, Imam SS, Iqbal B, Sultana Y, Mujeeb M, Iqbal Z. Development of transethosomes formulation for dermal fisetin delivery: Box–Behnken design, optimization, in vitro skin penetration, vesicles–skin interaction and dermatokinetic studies. Artificial cells, nanomedicine, and biotechnology. 2018 Nov 5;46(sup2):755-65.
8. Nayak D, Tawale RM, Aranjani JM, Tippavajhala VK. Formulation, Optimization and Evaluation of Novel

Ultra-deformable Vesicular Drug Delivery System for an Anti-fungal Drug. AAPS PharmSciTech. 2020 Jul; 21:1-10.
9. Fadeel DA, Al-Toukhy GM, Elsharif AM, Al-Jameel SS, Mohamed HH, Youssef TE. Improved photodynamic efficacy of thiophenyl sulfonated zinc phthalocyanine loaded in lipid nano-carriers for hepatocellular carcinoma cancer cells. Photodiagnosis and photodynamic therapy. 2018 Sep 1; 23:25-31.
10. Fadel M, Kassab K, Samy N, Abdelfadeel D, Yassin G, Nasr M. Nanovesicular Photodynamic Clinical Treatment of Resistant Plantar Warts. Current Drug Delivery. 2020.
11. Yoong C, Di Stefani A, Hofmann-Wellenhof R. Unusual clinical and dermoscopic presentation of a wart. Australas J Dermatol. 2009; 50: 228-229.
12. Aqil N, Nassiri A, Baybay H, Gallouj S, Sara E, Mernissi FZ. Warts under the dermoscope. SM Dermatolog J. 2019 Jun; 5(1):1030.
13. Piccolo V. Update on dermoscopy and infectious skin diseases. Dermatology Practical & Conceptual. 2020; 10(1).
14. Malicka J, Gryczynski I, Geddes CD et al. Metal-enhanced emission from indocyanine green: a new approach to in vivo imaging. J Biomed Opt 2003; 8:472–8.
15. Genina EA, Bashkatov AN, Sinichkin YP et al. In vitro and in vivo study of dye diffusion into the human skin and hair follicles. J Biomed Opt 2002; 7:471–7.
16. Jang MS, Doh KS, Kang JS, Jeon YS, Suh KS, Kim ST. A comparative split-face study of photodynamic therapy with indocyanine green and indole-3-acetic acid for the treatment of acne vulgaris. British Journal of Dermatology. 2011 Nov; 165(5):1095-100.
17. Ibrahim NA, Fadeel DA, Sadek A, Fadel M, Tawfik A. Intralesional vitamin D3 versus new topical photodynamic therapy in recalcitrant palmoplanter warts Randomized comparative controlled study. Photodiagnosis and Photodynamic Therapy. 2020 Aug 28:101979.
18. Huang K, Xie Y, Li M, Liu D, Su J, Li F, Deng B, Li J, Shi W, Zhang J, Chen X. A comparative study: superficial shaving combined with photodynamic therapy versus cryotherapy in the treatment of recalcitrant plantar warts. Lasers in Surgery and Medicine. 2020 Jan 10.
19. Ohtsuki A, Hasegawa T, Hirasawa Y, Tsuchihashi H, Ikeda S. Photodynamic therapy using light-emitting diodes for the treatment of viral warts. The Journal of dermatology. 2009 Oct; 36(10):525-8.
20. Chong WS, Kang GY. Dramatic clearance of a recalcitrant acral viral wart using methyl aminolevulinate-red light photodynamic therapy. Photodermatology, photoimmunology & photomedicine. 2009 Aug; 25(4):225-6.

