

Utility of dermoscopy for evaluating the therapeutic efficacy of tacrolimus ointment plus 308-nm excimer laser combination therapy in localized vitiligo patients

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Abstract. The aim of the present study was to assess the function of dermoscopy in evaluating the therapeutic efficacy of tacrolimus ointment plus 308-nm excimer laser combination therapy in patients with localized vitiligo. A total of 147 patients with localized vitiligo (progressive disease, n=92; stable period, n=55) were enrolled and received combination therapy for 12 weeks. The condition of the skin lesions was monitored by dermoscopy and visual observation. At the initial visit, skin lesions were observed in 61 progressive and 19 stable patients. Residual perifollicular pigmentation was more abundant in progressive-stage patients than in stable-stage patients, whereas the presence of perilesional hyperpigmentation was obviously lower in patients with progressive vitiligo. After 12 weeks of combination therapy, marked differences in residual perifollicular pigmentation were identified between the progressive- and stable-stage patients. Dermoscopy and visual observation indicated that the 12-week treatment efficacy in patients with progressive disease was significantly higher than in those with stable disease and that assessment by dermoscopy was superior to visual observation at 8 or 12 weeks of treatment. Binary logistic regression analysis revealed that the disease stage, vitiliginous areas and disease course were risk factors associated with the treatment efficacy of the combination therapy. In conclusion, dermoscopy may be used as an effective means of vitiligo therapy assessment to provide an accurate and scientific evaluation of treatment efficacy for localized vitiligo patients.

Introduction

Vitiligo, an acquired, idiopathic disorder, is a long-term skin disease featuring melanocytic destruction and depigmented

maculae and/or patches in the skin. The patches of affected skin become white and usually have sharp margins (1,2). The incidence of vitiligo is 0.1-2% worldwide (3) and may be as high as 2-3% in certain populations, while females and males are equally affected (4,5). The pathogenesis of vitiligo has remained to be fully elucidated but appears to be associated with the interaction of genetic susceptibility as well as neurological and immunological risk factors (6,7). The course of vitiligo is unpredictable but is often progressive, which may have a severe impact on the quality of life of patients and result in marked emotional stress (8,9). Several treatments are available for vitiligo patients, including applied steroids, ultraviolet light and phototherapy (10,11). Due to the large body surface area affected, patients with extensive depigmentation may receive treatment with phototherapy, including ultraviolet A radiation, narrowband ultra-violet B radiation (NBUVB) and excimer laser (12-14).

The 308-nm excimer laser is a relatively novel treatment option, which has been reported to increase the precision and the capability of delivering higher-energy fluencies to the lesions in less time without affecting uninvolved skin (15,16). Of note, the wavelengths of the excimer laser and NBUVB are in close proximity (308 and 311 nm, respectively), indicating similar therapeutic effects (17); however, several studies demonstrated that in adult as well as in pediatric vitiligo patients, the effectiveness and safety of 308-nm excimer laser phototherapy are well established, with safety likely superior to that of NBUVB phototherapy (18-20), which may be explained by the ability of the excimer laser to emit coherent short pulses, as well as differences in phototherapeutic parameters, such as impulse frequency and intensity, thereby stimulating a deeper reservoir of melanocytes in hair follicles (17). Tacrolimus, a macrolide immunosuppressant from the fungus *Streptomyces tsukubaensis*, is a novel treatment for vitiligo (21). Recent studies have demonstrated that combination therapy with the 308-nm excimer laser and topical tacrolimus is more effective than either treatment alone (22,23).

As is known, digital imaging is a useful method for assessing vitiligo (24). Compared with conventional clinical digital imaging, dermoscopy, a non-invasive skin imaging technique, facilitates the visualization of the subsurface structures of the skin using optical magnification, liquid immersion or cross-polarized lighting (25). In addition, dermoscopy allows for visualization of morphological structures of the epidermis,

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which may improve the analysis of the clinicopathological features of pigmented skin lesions (26). A previous study indicated that dermoscopy had 89.6% sensitivity for malignant lesions (tested on 68 melanomas and 9 pigmented basal cell carcinomas) compared with 69.7% sensitivity by the observation using the naked eye (27). However, due to the complexity of patterns and interpretation, dermoscopy examination results also have limitations (25). In the present study, an analysis to assess the effectiveness of dermoscopy in evaluating the therapeutic efficacy of combination therapy with tacrolimus ointment and 308-nm excimer laser in vitiligo patients was performed.

