COMPARATIVE EFFICACY OF TOPICAL MINOXIDIL VERSUS TOPICAL MINOXIDIL COMBINED WITH 308-NM EXCIMER LASER THERAPY IN THE TREATMENT OF SCALP ALOPECIA AREATA: A DERMOSCOPIC, HISTOLOGICAL AND BIOCHEMICAL STUDY

Osama I. Ramadan[1], Osama M. Elshahat[2], Ezz-Eldin E. Abd-Allah[1], Tamer A. Gomaa[1], Fatma M. Abd-Allah[1], Medhat M. Darwish[3], Ahmed E. Moustafa[3], Salah El-Din S. Elsemary[4], Alaa El Deen S. Elsagheir[5], Moaaz M.Y Awad [6,7], Reda M.A. Fayyad[8,9]

Article History: Received: 05.11.2022 **Revised:** 10.12.2022 **Accepted:** 15.12.2022

Abstract

Background: Alopecia areata is a chronic inflammatory disease with unknown etiology resulting in non-scarring hair loss. Till now there is no recommended treatment for alopecia areata.

Aim of the study: we aimed to evaluate the efficacy of topical minoxidil versus combination of topical minoxidil with 308- nm excimer laser in treating patchy alopecia areata.

Patients and methods: A randomized controlled trial was conducted among 60 patients diagnosed with scalp alopecia areata and assigned to two treatment groups: Group A (30 patients) received topical minoxidil 2%, and Group B (30 patients) received topical minoxidil 2% combined with 308-nm excimer laser therapy. Biochemical, histopathological, immunohistochemical study, and dermoscopic imaging were used to evaluate therapeutic response.

Results: A significant reduction in all alopecia areata-related dermoscopic findings were observed in both treatment modalities compared to both groups before and after therapy with significant efficacy in group B compared to group A. The microscopic examination of skin sections of Group A after 12 weeks revealed sparse inflammatory lymphocytic infiltration around hair follicle while, in Group B revealed irregular island of basaloid cells representing the telogen germinal unit**.** Immunohistochemical stained sections showed negative immunoreaction for CD8 T lymphocyte in both treated groups indicating decreased inflammatory lymphocytic infiltration. While, there were increased immunoreaction for PCNA in both treated groups indicating hair cell growth. Erythema and Itching were the insignificant adverse effects of both treatment modalities.

Conclusion: the combination of 308-nm excimer laser therapy with topical minoxidil has superior efficacy in achieving hair regrowth compared to topical minoxidil only.

Keywords: Alopecia Areata; Minoxidil; 308-nm Excimer Laser; Dermoscopic; Microscopic.

1 Histology Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt.
2 Department of Dermatology, Venereology and Andrology, Damietta Faculty of medicine, Al-Azhar University, Egypt.
3 Medical Biochemistry Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt.
4 Pathology Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt.
5 Anatomy and Embryology Department, Faculty of Medicine(Cairo), Al-Azhar University, Egypt.
6 Anatomy and Embryology Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt.
7 Anatomy Department, Batterjee Medical College, KSA.
8 Pharmacology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.
9 Pharmacology Department, Batterjee Medical College, KSA.

DOI: 10.31838/ecb/2022.11.12.57

INTRODUCTION

Alopecia areata (AA) is a common inflammatory autoimmune disease affecting ~2% of the population, irrespective of sex, gender, ethnicity and age [1]. Although AA can affect any body region where hair grows, the scalp is the most frequently influenced area [2]. There are many different patterns and degrees of severity for hair loss. Alopecia totalis, or total loss of scalp hair, and alopecia universalis, or total loss of body hair, are two AA variations. Patchy AA, diffuse AA, AA reticularis, AA ophiasis, AA sisaipho, and perinevoid AA are further clinical manifestations[3].

Alopecia areata is a genetically predisposed autoimmune illness that is T cell-mediated [2]. The pathogenesis of AA is principally influenced by the interferon-induced breakdown of the hair follicle-immune privilege and the activation

of autoreactive cytotoxic natural killer group (NKG)2D+CD8+ T cells[3]. Hair follicle (HF) is an immune privileged (IP) location that largely inhibits autoimmune reactions to autoantigens released in the bulge throughout the hair cycle and in the bulb during the anagen phase [4]. This is primarily achieved by downregulating the production of MHC class I molecules in anagen hair bulbs, which could prevent autoantigens from being presented to CD8 + T cells [2]. So, the collapse of the hair follicle (HF)-immune-privileged (IP) site is a major precondition for the development of AA[1].

The diagnosis of AA is frequently made clinically, and active disease is characterized on dermoscopy by yellow dots, black dots, "exclamation marks" or tapering hairs, and damaged hairs. The occurrence of vellus hair is common in late or inactive phases of AA [5].

Numerous therapeutic approaches have been used to treat AA, including topical, systemic, and intralesional steroids, contact immunotherapy, and Janus kinase (JAK) inhibitors[6]. However, there is currently no known treatment for AA due to its unknown molecular aetiology. The European Medicines Agency (EMA) and Food and Drug Administration (FDA) have not approved the use of glucocorticoids (topical, intralesional, and systemic), contact immunotherapy, and other immunosuppressive drugs (methotrexate, azathioprine, cyclosporine) for the treatment of severe instances of AA[7].

The majority of conventional medical therapies available today have a low success rate and are frequently accompanied by a high risk of side effects[8]. But, the aim of treatments is to inhibit the progression of the disease and stimulate hair regrowth [9].

Minoxidil, a vasodilator used to treat hypertension, has been widely used to treat hair loss problems, including AA, when it was discovered to be connected with hair growth [10]. Topical 5% minoxidil has been shown to be successful in treating children and adults with patchy AA, according to a meta-analysis. However, for extensive AA, topical minoxidil may not be enough to fully restore hair growth [11].

