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# Treatment of Non-Segmental Vitiligo in The Resistant Localizations with NB-UVB after Application of Erbium: YAG Laser Plus Pimecrolimus in Comparison with NB-UVB after Application of Erbium: YAG Laser Plus Topical Steroid

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## ABSTRACT

**Background:** Vitiligo is an acquired depigmentation condition. It affects around 0.1%-2% of the general population, regardless of ethnicity, gender, or socioeconomic status of the affected individuals. Vitiliginous patches contain either reduced melanin or no pigment at all.

**Objective:** This study aimed to evaluate the efficacy of Erbium: YAG (Er: YAG) laser plus pimecrolimus and narrowband ultraviolet B (NB-UVB) (on the right side) in comparison with Er: YAG laser plus topical steroid and NB-UVB (on the left side of the same patient) in treatment of vitiligo in the resistant localizations.

**Patients and methods:** The study comprised thirty patients with persistent non-segmental vitiligo and at least two symmetrical lesions on bony prominences and/or extremities. Patients were selected from the Outpatient Dermatological Clinic of Mataria Teaching Hospital.

**Results:** Regarding the area of the lesion on the right side, there was statistically significant difference between the area of the lesion before and after treatment at the 1st, 2nd, 3rd and 6th month of treatment. On the left side, there was also statistically significant difference between the area of the lesion before and after treatment at the 1st, 2nd, 3rd and 6th month of treatment. The higher percentage of improvement was seen in the knees followed by the elbows, while the lateral malleolus has 0% of improvement on both the right and left sides.

**Conclusion:** From this study we concluded that skin ablation by Er: YAG laser followed by NB-UVB in combination with either pimecrolimus or hydrocortisone is one of the alternative treatment modalities in treatment of stable, resistant vitiligo patches with almost no difference between the two treatment lines.

**Keywords:** Non-segmental vitiligo, Erbium: YAG laser, Pimecrolimus, Topical steroid, NB-UVB phototherapy.

## INTRODUCTION

A frequent acquired pigmentary condition caused by the death of melanocytes is vitiligo [(1)]. Despite the abundance of therapy approaches, it remains a significant dermatological issue. It has an impact on 1% of people worldwide [(2)]. It is still unclear exactly how illnesses develop. Regarding the genesis of vitiligo, there are three basic theories: the neurological theory, autocytotoxicity, and autoimmune [(3)].

Although vitiligo has been treated with a variety of techniques, there is currently no reliable, safe cure for this condition [(4)]. The main issues with the existing therapies include recurrence, treatment complications, and resistance to therapy [(5)]. The greatest way to demonstrate the importance of medical care for vitiligo is using growing patches. Surgical methods, either in isolation or in conjunction with medicinal care, represent the most favorable alternative after the lesions stabilize [(6)]. Using a combination of UV sources and topical medicines such as pimecrolimus or tacrolimus improved the rate of repigmentation [(7)]. Despite these advancements, bony prominences and extremities are often resistant to all available treatment modalities [(8)].

The rate of repigmentation in vitiligo lesions is greatly increased by the ablative Er: YAG laser. The high incidence of adverse effects and low tolerance severely restrict its use in current practice alone, despite a high rate of repigmentation in such challenging locations [(8)]. Furthermore, powerful topical prednisone and narrowband ultraviolet B (NB-UVB) are regarded as the first-line treatments for localized and widespread vitiligo respectively [(9)]. However, some of the negative effects of topical steroids included atrophy, telangiectasia, infection, and intradermal bleeding. As a result, this treatment is not advised [(10)]. Topical calcineurin inhibitors are almost as effective as topical steroids and is better for sensitive areas because, unlike corticosteroids, it does not cause skin atrophy [(11)].

The aim of the study was to evaluate the efficacy of Er: YAG laser plus pimecrolimus in comparison with Er: YAG laser plus topical steroid on the outcome of NB-UVB in treatment of non-segmental vitiligo in the resistant localizations.

## PATIENTS AND METHODS

The study included thirty patients having stable non segmental vitiligo with at least 2 symmetrical lesions located on bony prominences and/or extremities. Patients were selected from the Outpatient Dermatological Clinic of Mataria Teaching Hospital.

**Inclusion criteria:** Patients should have a minimum of 2 symmetrical patches of vitiligo in resistant localizations and the vitiligo should be stable (lack of progression of old lesions within the last year and no new lesions developing within the same period) [(12)]. All patients should stop any local or systemic medication for at least 1 month prior to the laser



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session as a washout period [13]. Patients should be above 12 years old.

**Exclusion criteria:** Patients with active infection or bleeding disorders. Pregnant women. Patients on systemic steroids or immunosuppressive. Personal history of skin cancer or photodermatosis. Personal history of keloid or hypertrophic scar.

**MATERIALS:**

- **Apparatuses:**

**I-Laser apparatus:**

**- Type:** The laser used was Fotona SP spectro Er: YAG laser (Fotona SP. spectroline, Slovenia, EU.) associated with air cooling system.

**- Emission parameters:** The parameters were energy of 1200 mJ with a **spot size** of 3 mm.

**II- NB-UVB unit:**

- **Type:** The NB-UVB unit used was Waldmann UV- 100 unit, with a TL-01 lamps emitting maximum wavelength of 311nm (Waldmann GmbH, Schwenningen, Germany).

- **Dose:** The beginning dose was 0.21 J/cm$^2$, regardless of skin type, and raised by 20% each session.

- **Goggles:**

**- Laser goggles:**

**For doctor:** red transparent goggles with protection spectrum from 2000 to 3000 nm were used for doctors' protection.

**For patient:** Another opaque titanium goggles used for patient protection.

- **NB-UVB Goggles**: protective opaque (black) goggles used for patient protection during NB-UVB session.

- **Digital camera:** the digital camera used for documentation was Nikon digital camera (Nikon digital camera, Coolpix P90, Tokyo, Japan).

**METHODS**

**The followings were done for each patient:**

1. Full **history** was taken including personal history, duration of the disease, family history of vitiligo, history of associated diseases and previous therapy received.
2. **Examination** to assess the extent and distribution of the lesions.
3. **Treatment protocol:** in each patient 2 symmetrical lesions in resistant localizations were chosen. One session of Er: YAG laser was first performed on both sides. After 48 hours, pimecrolimus 1% cream was applied once daily for 12 weeks on the right side **(treatment I)** and hydrocortisone butyrate 0.1% cream was applied once daily; 3 weeks every 4 weeks for 12 weeks on the left side **(treatment II).** Concomitantly, NB-UVB two sessions per week was done on both sides for 12 weeks. Photos were taken before the treatment and at the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ month throughout the treatment period and also at the 6$^{th}$ month after treatment to confirm the stability of the response.

**I. Area chosen for treatment:**

- The site: 2 symmetrical lesions seen on bony prominences or extremities (Resistant localizations for conventional treatment) were chosen and marked in each patient.
- The size: The average size of the treatment area was 3×3 cm.

**II. Followed precautions:**

- Sterilization with povidone iodine 7.5% and 70% alcohol to remove the color of povidone iodine.
- Goggles were used for both patient and doctor.