Materials and methods

Study subjects. Between October 2015 and October 2016, 147 eligible patients treated at Dong Guan People's Hospital (Dongguan, China) were enrolled as study subjects. All patients were diagnosed with localized vitiligo based on the diagnostic criteria of chloasma and vitiligo established by the Pigmented Dermatoses Group of the Dermatoses Committee of the China Society of Integrated Traditional Chinese and Western Medicine in 2010 (28). Of the 147 patients, 87 were male and 60 were female; the age of the patients ranged from 4 to 58 years (average age, 32.11 ± 16.26 years), and the course of the disease ranged from 2 weeks to 10 years. A total of 92 vitiligo patients were in the progressive stage (57 males and 35 females; age range, 4-56 years; average age, 29.34 ± 12.07 years), and the vitiliginous areas covered 0.6-10% of the body surface (average, $5.37 \pm 2.08\%$). The other 55 vitiligo patients were in the stable stage (30 males and 25 females; age range, 7-58 years; average age, 31.98 ± 16.77 years), and the vitiliginous areas covered 2.7-9% of the body surface (average, $5.25 \pm 1.73\%$). Vitiliginous areas were mainly present on the face, neck and torso. Among the 92 progressive-stage patients, vitiliginous areas were present on the face in 33, on the neck in 18 and on the torso in 41. Among the 55 stable-stage patients, vitiliginous areas were present on the face in 18, on the neck in 13 and on the torso in 24. No significant differences in age, sex, proportion of vitiliginous area or the location of vitiliginous areas were observed between the progressive-stage and stable-stage patients (all $P > 0.05$; Table I). Stable vitiligo was defined as static lesions of vitiligo without any new lesions or extension of previously existing lesions occurring over the past six months. By contrast, progressive vitiligo was defined as the recent appearance of new lesions or enlargement of existing lesions. Patients selected for inclusion had localized vitiligo and a vitiliginous area of $<10\%$ of the body surface. Patients were excluded if they had: i) Segmental or generalized vitiligo; ii) skin lesions combined with other skin diseases, or scar diathesis; iii) poor compliance or a history of allergy to ultraviolet rays or tacrolimus; iv) heart, liver or kidney photosensitization, or autoimmune diseases; v) a history of cataract or glaucoma; vi) had received any systemic or local therapy over the past 2 months; or vii) were pregnant or lactating if females. All subjects or their family members signed an informed consent form including publication of the data and figures, and the experiment was performed with the approval of the ethics committee of Dong Guan People's Hospital (Dongguan, China).

Treatment schedule. All patients received laser therapy twice weekly combined with external tacrolimus ointment twice daily for 12 weeks. The laser used was a 308-nm xenon chloride excimer laser (HONKON-308VAL; Beijing Honkon Technologies Co., Ltd., Beijing, China). The fixed technical variables were as follows: Laser wave length, 308 ± 2 nm; wave crest, 308 nm; single pulse energy, 3 mJ/cm^2 ; pulse frequency, 200 Hz; power density, 50 mw/cm^2 ; light output window of therapy area, 50×60 , 30×50 , 30×40 and $20 \times 30 \text{ mm}^2$; diameter of the spot sizes, 10 mm; and time of therapy, 1-99 sec. First, the minimal erythema dose (MED) of each subject was assessed and a suitable light output window was selected based on the lesion condition, with an initial dose of 1-2 times the MED. Care was taken to avoid exposing normal skin to the light output window. The initial dose was decreased by 1 MED for lesions in wrinkled skin and the area around the ankle joint but increased by 1 MED for patients with normal, darker and thicker skin. Each dose was increased by 15-25% of the previous dose until the maximum safe dose for treatment was reached (3 J/cm^2 for the head, face and neck and 4.5 J/cm^2 for the torso). If local erythema persisted, the dose was reduced 10-20% from the previous dose; if a painful erythematous skin lesion or blister appeared, laser treatment was discontinued one or two times. Tacrolimus ointment (0.1% for adults or 0.03% for children; Astellas Pharma Tech, Toyama, Japan) was applied and patients returned for visits once a week.