Effective topical medicine distribution may be challenging due to the stratum corneum's barrier function. So, researchers have investigated the effect of combined laser therapy on the delivery of topical drugs for alopecia areata. The stratum corneum can be removed using lasers in a painless and secure manner [12].

Recently, 308 excimer laser/light treatment has been used to treat AA with notable improvements and fewer side effects due to induction of apoptosis in affected T-cells. A lower cumulative UV exposure, a shorter treatment period, and the ability to target specific lesions without damaging the surrounding healthy skin are all benefits of excimer therapy [13].

The purpose of this study was to determine the efficacy of topical minoxidil versus topical minoxidil combined with 308 excimer laser therapy in the treatment of alopecia areata.

PATIENTS AND METHODS:

A randomized controlled trial was conducted among 60 patients diagnosed as scalp alopecia areata. The study was carried out at the Dermatology Department at Damietta Faculty of medicine Al-Azhar University from August 2022 till November 2022, patients who participated were chosen randomly from the dermatological outpatient clinic of Al-Azhar University Hospital Damietta.

- **Inclusion and exclusion criteria**

The inclusion criteria include patients diagnosed with localized alopecia areata (<3 patches) who were between the ages of 16 and 60 years and who had not received systemic treatment for alopecia areata in the previous three months. While, the exclusion criteria include, alopecia areata totalis or universalis, extensive alopecia areata (<3 patches), Pregnant or lactating women, people with serious medical or dermatological disorders, and people who had contraindications to laser or minoxidil.

- **Ethical considerations**

The protocol of this study was approved by the medical ethical committee, Damietta Faculty of Medicine, Al-Azhar University, Egypt (DFM-IRB 0001267-22-08-013). Every patient who participated in the study gave their verbal and written consent before beginning, after explaining all concerns about this study. Through the use of a computer-generated randomization process, all qualified participants were equally distributed into Groups A received topical minoxidil only and group B received topical minoxidil combined with a 308-nm excimer laser.

- **Treatment protocol**
- **Topical minoxidil 5% gel**

Minoxidil 5% gel was purchased in the form of (Minoxidil Forte® 5% topical gel 60 gm; Pharmacare Egypt Co., Cairo, Egypt), Patients were instructed to clean the lesions by thermal water before application of the drug and to apply topical medication as a thin layer over the entire affected patch with rubbing twice daily for 12 weeks.

- **Laser therapy**

The 308-nm excimer laser (XTRAC®; Ultra-PhotoMedex) was used in combination with topical minoxidil to treat AA. laser-treated patches were exposed to radiation at regular intervals once weekly for 3 months (12 sessions). Depending on the skin type, the initial radiation exposure was between 100 and 200 mJ. If an adverse event didn't happen, the dose of following radiation was increased by 100 mJ for each session. When the

erythema persisted for 24-48 hours, the dosage was maintained. The dosage was decreased by 100 mJ in the following session if the erythema persisted for more than 48 hours or was accompanied by itching. Additionally, the next course of treatment was delayed when erythema became painful or was accompanied with vesicle or bullae [14].

- **Demographic, clinical and Dermoscopic examinations**

Both demographic and clinical data of the participants were recorded through history taking including the following: personal history, present history, including the onset, course, and duration of AA; predisposing factors; any additional skin diseases; associated systemic or autoimmune diseases; past history, including a history of surgery, trauma, and chronic disease; family history of AA; and drug history, including any topical or systemic treatments used for this current or prior attacks of the disease.

Dermatological examination: Type of alopecia areata (localized patchy) and distribution of lesions; Dermoscopic examination (Dermlite DL4): before starting treatment (baseline) and during the treatment period to detect Signs of activity of the disease include yellow dots, Exclamation mark hairs, broken hairs, black dots and tapering hairs, Signs of improvement of the disease includes short vellus hairs and terminal hair; A photograph was taken: before starting treatment (baseline), during the three months of treatment period and after 3 months of treatment.

- **Biochemical profile**

All the blood samples (10 ml of peripheral venous blood) from all participants were collected using biochemistry tubes (BD Vacutainer® SST™ II Advance serum tube, 367955 - 13×100 mm×5 ml, BD-Belliver Industrial Estate, Plymouth PL6 7BP, UK) in the morning after an overnight fast. Fasting blood glucose (FBG), Total leucocytic count (TLC), alanine transaminase (ALT), aspartate aminotransferase (AST), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)and triglycerides (TG) were measured using the enzymatic method.

The accepted normal ranges of the laboratory parameters in this study were as follows: FBG: 74–109 mg/dl; TLC: 6000-1100 10^3/uL; ALT: 0-40 U/L; AST: 0-40 U/L; TG: 0–200 mg/dl; LDL-C: 0–100 mg/dl; HDL-C: 40–60 mg/dl; and Very LDL-C: 0–40 mg/dl. For each parameter, a value higher than the upper limit was categorized as "elevated".

- **Histopathological and Immunohistochemical study:**

Four-millimeter punch biopsies were taken from the margins of the most recent patch of AA before and after treatment (at 6^{th} & 12^{th} weeks), embedded in paraffin block and prepared for the following: Hematoxylin and eosin stain to assess the histopathological changes and Immunohistochemical staining was performed using the labeled streptavidin–biotin complex method and stained for CD8 +T lymphocyte to detect the numbers and function of intra-follicular inflammatory cells & proliferating cell nuclear antigen (PCNA) to detect cell proliferation in the epidermis and hair follicles [15].

Light microscopy was used to examine the produced slides. Photographs of the images and assessments of the immunological expression of CD8 and PCNA were made using a Raywild E5 microscope with a Raywild M-300 digital camera.

STATISTICAL ANALYSIS:

The mean±SE is used to represent data. The statistical program SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The one-way ANOVA was used for comparison between the two groups with considered statistical significance at $P < 0.05$.

