THE EFFECT OF 1550 nm Er:GLASS FRACTIONAL LASER ON INSULIN-LIKE GROWTH FACTOR 1 AND WNT/β-CATENIN PATHWAY EXPRESSION IN ANDROGENETIC ALOPECIA

BY

MISS NAWAPORN UNGPRAPHAKORN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE
THAMMASAT UNIVERSITY
ACADEMIC YEAR 2016

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ABSTRACT

Androgenetic alopecia is a non-scarring hair loss problem that caused by multiple etiologies, including: genetic, hormone androgen through micro-inflammation of the perifollicular area. It is trend to be more concerned in individuals as the increasing in prevalence. The standard treatment of the androgenetic alopecia is a topical minoxidil and systemic 5α-reductase inhibitor which is still limited by its efficacy and side effects. Many new treatment modalities are introduced to treat the androgenetic alopecia. One of the new effective treatment includes 1550 nm fractional Er:Glass laser. The effect from fractional laser may accelerated the transition period of telogen phase to anagen phase. Fractional laser creates the microscopic thermal wound on irradiate area and can penetrate depth into deep dermis layer. The wound healing process is thought to be one of the mechanism that stimulating the new hair growth. Many cytokines were induced into the healing area for promoting the cell proliferation included hair stem cell.

In this study we would like to determine the mRNA level of IGF-1 and Wnt/β-catenin in androgenetic alopecia patients at before and after treatment with 1550 nm fractional Er:Glass laser.

Twenty three patients both male and female who were diagnosed an androgenetic alopecia with Hamilton-Norwood stage III-IV (include type III vertex) or female pattern hair loss Ludwig type I-II were enrolled in this study. Patients received 14 session of treatments with 1550 nm fractional erbium-glass laser (MOSAIC, Lutronic, Bangkok, Thailand) at 2 weeks interval using the same parameter with 2 x 12 mm tip, 6 mJ pulse energy, 300 spot/cm^2 density, static mode 2-4 passes on the balding area which involve fronto-vertical and parietal area of the scalp. Global photograph and target photograph were taken every month for clinical assessment in hair density and hair shaft diameter. Scalp biopsy was done in each patient at baseline and 24 hours after third laser treatment with 2 mm punch biopsy for RT-PCR evaluating mRNA expression level of IGF-1 and WNT10A (Wnt/β-catenin pathway). Histology were collected on baseline 1^{st} month, 2^{nd} month and 3^{rd} month of study. The All adverse effects were reported during the study.

RT-PCR of scalp tissue from ten patients were performed, at baseline mean of IGF-1 mRNA level is 4.85 ± 3.68 and mean of WNT10A mRNA level is and 3.22 ± 3.26. Then twenty four hours after third laser treatment (1 month) mean of IGF-1 mRNA is 6.14 ± 12.32 (P=0.445) and mean of WNT10A mRNA is 3.45 ± 6.59 (P=0.889). The trend of hair density and hair shaft diameter were significantly increased through 6 months of study.

Conclusion At 24 hours after treatment with 1550 nm Er:Glass laser in androgenetic alopecia patients, WNT10A and IGF-1 do not always increasing in level of expression compared with baseline. The mechanisms that 1550 nm Er:Glass laser induce the new hair growth may not limit to Wnt/β-catenin or IGF-1.

Keywords: Androgenetic alopecia, female pattern hair loss, fractional laser, Wnt/β-catenin, IGF-1

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
α	Alpha
β	Beta
γ	gamma
%	Percent
µl	Microliter(s)
µm	Micrometer(s)
º	Degree(s) of arch
ºC	Degree(s) Celsius
/	Per
AA	Alopecia areata
AFL	Ablative fractional laser
AGA	Androgenetic alopecia
ASC 2-P	L-ascorbic acid 2-phosphate
ATP	Adenosine Tri-Phosphate
BDNF	Brain-derived nerve factor
bFGF	Basic fibroblast growth factor
cm	Centimeter
cm^2	Centimeter square
CO_2	Carbon dioxide
CPA	Cypoterone acetate
DHT	Dihydrotestosterone
DP	Dermal papilla
EGF	Epidermal growth factor
EG-VEGF	Endocrine gland vascular endothelial growth factor

Er	Erbium
ER:YAG	Erbium-doped yttrium aluminium garnet
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FPHL	Female pattern hair loss
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
J	Joule(s)
KGF	Keratinocyte growth factor
LLLT	Low-level light therapy
mJ	Millijoule(s)
mm	Millimeter(s)
MPHL	Male pattern hair loss
mRNA	Messenger ribonucleic acid
MTZ	Microscopic thermal zone
MNC	Micro necrotic column
NAFL	Non-ablative fractional laser
ND:YAG	Neodymium-doped yttrium aluminium garnet
NGF	Nerve growth factor
nm	Nanometer
NT-3	Neurotrophic factor-3
OPD	Out-patient department

PSA	Prostate specific antigen
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase polymerase chain reaction
TGF	Transforming growth factor
TNF	Tumor necrosis factor
UV	Ultra violet
VEGF	Vascular endothelial growth factor
W	Watt(s)
Wnt	Wingless-type MMTV integration site family member
WNT10A	Wnt family member 10A
Wnt10b	Wnt family member 10B

CHAPTER 1
INTRODUCTION

1.1 Background and rationale

Androgenetic alopecia is a common non-scarring hair loss disorder in Thailand. In 2002, the prevalence of androgenetic alopecia is about 38.52% and it seems to be more frequency as the time goes by. Androgenetic alopecia is under the influence of androgen, genetic and micro-inflammation both male and female, but the standard treatment of this problem is still limited by its efficacy and side effects. The clinical manifestation is hair miniaturization of terminal hair, increase ratio of telogen hair, shorten hair life and thinning hair. In balding area IGF-1, VEGF (vascular endothelial growth factor) protein is down-regulated while BDNF (brain-derived nerve factor), NT-3 (neurotrophic factor), β-NGF are all up-regulated. Currently, new treatment modalities have been launched for androgenetic alopecia included 1550 nm Er:Glass fractional laser. Many dermatologists are interested in 1550 nm Er:Glass fractional laser treatment in androgenetic alopecia because of its efficacy. By the way, the mechanism of the 1550 nm Er:Glass fractional laser is still unknown, in this study we try to determine IGF-1 and Wnt/β-catenin level in balding area before and after treatment with 1550 nm Er:Glass fractional laser. Our results will help dermatologists understanding in mechanism of the 1550 nm Er:Glass fractional laser, which is one of the best options for androgenetic alopecia treatment modalities.

1.2 Research question

Androgenetic alopecia is a non-scarring hair loss problem that caused by many etiology since genetic, hormone androgen through micro-inflammation of the perifollicular. It is trend to be more concerned in individuals as the increasing in prevalence. The standard treatment of the androgenetic alopecia is still limited by its efficacy and side effects. Many new treatment modalities are introduced to treat the

androgenetic alopecia for the purpose of reduce in systemic side effects, increase efficacy of the treatment and prolong anagen phase.

One of the new effective treatment includes 1550 nm fractional Er:Glass laser, but the mechanism of how this laser stimulate the new hair growth is still unknown. Wound healing process are thought to be the main stream of new hair growth stimulation from fractional laser. In healing process many cytokines and growth factors are pooled into the wound area. The Wnt/β-catenin pathway which known as the initiating pathway of cell proliferation, might be activate after laser irradiation. In addition to the initiation pathways, another outstanding factor which known as anagen maintenance factor and telogen-anagen accelerator is IGF-1. Conducting this study will bring us to understand more in the mechanism of the 1550 nm Er:Glass fractional laser treatment in androgenetic alopecia. By compare mRNA level expression of WNT10A and IGF-1 at baseline and after treatment with 1550 nm Er:Glass fractional laser. Additionally, this study will demonstrate the clinical presentation and histopathology report after treat with 1550 nm Er:Glass fractional laser.

1.3 Specific objective

The primary objective is to determine mRNA level of IGF-1 and Wnt/β-catenin in Androgenetic alopecia at before and after the treatment of 1550 nm fractional Er:Glass laser.

The secondary objective is to evaluate the clinical efficacy of 1550 nm fractional Er:Glass laser treatment in androgenetic alopecia.

1.4 Hypothesis

Upregulation of Wnt/β-catenin and IGF-1 mRNA level might be observed in balding scalp area after treat with 1550 nm fractional Er:Glass laser in androgenetic alopecia.

1.5 Keywords

Androgenetic alopecia
Female pattern hair loss
Fractional laser
Wnt/β-catenin
IGF-1

1.6 Operation definition

Androgenetic alopecia, MPHL Hamilton-Norwood stage III-IV (include type III vertex) or FPHL Ludwig type I-II

1.7 Ethical consideration

All the patients were informed about the objectives, methods and expected benefits of this study and the possible adverse events or any inconvenience during the study were clearly informed. The patients have the right to withdraw from this study without deprivation of physician bias for continue the standard treatment of androgenetic alopecia. The data of subjects was confidentially and primarily concerned. Approval of this study was obtained from the Human Ethics Committee of Thammasat University.

1.8 Limitation

The limitations are number of cases, patient compliance, duration of study, financial condition

1.9 Expected benefit and application

Androgenetic alopecia is a most common non scaring hair loss disorder that influence by ages, hormone, environment factor and also micro-inflammation. Even

this condition is not a life threatening, but has a great impacts in individual self-esteem through the psychological health. The standard treatment of AGA is topical minoxidil and 5α-reductase inhibitor which is limited by its efficacy and systemic side effect while other therapy are all off label used. The new modalities of AGA treatment aiming to create maximize therapeutic effect with less to least side effect. One of the eye catching treatment in AGA is fractional laser that some papers reported the clinical efficacy in hair loss disorder and less systemic side effect. Our study purpose is to prove to the mechanism of how fractional laser stimulate the hair growth, evaluate the clinical efficacy and side effect of this treatment modality for the near future suitable treatment of hair loss condition.

1.10 Obstacles and strategies to solve the problems

This study took a long period of time and got many session for patients, some session was skip by few patients because of their inconvenience. This problem was solved by the patient-doctor relationship and only one session skipped is not much interfered the other part of data results.

One patients loss from the study due to his inconvenience the treatment take a long period and on session of treatment took about 30 minutes, this quite disturbed his work.

The laboratory part got many process and delicate handheld, it must be under supervised.