Dermoscopy monitoring. Dermoscopy was performed using an electron microscope (Dr Camscope; Somatech Inc., Seoul, Korea) with a x30 lens via the immersion dermoscopy method. Dermoscopy of skin lesions of all patients was performed by skilled professional personnel. For each patient, one vitiliginous area was selected to observe the condition of the lesion and fully exposed lesions underwent dermoscopy at the initial visit. To avoid omitting detailed information on the lesions, the physician carefully scanned and observed the lesions of the patients from top to bottom and from left to right. After 4, 8 and 12 weeks of treatment, dermoscopy of the same skin lesions was performed by the same physician. The information on the observed lesions at the initial visit of each patient was carefully recorded to ensure that follow-up dermoscopy monitoring was performed in the same vitiliginous area.

Assessment of efficacy and adverse reactions. The therapeutic effect index was determined as follows: i) Residual perifollicular pigmentation: The pilosebaceous orifice in the vitiliginous areas and the surrounding non-interrupted pigmentation had a homogeneous appearance with a single diffuse distribution usually seen in peripheral lesions compared with central lesions. Observation of one or multi-residual perifollicular pigmentation areas by dermoscopy was evaluated as 'yes'; by contrast, absence of residual perifollicular pigmentation or observation of typical pigment residue was rated as 'no'. ii) Angiotelectasis: Punctate, linear or mesh angiotelectasis was identified in skin lesions. iii) Early formation of pigmentation islands: The occurrence of black and hair follicle pigmentary units is difficult to observe with the naked eye, and mutual integration of the two residual perifollicular pigmentations may indicate early signs of recovery. Pigmentation islands contain two main forms, the marginal type and the central

Table I. Comparison of general data between patients with progressive-stage and stable-period vitiligo at baseline.

Parameter	Progressive stage (n=92)	Stable period (n=55)	χ^2/t	P-value
Sex			0.783	0.376
Male	57 (61.96)	30 (54.55)		
Female	35 (38.04)	25 (45.45)		
Age (years)	32.18±16.05 (3-56)	31.98±16.77 (5-58)	0.072	0.943
Vitiliginous area (%)	5.37±2.08 (0.5-10)	5.25±1.73 (2.5-9)	0.569	0.572
Location of vitiliginous areas			0.375	0.829
Face	33 (35.86)	18 (32.73)		
Neck	18 (19.57)	13 (23.64)		
Torso	41 (44.57)	24 (43.63)		

Values are expressed as the mean ± standard deviation (range) or as n (%).

type, which refer to the occurrence of pigmentation islands in the normal skin at the edge of white spots and at the center of white spots, respectively. iv) Perilesional hyperpigmentation: Darker pigmentation was observed around the skin lesions. The evaluation criteria for dermoscopy efficacy were as follows: Dermoscopy images from the initial visit were compared with those obtained after 4, 8 and 12 weeks of treatment to evaluate the efficacy by referring to standards of clinical efficacy with the naked eye. The standards of clinical efficacy were examined by the naked eye (28): i) Fading away of all vitiligo and return to normal skin color was regarded as a 'cure'; ii) partial fading or reduction of vitiliginous areas and return of >50% of skin lesions to normal skin color was regarded as 'effective'; iii) partial fading or reduction of vitiliginous areas was regarded as an 'improvement'; iv) no pigment regeneration in vitiliginous areas or larger vitiliginous areas was regarded as 'ineffective'. The effective rate was determined as follows: Effective rate=[(n_{cure}+n_{effective})/n_{total}] \times 100%, and the efficacy was calculated as follows: Efficacy=[(n_{cure}+n_{effective}+n_{improvement})/n_{total}] \times 100%.