**III. Treatment steps:**

- After sterilization, injection of lidocaine hydrochloride 2% solution without epinephrine for local anesthesia, as to balloon out the lesion.
- The working field is carefully dried out with sterile gauze.
- One session of Er: YAG laser was first performed on both sides, the parameters were Energy of 1200 mJ with a spot size of 3 mm and a total of 3 to 7 passages were performed until a pin point bleeding was achieved according to the skin thickness of the affected area (Figure 1).

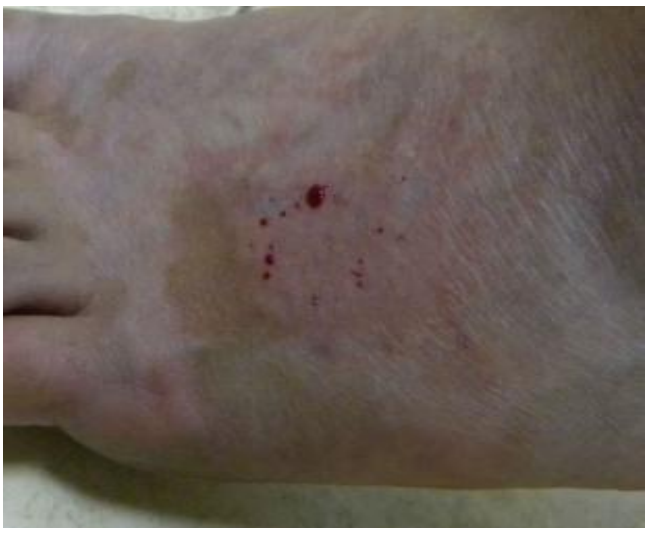


**Figure (1):** The pin point bleeding was achieved by 3 to 7 passages of Er: YAG laser according to the skin thickness of the affected area.

- Hemostasis was achieved by compression using a typical saline-soaked piece of gauze on the work area.
- A daily dressing by Gentamicin 0.1% to prevent secondary bacterial infection for the first two days of treatment.

Then the patients were recommended for a 12-week application of treatment that involved using 1% pimecrolimus cream once a day on the right side, and hydrocortisone butyrate 0.1% cream once daily on the left side for three periods of 3 weeks followed by a one week steroid-free interval for 12 weeks [8].

- NB-UVB began on both sides twice a week for 12 weeks, but not on consecutive days. The beginning dose was 0.21 J/cm$^2$, regardless of skin type, and was raised by 20% with each session, subject to the incidence of erythema. If erythema lasting greater than 48 hours was detected, in such case the last (previous) safe narrow band dose will be applied after clearance of erythema.

- Clinical assessment was done at the 1$^{st}$ , 2$^{nd}$ , 3$^{rd}$ month and also at the 6$^{th}$ month after the laser session for:
  - Therapeutic efficacy (repigmentation).
  - Side effects on laser sites as pain, keloid, koebner phenomena, hyperpigmentation, scar formation, and side effects of topical corticosteroid such as delayed healing, skin atrophy and telangectasia.

**IV. Digital photographs were taken for assessing and documenting the results. Photography was done under standard conditions:**
  - Fixed distance between person and camera.
  - Fixed source of illumination and so the room.
  - The same camera was used throughout the study using a digital camera.
- Photos were taken at the baseline and at the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ month throughout the treatment period and also at the 6$^{th}$ month to confirm the stability of the response to the treatment.
- The captured photos were analyzed using image analysis program (image tool 3.1 software program) comparing changes of area regarding the extent of repigmentation as well as the depth of pigmentation by assessment of optical density.
- Calibration of the program was performed using a known area (square paper) included in the same photo as a drawing scale for measurement of the selected area (Figure 2).
- The obtained data were exported to Microsoft Excel sheet (Figure 3).
- This assessment was carried out before starting therapy, one month, two months, three months, and six months after treatment.

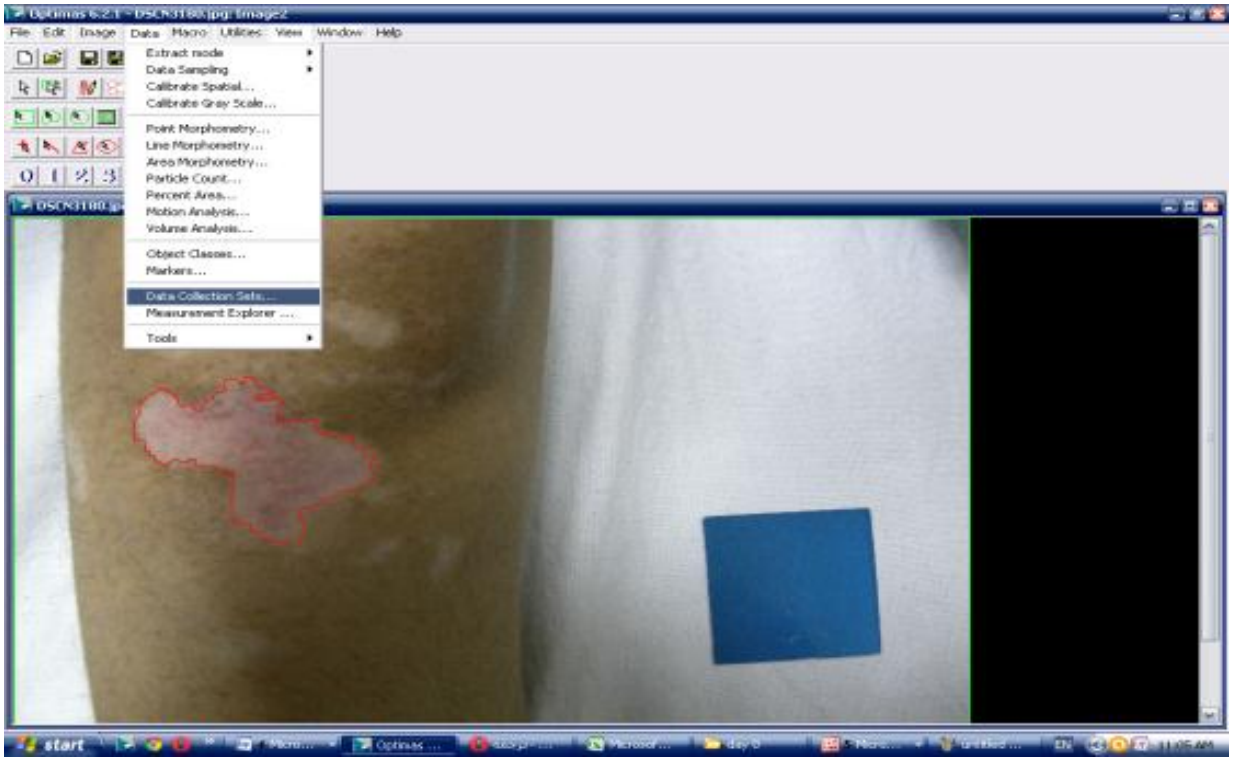



**Figure (2):** Measuring surface area of selected lesion using image tool program (the patient in the photo after 1 month of treatment).