Table 1.1 Administration and time schedule

	2016									2017						
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Resaerch proposal																
Research ethics																
Experiment																
Data analysis																
Manuscript preparation																
Publication																

CHAPTER 2
REVIEW OF LITERATURE

Human hair plays a role in many importance functions such as insulation, protection as well as social interaction. Human hair is individualized in racialism and has a big role in social interaction, communication through sexual attraction.

Loss of hair (alopecia) or any diseases that lead to hair loss are often impact the person by diminished sense of person well-being, self-esteem leading to depressive mode and withdrawal themselves from the society.

Androgenetic alopecia (AGA) is one of a non-scarring hair loss disorders which has been described as the most common hair loss disorder in both men and women. Male pattern hair loss (MPHL) and female pattern hair loss (FPHL) are believed to be an androgen-dependent, genetically determined trait. It's characterized by a progressive decline in the duration of anagen, an increase in the duration of telogen and miniaturization of the scalp hair follicles. The distribution of affected area in male pattern hair loss (MPHL) and female pattern hair loss (FPHL) is different and individualized classified. In men, the baldness's depend on family history, ethnic variation whereas women pattern hair loss seems to be aged-related increase in frequency and severity.

Male pattern hair loss is grading in the Hamilton-Norwood classification scales ranges from type I to VII, female pattern hair loss (FPHL) is classified by Ludwig that characterized by a diffuse hair loss on the crown and persistence of the frontal hair line scale ranges from I to III.

Nowadays the standard treatment of Androgenetic alopecia is topical minoxidil and oral finasteride which limited used and low efficacy (1). Further treatment of androgenic alopecia also notable like low-level laser therapy, hormonal therapy and hair restoration (2).

Many studies have been searched for the new therapy of common hair loss disorders such as alopecia areata and androgenetic alopecia. It seems like fractional photothermolysis laser has been coming to be new choice of treatment.

In 2010, there was a report of fractional laser therapy in multiple alopecia areata which had been failed to conventional treatment such as topical 5% minoxidil, topical steroid and intra-lesion steroids for 2 years. In this case they treated the patient with fractional laser therapy (Mosaic^{TM} Lutronic, Inc., Gyeonggi, Korea) weekly for 24 weeks with a pulse energy of 10-15mJ, and a density of 300 MTZ/cm^2/pass and 2 passes per session. After 1 month, hair growth was observe, 30 to 40% (mostly pigmented terminal hair) covered at 3 months and after 6 months of fractional laser treatment session hair growth was completely cover the lesions. During the follow up time, a period of 6 months no relapsing of alopecia areata was occurred (3).

In 2011, Twenty Korean men with MPHL were enrolled in the pilot study of a 1,550-nm fractional erbium glass laser (Mosaic, Lutronic, Seoul, Korea) effect on hair cycle. This study was designed as half split; right side of the frontal scalp was treated with 1,550-nm fractional laser while left sided was left untreated as a control. The participants underwent the laser treatment for 5 sessions at 2 weeks interval with an energy of 5mJ, total density 300 spots/cm^2 without anesthesia. After photothermolysis treatment, hair density and hair growth rate are improved. At 24 hours after first treatment Wnt10a was highly expressed RT-PCR (tissue biopsies were taken from 3 participants). At 1 month, histological findings showed increasing in number of anagen hair follicles and anagen to telogen ratio (tissue biopsies were taken from 5 participants) (4).

In 2011, A group of south Korean female pattern hair loss patients (28 person) were received 10 treatments with a 1550 nm fractional laser therapy (Mosaic, Lutronic Co., Ltd, Seoul, South Korea) at 2 – weeks intervals using the same parameters (5-10 mm tip, 6mJ pulse energy, 800 spot/cm^2 density, static mode). The result showed significant increasing in hair density and hair thickness in 5 months. Mean % change from baseline of “hair density” is 57% and “hair shaft thickness” is 77% orderly which P-value < 0.001(5).

In 2012, 17 patients with various hair loss disorders both scarring and non-scarring alopecia were treated with non-ablative fractional laser (NAFL) using 1,550-nm erbium-glass Mosaic^{TM} laser: (Lutronic Corporation, Goyang, Korea) and/or ablative fractional laser (AFL) using a 10,600 nm Mosaic eCO2^{TM} laser (Lutronic Corporation). Two passes of the NAFL treatment was performed in static operating

mode with fluence of 6-8mJ, density of a 300 spots/cm^2/pass, without local anesthesia. For AFL, treated with fluence of 30 to 50mJ, density of a 150 spots/cm^2/pass, in static operating mode (spot diameter of 120 µm; percent coverage of 8.1% to 10.2%) was performed without local anesthesia on affected area. An epidermal cooling device (Zimmer Medizin Systems, Irvine, CA) was used during AFL treatments for pain relief. Seven patients were treated with NAFL alone, 6 with NAFL and AFL combination therapy and AFL alone for 4 patients. The data were retrospectively reviewed, the result show the clinical effectiveness of both non-ablative and ablative fractional laser. The responder group, 12 out of 17 patients (70.6%) has showed the clinical response with NAFL and/or AFL therapy, while non-responder group did not improve or worsen after the laser for 5 patients (29.4%) (6).

In 2016, thirty-two turkey people (n=96) with long standing alopecia areata were received 3 different treatment on three hair loss patches in the same person. First patch was treated with Nd:YAG laser (laserscope Lyrai, San jose, CA) pulse duration of 30 milliseconds and an energy of 10 J/cm^2 at 2 to 8 weeks intervals for total of 2-3 sessions. The second patch, they used fractional laser (eCO_2; Lutronic, Seoul, South Korea) with a power of 30 W, 120µm probe diameter, pulse energy range of 10-45mJ/cm^2 and density range of 75-100 spot/cm^2/pass at 2 or 4 weeks intervals for total 3 to 6 sessions. The third patch was served as a control patch, no laser treatment application. The result showed no statistically significant of the initial and final mean hair count among 3 groups of treatment. The initial mean hair count of the 3 patches was similar, Nd:YAG laser is 58.38 at p-values 0.4, Fractional CO_2 laser is 64.25 at p-values 0.17 and control patch is 62.06 at p values 0.2. The final means hair count for, Nd:YAG laser is 62.41 at p-values 0.4, Fractional CO_2 laser is 70.69 at p values 0.17 and control patch is 69.06 at p-values 0.2. The discussion of this unsatisfied study result are mentioned about the case they selected were a long standing disease, this might me the reason why there was no improvement (7).

The mechanism of how fractional laser act to hair follicle is still unclear. For years hair regrowth after wound healing had been discussed (8-10). Mice's keratinocyte IGF-1 overexpression improved wound healing and stimulated hair follicle formation and cycling reported by Semenova et al (11). From the previous study, they believed that wounding from fractional laser induce cytokines, increased

blood flow, growth factors and may direct altered dermal papilla. Many growth factors are taken a role in wound healing process, since the FGF family, EGF, IGFs, HGF, TGF-β, VEGF, NGF as well as interleukins which all known to be as the key factor of hair growth and hair cycle (12, 13).

In animal models Wnt/β-catenin pathway was activated after ablative fraction lasers had been done on telogen phase hair area of the mice, which is well known as one of the mechanism of new hair growth. The study showed that mRNA expression of Wnt10b had increased continued until 9 days after treatment. In addition to wnt10b, various growth factors are intermediately detected after fractional laser treatment was done such as VEGF, transforming growth factor β1 (TGF- β1), keratinocyte growth factor (KGF) (14).

The role of Wnt/β-catenin pathway inducing anagen reentry was proved by a group of China researchers in 2015. The mice with telogen hair phase on dorsal skin were treated with ablative fractional laser 2,490-nm Er:YAG laser (Pixel, Alma Lasers Ltd, Caesarea, Israel) with microlens 9 x 9 (81) dots (pixels), 1200mJ/cm^2, 2 passes on target area weekly for 3 session in laser group. The mice in second group were treated with 5% Minoxidil Tincture (Wanma Group, Zhejiang, China) apply on target area every day. For the third group, laser (parameter and interval as same as laser group) and 5% minoxidil were combined together and controlled group for the last one. At 24 hours after first treatment in each group Wnt10b and β-actin in treatment group were significantly increased in level compared with controlled group. The highest expression was belong to laser and 5% minoxidil combination group. Histology on day 15 after first treatment showed that the hair follicles number were increased and larger hair bulb in treatment groups compared with controlled group. The period from telogen turning to anagen in combined laser and 5% minoxidil group was significantly shorter than the controlled group (15).

In April 2015, there is a study of irradiation parameters of nonablative fractional laser in murine hair follicle regeneration. Researchers from Taiwan used a 1,550-nm fractional erbium-glass laser (Fraxel RE:STORE (SR1500) Laser system, Solta Medical, U.S.A.) treated dorsal skin with telogen hair phase of mice with various beams energy and densities range from 5-35mJ pulse energy and 500-3500 densities spot per cm^2 to evaluate the proper parameter that can induce anagen reentry. At

minimum pulse energy, 5mJ is failed to promote anagen though highest densities of 3500 MTZ/cm^2 was used get along with 5mJ pulse energy, anagen reentry is not observed. Minimal laser parameter required for promoting anagen reentry is about 10mJ with 1600 MTZ/cm^2 densities without developing erythema, ulcer or scar formation. At higher pulse energy and densities anagen reentry was well promoted but some erythema, ulcer even scar were noted. The molecular study showed that inflammatory cytokines including TNF-α, IL-1β and IL-6 at pulse energy 15mJ were all up regulated especially during day 1 to day 3 then they started to decrease in level, with higher densities the inflammatory cytokines trended to increase more expression. At 1600 MTZ/cm^2 TNF-α, IL-1β and IL-6 were observed increasing in expression until day 5. The association between histology and molecular study suggest that moderate but transient inflammatory process can induce new hair regrowth but not for intense and persist inflammation which can cause ulcer and scar (16).

Hair has their regenerating system, the hair cycle is consist of 3 stages: anagen, catagen and telogen (17). The anagen phase is the phase that hair follicle stem cell has ability to regenerate the new hair follicle (18). Hair bulge contains hair follicle stem cells which locate in the middle part of hair follicle (19). Turning to anagen phase, hair follicle stem cells proliferate and regenerate transit amplifying cells, for differentiate to the new hair shaft (20). During this phase many molecular signals have been influenced in the regulation of hair cycle including to Wnt/β-catenin pathway. The importance of wnt/β-catenin pathway is this pathway take part as primary initiator of anagen phase (21).