Figures and Tables

Figure 1. TEM photo of Transetho-ICG showing spherical vesicles with dark membrane due to the presence of ICG (X 2500)

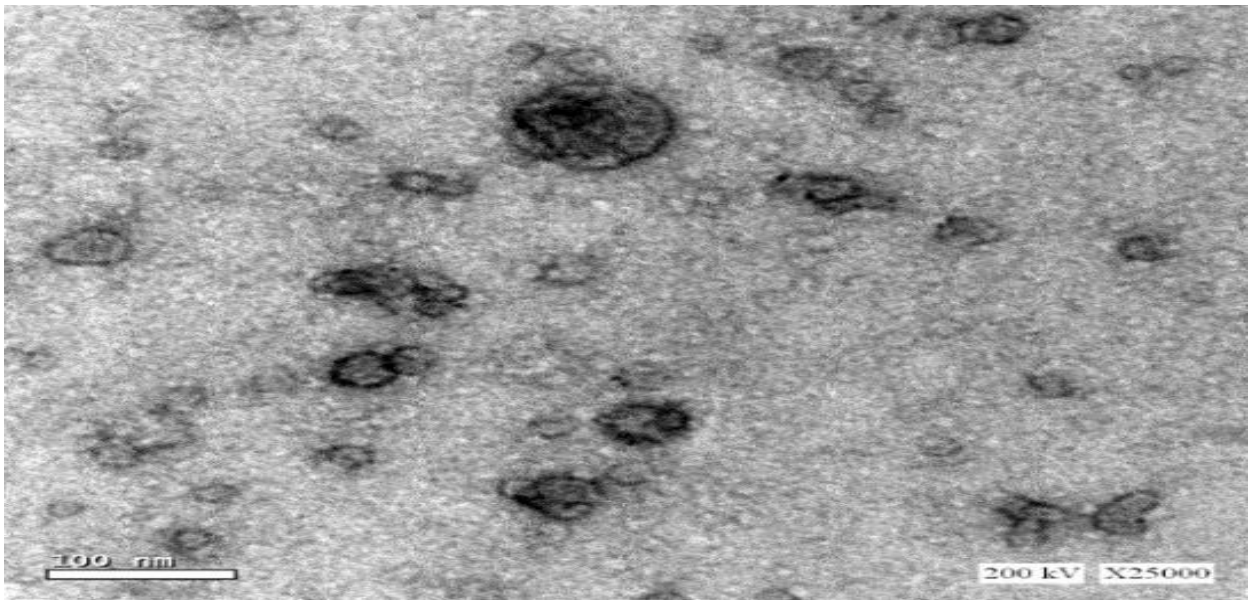
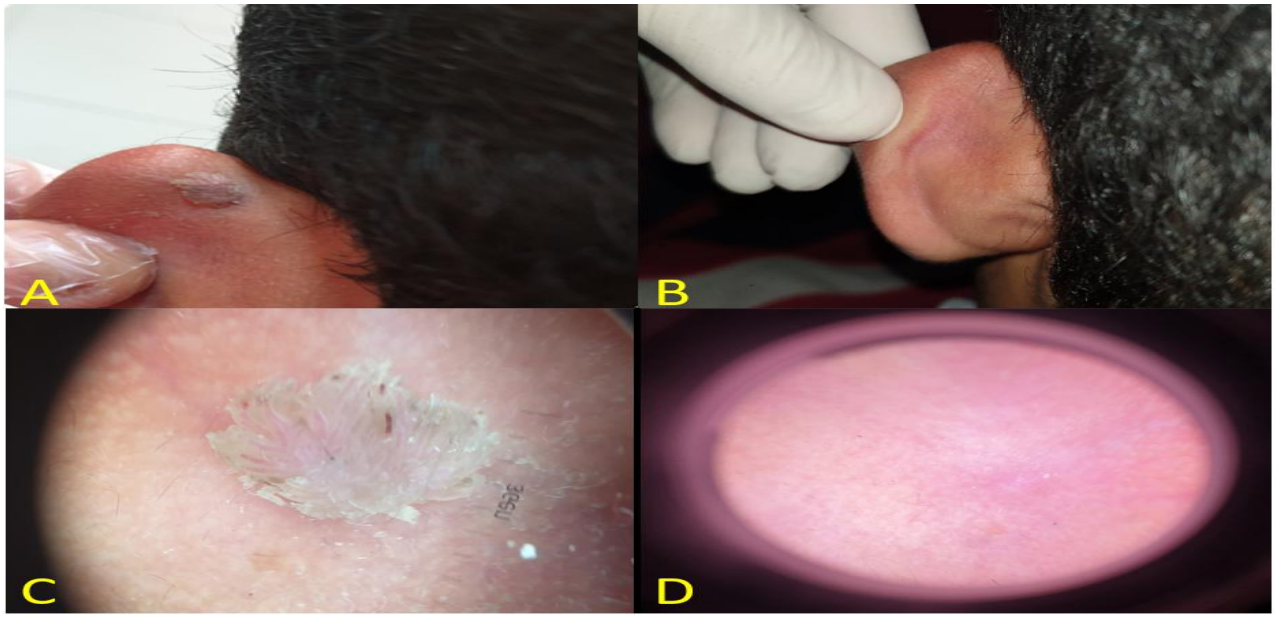