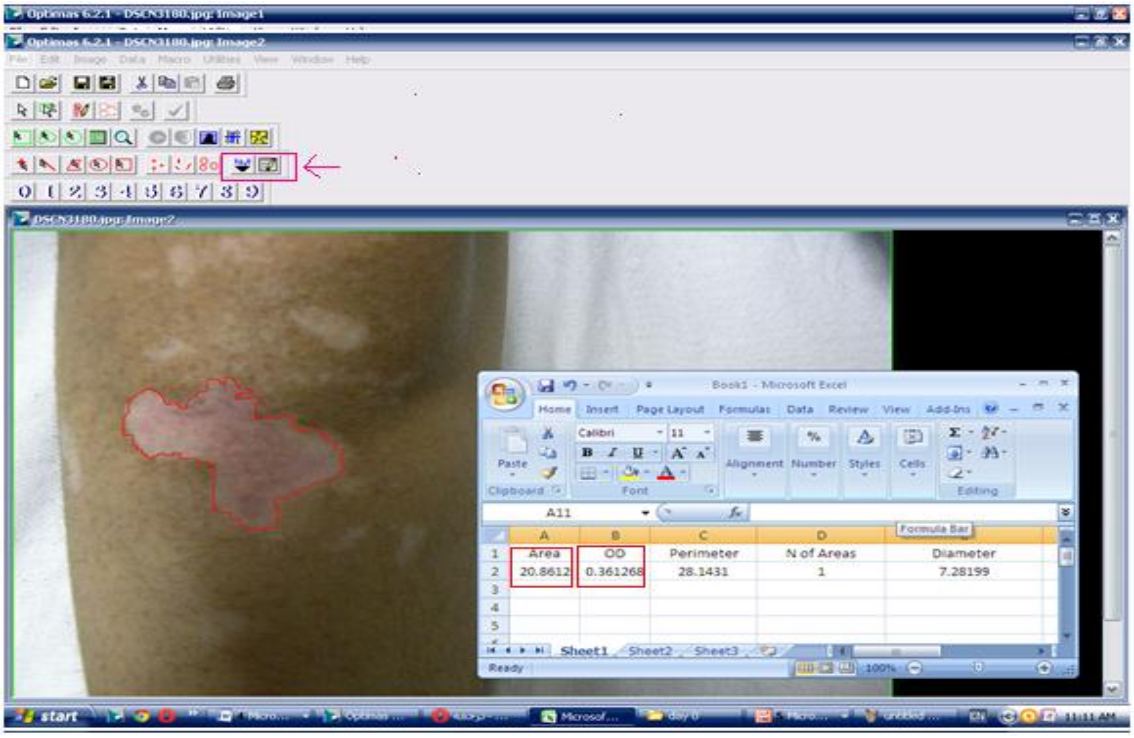



**Figure (3):** Notice the area and optical density measured were exported to an excel sheet using the buttons above, which the arrow pointed to it.

Quantitative evaluation of the response was also performed in a numerical percentage by subtracting post-treatment surface area ( at 6 months) from pre-treatment surface area and calculating the percentage of change in the surface area of lesions, which gave a quantitative evaluation of the response in a numerical percentage [(14)]. In addition, this numerical percentage of improvement was given a score translated to a qualitative response as followings:
**Excellent** result for 75-100% extent of repigmentation. **Good** result for 51-75% extent of repigmentation. **Fair** result for 26-50% extent of repigmentation. **Poor** result for 1-25% extent of repigmentation, and **No** response for 0 or < 1% extent of repigmentation [(8, 15)].

**Ethical approval:** This study was approved by the Mataria Teaching Hospital and Al-Hussein Hospital Ethics Committee. Following receipt of all information, signed consent was provided by each participant. The study adhered to the Helsinki Declaration throughout its execution.

***Statistical analysis***

Using SPSS version 15.0 for Windows®, the gathered data were coded, processed, and examined. Descriptive statistics: Range and Mean ± SD for numerical data with parametric distributions, and Median and IQR for data without. Quantity and proportion of data that are not numerical. Analytical statistics: To determine the statistical significance of the difference in a non-parametric variable between two research groups, the Mann-Whitney test (U-test) was employed. To identify variations in therapy over several test attempts, the Freidman test was employed. Wilcoxon In order to determine if the populations mean ranks of two related samples, matched samples, or repeated measurements on a single sample differ, the signed rank test was utilized. The Fisher's exact test was utilized when the predicted count was less than 5 in more than 20% of the cells, while the $X^2$-test was utilized to investigate the link between two qualitative factors. When the p-value was equal to or less than 0.05, it was deemed significant.

**RESULTS**

This study included 30 patients, with stable non-segmental vitiligo. They were 17 males and 13 females and their ages ranged from 13 to 62 years, with a mean of 31.7 years. Regarding the skin type, there were 23 patients with skin type III, 4 patients with skin type IV, and 3 patients with skin type II according to Fitzpatrick's classification. All patients had at least 2 symmetrical lesions located on bony prominences and/or extremities (resistant localizations).

About one third of the patients had vitiligo lesions on the elbow (30.0%), while 23.3% had the lesion on the dorsum of feet, 20.0% on the knee, 16.7% on the dorsum of hands, and only 10.0% had the lesions on the lateral malleolus. The mean disease duration was 6.55 ± 4.26 years and the majority of the patients (96.7%) had no associated autoimmune disease, while only one patient (3.3%) of the studied patients had autoimmune disease in the form of thyroiditis. Table (1) showed the demographics and patients parameters.

**Table (1):** Description of personal and medical history of studied cases

| Patient Characteristics | | | |
|---|---|---|---|
| **Age (Years)** | **No.** | **%** | **P-value** |
| ≤ 20 Ys | 9 | 30.0 | 1.000 |
| 21-40 Ys | 12 | 40.0 | |
| 41-70 Ys | 9 | 30.0 | |
| **Age (Years)** | **Mean ± SD** | | |
| | 31.7 ± 16.3 | | |
| **Sex** | **No.** | **%** | **P-value** |
| Male | 17 | 56.7 | 0.465 |
| Female | 13 | 43.3 | |
| **Skin Type** | **No.** | **%** | **P-value** |
| 2 | 3 | 10.0 | 0.000** |
| 3 | 23 | 76.7 | |
| 4 | 4 | 13.3 | |

| Disease Characteristics | | | |
|---|---|---|---|
| **Site** | **No.** | **%** | **P-value** |
| Dorsum of feet | 7 | 23.3 | 0.504 |
| Dorsum of hands | 5 | 16.7 | |
| Elbow | 9 | 30.0 | |
| Knee | 6 | 20.0 | |
| Lateral Malleolus | 3 | 10.0 | |
| **Disease Duration (Years)** | **Mean ± SD** | | |
| | 6.55 ± 4.26 | | |
| **Assosciated Autoimmune Disease** | **No.** | **%** | **P-value** |
| No | 29 | 96.7 | 0.000** |
| Yes (Thyroiditis) | 1 | 3.3 | |

(**) Statistically Significant at P<0.01

Regarding the lesion area on the right side, there was significant reduction of the vitiliginous area measured before and after treatment (P<0.01). This significant difference was more obvious in the 2nd, 3rd and 6th month when compared with the area before treatment (P<0.05). On the other hand, the progressive improvement continued until the 3rd month and no more improvement was observed after 3 months of treatment (Table 2).