RESULTS:

Socio-demographic and history distribution among the studied groups

There is no statistically significant difference between studied groups as regard age, sex, family history, stress and previous treatment (table. 1).

Table (1): Socio-demographic and history distribution among the studied groups

Parameters / Groups	Age/years (mean±SD)	Sex		P value	Family history		Stress		Previous treatment
		male	female		+ve	-ve	+ve	-ve	
Minoxidil only group N=30 (%)	18–60 (30 ± 8.47)	17 (56.6)	13 (43.4)	0.012*	0	30 (100)	18 (60)	12 (40)	0
Minoxidil with Laser group N=30 (%)	19-58 (33.5 ±7.65)	20 (66.6)	10 (33.3)	0.034*	0	30 (100)	17 (33.3)	13 (66.7)	0
P value	0.527	0.624	0.328			-----		0.519	

Data are shown as mean ± SD and (n=10).
***Statistically significantly at p <0.05.**

Clinical evaluation

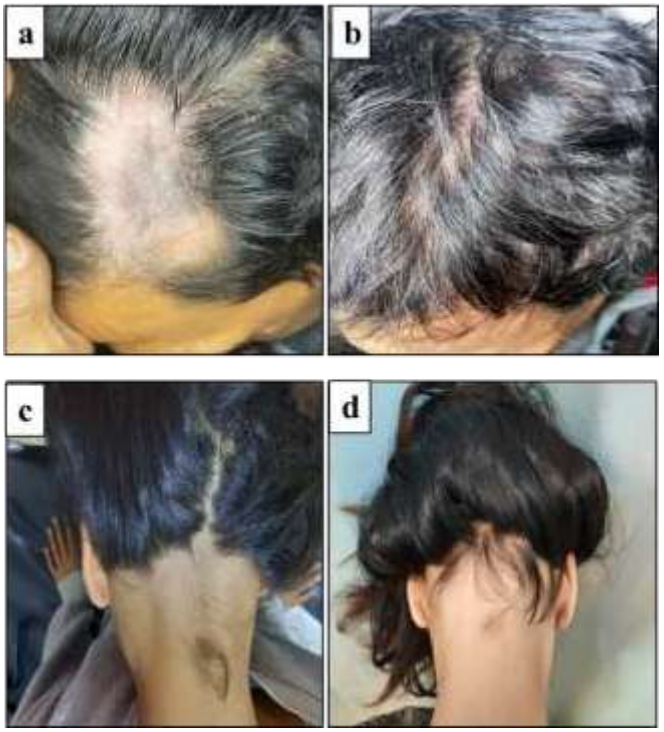

Fig. 1: Showing excellent improvement in patient with alopecia areata in group A, (**a**: before treatment, **b**: after treatment); Excellent improvement in a patient with alopecia areata in group B (**c**: before treatment, **d**: after treatment).

Dermoscopic findings

Compared to the baseline, all treatment groups showed significant post-treatment reduction in black dots (BD), Exclamation mark hair (EMH), and vellus hair (VH) and increase in terminal hair with significant improvement in treated group with combined topical minoxidil with Laser group compared to treated group with Minoxidil only.

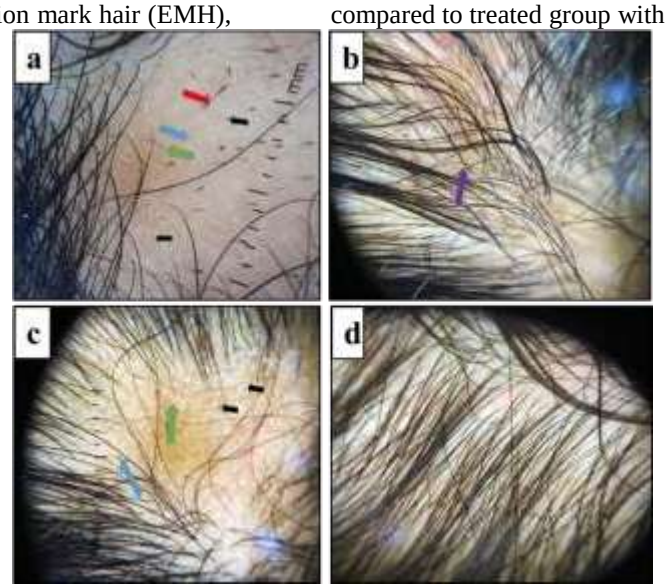

Fig. 2: Dermoscopic images of alopecia areata patients. **a** and **c** Before treatment, showing red arrow (exclamation mark), black arrow (black dots), blue arrow (vellus hair) purple arrow (terminal hair). **b** and **d** After treatment, showing disappearance of all features of AA activity and appearance of terminal hair (purple arrow) and short vellus hair (blue arrow).

There is no statistically significant difference between studied groups as regard dermoscopic finding at baseline (**Table 2)**.

Table (2) Comparison of dermoscopic findings at base line among the studied groups.

Dermoscopy at baseline	Minoxidil only group N=30 (%)	Minoxidil with Laser group N=30 (%)	P value
Black dot	19(63.3)	18(60)	p=0.728
Yellow dot	23(76.6)	24(80)	p=0.148
Broken hair	9(30)	8(26.6)	p=0.715
Exclamation mark	16(53.3)	17(56.6)	p=0.354
Vellus hair	13(43.3)	14(46.6)	p=0.647
Terminal hair	2(6.6)	3(10)	p=0.258

***Statistically significantly at p <0.05.**

There were statistically significant decreased activity signs of the disease and increased improvement signs in both treated groups with either minoxidil alone or minoxidil combined with Laser after 6 weeks of follow up in comparison to both groups at baseline. Also, there were statistically significant decreased activity signs of the disease and increased improvement signs in the treated group with minoxidil combined with Laser after 6 weeks of follow up in comparison to minoxidil alone (**Table 3)**.