Statistical analysis. Data were evaluated by SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Differences in measurement data between groups were compared with Student's t-test, and comparison of the effective rate and further parameters between the two groups was performed using the χ^2 test. Binary logistic regression analysis identified risk factors associated with the combination therapy of tacrolimus ointment with 308-nm excimer laser for vitiligo patients. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Observation results for dermoscopy on vitiligo patients in different stages. The observation results by dermoscopy revealed characteristics of vitiligo skin lesions in 61 patients in the progressive-stage group and 19 patients in the stable-stage group. More progressive-stage patients than stable-stage patients had residual perifollicular pigmentation ($\chi^2=23.180$, $P<0.001$), while the presence of perilesional hyperpigmentation was obviously lower in patients with progressive vitiligo

than in those with stable vitiligo ($\chi^2=32.780$, $P<0.001$). No significant differences were observed in angioectasis or the early formation of pigmentation islands between the progressive- and stable-stage vitiligo patients (both $P>0.05$; Table II).

Dermoscopy observation results of skin lesions after combination therapy with tacrolimus ointment and 308-nm excimer laser. Fig. 1A and B display representative the visual observation results for skin lesions in a vitiligo patient at the initial visit and after 12 weeks of combination therapy with tacrolimus ointment and 308-nm excimer laser, and the dermoscopy results at the same time-points are presented in Fig. 1C and D. Perilesional hyperpigmentation, angioectasis and early reservoirs of pigmentation may be considered as the most important dermoscopic features of vitiligo.

In terms of patients treated with topical tacrolimus, angioectasis was not observed on the face, but on the body. After 4, 8 and 12 weeks of combination therapy, skin lesions were found in 57, 36 and 26 patients with the progressive vitiligo, and in 18, 12 and 11 cases with stable vitiligo, respectively (Table III). After 4 weeks of combination therapy, the occurrence of residual perifollicular pigmentation, angioectasis and early reservoirs of pigmentation were significantly higher in the progressive-stage vitiligo patients than in those in the stable stage (all $P<0.001$). In addition, more patients in the progressive-stage group had residual perifollicular pigmentation and angioectasis than those in the stable-stage group after 8 weeks (all $P<0.05$). Furthermore, at 12 weeks, a significant difference in the presence of residual perifollicular pigmentation between the progressive- and stable-stage patients was observed ($P=0.025$).

Evaluation of the efficiency of combination therapy with tacrolimus ointment and 308-nm excimer laser by dermoscopy. Eight patients presented with erythema and a burning sensation, but these symptoms did not affect the combination therapy, and no medical treatments were administered. The efficiency of the combination therapy at 4 and 8 weeks did not significantly differ between the progressive-stage and stable-stage groups when assessed by either visual observation or dermoscopy (all $P>0.05$). At 12 weeks, the efficiency of combination

Table II. Dermoscopy observation results of patients with progressive-stage and stable-period vitiligo at baseline.

Description of skin lesions	Progressive stage (n=92)	Stable period (n=55)	χ^2	P-value
Residual perifollicular pigmentation				
Yes	56 (60.87)	11 (20.00)	23.180	<0.001
No	36 (39.13)	44 (80.00)		
Angiotelectasis				
Yes	5 (5.43)	2 (3.64)	0.246	0.620
No	87 (94.57)	53 (96.36)		
Early formation of pigmentation islands				
Yes	2 (2.17)	1 (1.82)	0.022	0.883
No	90 (97.83)	54 (98.18)		
Perilesional hyperpigmentation				
Yes	1 (1.09)	19 (34.55)	32.780	<0.001
No	91 (98.91)	36 (65.45)		

Values are expressed as n (%).

therapy in the progressive-stage group was markedly higher than that in the stable-stage group as assessed by direct visual and dermoscopy observation (dermoscopy, 92.39 vs. 80.00%; visual, 76.09 vs. 60.00%; both $P<0.05$). After 8 and 12 weeks of combination therapy, the efficiencies in progressive-stage patients assessed by dermoscopy observation were significantly higher than those determined by visual observation (8 weeks, 51.09 vs. 35.87%; 12 weeks, 92.39 vs. 76.09%; both $P<0.05$), and the efficiencies in stable-stage patients assessed by dermoscopy observation were significantly higher than those determined by visual observation at 12 weeks (80.00 vs. 60.00%, $P<0.05$), as illustrated in Fig. 2.