**Table (2):** Comparison between area of the lesion on Rt. side before and after treatment with Er: YAG laser plus pimecrolimus (n=30)

| Follow up | Mean | SD | Friedman Test | P-value | Wilcoxon Sign Rank Test |
|---|---|---|---|---|---|
| **Area Rt (Before)** | 17.45 | 11.70 | 22.417 | 0.000** | (0,1), (0,2), (0,3) , (0,6) (1,2), (1,3), (1,6) |
| **Area Rt_1M** | 16.91 | 11.91 | | | |
| **Area Rt_2M** | 16.37 | 12.20 | | | |
| **Area Rt_3M** | 16.15 | 12.27 | | | |
| **Area Rt_6M** | 16.15 | 12.27 | | | |

(**)Statistically Significant at P<0.01

Regarding the area of the lesion on the left side, there was significant reduction of the vitiliginous area measured before and after treatment (P<0.01). This significant difference was also more obvious in the 2nd, 3rd and 6th month when compared with the area before treatment (P<0.05). On the other hand, the progressive improvement continued until the 3rd month and no more improvement was observed after 3 months of treatment (Table 3).

**Table (3):** Comparison between lesion area on Lt. side before and after treatment with Er: YAG laser plus hydrocortisone (n=30)

| Follow up | Mean | SD | Friedman Test | P-value | Wilcoxon Sign Rank Test |
|---|---|---|---|---|---|
| **Area Lt (Before)** | 16.4530 | 11.03258 | 18.061 | 0.001** | (0,1), (0,2), (0,3) , (0,6) (1,2), (1,3), (1,6) |
| **Area Lt_1M** | 15.3727 | 11.10771 | | | |
| **Area Lt_2M** | 14.6833 | 11.10869 | | | |
| **Area Lt_3M** | 14.6167 | 11.22255 | | | |
| **Area Lt_6M** | 14.6098 | 11.22373 | | | |

(**) Statistically Significant at P<0.01

When comparing between the two administered treatments (Er: YAG laser plus pimecrolimus) in the Rt side and (Er: YAG laser plus hydrocortisone) in the Lt side of the studied patients, the mean difference in the lesion area (reduction in the area when compared to the area before treatment) was more in the Lt side than in the Rt side. The obtained values after the 1st, 2nd, 3rd and 6th months were as follow (0.55 ± 1.2 versus 1.08 ± 2.5), (1.09 ± 2.5 versus 1.77 ± 3.8), (1.3 ± 3.0 versus 1.8 ± 4.0), (1.3 ± 3.0 versus 1.8 ± 4.0) respectively. However, such difference was **statistically insignificant** (p>0.05) (Table 4).

**Table (4):** Comparison between 2 studied treatments (Er: YAG laser plus pimecrolimus) and (Er: YAG laser plus hydrocortisone) as regards the area of the lesion

| Mean difference (reduction) in the lesion area | Er:YAG laser plus pimecrolimus (Rt Side) | Er:YAG laser plus hydrocortisone (Lt Side) | t test | P-value |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | | |
| **After 1 Month** | 0.55± 1.2 | 1.08 ± 2.5 | -1.03 | 0.304 |
| **After 2 Months** | 1.09 ± 2.5 | 1.77 ± 3.8 | -.811 | 0.421 |
| **After 3 Months** | 1.3 ± 3.0 | 1.8 ± 4.0 | -.579 | 0.565 |
| **After 6 Months** | 1.3 ± 3.0 | 1.8 ± 4.0 | -608 | 0.546 |

Regarding the percentage of improvement, the mean percentage of improvement on the Rt. side was 11.90 ± 23.097% while on the Lt. side was 13.54 ± 24.74% such difference was **statistically insignificant** (p>0.05) (Table 5).

**Table (5):** Comparison between Rt and Lt sides as regard the mean percentage of improvement.

| Side | Mean | Std. Deviation | t test | P value |
|---|---|---|---|---|
| **Rt** | 11.9023 | 23.09719 | -0.266 | 0.791 |
| **Lt** | 13.5439 | 24.74821 | | |

The overall response to treatment showed that over 50% repigmentation (including excellent and good response) was seen in 2 patients (6.67%) on their Rt side and 3 patients (10%) on their Lt side. Such difference was statistically insignificant ($\chi^2$=0.200, P=0.655 and p > 0.05). An excellent response (>75% repigmentation) was seen in 1 patient only (3.33%) on his Rt side and 2 patients (6.67%) on their Lt side and good response (51-75% repigmentation) was seen in 1 patient (3.33%) on his Rt side and also 1 patient (3.33%) on his Lt side. On the other hand, fair response (26-50% repigmentation) was seen on 4 patients (13.33%) on their Rt side and 3 patients (10%) on their Lt side. A poor response (1-25% repigmentation) was seen on 8 patients (26.67%) on their Rt side and 6 patients (20%) on their Lt side and no response (1>% repigmentation) was seen on 16 patients (53.33%) on their Rt side and 18 patients (60%) on their Lt side. There was a statistically insignificant difference between the Rt and Lt side as regard the mean percentage of improvement (p>0.05) (Table 6).

**Table (6):** Description of percentage of patients and grade of repigmentation on the Rt and Lt sides (qualitative assessment to the treatment).

| score of improvement | | Rt | Lt | χ2 test | P-value |
|---|---|---|---|---|---|
| **Excellent >75%** | **count** | 1 | 2 | 0.905 | 0.924 |
| | **%** | 3.33% | 6.67% | | |
| **Good 51%-75%** | **count** | 1 | 1 | | |
| | **%** | 3.33% | 3.33% | | |
| **Fair 26%-50%** | **count** | 4 | 3 | | |
| | **%** | 13.33% | 10% | | |
| **Poor 1-25%** | **count** | 8 | 6 | | |
| | **%** | 26.67% | 20% | | |
| **None 0%** | **count** | 16 | 18 | | |
| | **%** | 53.33% | 60% | | |

Over 50% repigmentation was 6.67% on the Rt side versus 10% on the Lt side, which was statistically insignificant. However, optical density showed **statistically insignificant** difference between both modalities of treatment (Rt side versus Lt side) ($p>0.05$). The mean optical densities after 1st, 2nd, 3rd and 6th months of treatment on the Rt and Lt side were 0.32 ± 0.09 versus 0.32 ± 0.09, 0.32 ± 0.10 versus 0.32 ± 0.09, 0.34 ± 0.13 versus 0.33 ± 0.10 and 0.32 ± 0.10 versus 0.32 ± 0.10 respectively **(**Table 7).