Table (3): Comparison of dermoscopic findings after 6 weeks follow up among the studied groups and compared to baseline.

Group	Minoxidil only group N=30 (%)		Minoxidil with Laser group N=30 (%)	
Time of dermoscopy	**At base line**	**After 6 weeks**	**At base line**	**After 6 weeks**
Black dot	19(63.3)	8(0.0)[a]	18(60)	7(20) [ab]
Yellow dot	23(76.6)	10(33.3)[a]	24(80)	14(40) [ab]
Broken hair	9(30)	5(16.66)[a]	8(26.6)	4(13.33) [ab]
Exclamation mark	16(53.3)	5(16.66)[a]	17(56.6)	3(10) [ab]
Vellus hair	13(43.3)	11(36.6)[a]	14(46.6)	9(30)[ab]
Terminal hair	2(6.6)	19(63.3)[a]	3(10)	21(70) [ab]

[a]Statistically significant from the same group at baseline (p <0.05).
[b]Statistically significant from the treated group with minoxidil only After 12 weeks (p <0.05).

There were marked statistically significant decreased activity signs of the disease and increased improvement signs in both treated groups with either minoxidil alone or minoxidil combined with Laser after 12 weeks of follow up in comparison to both groups at baseline. Also, there were marked statistically significant decreased activity signs of the disease and increased improvement signs in the treated group with minoxidil combined with Laser after 12weeks of follow up in comparison to minoxidil alone (**Table 4)** .

Table (4): Comparison of dermoscopic findings after 12 weeks follow up among the studied groups and compared to base line.

Group	Minoxidil only group N=30 (%)		Minoxidil with Laser group N=30 (%)	
Time of dermoscopy	**At base line**	**After 12 weeks**	**At base line**	**After 12 weeks**
Black dot	19(63.3)	1(0.0)[a]	18(60)	6(20) [ab]
Yellow dot	23(76.6)	7(33.3)[a]	24(80)	12(40) [ab]
Broken hair	9(30)	5(16.66)[a]	8(26.6)	4(13.33) [ab]
Exclamation mark	16(53.3)	6(20)[a]	17(56.6)	5(16.6) [ab]
Vellus hair	13(43.3)	9(30)[a]	14(46.6)	8(26.6)[ab]

Terminal hair	2(6.6)	22(73.3)[a]	3(10)	25(83.3) [a]

[a]Statistically significant from the same group at baseline (p <0.05).
[b]Statistically significant from the treated group with minoxidil only After 12 weeks (p <0.05).

Histopathological & immunohistochemical results at base line and after 12 weeks of treatment

Light microscopic examination of skin sections at base line before treatment showed perifollicular inflammatory lymphocytic infiltration, the telogen germinal unit consists of trichilemma that was somewhat convoluted and surrounded by palisading basaloid cells **(Fig. 3a).** Immunohistochemical stained sections showed strong positive immunoreaction for CD8 T Lymphocyte (**Fig. 3b).** Immunohistochemical stained sections showed mild positive immunoreaction for PCNA (Fig. 3c).

Light microscopic examination of skin sections after 12 weeks of topical minoxidil only treatment showed sparse inflammatory lymphocytic infiltration around hair follicle Fig. (3d); While in combined treatment with minoxidil and laser group, there was irregular island of basaloid cells which represents the telogen germinal unit **(fig. 3g).** Immunohistochemical stained sections showed negative immunoreaction for CD8 T lymphocyte in both treated groups (Fig. 3b,e,h). Immunohistochemical stained sections showed strong positive immunoreaction for PCNA in both treated groups **(fig. 3f,i).**