Multivariate binary logistic regression analysis. Multivariate binary logistic regression analysis was performed with the efficiency of dermoscopy observation (effective or non-effective) as a dependent variable, and with the disease stage, age, sex as well as other parameters that affect efficiency as independent variables (Table IV). The results demonstrated that the disease stage, vitiliginous area and course of disease were risk factors associated with the treatment efficacy of tacrolimus ointment with 308-nm excimer laser (all $P<0.05$).

Discussion

Vitiligo is a common depigmentation disorder; its pathogenesis has remained to be fully elucidated, while the major theories include autoimmunity and toxicity in melanocytes mediated by oxidative stress (29,30). The efficacy of previous treatment options for vitiligo is not significant, particularly for leukoplakia on the exposed parts of the face and neck, which may induce great psychological stress in vitiligo patients (8). Kim *et al* (31) demonstrated that excimer laser treatment of vitiligo patients with leukotrichia had a poor clinical outcome of compared with those without leukotrichia. In this regard, early diagnosis and treatment as well as timely monitoring of affected patients may help prevent

the development and progression of the disease. However, in clinical practice, it is difficult to distinguish evolving vitiligo lesions from hypopigmentation or depigmentation induced by other causes. The present study performed an analysis to evaluate the function of dermoscopy in the evaluation of the therapeutic efficacy of combination therapy with tacrolimus ointment and 308-nm excimer laser in vitiligo patients. It was determined that dermoscopy may be used as an effective means of vitiligo therapy outcome assessment to provide an accurate and scientific evaluation of the treatment efficacy. As a noninvasive observation method, dermoscopy may detect subtle changes in the structure of the skin surface and lower epidermis as well as skin changes that cannot be observed by the naked eye (32). At present, dermoscopic examination is widely used in the diagnosis and differentiation of depigmentation disorders. Previous studies have demonstrated that examination by dermoscopy may effectively detect subtle changes in pigmentation, which may be helpful in the early diagnosis of vitiligo patients (33,34). In the present study, dermoscopy to was applied detect the recovery of pigmentation after combination therapy in vitiligo patients at different stages under continuous observation. The most important result was that, compared with the stable-stage patients, more progressive-stage patients had residual perifollicular pigmentation, while fewer patients had perilesional hyperpigmentation. In accordance with the present findings, a previous study by Meng *et al* (35) reported that 91.9% of progressive and 62.9% of stable vitiligo patients presented with residual perifollicular pigmentation. Furthermore, to the best of our knowledge, the present study was the first to demonstrate that after 12 weeks of combination therapy, marked differences in the frequency of residual perifollicular pigmentation were present between the progressive- and stable- stage patients. A previous study indicated that, compared with stable patients, progressive-stage vitiligo patients had increased CD4⁺ T cells and decreased CD8⁺ T cells, which indicated that a greater T-cell imbalance may be present in vitiligo patients in the

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Table IV. Multivariate logistic regression analysis of the influencing factors for the treatment efficacy of tacrolimus ointment with a 308-nm excimer laser.