**Table (7):** Comparison between Rt and Lt side as regard optical density

| Optical Density | Er:YAG laser plus pimecrolimus Rt Side) | Er:YAG laser plus topical steroid (Lt Side) | Mann-Whitney U test | P-value |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | | |
| **After 1 Month** | 0.32 ± 0.09 | 0.32 ± 0.09 | 437.000 | 0.847 |
| **After 2 Months** | 0.32 ± 0.10 | 0.32 ± 0.09 | 441.000 | 0.894 |
| **After 3 Months** | 0.34 ± 0.13 | 0.33 ± 0.10 | 424.500 | 0.706 |
| **After 6 Months** | 0.32 ± 0.10 | 0.32 ± 0.10 | 450.000 | 1.000 |

There was a statistically insignificant difference between the Rt side and Lt side as regards the optical density ($p>0.05$). There was often **non-significant negative** correlation between changes in the lesion' area and optical density in Rt side and Lt side after the 1st, 2nd, 3rd and 6th months of treatment ($P>0.05$) (Table 8).

**Table (8):** Correlation between changes in the area and optical density in the Rt side and Lt side

| Optical Density | | Area Rt | Area Rt 1M | Area Rt 2M | Area Rt 3M | Area Rt 6M |
|---|---|---|---|---|---|---|
| **Optical density Rt (1M)** | **R** | -.136 | -.118 | -.108 | -.099 | -.099 |
| | **P** | .473 | .536 | .571 | .602 | .602 |
| **Optical density Rt (2M)** | **R** | -.166 | -.195 | -.232 | -.238 | -.238 |
| | **P** | .380 | .302 | .217 | .205 | .206 |
| **Optical density Rt (3M)** | **R** | -.145 | -.194 | -.257 | -.269 | -.269 |
| | **P** | .444 | .305 | .171 | .151 | .151 |
| **Optical density Rt (6M)** | **R** | -.160 | -.157 | -.161 | -.163 | -.162 |
| | P | .397 | .408 | .395 | .391 | .392 |
| | | **Area LT** | **Area LT 1M** | **Area LT 2M** | **Area LT 3M** | **Area LT 6M** |
| **Optical density Lt (1M)** | **R** | -.037 | .000 | .007 | .008 | .007 |
| | **P** | .845 | .997 | .969 | .968 | .970 |
| **Optical density Lt (2M)** | **R** | -.210 | -.226 | -.242 | -.239 | -.240 |
| | **P** | .264 | .229 | .197 | .203 | .201 |
| **Optical density Lt (3M)** | **R** | -.217 | -.274 | -.313 | -.315 | -.316 |
| | **P** | .250 | .143 | .092 | .090 | .089 |
| **Optical density Lt (6M)** | **R** | -.221 | -.224 | -.237 | -.234 | -.234 |
| | **P** | .240 | .234 | .207 | .214 | .212 |

As regard the percentage of improvement in the different anatomical sites, the higher percentage of improvement was seen in the knees followed by the elbows on both the right and left sides, as the mean percentages of improvement on the Rt. side for knees and elbows were 20.27 ± 28.3% and 16.67 ±32.2% respectively. On the other hand, that of the Lt. side were 35.87 ± 32.9% for knees and 16.02 ± 29.7% for elbows, while the lateral malleolus had 0 % of improvement on both the right and left sides (Table 9).

**Table (9):** Percentages of improvement of the lesion area in the different anatomical sites on each side separately

| Anatomical site | Rt. side | | Lt. side | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| **Dorsum of feet** | 5.5382 | 12.88980 | 3.3314 | 4.55456 |
| **Elbows** | 16.6771 | 32.29389 | 16.0244 | 29.75180 |
| **Hands** | 9.3114 | 13.75626 | 4.7036 | 10.31507 |
| **Knees** | 20.2750 | 28.30574 | 35.8767 | 32.89768 |
| **Lat. Malleolus** | 0.00 | 0.00 | 0.00 | 0.00 |

The higher percentage of improvement was in the knees followed by the elbows on the Rt and Lt sides. But, there wasn't statistically significant difference in the percentage of improvement between knees and other anatomical sites on the Rt side while on the Lt side there was **statistically significant difference** between knees versus each of dorsum of feet, hands and lateral malleolus (P<0.05) (Table 10).

**Table (10)** Comparison between percentages of improvement of knee versus each of dorsum of feet, elbow, hands and lateral malleolus

| Site | Rt. side | | | Lt. side | | |
|---|---|---|---|---|---|---|
| | Other anatomical sites | P-value | Post Hoc Test Sig. | Other anatomical sites | P- value | Post Hoc Test Sig. |
| Knee | Dorsum of feet | 0.275 | Non sig | Dorsum of feet | 0.017* | (1,2), (1,4), (1,5) |
| | Elbow | 0.776 | | Elbow | 0.112 | |
| | Hands | 0.453 | | Hands | 0.033* | |
| | Lat. Malleolus | 0.239 | | Lat. Malleolus | 0.036* | |

(*) Statistically Significant at P<0.05 on the lt side.

**The side effects recorded with both treatment modalities:**

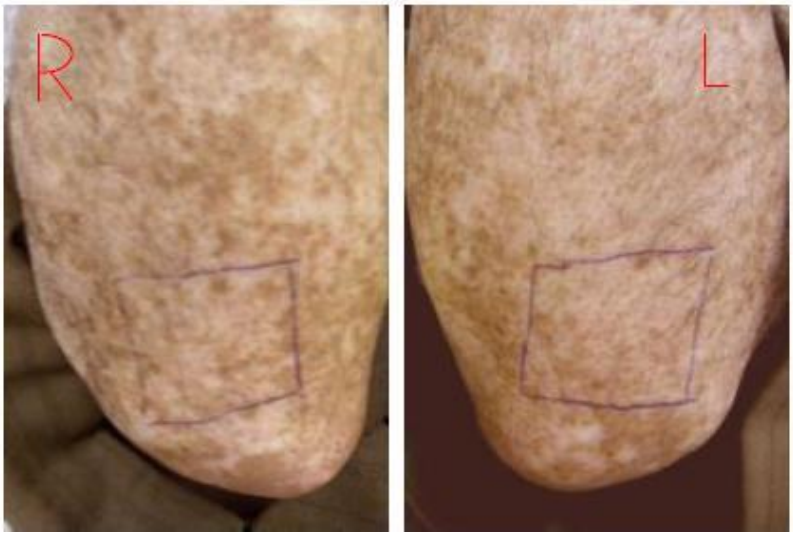
- Pain during the ablation process by Er: YAG laser, usually from the third pass onwards, was noticed in all patients, although it did not demand another injection of local anesthetic. They also experienced pain and serosanguinous oozing few days after the laser session.
- Delayed healing in patches treated by hydrocortisone after Er: YAG laser ablation (Lt. side treatment) was observed.
- Atrophy, telangiectasia and striae were not detected in corticosteroids treated areas.
- Nine patients showed erythema lasted over the 1st month and not observed in the last follow up visit at the 6th month.
- Crust formation observed in 7 patients but only 3 of them lasted to the 2nd month.
- Mild to moderate hyperpigmentation causing color mismatch, which persisted or resolved throughout the follow-up were shown in 4 patients with positive repigmentation results **(**Figures 4, **5**).
- Only one patient showed hypertrophy in the area of repigmentation.
- Koebner phenomenon was not detected in our study.