Recently in 2016, the pilot study of topical 0.2% methyl vanillate as an active ingredient derived from plant which is known as Wnt/β-catenin pathway activator. Twenty women with underlying of female pattern hair loss were given the spray with 0.2% methyl vanillate apply on thin hair area every other day for 6 months. After 6 months period of study, clinical data showed that mean hair counts, mean of hair mass index were significantly increased without unbearable side effect. Molecular studied from 10 participants for detecting WNT10B expression were done, 4 mm punch biopsied on scalp were sacrificed at baseline and 6 months. The mean of WNT10B expression was increased significantly around 32% compared to baseline. There are

some correlation between the up regulation of WNT10B expression and clinical presentation in androgenetic alopecia (22).

Currently, issues about cutaneous wound healing promote hair cycling by accelerated the time during anagen phase is widely acceptable (23). There are some associations between wound healing and hair follicle, normal wound healing process has influenced on hair follicle (24). In re-epithelization stage of wound healing, hair follicle stem cells migrate themselves into the epidermal defected area for proliferation and assist the wound healing (24). Furthermore some new hair follicles development have been observed after wounding in both animals and humans (25). There are also others interested molecular signaling in hair cycle named insulin – like growth factor (IGF)-1 (26). IGF-1 is founded in dermal papillae where the new hair growths are coming from. They also noted that in androgenetic alopecia patients have decreasing IGF-1 expression in dermal papillae. Because in balding area of androgenetic alopecia dermal papillae express more androgen receptor than non-balding area (27, 28). In androgenetic alopecia dermal papillae in balding area obviously express lower level of IGF-1 and its binding proteins (IGFBP-2, IGFBP-4) compare to non-balding area (29). IGF-1 is well known as an anagen maintenance, absence of IGF-1 could lead anagen phase prematurely enter to catagen phase (30).

In 2003, Tang reported the correlation between finasteride efficacy and expression of IGF-1 in follicular dermal papillae. Good clinical outcome from oral finasteride treatment in androgenetic alopecia patients represent up regulation of IGF-1 in dermal papillae (31).

In addition of a finasteride efficacy determination, IGF-1 level is widely selected for determination the treatment of hair growth. L-ascorbic acid 2-phosphate (Asc 2-P) promoting hair elongation by IGF-1 secretion from dermal papillae through phosphatidylinositol in vitro (32). The extract of Illicium anisatum also promotes hair growth by IGF-1 induction, KGF and VEGF in the hair follicles treatment promoting hair growth (33). Vascular endothelial growth factor D (VEGF-D) and endocrine gland-derived vascular endothelial growth factor (EG-VEGF) are significantly low in balding area (34). Normally VEGF help promoting angiogenesis and endothelial cell growth so these factor family are found to be up-regulated DP cells in during anagen phase. In

contrast, BDNF (brain-derived nerve factor), NT-3 (neurotrophic factor), β-NGF are all up regulated express from DP cell in balding area of androgenetic alopecia (34). The neurotrophic factors family are not only function as a neurons surviving factors by preventing them from apoptosis but also have some effect on hair morphogenesis (35, 36). NGF and neurotrophins are found to accelerate catagen developing in hair cycle (37-40). In mice, BDNF inhibits hair shaft elongation and it's under control by androgen (39-41).

Androgenetic alopecia is a common hair loss problem among Asian people and the prevalence increased with age (42). In Thailand, the prevalence of male pattern hair loss is about 38.52% (43). Coming through 21st century, both men and women are more interested in taking care of their health and appearance. Hair loss are seem to be more concerned in both men and women, its trend to be more detected androgenetic alopecia in the dermatology department. But the standard treatments are still limited by their efficacy and side effects. The ideal treatment of the androgenetic alopecia is shorten the time of treatment, local effect, less to least side effect and prolong the normal hair character duration without treatment. Many new treatment modalities that trend to meet the ideal efficacy have been introduced to treat this non-scarring hair loss problem include 1550 nm fractional Er:Glass laser. In this study we would like to evaluate the mechanisms of this 1550 nm fractional Er:Glass laser treatment in androgenetic alopecia by compare the protein level of IGF-1 and Wnt/β-catenin at before and after treatment with the 1550 nm fractional Er:Glass laser.

CHAPTER 3

ANDROGENETIC ALOPECIA

Androgenetic alopecia is a common non scarring hair loss disorder that effect both physical and mental individualized. This hair loss condition can be found in both male and female by using the term male pattern hair loss and female pattern hair loss. Androgen hormone, micro-inflammation (pollution, UV radiation, infection) and genetics are the key factors which initiating hair loss.

The characteristics of androgenetic alopecia is found to have diminish of anagen hair phase and increase in telogen hair phase. Moreover the affected scalp transform are occurred by the replacement of vellus hair instead of terminal hair (44). Theses result in a look of thinning hair by volume reduction, usually involved in vertex and fronto-parietal area.

3.1 Clinical manifestation

3.1.1 Male pattern hair loss

The severity of male pattern hair loss is categorized by Hamilton Norwood stage, there are 8 stages. Frontal recession of hairline presented in stage II and III while stage IV, V and VI diffuse hair loss involved both frontal and vertex of scalp. Type VII and VIII only hair around back (occipital area) and side (parietal area) were left balding area cover frontal and vertex area.

Type I: No recession of frontoparietal region of hairline, individualized hairline variation.

Type II: There is a symmetrical triangular recession of frontoparietal hairline which not exceed 3 cm anterior border of a line created down from the crown to external auditory meatus or not exceed the preauricular area.

Type III: Progressive deep symmetrical recession of temporal area which limited at auricular area or not exceed the created coronal line on external meatus of the ear.

Type III vertex: Vertex hair become thinning with frontal recession degree doesn't not exceed type III area.

Type IV: The symmetrical recession of frontal area become bald or sparsely cover with hair. Deep recession go across the pointed line. All anterior part may involve with baldness. Vertex area also become sparsely cover with hair or bald. Two area of baldness are separated by the band of dense hair across the crown.

Type V: The two baldness area are still separated but by the narrow and thin hair band. Both anterotemporal and vertex baldness area become larger.

Type VI: Only sparse of hair on the band was left. Largely involve in hair loss, both anterotemporal and vertex area baldness were connected.

Type VII and VIII: Sparsely horseshoe-shaped hair or totally denuded scalp (45).

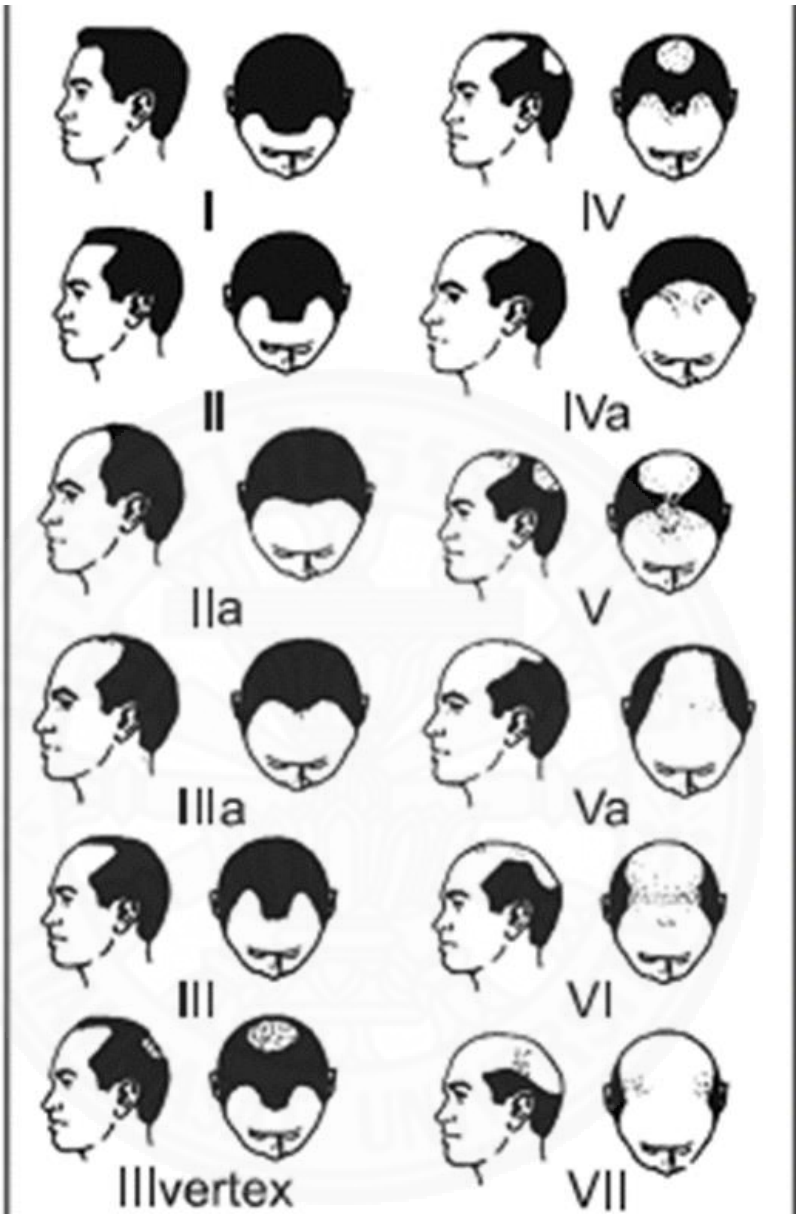

Figure 3.1 The Norwood-Hamilton classification of male balding defines two major patterns and several less common types. Thinning starts in both temples as well as the crown/vertex and slowly progresses to encompass the entire top of the scalp. (46)

3.1.2 Female pattern hair loss

Female pattern hair loss severity was classified by Ludwig, characterize with diffuse hair loss on vertex area or crown categorized into 3 stage. Differently from male pattern hair loss, in FPHL the frontal hair are persistence. Furthermore, Christmas tree pattern is described by Olsen as one of a FPHL characteristics. Diffuse hair loss over the crown area through the front of scalp.

Ludwig classification

Grade I: Thinning hair on crown area which is acceptable and limit posterior to frontal hair line 1 to 3 cm.

Grade II: Obviously thinning of hair on crown area within 1 to 3 cm posterior to frontal hair line.

Grade III: Completely denude of scalp within the area of 2 previous grading area (45).