Figure 2. Cutaneous wart on the left ear **A**- before starting therapy **B**- 1 week after completing treatment sessions **C**-



dermoscopical features before treatment **D**- dermoscopical features after completing treatment sessions

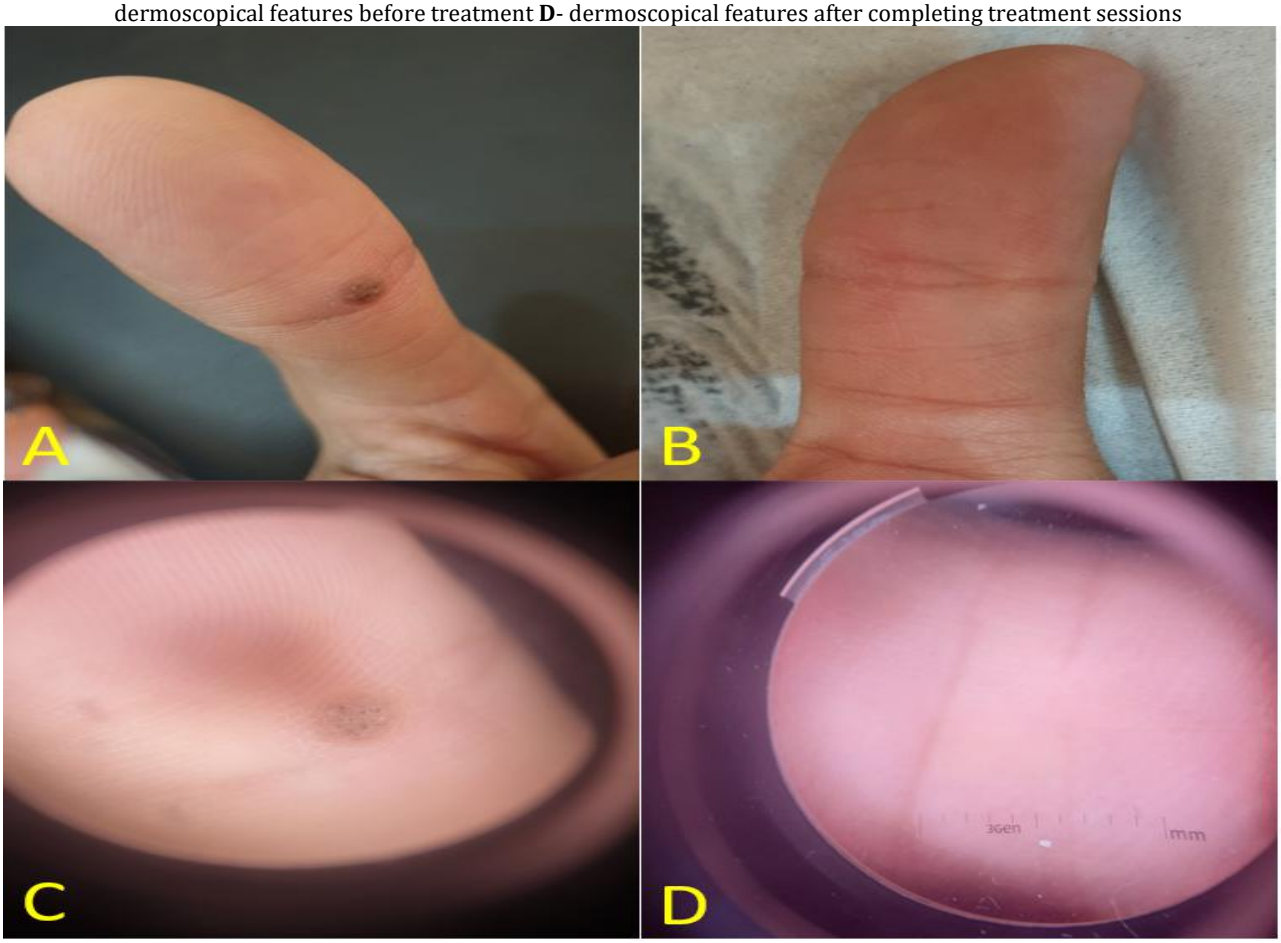


Figure 3. Cutaneous wart on the scalp **A**- before starting therapy **B**- 1 week after completing treatment sessions **C**-dermoscopical features before treatment **D**- dermoscopical features after completing treatment sessions

Figure 4. Cutaneous wart on the thumb of right-hand **A**- before starting therapy **B**- 1 week after completing treatment

sessions **C**-dermoscopical features before treatment **D**- dermoscopical features after completing treatment sessions