**As regards the results of follow up cases:**

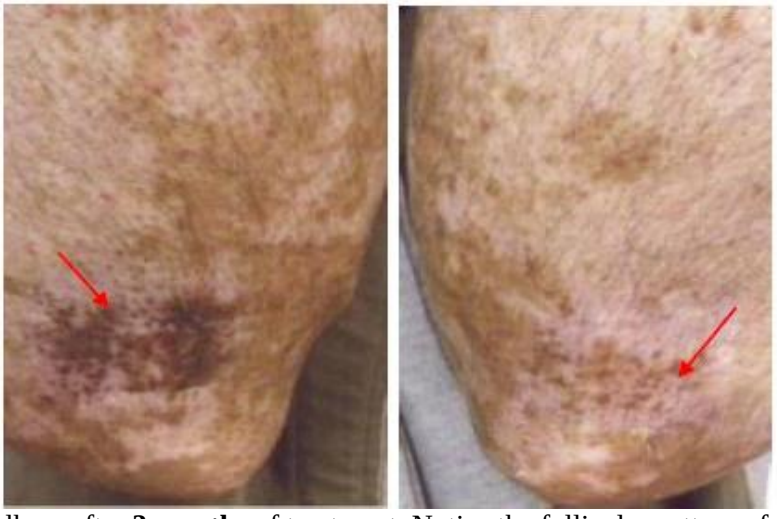
- Repithelization after Er: YAG laser taken about 2 weeks, with delayed healing of patches treated by corticosteroids after laser (Lt side treatment).
- It was noted that 6-8 weeks were the average of appearance of repigmentation and after 10 weeks patches with poor or no response were non-responsive and those with good response continued repigmentation.
- There was stability in the results till the last visit of follow up in the $6^{th}$ month.
- Some of treated lesions healed by a transient slate blue color**.**

- The results were very good when the patch of laser session has specks of residual pigment.
- The pattern of repigmentation was usually formation of small brown perifollicular macules, then grew and consolidated. This began at the borders and progressed centrally (Figure 5). Nevertheless, in other areas, repigmentation happened diffusely from the start.

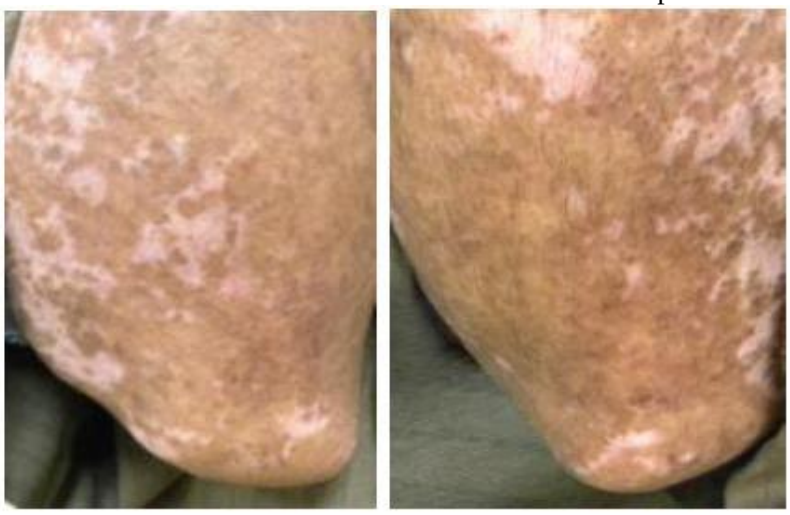
**In all the following pictures the Rt. column represented the Rt side (Er: YAG laser plus pimecrolimus and NB-UVB) and the Lt. column represented the Lt side (Er: YAG laser plus hydrocortisone and NB-UVB)**




**(a)** Rt and Lt elbow **before** treatment with Er: YAG laser.

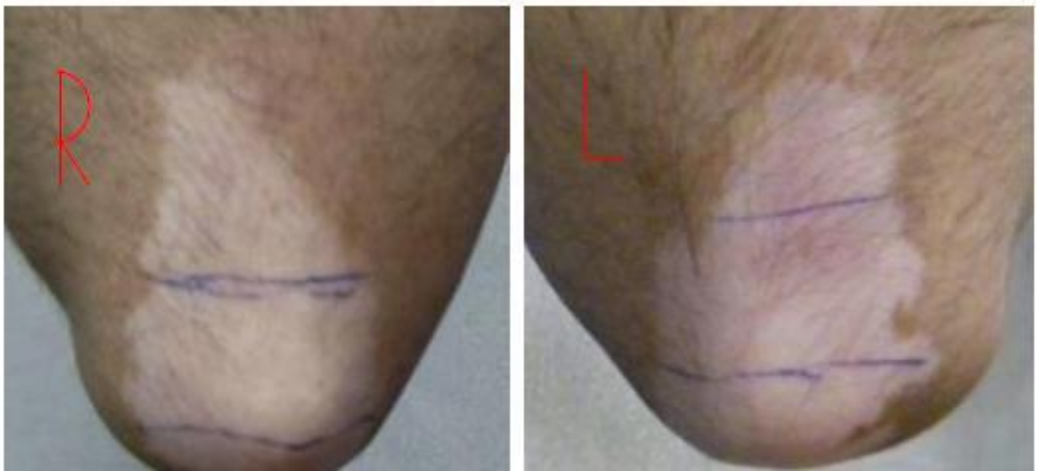


**b)** Rt and Lt elbow after **3 months** of treatment. Notice the follicular pattern of repigmentation

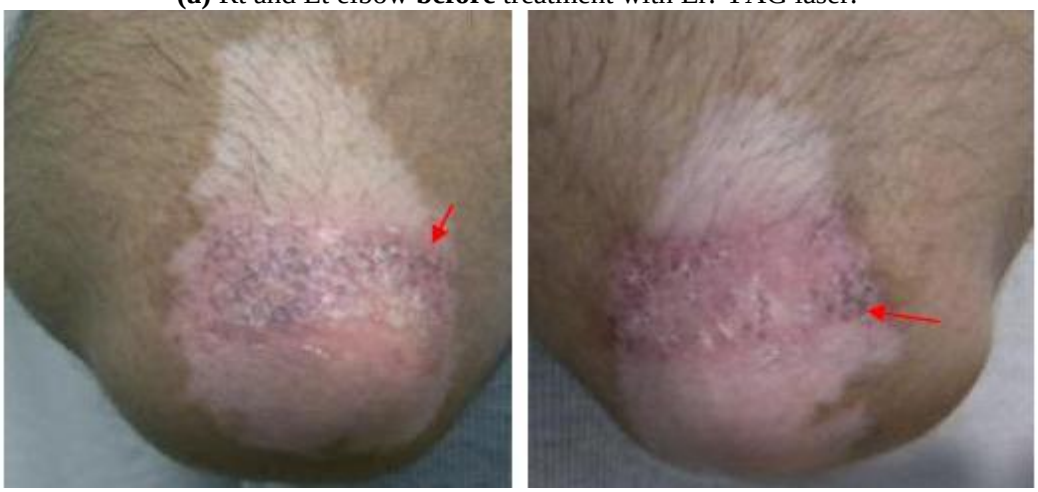


**(c)** Rt and Lt elbow after **6 months** of treatment. Notice that hyperpigmentation resolved, and the repigmentation exceeded the area of laser session.