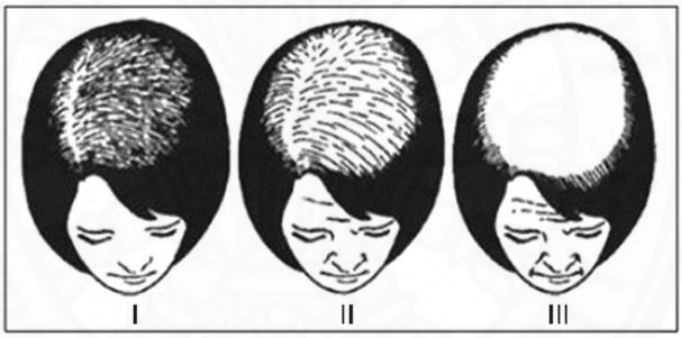

Figure 3.2 The Ludwig pattern of hair loss (3-point). There are three main classes, each with increasing hair loss (47)

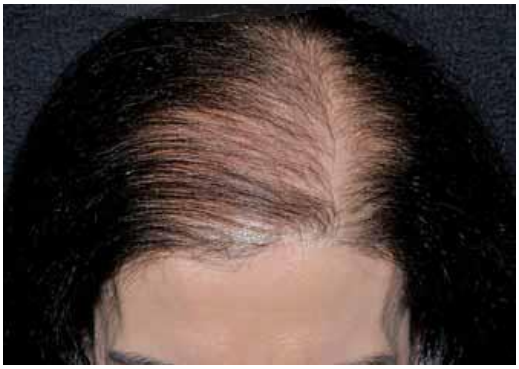

Figure 3.3 Female Pattern Hair Loss. Diffuse thinning of the hairs in the frontal and parietal regions, preserving the anterior hair implantation line. (48)

3.2 Histopathology

The horizontal section from 4 mm punch biopsied is a standard for diagnosed the androgenetic alopecia in order to assess the hair follicle numbers. From horizontal section, increasing in miniaturized hair proportion compared to terminal hair are found. The anagen:telogen ratio is decreased and follicle numbers in advanced case may found to be reduced. There are some inflammation infiltrate around hair follicle by lymphocyte and fibrosis may occur in a very late stage or poor prognosis case.

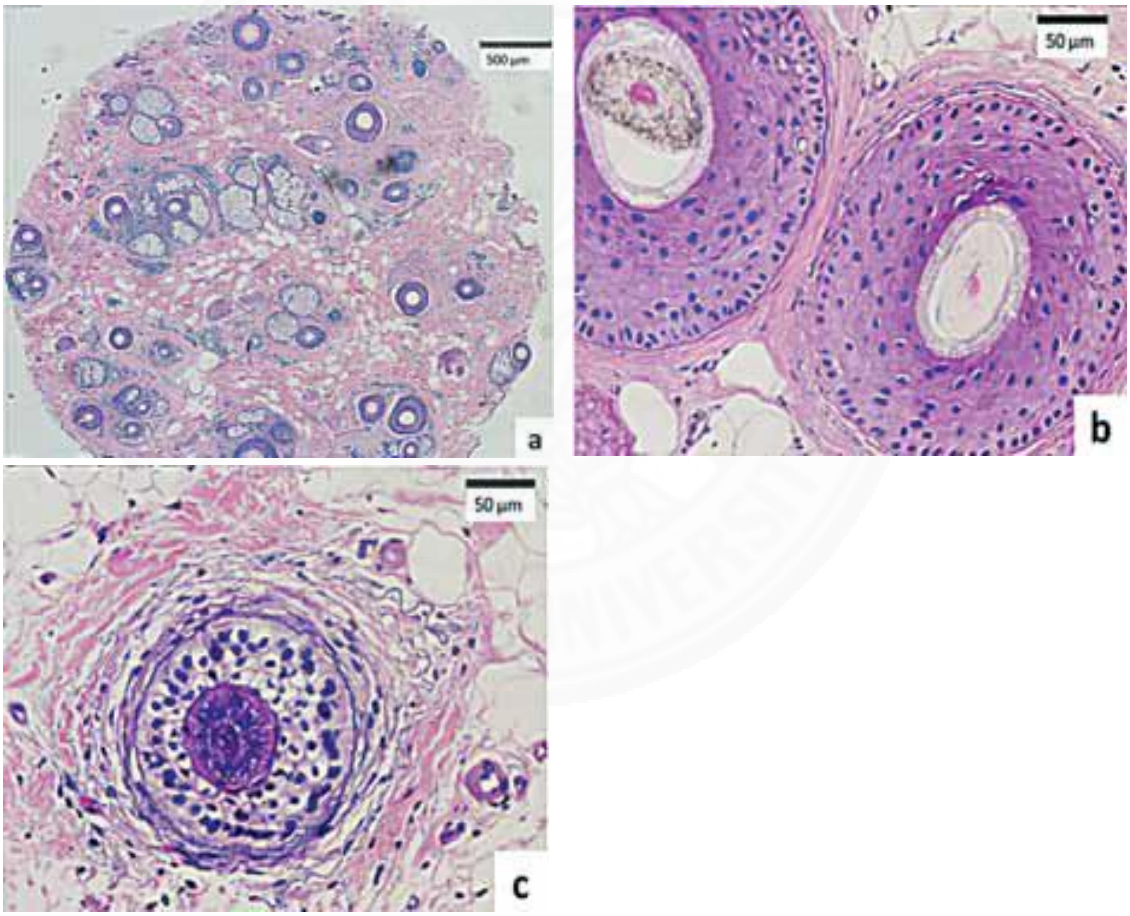

Figure 3.4 Histopathological examination of FPHL. a) Transverse section evidencing wide variability in diameter of the follicles. b) Terminal follicle in detail. c) Miniaturized follicle, Perifollicular fibrosis and sparse mononuclear inflammatory infiltrate in detail. (48)

3.3 Laboratory investigation

There is no specific laboratory investigation for diagnosed androgenetic alopecia. The investigation may be helpful in other hair loss condition suspected case for example hyperandrogenism which patient would come with some clue of that symptoms such as hirsutism, acne, oily skin, menstrual cycle problem, infertility get along with hair loss. Even useful work up for the cause of telogen effluvium in order to maximize the treatment response.

3.4 Dermoscopy

Dermoscope is a non-invasive tool used widely by dermatologist. From dermoscope, the variation thickness of hair shaft are found, increasing in miniaturization of hair especially on affected area such as frontal and vertex. The number of hair per follicular unit also reduced.

Brown patch around follicular opening with slightly atrophic change called peripilar sign can be found in early stage of FPHL, it is correlated with inflammatory cell infiltrated in histology. In advanced case, sebum and keratin are plug in dilated follicle result in yellow dot on dermoscope. Honey comb appearance may occur from pigment alteration from aging process or even UV radiation.

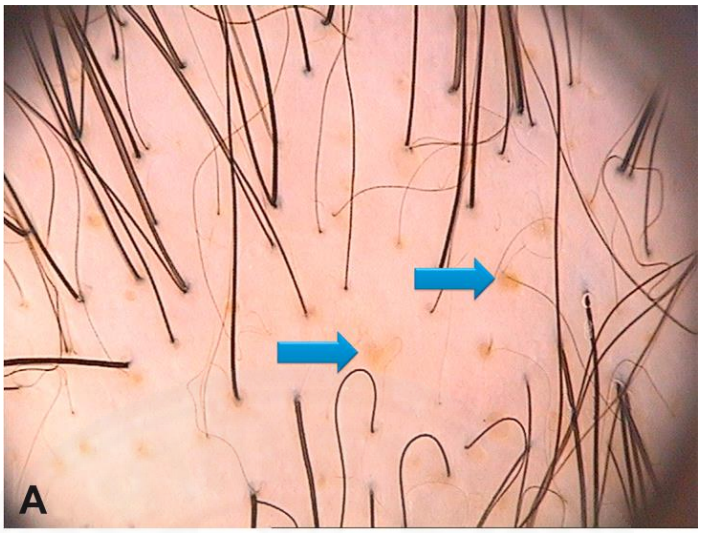

Figure 3.5 Yellow dots in female pattern hair loss. (49)

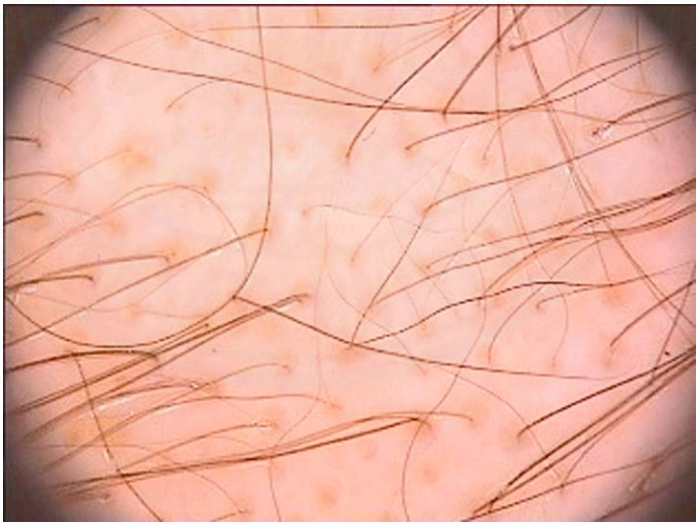

Figure 3.6 Trichoscopy of patterned hair loss reveals hair shaft thickness heterogeneity, multiple vellus hairs, and a predominance of follicular units with only 1 hair. (Original magnification:320.) (49)

3.5 Pathophysiology of androgenetic alopecia

In androgenetic alopecia many factors are involved in the pathophysiology but it is not completely elucidated. The main factors that have been widely talked about are genetics, androgen hormone, micro-inflammation that result from environmental factor.

From hair cycle, anagen hair phase is the main stage on normal scalp cover around 80-90% of entire area while telogen and catagen hair phase always do not excess 20% of entire hairs (10-20% of telogen phase and 1-2 % of catagen). Normally anagen hair last on scalp 2 to 8 years then turn into catagen that last only 2 to 3 weeks and finally become telogen that last only 3 months before fall out as a catagen.

In androgenetic alopecia, anagen period become shortening and early turn into telogen, that result in increasing the proportion of telogen (17, 50). According to testosterone conversion into dihydrotestosterone (DHT) by 5α-reductase enzyme, the hair follicle become miniaturization and early termination of anagen was resulted from 5α-reductase that acts on hair follicle receptor (44). Not only anagen phase shortening time that effect the baldness but also time lengthening from telogen to new anagen. These changes alter the hair cycle, by reduce the anagen-maintaining factor and promoting the apoptosis of cell (51, 52).

In androgenetic alopecia scalp, IGF-1 and VEGF are down regulated whereas BDNF, NT-3 and β-NGF are upregulated. IGF-1 and VEGF are known as anagen-promoting factors include Wnt which is known as the initiation pathway of new hair growth (12, 13).