Variable	B	SE	Wald	df	P-value	Exp (B)	95% CI
Group (Stable vs. Progressive)	-3.075	1.087	8.004	1	0.005	0.046	0.005-0.389
Sex (Female vs. Male)	-1.614	1.044	2.390	1	0.122	0.199	0.026-1.541
Age	0.053	0.028	3.445	1	0.063	1.054	0.997-1.115
Vitiliginous area	-1.370	0.398	11.866	1	0.001	0.254	0.117-0.554
Course of disease	-0.010	0.005	3.971	1	0.046	0.990	0.980-1.000
Location of vitiliginous areas			2.778	2	0.249		
Face vs. torso	1.043	1.084	0.926	1	0.336	2.838	0.339-23.762
Neck vs. torso	-0.937	1.118	0.703	1	0.402	0.392	0.044-3.503

B, regression coefficient; SE, standard error; Wald, χ^2 value; df, degrees of freedom; Exp (B), odds ratio; 95% CI, 95% confidence interval.

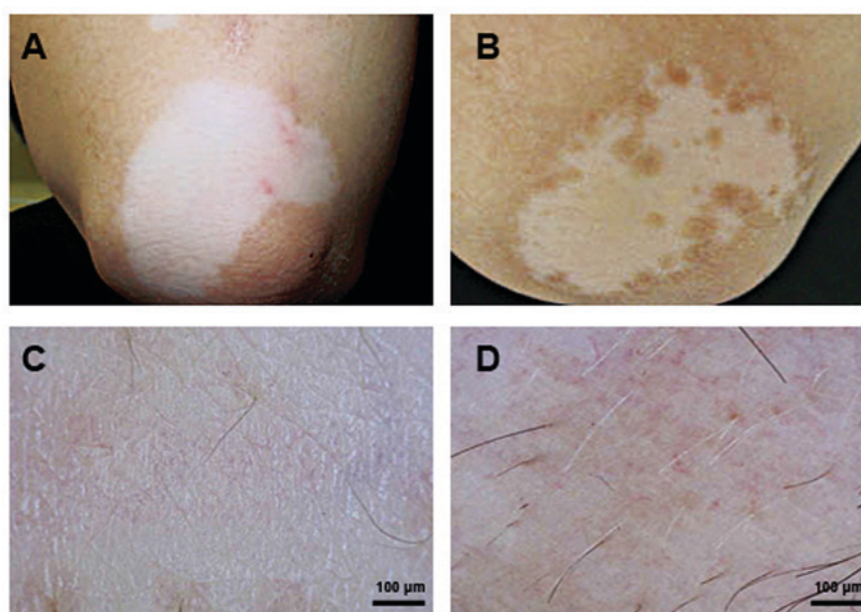


Figure 1. A 23-year-old woman presented with localized vitiligo on the left elbow. After 12 weeks of combination therapy (tacrolimus ointment and 308-nm excimer laser), the vitiliginous area showed a residual perifollicular pigmentation. (A and B) Images of skin lesions subjected to visual observation (A) at the initial visit and (B) after combination therapy for 12 weeks. (C and D) Dermoscopy images (C) at the initial visit and (D) after combination therapy for 12 weeks (magnification, x200; scale bar, 100 μ m).

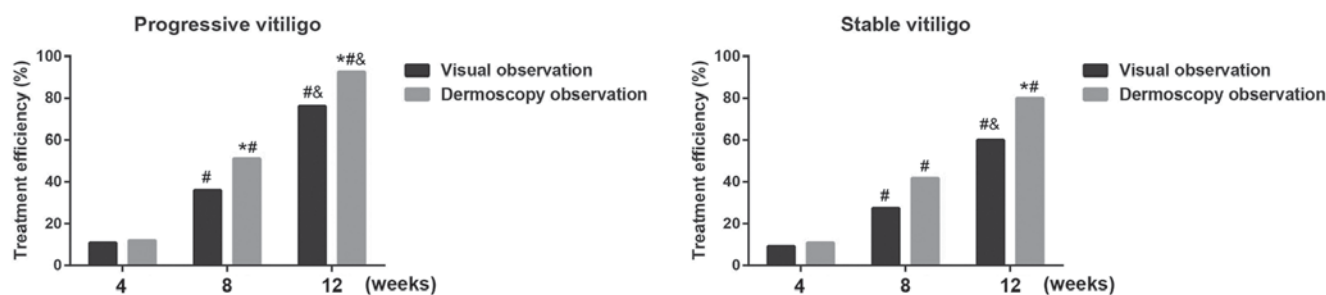


Figure 2. Comparison of the efficiency of combination therapy with tacrolimus ointment plus 308-nm excimer laser as observed by dermoscopy and visual observation. * $P < 0.05$ compared with visual observation; # $P < 0.05$ compared with dermoscopy and visual observation at 4 weeks; & $P < 0.05$ compared with dermoscopy and visual observation at 8 weeks.