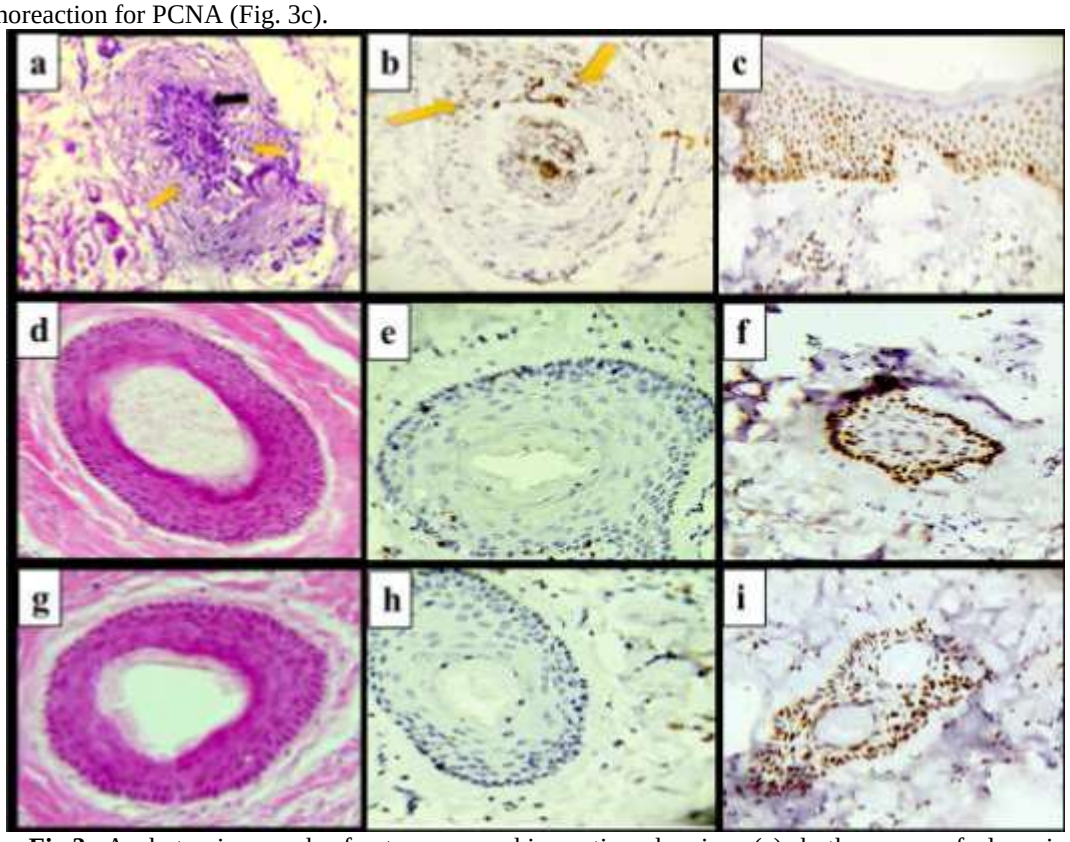

Fig.3: A photomicrograph of a transverse skin section showing: (a): both groups of alopecia areata before treatment at base line appear with irregular island of basaloid cells which represents the telogen germinal unit (black arrow) and perifollicular inflammatory lymphocytic infiltration (yellow arrows), Increased number of the telogen hairs (arrows); (d,g): treatment with either minoxidil only or minoxidil with Laser respectively showed Increased number of anagen hair follicles in both treated groups (Hx.&E.× 400). (b) Immunohistochemical stained sections showed marked increase in positive immunoreaction for CD8+T Lymphocyte in both groups of alopecia areata before treatment at base line; (e,h): while there were marked decrease of immunoreaction for CD8+T Lymphocyte after treatment with either minoxidil only or minoxidil with Laser respectively (CD8+T. immune stain × 400). (c): Immunohistochemical expression of PCNA showed week expression in the peri-follicular extracellular matrix (ECM) of the anagen hair follicles in both groups of alopecia areata before treatment at base line, while in (f, i) showed strong expression of PCNA in the perifollicular ECM of the anagen hair follicles in AA group after treatment with either minoxidil only or minoxidil with Laser respectively (PCNA. immune stain × 400).

Comparison between the two studied groups according to Patient assessment of improvement

There is statistically significant higher degree of Patient assessment of very good

improvement among the treated group with Minoxidil combined with Laser group only versus Minoxidil alone after 6 weeks of follow up; significant marked increase in excellent improvement was detected among treated group with minoxidil combined with Laser group versus Minoxidil only group after 12 weeks of follow up (Table 5).

Table (5): Patient assessment distribution among the studied groups

Degree of improvement	Minoxidil only group N=30 (%)	Minoxidil with Laser group N=30 (%)	P value
After 6 weeks			
Poor	3(10)	1(3.3)	
Fair	11(36.6)	3(10)	
Good	4 (13.3)	5(10)	p=0.005*
Very good	8(26.6)	12(40)	
Excellent	4 (13.3)	9(30)	
After 12 weeks			
Poor	1 (3.3)	0(0)	
Fair	3(10)	1(3.3)	
Good	4(23.3)	6(20)	p=0.004*
Very good	10 (33.3)	4(13.3)	
Excellent	12(40)	19(63.3)	

***Statistically significantly at p <0.05.**

Comparison between the two studied groups according to adverse effects

There is no statistically significant difference between Minoxidil only group versus Minoxidil with Laser group as regard burning sensation and itching **Table (8)**.

Table (6): Comparison between the two studied groups according to adverse effects.

Side effects	Minoxidil only group N=30 (%)	Minoxidil with Laser group N=30 (%)	P value
Erythema	**3(10)**	**6(20)**	**p=0.733**
Itching	**10(33.3)**	**11(36.6)**	**p=0.225**

***Statistically significantly at p <0.05.**

Biochemical profile in the various study groups

No statistically significant difference was present in the biochemical profile between both groups (Table 7).

Table 7: Biochemical profile in the various study groups

Groups / Parameters	Minoxidil only group	Minoxidil with Laser group	P value
FBG	**86.17±5.56**	**85.5±3.83**	**0.2881**
ALT	**23.74±5.73**	**26.18±2.43**	**0.1208**
AST	**27.24±3.27**	**24.84±4.25**	**0.2544**
Triglycerides	**97.33±13.39**	**89.33±5.56**	**0.1735**
LDL	**91.84±28.74**	**90.81±31.41**	**0.4852**
HDL	**51.25±19.14**	**54.67±11.27**	**0.5411**
Total Cholesterol	**160.37±29.12**	**163.74±32.64**	**0.0214**
TLC	**8500±500.56**	**7125±415.39**	**0.0474**

Data are shown as mean ± SD
***Statistically significantly at p <0.05.**

DISCUSSION

Alopecia areata (AA) is an autoimmune condition that affects 2.1% of people and is marked by repeated patches of hair loss that come and go. It can happen at any age, but is most frequently recognized as circular areas of hair loss [12]. No medicine has ever been proven to be consistently successful or to fully cure AA[14] . In the present

study we aimed to evaluate the efficacy of topical minoxidil versus combination of topical minoxidil with 308- nm excimer laser in treating patchy alopecia areata.

In the present study, a higher incidence of statistically significant predominance was observed in males than females, as 61.6% were males and 38.4% were females. The gender distribution of AA patients has been examined in various research, and inconsistent findings have been found. In agreements to our results, higher prevalence of alopecia areata in men was also reported in several studies [16, 17]. In contrary to our results several researches [18, 19] found female predominance than males. This difference could be due to variation of sample size, different age group and different inclusion criteria.