Miniaturized hair is one of the key characteristics in this non scarring hair loss condition. The thickness of hair determined from the volume of dermal papilla, as dermal papilla volume was decrease in AGA, the hair shaft diameter become smaller (53). Dermal papilla volume decrease mechanism is still unclear there are some evidence show that androgen interfere the Wnt signaling activity of hair cycle, microinflamation around hair follicle also dose it too (44, 52). The process of becoming vellus hair is same as hair miniaturization, just only a small difference; vellus hair do not develop a piloerector muscle like normal hair.

Anagen-promoting factors	Factors promoting follicle apoptosis
bFGF	FGF5
FGF7	IL-1α
HGF	PGD_2
IGF-1	TGF-β1
PGE2	TNF-α
VEGF	
Wnt	

bFGF, basic fibroblast growth factor; FGF5, fibroblast growth factor 5; FGF7, fibroblast growth factor 7; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; IL-1 α, interleukin-1 alpha; PGD2, prostaglandin D2; PGE2, prostaglandin E2; TGF-β1, transforming growth factor beta1; TNF-α, tumor necrosis factor α; VEGF, growth factor of the vascular endothelium; Wnt, Wnt signaling pathway.

Figure 3.7 Main factors associated with the transition from the anagen hair phase to the catagen hair phase. (48)

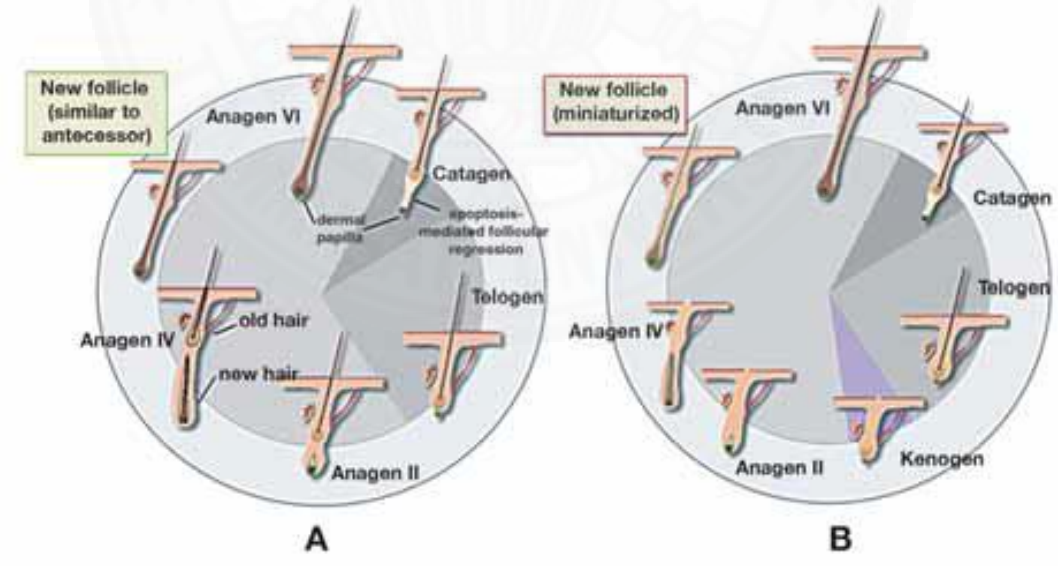

Figure 3.8 Hair cycle. A – Normal cycle of the follicle. B – Alterations occurring in baldness: shortening of the anagen phase, increase in the latency period (kenogen phase) and hair follicle miniaturization. These alterations may occur together or individually both in FPHL and MPHL. (48)

3.6 Treatment of androgenetic alopecia

The only FDA approved treatment of androgenetic alopecia is topical minoxidil and systemic 5 alpha reductase inhibitor. (1)

3.6.1 Minoxidil

Topical minoxidil in 2% and 5 % concentration in a lotion or foam preparation are used in androgenetic alopecia. The mitogenic effect of minoxidil induced proliferation of hair follicles. The mechanism of how minoxidil works still unclear, calcium homeostasis of cells is in charge of this.

Apply minoxidil solution twice daily on dry scalp. It may take 4-6 months to work and life-long use.

Side effect of topical minoxidil is contact dermatitis, facial hypertrichosis can be found. Mostly contact dermatitis are from irritant contact dermatitis of vehicle of minoxidil such as propelene glycol. Minoxidil has less systemic absorption, but tachycardia and lowering blood pressure had been report, described with caution in hypotension and cardiovascular problem case (54, 55).

3.6.2 Systemic 5α-reductase inhibitor

3.6.2.1 Finasteride

Finasteride is a selective 5α-reductase type 2 inhibitor, inhibit conversion of testosterone to DHT on scalp (sebaceous gland). Recommend dose for male pattern hair loss is 1 mg daily. Finasteride is an only FDA approved Systemic 5α-reductase inhibitor for androgenetic alopecia in men.

Precaution during administered of finasteride is 50% decreasing of PSA level due to volume reduction of prostate gland. This should be considering during health check-up of patient who received finasteride.

Only minimal side effect that can be found included, decreased in libido, erectile dysfunction and decreased in ejaculation volume. (56)

3.6.2.2 Dutasteride

Dutasteride is a non-selective 5α-reductase inhibitor. Normally 0.5 mg dutasteride is used for benign prostrate hypertrophy treatment, in androgenetic alopecia is not approved by FDA. (57, 58)

3.6.3 Hormonal therapy

3.6.3.1 Cyproterone acetate (CPA)

Synthetic derivative of 17-hydroxyprogesterone acts as androgen receptor antagonist. CPA is described combined with oral contraception pill for female pattern hair loss.

Side effect of CPA is menstrual cycle irregular, breast tender, loss of libido, nausea, weight gain and depression.

3.6.3.2 Spironolactone

A competitive aldosterone antagonist with antiandrogenic effect by blocking DHT interaction. Spinorolactone can used in FPHL but not in men.

Precaution in those who has renal problem due to its potassium sparing function and contraindicated for abnormal uterine bleeding, pregnancy and family history of breast cancer.

3.6.3.3 17α-and 17β-estradiol

In Europe 17α-and 17β-estradiol is commercial available for FPHL due to induce increasing of testosterone conversion to estrogen in hair follicles.

3.6.4 Low-level light therapy

Low-level light therapy (LLLT) is an optional treatment for androgenetic alopecia. It is non-invasive and less systemic side effect, only local irritation can be found. After treated with LLLT hair density and hair shaft diameter improved. The mechanism of how LLLT stimulate the new hair growth is not clearly understood, vasodilatation and cytokine inducer was thought to be the main idea (59). The ATP production was increased after treated with LLLT then reactive oxygen species (ROS) and Nitric oxide were released and followed by vasodilation (60-63).

3.6.5 Hair restoration surgery

Permanent treatment for androgenetic alopecia using the graft of occipital area. Good cosmetics outcomes, favorable in patients with Hamilton-Norwood VI and VII.

CHAPTER 4
1550 nm ERBIUM:GLASS FRACTIONAL LASER

Fractional laser has played a role in skin rejuvenated for a while, new technology and instrument had been developed very quickly to serve the trend of global antiaging. From ablative to non-ablative laser and variety source of laser energy were introduce for the best treatment outcome, function and marketing cost.

1550 nm Erbium glass fractional laser is a non-ablative fractional laser which mainly used for skin rejuvenated and remodeling for original indication (64, 65). The fractional photothermolysis technology works by creating multiple micro laser beams that penetrated skin depth around reticular dermis layer or up to 900μm (66). Laser beam diameter is less than pore size, it is less than 200μm. Water is the target of this laser source energy, it is non-selective thermal heating. When laser beam are launched from the tip to skin, multiple micro necrotic column (MNC) are created on treated area from epidermis to reticular dermis (67).(Figure 4.1) This columns are the result from superheated thermal wounds, thermal from laser and wound healing process altered collagen remodeling at dermis layer and re-epithelization was occurred at epidermis (68, 69). The thermal from fractional laser cause collagen denatured within microscopic wound then new synthesis collagen was replaced in 3 months after laser treatment (70).

In photorejuvenation, after irradiated with 1550 nm non-ablative Er:Glass fractional laser, pro-inflammatory cytokines which included IL-1, TGF-β were up-regulation compare to before treatment. TGF-β signaling can induce cutaneous proliferation, collagen synthesis in wound healing process. More over TGF-β1 receptor was increased in expression by fibroblast and many growth factors were released (68). TGF-β signaling is the core of collagen remodeling (71). In inflammatory environment fibroblasts was promote by growth factors, cytokines and angiogenic factors that were released and pooled resulting in collagen remodeling, angiogenesis and proliferation (72-76). Insulin-like growth factor is one of the factor that can enhance fibroblast activity with the TGF-β signaling, fibroblast could increase their response to insulin like growth factor.

In this few years, fractional laser was used not only for its own indication, many cutaneous disorders were treated with this technology and outcomes seem to be satisfy (67). Hair loss disorder is one that, fractional laser both ablative and non-ablative were used as alternative treatment for both scarring and non-scarring hair loss disorders (3-7). Many study showed a very satisfied outcome especially in androgenetic alopecia cases. In androgenetic alopecia cases treated with low energy pulse, high density of laser beam array, after treated hair count and hair diameter are improved. The mechanism of how laser stimulated the new hair growth is thought to be part of wound healing process. In wound healing process, vasolidilatation was occurred inducing many cytokines, growth factors such as platelet-derived growth factor, fibroblast growth, vascular endothelial growth factor and insulin like growth factor for promoting healing and regenerating at the site of micro array wounds which may directly altered the dermal pappilla. The Wnt/β- catenin signal pathway is one of the pathway that thought to be the initiation of hair regrowth in. The Wnt signal is induced by the inflammatory process from wounding area. The thermal effect from laser beam might also altered dermal papilla where hair stem cell were resided too.