progressive stage (36), leading to disorders of the functional immune response (17). As is known, tacrolimus ointment and

308-nm excimer laser therapies are associated with significant changes in immune cells. Cather *et al* (37) reported that

tacrolimus had the ability to inhibit T-lymphocyte activation, and a study by Yang and Huang (38) revealed that 308-nm excimer laser treatment induced apoptosis of T lymphocytes, suggesting that combination therapy of tacrolimus ointment and 308-nm excimer laser may enhance these immunosuppressive effects for the treatment of vitiligo, particularly in progressive vitiligo patients. Furthermore, a previous study reported that melanocyte damage and local inflammation are important in the induction of CD8⁺ cytotoxic T lymphocyte-recognizing peptides derived from melanocyte protein, thus leading to the immune destruction of melanocytes (39). Of note, melanocytes were incompletely destroyed in lesions of progressive vitiligo patients, whereas stable vitiligo patients, who often have a long-term disease course, had a complete depigmentation disorder (24); therefore, the present study hypothesized that destruction of melanocytes may affect the immune imbalance, leading to differential sensitivity of combination treatments in different stages of vitiligo. Furthermore, Kang *et al* (40) demonstrated that during repigmentation, the melanocytes are mainly located on the edge of the leukoplakia and around the follicle and may represent early reservoirs of pigmentation. In the present study, dermoscopy revealed perifollicular repigmentation in several patients and early reservoirs of pigmentation on the edge of the leukoplakia in certain patients, consistent with this previous study.

As the second major novel finding of the present study, the efficiencies assessed by dermoscopy observation were significantly higher than those determined by visual observation in progressive-stage as well as stable-stage patients after 12 weeks of combination therapy. Thatte and Khopkar (33) reported that dermoscopy is able to effectively detect the presence of pigmentary changes, perifollicular hyperpigmentation and perilesional hyperpigmentation, and Kim *et al* (31) revealed that skin lesions confirmed by dermoscopy in patients with vitiligo prior to excimer laser treatment may be helpful in predicting the response to treatment, indicating that dermoscopy is better than routine histopathology in the diagnosis of vitiligo. Therefore, it may be concluded that dermoscopy, as a specific optical amplification method, allows for early detection of repigmentation conditions in patients with vitiligo more effectively than observation with the naked eye. This superior performance may be associated with the advantages of dermoscopy, which include a wide observation range, unlimited positions of observation and ease of changing the view. Tacrolimus ointment has been proven to be an effective and helpful alternative therapy for vitiligo patients, particularly those with lesions involving the head and neck (21,41-43). In the present study, no occurrence of angiotelectasis was observed on the faces of patients treated with topical tacrolimus, which was consistent with previous studies. During the course of treatment, dermoscopic examination facilitates the observation of the occurrence of angiotelectasis, which is helpful for clinicians to readjust medications and implement other necessary measures. The present study had certain limitations, as more clinical data should have been collected, the sample size should be have been expanded and a more comprehensive evaluation of the efficacy of therapy for vitiligo should have been performed.

In summary, the present results supported that dermoscopy is advantageous in monitoring the recovery of patients with vitiligo, and compared with observation by the naked eye, dermoscopy is more effective for evaluating the therapeutic efficacy of combination therapy with tacrolimus ointment and 308-nm excimer laser in vitiligo patients. Dermoscopy may be used as an effective means of vitiligo therapy outcome assessment to provide an accurate and scientific evaluation of the effects of vitiligo treatment.

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