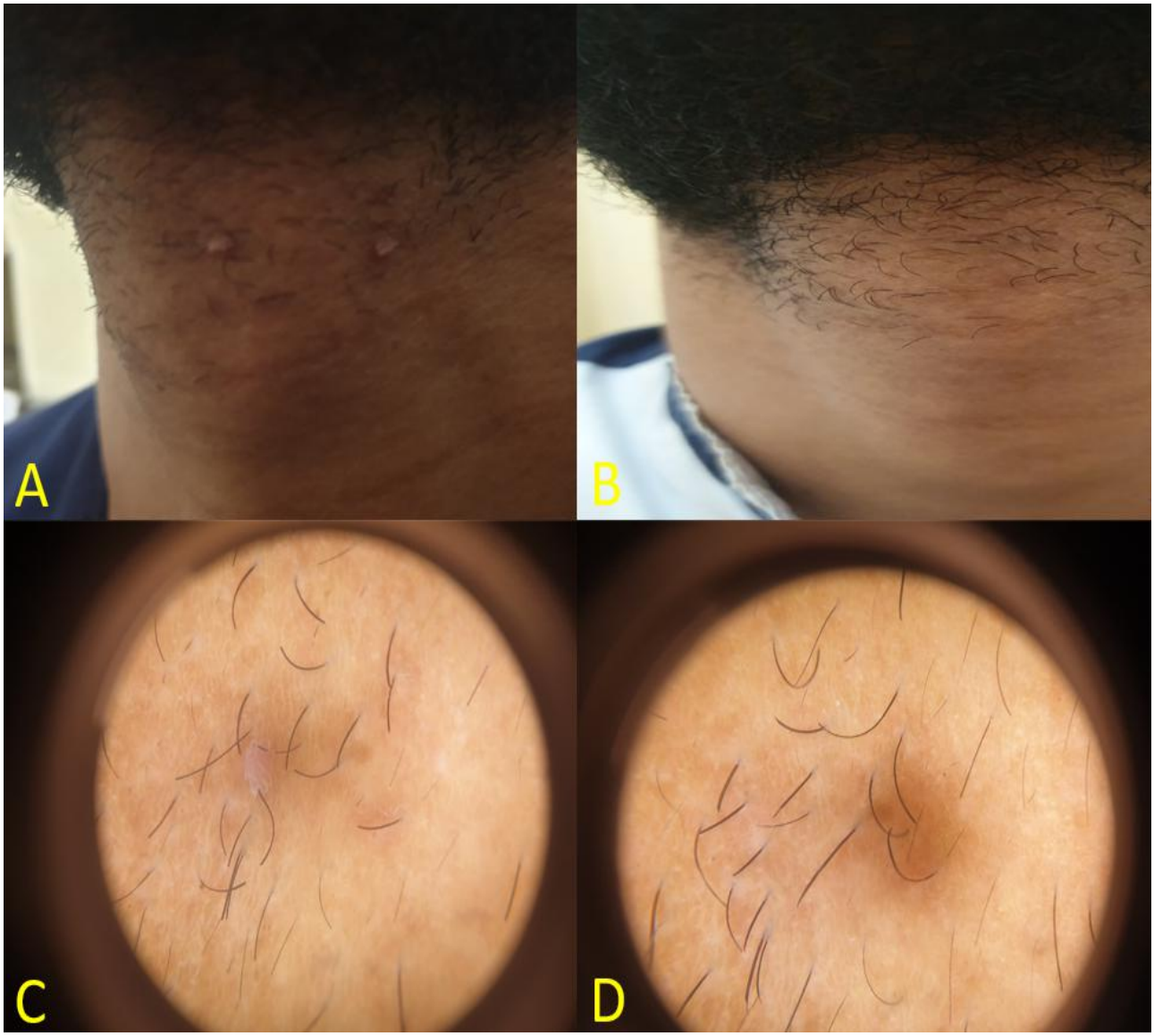


Figure 5. Cutaneous wart on the neck **A**- before starting therapy **B**- 1 week after completing treatment sessions **C**- dermoscopical features before treatment **D**- dermoscopical features after completing treatment sessions

Table 1. Demographic data of the study sample:

		No. = 30
Age	Median (IQR)	28.50 (20 - 39)
	Range	5 – 55
Gender	Female	11 (36.7%)
	Male	19 (63.3%)
No. of lesions	Mean ± SD	3.33 ± 1.52
	Range	2 – 6
Type	Common	21 (70.0%)
	Filiform	8 (26.7%)
	Plantar	5 (16.7%)
Site	Ear	1 (3.3%)
	Hand	16 (53.3%)
	Foot	4 (13.3%)
	Hand & Foot	1 (3.3%)
	Scalp	6 (20.0%)
	Neck	1 (3.3%)
	Face	1 (3.3%)
Duration of disease (months)	Mean ± SD	9.17 ± 4.65
	Range	2 – 24

Table 2. Treatment protocol and curative effects for cases under study

	No. = 30

No. of sessions	Mean ± SD	4.90 ± 1.09
	Range	3 – 6
Healing time (days)	Mean ± SD	34.30 ± 7.66
	Range	21 – 42
Curative effects	Poor	2 (6.7%)
	Good	9 (30.0%)
	Excellent	19 (63.3%)
Recurrence	Negative	29 (96.7%)
	Positive	1 (3.3%)

Table 3. Dermatology life quality index before and after treatment with degree of improvement

DLQI		**No. = 50**
Before	Mean±SD	19.50 ± 2.52
	Range	15 – 25
After	Mean±SD	5.43 ± 3.26
	Range	1 – 14
Degree of improvement (%)	Mean±SD	71.71 ± 17.23
	Range	22.22 – 95.83
Paired t-test		**18.670**