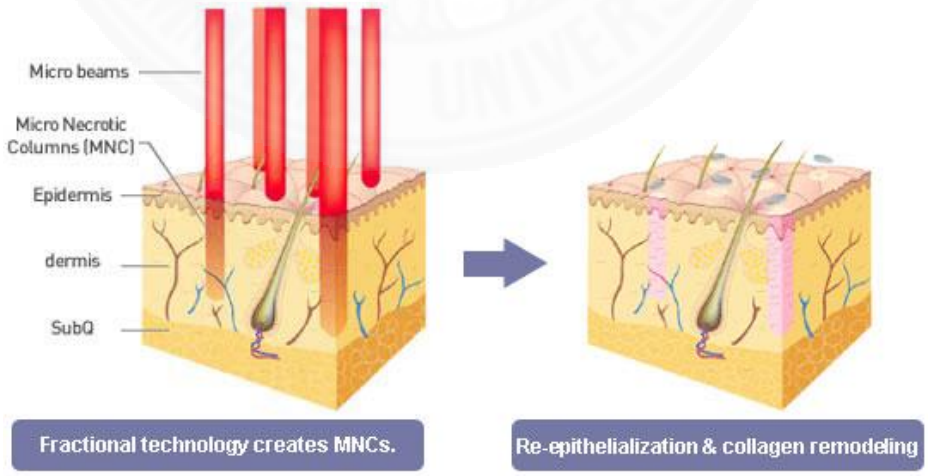

Figure 4.1 Microscopic thermal wound penetrated depth to reticular dermis created by fractional laser.

CHAPTER 5
RESEARCH METHODOLOGY

5.1 Study sample

5.1.1 Target population

Patients presenting to outpatients department (OPD) of Dermatology, Tobacco Monopoly Hospital, who had been diagnosed androgenetic alopecia. The fulfilling inclusion criteria were included in the study. This experimental study will be conducted at the Department of Dermatology Tobacco Monopoly Hospital between August 2016 and March 2017

5.1.2 Sample size

A total of 15 patients are recruited for this study. (5)

$$\text{N (Pair)} = \frac{[Z\alpha + Z\beta]^2 \sigma_{x-y}^2}{\mu_{x-y}^2}$$

α = 0.05 $Z\alpha = 1.96$

β = 0.2 Power = 0.8

μ = 0.86 $Z\beta = 0.84$

σ = SD of the within pair difference = 3.09

N (Pair) = 15 subjects per group

Drop out rate 10% = 17 subjects

5.1.3 Inclusion criteria

- Patient aged between 18-45 years old
- MPHL Hamilton-Norwood stage III-IV (include type III vertex) or FPHL Ludwig type I-II

5.1.4 Exclusion criteria

- Patient's refusal to participate the study.
- Patient taking other treatment for disease prior to the study periods;
  - Medical topical therapies within 6 months
  - Medical systemic therapies

    Finasteride within 1 year

    Dutasteride within 1year and 6 months
  - Non medication: shampoo stimulating hair growth treatment and another hair supplement within 3 months
  - Other alternative treatment for hair growth within 1 month
- Patient with contraindication of non-ablative fractional laser.
- FPHL patient that has a hyper-androgen condition

  (Patient information, history and physical examination).
- Patient with diabetes mellitus, nutritional deficiency, Autoimmune disease, immunocompromised conditions, HIV-infection and cancer.
- Pregnant and lactating women.
- Unreliable and poor compliance patient.
- Patient with previous hair restoration surgery.

5.1.5 Discontinuation criteria

- Patients' refusal to participate the study.
- Patient suffering serious adverse effect of laser treatment.
- Unreliable and poor compliance patient.

5.2 Research design

Semi-experimental before and after study conducted at Tobacco monopoly hospital between August 2016 and March 2017

5.3 Materials and methods

5.3.1 Data collection

The collected data include patient' personal history (age, gender, underlying disease, family history, age of disease onset, disease staging, site of lesions, hair thinning area, photographs of scalp), family history and physical examination will be done after they are fully informed of the nature of the study. The formal consent form will be taken from the participants.

Physical examination will be established regarding the balding area, hair count/cm^2, disease staging in MPHL Hamilton-Norwood stage II-III and FPHL Ludwig type I-II. Global photograph using digital camera (Sony DSC-RX100M3), while hair count per cm^2, hair density per cm^2 and hair shaft diameter using Dino-Lite microscope (AM7013MRZT(R4) Series) focused on balding area. Focus area of dino-lite microscope will be taken on the same point, by using the 2 mm punch biopsy scar as a center of landmark. The global photograph, hair count, hair density and hair shaft diameter will be done at baseline then once a month until 6 months, for evaluate hair density and clinical manifestation.

The skin biopsy will be collected at baseline of every patients with 2 mm punch biopsy for PCR detecting mRNA level of Wnt-β/catenin and IGF-1 and 4 mm punch biopsy for histopathology. The 2 mm skin biopsy will be done again at the 24 hours after third laser treatment or at week 4. The 4 mm punch biopsy will be done again at month 1, month 2 and month 3 after the first laser treatment, each group of timing contained 5 patients whose scalp biopsied will be revealed for histology. The second skin biopsy site will be done on 1 cm distance next to the prior skin biopsy wound for preventing scarring alopecia.

5.3.2 Intervention

23 patients were enrolled into the study and received the treatment with 1550 nm fractional erbium-glass laser (MOSAIC, Lutronic Co., Ltd, Seoul, South Korea) tip 2×12 mm, pulse energy 6mJ, 300/400 spot/cm^2 density, static mode 2-4 passes on the effect area for 14 session at 2 weeks interval until 24 weeks (6 months) without local anesthesia. The global photograph and hair count for hair density evaluation will be done every month. The side effect and complication from the laser was evaluated and noted.

Figure 5.1 1550 nm Er:Glass fractional laser irradiate on balding area with 2x12 mm tip, 6mJ pulse energy, 300 spot/cm^2 for 2-4 passes

Methodology

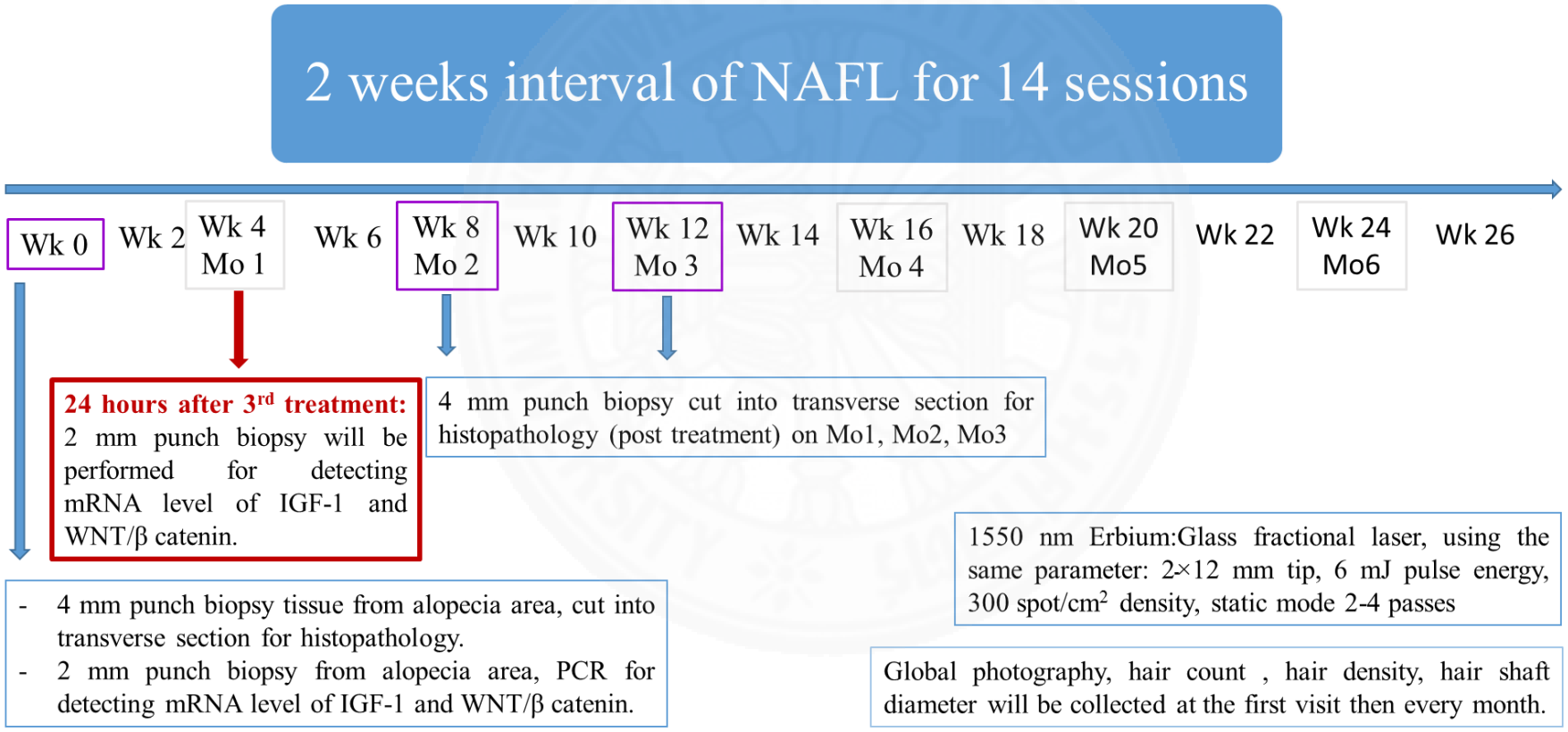

Figure 5.2 Methodology

5.3.3 Outcome measurement

5.3.3.1 Clinical manifestation: Hair regrowth

- Global photograph taking by Sony DSC-RX100M3 digital camera
- Target area for hair density per cm^2, hair shaft diameter per cm^2, hair count per cm^2 is taken by Dino-Lite microscope (AM7013MZT(R4)Series) at the same site every time using the 2 mm punch biopsy scar as a center of a landmark of the target area.

5.3.3.2 Histopathology analysis

Tissues from balding area from 15 patients was collected by 4 mm punch biopsy at baseline (before treatment) and then at 1^{st}, 2^{nd} and 3^{rd} months (each group of month contained 5 patients) of the study then cut into transverse sections for evaluate:

- Anagen:Telogen ratio
- Terminal hair
- Vellus hair
- Follicular unit

At each time points of scalp biopsied, local anesthesia (2% Xylocaine with adrenaline) was injected in to the target area before biopsied was done. The scalp tissues were fixed in 10% formaldehyde solution then embedded in paraffin block. Every specimens were cut into 3μm in transverse section for hematoxylin and eosin staining.

5.3.3.3 Laboratory analysis

Tissues from balding area of the patient scalp will be collected by 2 mm punch biopsy at baseline (before treatment) and then at 24 hours after third laser (1st month) treatment for detecting mRNA expression of WNT10A and IGF-1.