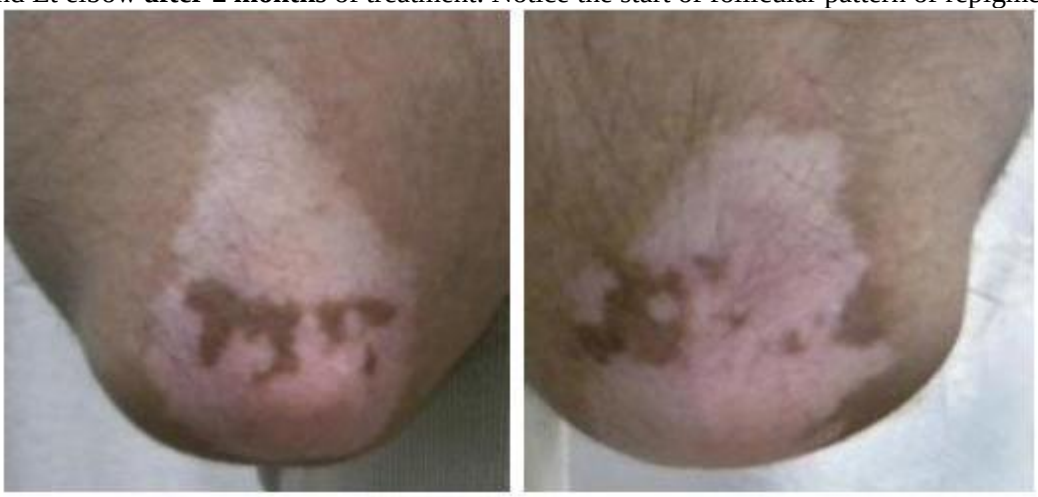
**Figure (4):** Rt and Lt elbow before and after different stages of treatment (a), (b) and (c)




**(a)** Rt and Lt elbow **before** treatment with Er: YAG laser.



**(b)** Rt and Lt elbow **after 2 months** of treatment. Notice the start of follicular pattern of repigmentation.



**(c)** Rt and Lt elbow **after 3 months** of treatment.

**Figure (5):** Rt and Lt elbow before (a), after 2 months (b), and 3 months (c) of treatment.

## DISCUSSION

The current study evaluated the effect of one session of Er: YAG laser plus pimecrolimus (on the right side) in comparison with Er: YAG laser plus hydrocortisone cream (on the left side of the same patient) on the outcome of NB-UVB phototherapy in vitiligo lesions at resistant localizations. Two symmetrical lesions in resistant localizations were chosen in each patient. Erbium YAG laser session was first performed on both sides. After 48 hours, pimecrolimus 1% cream was applied once daily for 12 weeks on the right side **(treatment I).** Hydrocortisone butyrate 0.1% cream was applied once daily for 3 weeks plus one week rest and repeating this regimen for 3 cycles on the left side **(treatment II).** Concomitantly; Narrowband UVB two sessions per week was done on both sides for 12 weeks. Photos were taken before the treatment then at the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ month throughout the treatment period and lastly at the 6$^{th}$ month after

treatment to confirm the stability of response. Assessment was done through an objective method using image analysis program through which area measurement as well as optical density assessment was carried out with serial recording of those parameters at pretreatment, 1st, 2nd, 3rd and 6th month after treatment. Percentages of change in the surface area of the lesions were also calculated and those percentages of repigmentation were graded to 5 grades (excellent >75%, good 51%- 75%, fair 26%-50%, poor 1-25%, none 0%). Those measurements were used in comparing progressive difference (improvement) for each side separately, then comparing right and left side.

In all other studies, the assessment was done through 2 dermatologist blinded to the treatment options, to evaluate either the photos or the patient's lesion and the response to the treatment was graded to 5 grades similar to those in our study. While, in our study the assessment method was objective with accurate estimation of change in the vitiliginous area as well as change in the color with exclusion of erythema.

Our study's findings demonstrated that **on the right side**, the percentage of improvement was over 50% repigmentation in 2 patients out of 30 patients (6.67%), and 26-50% repigmentation was seen in 4 patients (13.33%). A poor response (1-25% repigmentation) was seen in 8 patients (26.67%) and no response (1>% repigmentation) was seen in 16 patients (53.33%). This qualitative assessment was done based on the method used to assess the area of the lesion. There was **statistically significant difference** between the area of the lesion before and after treatment at the 1st, 2nd, 3rd and 6th month of treatment on the right side and there was also **statistically significant difference** between the area after 1st month of treatment versus the 2nd, 3rd and 6th month of treatment, which confirms the clinical observation of the average time of appearance of regimentation, which was between 1st and 2nd month of treatment (6-8 weeks) and continued to the 3rd and 6th month as the mean area of the lesion was found to be more reduced in the 3rd and 6th month.

Concerning the optical density on the right side, there was **statistically significant difference** between the optical density of the lesion before and after treatment at the 1st, 2nd, 3rd and 6th month of treatment. On the other hand, the mean optical density of the lesion was found to be more increased in the 3rd month when compared to the optical density before treatment, at 1st, 2nd and 6th month of treatment but this elevation was statistically insignificant. This may be explained by the transient hyperpigmentation, which was more obvious in the 3rd month and resolved in many patients on the 6th month. Up to our knowledge, no further studies done to assess Er: YAG laser in combination with pimecrolimus and NB-UVB in the resistant or non-resistant localizations of vitiligo.

**Farajzadeh *et al.*** [5] in a placebo-controlled trial compared the effectiveness of pimecrolimus cream 1% alone to pimecrolimus 1% coupled with microdermabrasion in the treatment of non-segmental pediatric vitiligo, and only 6.7% of the patches investigated were acrofacial. At the third month of follow-up, 60.4% of all patches treated with combination treatment showed repigmentation of more than 50%, compared to 32.1% and 1.7% for pimecrolimus alone and placebo, respectively. Their results are consistent with the results of our study, which might be related to the distinct age range of their study (childhood only). Moreover, the lesions in our study were in the resistant localizations.

**On the left side**, the results of our study showed that the percentage of improvement was over 50% repigmentation in 3 patients out of 30 patients (10%), and 26-50% repigmentation was seen in 3 patients (10%). A poor response (1-25% repigmentation) was seen in 6 patients (20%) and no response (1>% repigmentation) was seen in 18 patients (60%). There was **statistically significant difference** between the area of the lesion before and after treatment at the 1st , 2nd ,3rd and 6th month of treatment on the left side and there was also **statistically significant** difference between the area after 1st month of treatment versus the 2nd, 3rd and 6th month of treatment, which also confirms that the appearance of regimentation was between 1st and 2nd month of treatment (6-8 weeks) as clinically observed and continued to the 3rd and 6th month as the mean area of the lesion was found to be more reduced in the 3rd and 6th month.