As regard age distribution in our study we found that, 85% of cases were >20 years old and 15% were ≤20 years old. Median age of the studied cases was 32.5 years which was higher than expected because cases were selected above the age of 17 as the nature of the two modalities of treatment used in this study required cooperative patients, this was conistant to a recent study findings in treatment of alopecia areata with topical application of triamcinolone acetonide after fractional carbon dioxide laser [20].

In our study, all patients received topical minoxidil 5% gel, this was in harmony with the previous researches as topical minoxidil has been shown to promote vasodilation and promote hair growth in AA patients [21, 22].

As regard the dermoscopic findings in our study, we found that the most frequent dermoscopic features in AA were short vellus hairs, YD, BD, BH, and EMH. This was in harmony with the findings of previous researches[23, 24]. Compared to the dermoscopic findings at the baseline, all treatment groups showed significant post-treatment reduction in BD, BH, EMH, and YD and increase in terminal hair with significant improvement in treated group with combined topical minoxidil and Laser compared to treated group with Minoxidil only group. This was in agreement to the results of a previous research[5] on either minoxidil alone or combined with 308-nm laser therapy which revealed that the dermoscopic findings in patients who had received 308-nm excimer lamp with minoxidil therapy was better than on the patients who had not accepted other treatments before.

In this study, the histopathological findings of skin biopsy of both groups of alopecia areata before treatment at base line showed irregular island of basaloid cells which represents the telogen germinal unit and perifollicular inflammatory lymphocytic infiltration, Increased number of the telogen hairs. In accordance to our results, several studies [24, 25] found that the baseline samples from AA lesions revealed considerably less anagen hair follicles prior to therapy.

While, the skin biopsy after treatment with either minoxidil only or minoxidil with Laser in this study showed Increased number of anagen hair follicles in both treated groups. Similar to our results, a previous study[26] on the role of low-level light therapy in combination with minoxidil 2% to promote sustainable hair regrowth activity in treatment of alopecia.

Fractional carbon dioxide laser alone and as an assisted drug delivery for treatment of alopecia areata found that in post-treatment biopsies compared to baseline, all groups had significantly more anagen follicles and significantly less telogen/catagen follicles. Furthermore, when the number of post-treatment telogen/catagen follicles was compared to those from healthy controls, no obvious variations were seen.

In this study, we found that there were marked increase in positive immunoreaction for CD8+T Lymphocyte in both groups of alopecia areata before treatment at base line. Similar to our results, multiple studies[27, 28] revealed that CD8+ T lymphocytes have an effector role in the development of alopecia areata as the majority of the intrafollicular T-cell infiltration is made up of CD8+ T cells. This could be due to the cytotoxic Fas/Fas ligand and granzyme B pathways present in the inflammatory intrafollicular T cells of alopecia areata [29].

In contrast, there were marked decrease of immunoreaction for CD8+T Lymphocyte after treatment with either minoxidil only or minoxidil with Laser respectively. Similarly to our results in the Dundee[30] experimental bald rat, depletion of CD8+ T cells was a sign of recovery of alopecia areata. Along similarity, individual researchers have considered using a 308-nm excimer light to treat AA by causing T cells to undergo apoptosis [31]. Also, a previous research[5] showed effectiveness and contributing factors in the treatment of alopecia areata with a 308-nm excimer lamp and minoxidil by preventing T cells from attacking hair follicles and the function of the hair follicle will be restored and the hair growth will be promoted.

As regard immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in this study, we found that there were marked increase in positive immunoreaction for PCNA in both treated groups of alopecia areata after treatment compared to both groups before treatment at baseline indicating that treatment with either minoxidil alone or combined with laser caused cell growth in the skin. Similar to our results, previous researches on animal and human studies revealed marked increase in numbers of PCNA-positive cells in either control groups or after treatments of alopecia with different modalities compared to those in the alopecia groups before treatment[32, 33]

As regard the lipid profile, there were no

statistical difference between both AA treated groups similar to our results, the statistics of a previous study [34] on patients with alopecia areata revealed that there was no significant difference between the study groups in the existence of dyslipidemia. In contrary to our results, several studies[35, 36] have shown that alopecia and dyslipidemia are related. This difference could be due to that those studies were carried on patients with androgenic alopecia, which differs from AA in its etiology.

Also, our study did not exhibit any statistical difference between both AA groups after treatments as regard the biochemical profile (FBG, TLC, ALT and AST) indicating the safety of both treatment modalities. This was in accordance to the results of previous studies on alopecia and its treatments, they found no elevation in either FBG, transaminases or leucocytes [34, 37, 38].

In accordance to the safety of the used modalities in treatment of AA in this study over the course of a 12-week therapy period, we found no major adverse effects except from non significant erythema, and itching, which had no negative effects on the patients' day-to-day activities. This was in agreements to the results of a similar study on the effectiveness and contributing factors in the treatment of alopecia areata with a 308-nm excimer lamp and minoxidil[5].

In this study, there were statistically significant higher degrees of patients assessment of improvement among both treated group with Minoxidil only and Minoxidil combined with Laser group ; significant increase in very good and excellent improvement was detected among the treated group with Minoxidil combined with 308-nm Laser group compared to minoxidil only group either after 6 weeks or 12 weeks of treatment. In agreement to our results previous researches analyzing the therapeutic effects of ultra-pulsed carbon dioxide fractional laser in combination with minoxidil on alopecia areata was more successful than using topical minoxidil alone in the treatment of AA[21, 39].

The significant improvement in treatment of AA with topical minoxidil combined with a 308-nm excimer laser compared to treatment with minoxidil alone could be interpreted by permission of topical minoxidil to bypass the epidermal barrier by creation of microchannels in the epidermis by a 308-nm excimer laser to reach the hair follicle, which is located in the dermis and subcutaneous fat beneath the stratum corneum (around 10-15 M thick) and is made up of stratified keratinocytes inside a complex matrix [20].