Before biopsied was done every patients were injected with local anesthesia (2% Xylocaine with adrenaline) into the target area.

qRT-PCR: After 2 mm punch biopsied was done, each scalp tissue was placed in a collector tube and stored at -80ºC until molecular process was started. RNA isolation process was done by using RNeasy® Mini Kit (QIAGEN™, Hilden, Germany). RT-PCR was performed on a BIO-RAD Real-Time PCR system (BIO-RAD™, California, U.S.A). Data were calculated relatively to expression of reference gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). Primers and probes for human WNT10A (Hs.121540), human IGF-1 (Hs.160560) and GAPDH (Hs.544577) were obtained from BIO-RAD™ (California, U.S.A.).

Figure 5.3 Commercial reagent primers and probes BIO-RAD™ (California, U.S.A.)

RNA extraction: 2 mm biopsied scalp tissues were disrupted until homogenized to the buffer from RNeasy® Mini Kit (QIAGEN™, Hilden, Germany), then placed into the tubes from commercial kit known as QIAshredder which can purified RNA from buffer and other reagent follow the process of the commercial kit .

Figure 5.4 Commercial RNA extraction kit - RNeasy® Mini Kit (QIAGEN™, Hilden, Germany)

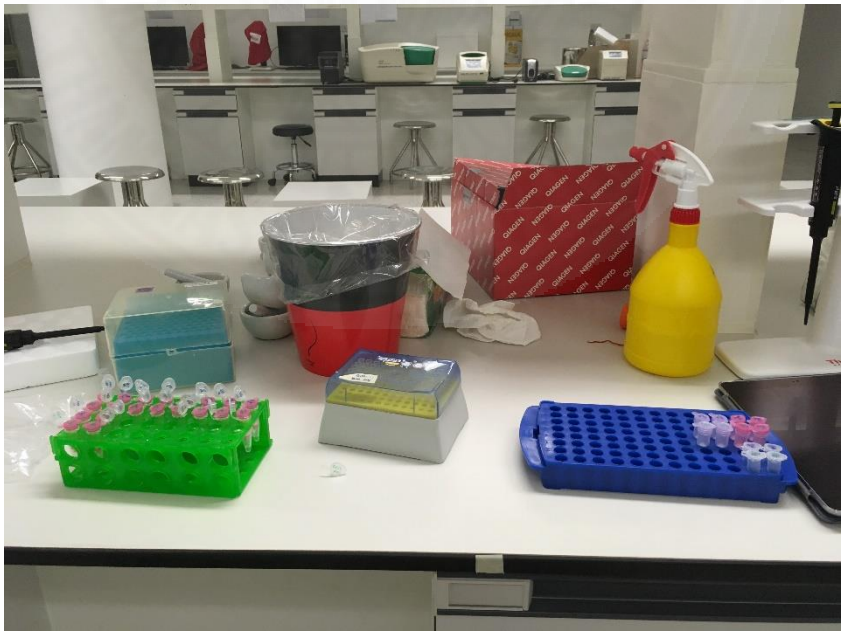

Figure 5.5 RNA extraction laboratory process.

Real time PCR: After RNA extraction was done, 2 µl volume of RNA was mixed into this following agents in the tubes.

- Probe RT-PCR master mix 10 µl
- QN Probe RT-Mix 0.2 µl
- IGF-1 primer (Hs.160560) 1 µl or
  WNT10A primer (Hs.121540) 1 µl
- Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) 1 µl
- RNase free water 5.8 µl

After mixing well the tubes were gently closed with the cover and place in the PCR machine (BIO-RAD Real-Time PCR system, BIO-RAD™, California, U.S.A). The reactions were preheated to 94ºC for optimized temperature to amplify the reactions in the thermal cycler.

Denaturation	94ºC for 2 minutes
25 cycles:	
Denaturation	94ºC for 1 minutes
Annealing	60ºC for 1 minutes
Extension	72ºC for 2 minutes
Final extension	72ºC for 5 minutes
Hold	4ºC

Figure 5.6 PCR cycle and PCR machine (BIO-RAD Real-Time PCR system, BIO-RAD™, California, U.S.A)

5.3.3.4 Clinical improvement assessment score

Two blinded dermatologist evaluated the clinical response and improvement after treated with 1550 nm fractional erbium-glass laser at 3rd month and 6th month compared to baseline. Global photographs of participant scalp which had taken every month were the assessment tool for two blinded dermatologists.

The point that used for evaluate the improvement is 7-point global assessment scale which is (-3) to 3 score. The value of each score are significantly decreased hair thickness (-3), moderately decreased hair thickness (-2), slightly decreased hair thickness (-1), no hair thickness change (0), slightly increased hair thickness (+1), moderately increase hair thickness (+2) and significantly increased hair thickness (+3).

The participants are also given the 7-point global assessment scale for scoring their satisfaction after all laser treatment sessions (14 session) were done on 6th month.

5.4 Data analysis

The statistical analysis in this study was performed by using STATA/SE version 13. The quantitative data was presented in mean ± SD and median (IQR). The quantitative data analysis was done by Paired t-test and ANOVA test in mean ± SD and Wilcoxon Signed Ranks test was applied in median (IQR). The qualitative data was presented in percentage and analyzed by Chi square test.

CHAPTER 6
RESULTS

6.1 Baseline characteristics

Twenty three participants with androgenetic alopecia included 16 males (69.6%) and 7 females (30.4%) were enrolled into the study. The average age of the participants is 39.65 ± 7.5 years old. Severity of hair loss among participants, there are 6 patients with Hamilton-Norwood stage 3 (26.1%), 5 patients with Hamilton-Norwood stage 3V (21.7%), 5 patients with Hamilton-Norwood stage 4 (21.7%) for male pattern hair loss and 7 females with Ludwig stage 2 (30.4%). (Table 6.1)

Table 6.1 Patient characteristics

	Total (n=23)
Age (years)	39.65 ± 7.5
Gender	
Female	7 (30.4%)
Male	16 (69.6%)
Stage	
Hamilton-Norwood 3	6 (26.1%)
Hamilton-Norwood 3V	5 (21.7%)
Hamilton-Norwood 4	5 (21.7%)
Ludwig 2	7 (30.4%)

6.2 Clinical result

6.2.1 Hair count

6.2.1.1 Significant increase of terminal hair density after treated with 1550 nm Er:Glass fractional laser

At baseline terminal hair count per cm^2 mean is 70.43 ± 26.88, after treated with 1,550 nm Er:Glass fractional laser, terminal hair count trend to increased continuously since 2nd month (73.18 ± 23.78, $p = 0.708$) and 3rd month (86.82 ± 28.18, $p = 0.057$). On 4th month terminal hair count became statistical significant increased from baseline, mean of 4th month terminal hair count is 93.91 ± 29.96 ($p = 0.001$) and still significantly increased on 5th month compared to baseline which mean is 101.74 ± 27.08 ($p < 0.001$). (Figure 6.1)

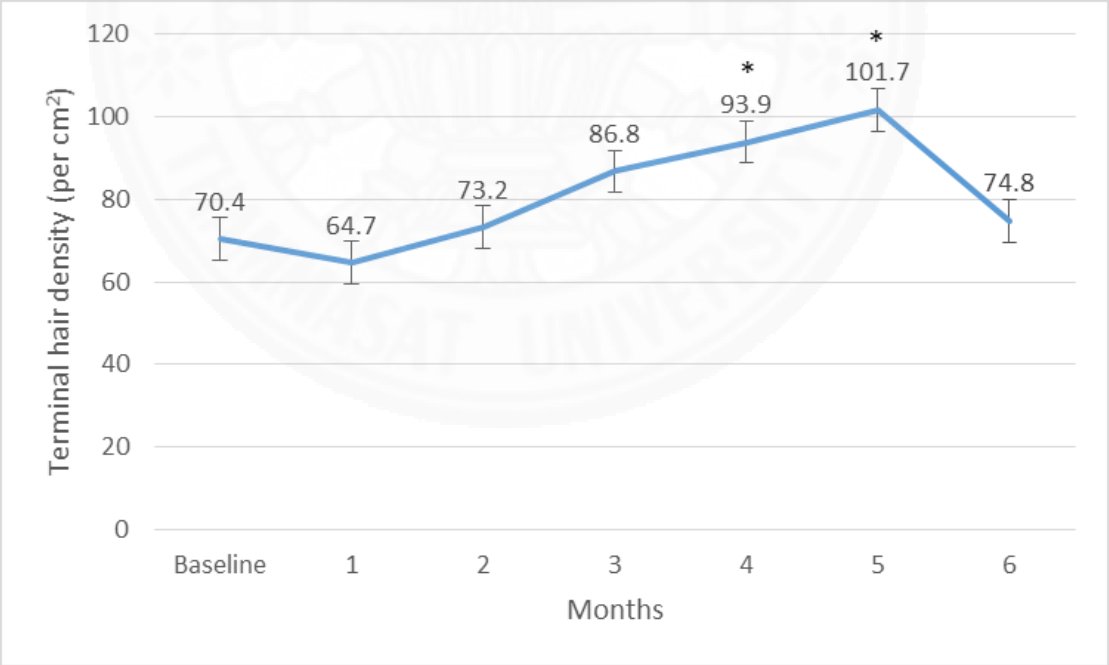

Figure 6.1 Terminal hair density mean ± SD, *$p < 0.05$ corresponds to Paired t test.