As regards the optical density on the left side, there was **statistically significant difference** between the optical density of the lesion before and after treatment at the 1st, 2nd, 3rd and 6th month of treatment, and the mean optical density of the lesion was found to be more increased in the 3rd month when compared to the optical density before treatment, at 1st , 2nd and 6th month of treatment but this elevation was also statistically insignificant and may be also explained by the transient hyperpigmentation, which was more obvious in the 3rd month and resolved in many patients on the 6th month.

Our results **on the left side** are similar to these of **Shin *et al.*** [16] who evaluated the impact of laser ablation using two sessions of fractional $CO_2$ laser (instead of Er: YAG laser) in conjunction with NB-UVB to cure vitiligo lesions resistant to prior therapy, and many of them were on the hands and feet. They also found that 1 patient out of 10 patients (10%) achieved over 50% of repigmentation (as also 10% in our study). They preferred fractional $CO_2$ laser over ablative Er: YAG laser to decrease the time of wound healing and decrease the risk of scar formation.

**Vachiramon *et al.*** [17] investigated the impact of fractional $CO_2$ laser as an ablative laser (1- week interval for 10 sessions) in combination with NB-UVB and topical steroids (0.05% clobetasol propionate twice daily throughout the study period, 10 weeks) for one side compared to only NB-UVB with topical steroids

on the other side. This treatment targeted resistant vitiligo of the hands as a difficult localization. They discovered that the repigmentation rate of vitiliginous lesions on hands is improved in some patients when fractional $CO_2$ laser treatment is combined with NB-UVB phototherapy and topical steroids. Specifically, 7.7% of patients showed excellent response (>75% improvement), 15.4% showed good response (51-75% improvement), and 42.3% of patients showed no response at all. There was also another study tried triple combination treatment with fractional $CO_2$ laser followed by topical betamethasone solution and NB-UVB therapy for refractory vitiligo on extremities and/or bony prominences done by **Li *et al.* [18],** but the topical steroids used in their study was more stronger than the used hydrocortisone butyrate 0.1% in our study and the total period of treatment application was 6 months. This may be the cause of their results as over 75% re-pigmentation was seen in 8% of the patients of the treatment side (compared to 6.67% in our study) and 32% achieved repigmentation from 51%-75% (3.33% only in our study), but on the control side (without topical steroid treatment), only 8% of their patients showed repigmentation more than 50%.

Our results also are comparable with those of **Bayoumi *et al.* [8]** who found that 45.9% of patients had more than 50% repigmentation in response to the combination of ablative Er: YAG laser, topical steroids and NB-UVB compared to 10% of the patients in our study. While, only 4.2% of his patients had more than 50% repigmentation in response to NB-UVB plus topical steroid. This result was after a short follow up period, only one month after treatment in addition to the different method of assessment as the method used in all the last studies was using 2 dermatologist while in our study the method was objective assessment.

**Anbar *et al.*[19],** evaluated the effects of combining Er: YAG laser resurfacing with topical 5-flurouracil on periungual vitiligo. The mean total response to therapy in the treated group was 47.8%, whereas in the control group it was 1.1%. This result is superior to our results as in our study the mean percentage of improvement was 11.9 % on the right side and in 13.5% on the left side. **Anbar *et al.* [20]** also studied treatment of non-segmental vitiligo in different body parts (excluding the extremities) using skin ablation by Er: YAG laser followed by 5-FU and NB-UVB on one side of the patient in comparison with 5-FU and NB-UVB on the other side of the same patient and concluded that about 78.1% of the patients experienced a moderate to marked repigmentation response in the side of Er: YAG laser compared to 23.4% in the other side. They suggested that 5-FU hyperpigmentation might be caused by higher amounts of melanocyte-stimulating hormone, direct melanocyte stimulation, an increase in the number of melanosomes in keratinocytes, and melanocyte activation.

The result of the current study showed that there was **no statistically significant difference** between right and left sides as regards the area of the lesion as well as the optical density, inspite of the mean area of the lesion was more reduced in the Lt side more than in the Rt side. So we suggest that we can choose the line of treatment according to the compliance of the patient and the side effects that may result from the treatment.

In our research, the pattern of repigmentation was mainly through follicular repigmentation and from the periphery of the lesion inwards. This suggested that the mechanism by which the Er: YAG laser induces repigmentation is either by (a) induction of melanocyte follicular reservoir migration to the epidermal surface during wound healing and propagation centrifugally around the hair follicle in hairy skin, (b) by stimulation of peripheral epidermal melanocytes at the periphery of ablated area during surface epithelialization, with migration of pigment for a few millimeters towards the center in non-hairy skin. **Sethi *et al.* [13]** and **Anbar *et al.*[20]** previously suggested these mechanisms. The ablative methods induces production of proinflammatory cytokines e.g. prostaglandin D2 (PGD2), leukotrienes (LT), thromboxane B2 (TXB2) and endothelin-1 (ET-1), which might serve as mitogens for melanocytes and have an effect on melanogenesis and pigment cell migration [16, 17].

Due to the ablation by Er: YAG laser, another suggested mechanism is increased penetration of the topically applied medical treatment and the penetration of the UV radiations after removal of affected keratinocytes by Er: YAG laser allowing a higher exposure of the dermis, with stimulation of the melanocyte stem cells that are located in the dermis of glabrous skin. Ease of penetration may cause a significant increase of repigmentation in areas treated by Er: YAG laser and topical steroids [8]**. Farajzadeh *et al.*[5]** also suggested that the good results of combination of dermabrasion (which here replaced by the Er: YAG laser) and pimecrolimus are due to increased pimecrolimus absorption after skin ablation, which enhances the immune modulation effect of pimecrolimus.

Above 50% repigmentation was seen in our study in either the knee or the elbow, while the lateral malleolus had 0% of repigmentation on both sides. This may be due to the relatively higher number of hair follicles in the knee and elbow in comparison with other resistant localizations like the lateral malleolus as evidenced by the follicular pattern of repigmentation in the knee and elbow. Also, the good clinical response (>50% repigmentation) was not related to the age, sex, skin type and the disease duration of the patients. The blue coloration, which was observed in some treated lesions that healed by a slate blue color, our explanation it may be due to the trauma induced by Er: YAG laser, which may be resulted in basement membrane destruction with pigmentary incontinence and localization of pigment in the deeper layer of dermis, which gives the blue discoloration. This was also seen in the study of **Anbar *et al.* [20].** This situation coincides

histologically with a lack of epidermal melanocytes and abundant dermal melanophages, because the skin melanin is situated in the deeper dermal layer **[21].** The founded blue coloration in our study may explain the increased optical density without real repigmentation in the form of decrease in the area of vitiliginous patch.

## CONCLUSION

From this study we concluded that skin ablation by Er: YAG laser followed by NB-UVB in combination with either pimecrolimus or hydrocortisone is one of the alternative treatment modalities in treatment of stable, resistant vitiligo patches with almost no difference between the two treatment lines. On the other hand, pain during the procedure and the healing process, which delayed specially with hydrocortisone and also the side effects of the treatment lines may impair the satisfaction of the patients in addition to the precise selection of the stable patient to avoid Koebner phenomenon is strongly limit the use of Er: YAG laser ablation specially in those with short durations of disease stability.

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