P-value		**<0.001 (HS)**

P-value >0.05: Nonsignificant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

Table 4. Degree of improvement of dermatology life quality index in relation to the curative effects of treatment

		Curative effects			**Test value•**	**P-value**	**Sig.**
		Poor	**Good**	**Excellent**			
		No. = 2	**No. = 9**	**No. = 19**			
DLQI (Before)	Mean±SD	19.00 ± 1.41	19.67 ± 2.74	19.47 ± 2.59	0.056	0.945	NS
	Range	18 – 20	15 – 24	15 – 25			
DLQI (After)	Mean±SD	13.00 ± 1.41	7.89 ± 2.03	3.47 ± 1.31	49.729	0.000	HS
	Range	12 – 14	4 – 10	1 – 6			
Degree of improvement (%)	Mean±SD	31.11 ± 12.57	59.39 ± 10.97	81.81 ± 7.02	44.375	0.000	HS
	Range	22.22 – 40	46.67 – 80	72.22 – 95.83			
Paired t-test		**3.000**	**11.348**	**23.392**			
P-value		**0.205 (NS)**	**<0.001 (HS)**	**<0.001 (HS)**			

P-value >0.05: Nonsignificant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

Table 5. Comparison among types of all (100) lesions regarding the curative effects

Curative effects	**Common**		**Filiform**		**Plantar**		**Test value**	**P-value**	**Sig.**
	No.	**%**	**No.**	**%**	**No.**	**%**			
Poor	9	13.4%	0	0.0%	0	0.0%	12.301	0.015	**S**
Good	23	34.4%	2	9.1%	4	36.4%			
Excellent	35	52.2%	20	90.9%	7	63.6%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS) *: Chi-square test