From the above findings we hypothesize that combination of 308-nm laser therapy with topical minoxidil could be more effective in treatment of alopecia areata more than treatment with topical minoxidil alone.

LIMITATIONS OF THE STUDY:

Small sample size and a short follow-up time.

REFERENCES

1. Al-Eitan LN, Alghamdi MA, Al Momani RO, Aljamal HA, Abdalla AM, Mohammed HM. Genetic predisposition of alopecia areata in jordanians: A case-control study. Heliyon. 2022 Mar 24;8(4):e09184. doi: 10.1016/j.heliyon.2022.e09184.
2. Zhou C, Li X, Wang C, Zhang J. Alopecia areata: an update on etiopathogenesis, diagnosis, and management. Clin Rev Allergy Immunol. 2021 Dec;61(3):403-423. doi: 10.1007/s12016-021-08883-0.
3. Richmond JM. Immunopathogenesis of alopecia areata. Curr Res Immunol. 2021 Feb 6;2:7-11. doi: 10.1016/j.crimmu.2021.02.001.
4. Rajabi F, Drake L, Senna M, Rezaei N. Alopecia areata: a review of disease pathogenesis. Br J Dermatol. 2018 Nov;179(5):1033-1048. doi: 10.1111/bjd.16808.
5. Li A, Meng X, Xing X, Tan H, Liu J, Li C. Efficacy and Influence Factors of 308-nm Excimer Lamp with Minoxidil in the Treatment of Alopecia Areata. Lasers Surg Med. 2020 Oct;52(8):761-767. doi: 10.1002/lsm.23210.
6. Sterkens A, Lambert J, Bervoets A. Alopecia areata: a review on diagnosis, immunological etiopathogenesis and treatment options. Clin Exp Med. 2021 May;21(2):215-230. doi: 10.1007/s10238-020-00673-w.
7. Fukuyama M, Ito T, Ohyama M. Alopecia areata: Current understanding of the pathophysiology and update on therapeutic approaches, featuring the Japanese Dermatological Association guidelines. J Dermatol. 2022 Jan;49(1):19-36. doi: 10.1111/1346-8138.16207.
8. Ramírez-Marín HA, Tosti A. Evaluating the therapeutic potential of ritlecitinib for the treatment of alopecia areata. Drug Des Devel Ther. 2022 Feb 17;16:363-374. doi: 10.2147/DDDT.S334727.
9. Cranwell WC, Lai VW, Photiou L, Meah N, Wall D, Rathnayake D, et al. Treatment of alopecia areata: an Australian expert consensus statement. Australas J Dermatol. 2019 May;60(2):163-170. doi: 10.1111/ajd.12941.
10. Suchonwanit P, Thammarucha S, Leerunyakul K. Minoxidil and its use in hair disorders: a review. Drug Des Devel Ther. 2019 Aug 9;13:2777-2786. doi: 10.2147/DDDT.S214907.
11. Freire P, Riera R, Martimbianco A, Petri V, Atallah A. Minoxidil for patchy alopecia areata: systematic review and meta- analysis. Eur Acad Dermatol Venereol. 2019 Sep;33(9):1792-1799. doi: 10.1111/jdv.15545.
12. Algowil MEA, Kandel AH, Gharib KM. Lasers

uses in the treatment of Alopecia Areata. The Egyptian Journal of Hospital Medicine. 2022;87(1):2037-41. doi: 10.21608/ejhm.2022.232257.
13. Al Hamzawi NK, Al Baaj MS. Overview of the Role of 308 Monochromatic Excimer Phototherapy for the Treatment of Alopecia Areata. Alopecia Management-An Update: IntechOpen; 2022. doi: 10.5772/intechopen.108531.
14. Kianfar N, Dasdar S, Mahmoudi H, Abedini R, Fahim S, Hosseini SA, et al. Comparison of the efficacy and safety of 308- nm excimer laser with intralesional corticosteroids for the treatment of alopecia areata: A randomized controlled study. Lasers Surg Med. 2022 Apr;54(4):502-510. doi:10.1002/lsm.23501.
15. Lim Y, Kim S, Kim H, Li K, Kim M, Park KC, et al. Potential relationship between the canonical Wnt signalling pathway and expression of the vitamin D receptor in alopecia. Clin Exp Dermatol. 2014 Apr;39(3):368-75. doi: 10.1111/ced.12241.
16. Al-Ajlan A, Alqahtani ME, Alsuwaidan S, Alsalhi A. Prevalence of alopecia areata in Saudi Arabia: cross-sectional descriptive study. Cureus. 2020 Sep 10;12(9):e10347. doi: 10.7759/cureus.10347.
17. Kavak A, Yeşildal N, Parlak A, Gökdemir G, Aydoğan I, Anul H, et al. Alopecia areata in Turkey: demographic and clinical features. J Eur Acad Dermatol Venereol. 2008 Aug;22(8):977-81. doi: 10.1111/j.1468-3083.2008.02699.x.
18. Uzuncakmak TK, Engin B, Serdaroglu S, Tuzun Y. Demographic and clinical features of 1,641 patients with alopecia areata, alopecia totalis, and alopecia universalis: a single-center retrospective study. Skin Appendage Disord. 2021 Jan;7(1):8-12. doi: 10.1159/000510880.
19. Harries M, Macbeth A, Holmes S, Chiu W, Gallardo W, Nijher M, et al. The epidemiology of alopecia areata: a population- based cohort study in UK primary care. Br J Dermatol. 2022 Feb;186(2):257-265. doi: 10.1111/bjd.20628.
20. Omar MM, Obaid ZM, Sayedahmed OME. Comparative study between topical application of triamcinolone acetonide after fractional carbon dioxide laser versus microneedling in the treatment of resistant alopecia areata. Dermatol Ther. 2022 Dec;35(12):e15913. doi: 10.1111/dth.15913.
21. Wang W, Gegentana, Tonglaga, Bagenna, Li Y. Treatment of alopecia areata with nonablative fractional laser combined with topical minoxidil. J Cosmet Dermatol. 2019 Aug;18(4):1009-1013. doi: 10.1111/jocd.12883.
22. Hu Y, Wang R, Zheng X, Tang H, Huang L. Clinical observation on the efficacy of CO2 fractional laser combined with 5% minoxidil tincture in the treatment of 38 patients with alopecia areata. Chin J Derm Venereol. 2014;28(06):587-9. doi:10.13735/j.cjdv.1001-7089.2014.0587.
23. Inui S. Trichoscopy for common hair loss diseases: algorithmic method for diagnosis. J Dermatol. 2011 Jan;38(1):71-5. doi: 10.1111/j.1346-8138.2010.01119.x.
24. Genedy RM, Badran FK, Tayae EM, Sabra HN. Lesson to learn from cellular infiltrate in scalp biopsy of alopecia areata. Am J Dermatopathol. 2021 Dec 1;43(12):e158-e164. doi: 10.1097/DAD.0000000000001929.
25. Herz- Ruelas ME, Gomez- Flores M, Miranda- Maldonado I, Welsh E, Ocampo-Candiani J, Welsh O. Escalating dosimetry of UVA- 1 in the treatment of alopecia areata. Int J Dermatol. 2017 Jun;56(6):653-659. doi: 10.1111/ijd.13572.
26. Mahe YF, Cheniti A, Tacheau C, Antonelli R, Planard- Luong L, de Bernard S, et al. Low-Level Light Therapy Downregulates Scalp Inflammatory Biomarkers in Men With Androgenetic Alopecia and Boosts Minoxidil 2% to Bring a Sustainable Hair Regrowth Activity. Lasers Surg Med. 2021 Nov;53(9):1208-1219. doi: 10.1002/lsm.23398.
27. Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS. Mediation of alopecia areata by cooperation between CD4+ and CD8+ T lymphocytes: transfer to human scalp explants on Prkdcscid mice. Arch Dermatol. 2002 Jul;138(7):916-22. doi: 10.1001/archderm.138.7.916.
28. Gilhar A. Alopecia areata transferred to human scalp explants on SCID mice with T-lymphocyte injections. J Clin Invest. 1998 Jan 1;101(1):62-7. doi: 10.1172/JCI551.
29. Bodemer C, de Prost Y, Peuchmaur M, Fraitaig S, Chatenoud L, Brousse N. Role of cytotoxic T cells in chronic alopecia areata. J Invest Dermatol. 2000 Jan;114(1):112-6. doi: 10.1046/j.1523-1747.2000.00828.x.
30. McElwee K, Spiers E, Oliver R. In vivo depletion of CD8+ T cells restores hair growth in the DEBR model for alopecia areata. Br J Dermatol. 1996 Aug;135(2):211-7. doi: 10.1111/j.1365-2133.1996.tb01149.x.
31. McMichael AJ, editor Excimer laser: a module of the alopecia areata common protocol. J Investig Dermatol Symp Proc. 2013 Dec;16(1):S77-9. doi: 10.1038/jidsymp.2013.31.
32. Hong G-L, Lee H-J, Kim Y-J, Kim K-H, Jung J-Y. Stauntonia hexaphylla Extract Ameliorates Androgenic Alopecia by Inhibiting Androgen Signaling in Testosterone-induced Alopecia Mice. Iranian Journal of Pharmaceutical Research: IJPR. 2022;21(1). doi: 10.5812/ijpr-133333.
33. El-Domyati M, Attia S, Saleh F, Bassyouni M, El-Fakahany H, Abdel-Wahab H. Proliferation, DNA repair and apoptosis in androgenetic

alopecia. J Eur Acad Dermatol Venereol. 2009 Jan;23(1):7-12.. doi: 10.1111/j.1468-3083.2008.02937.x
34. Nasimi M, Shakoei S, Abedini R, Ghandi N, Faghihi Z. A cross-sectional study of metabolic syndrome in patients with alopecia areata. Indian J Dermatol Venereol Leprol. 2021 May-Jun;87(3):427-429. doi: 10.25259/IJDVL_726_19.
35. Arias-Santiago S, Gutierrez-Salmeron MT, Buendia-Eisman A, Giron-Prieto MS, Naranjo-Sintes R. A comparative study of dyslipidaemia in men and woman with androgenic alopecia. Acta Derm Venereol. 2010 Sep;90(5):485-7. doi: 10.2340/00015555-0926.
36. Matilainen V, Koskela P, Keinänen-Kiukaanniemi S. Early androgenetic alopecia as a marker of insulin resistance. Lancet. 2000 Sep 30;356(9236):1165-6. doi: 10.1016/S0140-6736(00)02763-X.
37. AlMarzoug A, AlOrainy M, AlTawil L, AlHayaza G, AlAnazi R, AlIssa A, et al. Alopecia areata and tofacitinib: a prospective multicenter study from a Saudi population. Int J Dermatol. 2022 Jul;61(7):886-894. doi: 10.1111/ijd.15917.
38. Crispin MK, Ko JM, Craiglow BG, Li S, Shankar G, Urban JR, et al. Safety and efficacy of the JAK inhibitor tofacitinib citrate in patients with alopecia areata. JCI Insight. 2016 Sep 22;1(15):e89776. doi: 10.1172/jci.insight.89776.
39. Zhang J, Wei J, Zhen R, Zhao X. Effect of ultra-pulsed carbon dioxide fractional laser combined with minoxidil in the treatment of alopecia areata. Chinese J Aesthetic Med. 2022;31(01):49-53. doi: 10.15909/j.cnki.cn61-1347/r.004838.