6.2.1.2 Increasing intermediate hair count after treated with 1550 nm Er:Glass fractional laser

At baseline intermediate hair count per cm^2 mean is 88.7 ± 37.09 and the mean was dropped on 1st month to 71.67 ± 40.18 (p = 0.323). On 2nd month of study, intermediate hair count was significantly reduced compare to baseline which mean is 69.09 ± 33.65 (p = 0.043). After 2nd month the intermediate hair count trend to increase in number, mean on 3rd month is 77.73 ± 25.06 (p = 0.190), 75.65 ± 26.43 for 5th month (p = 0.168) and 74.78 ± 28.1 (p = 0.589) for 6th month respectively. (Figure 6.2)

The result showed that over entire 6 months of study, the intermediate hair count trend to be increased with P-value = 0.249 correspond to ANOVA test. (Table 6.2)

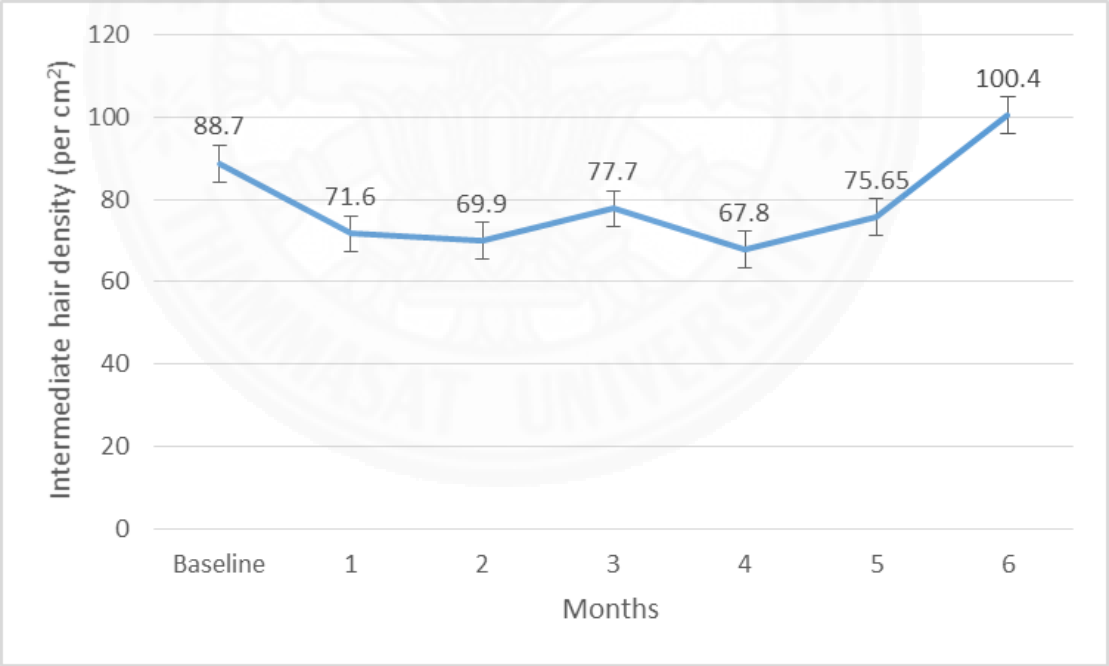

Figure 6.2 Intermediate hair density mean ± SD, $p < 0.05$ corresponds to Paired t test.

6.2.1.3 Significant increased non-vellus hair count (terminal hair and intermediate hair count) after treated with 1550 nm Er:Glass fractional laser

At baseline non-vellus hair count per cm^2 mean is 159.13 ± 28.91. On 1st month non-vellus hair count mean was dropped to 132.63 ± 43.44 (p = 0.058) compared to baseline. After 1st month of study the mean of non-vellus hair count slightly increased continuously on 2nd month mean is 142.27 ± 39.87 (p = 0.084), 3rd month mean is 164.55 ± 26.68 (p = 0.577) and 5th month mean is 177.39 ± 37.2 (p = 0.061) compared to baseline in order. (Figure 6.3)

For over entire study of 6 months, the result showed that the non-vellus hair count trend to increase significantly toward the study with P-value = 0.001 corresponds to ANOVA test. (Table 6.2)

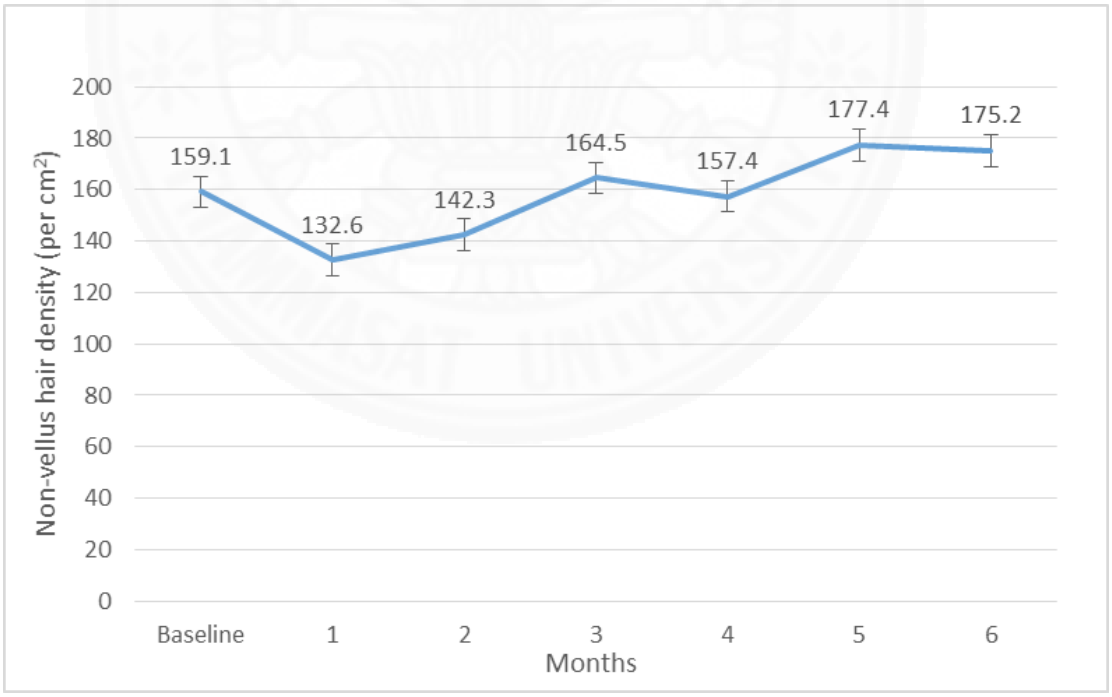

Figure 6.3 Non-vellus hair density mean ± SD, $p < 0.05$ corresponds to Paired t test.

6.2.1.4 Significant increased total hair count (terminal hair, intermediate hair and vellus hair) after treated with 1550 nm Er:Glass fractional laser

At baseline total hair count per cm^2 mean is 167.83 ± 31.33. On 1st month total hair count mean significantly fall from baseline to 144.21 ± 44.14 ($p = 0.04$) compared to baseline. After 1st month the mean of total hair count slightly increased continuously on 2nd month mean is 153.18 ± 45.29 ($p = 0.198$), 3rd month mean is 169.09 ± 28.44 ($p = 0.873$), 4th month mean is 162.61 ± 41.26 ($p = 0.624$), 5th month mean is 182.61 ± 33.06 ($p = 0.093$) and 6th month mean of total hair count is 175.22 ± 34.89 compared to baseline in orderly. (Figure 6.4)

For over entire study of 6 months, the result showed that the total hair count trend to increase significantly toward the study with P-value = 0.019 corresponds to ANOVA test. (Table 6.2)

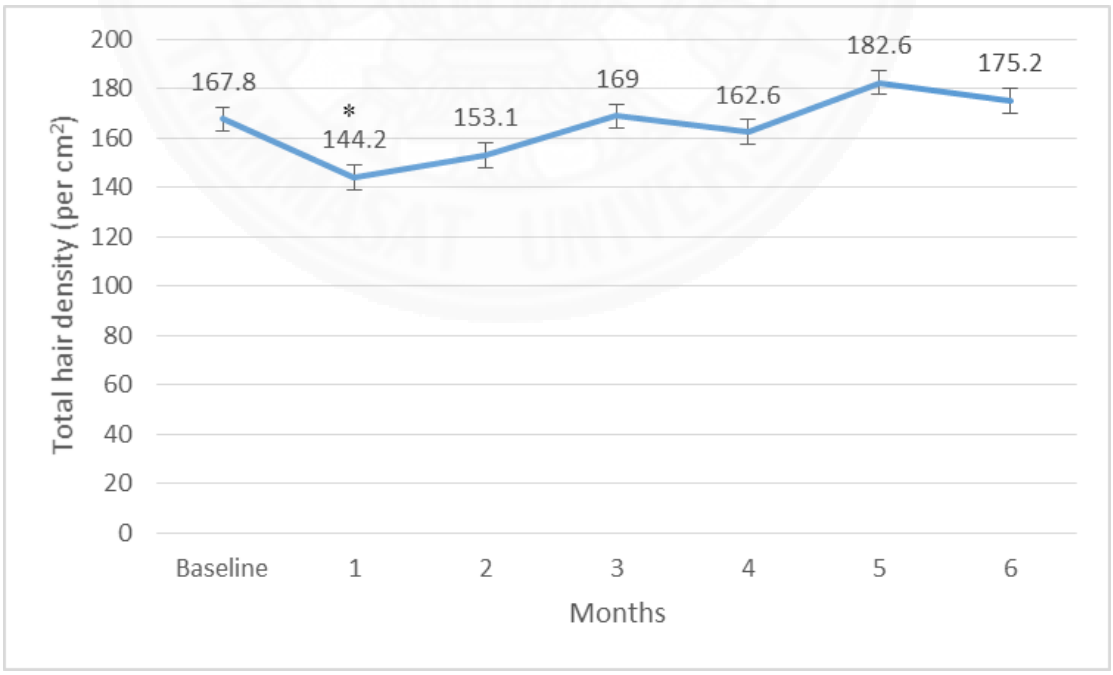

Figure 6.4 Total hair density mean ± SD, *p < 0.05 corresponds to Paired t test.

Table 6.2 Hair density through 6 months

	mean ± SD	%Change of baseline	(t)p-value (6 mo)
Terminal hair count (cm^2)			
Baseline	70.43 ± 26.88	Reference	
Months 1	64.74 ± 18.96	5.1 ± 59.9	0.205
Months 2	73.18 ± 23.78	32.2 ± 109.4	0.708
Months 3	86.82 ± 28.18	58.4 ± 129.7	0.057
Months 4	93.91 ± 29.96	53.5 ± 80.2	0.001*
Months 5	101.74 ± 27.08	80.5 ± 134.6	<0.001*
Months 6	74.78 ± 28.1	31.6 ± 93.1	0.589
p-value(r)			0.005*
Intermediate hair count (cm^2)			
Baseline	88.7 ± 37.09	Reference	
Months 1	71.67 ± 40.18	1.1 ± 73.4	0.323
Months 2	69.09 ± 33.65	-10.6 ± 60.4	0.043*
Months 3	77.73 ± 25.06	1.2 ± 50.5	0.190
Months 4	67.83 ± 29.07	-7 ± 63	0.071
Months 5	75.65 ± 26.43	-0.6 ± 54	0.168
Months 6	100.43 ± 32.26	33.4 ± 76.3	0.224
p-value(r)			0.249
Non vellus hair count (cm^2) (terminal + intermediate)			
Baseline	159.13 ± 28.91		
Months 1	132.63 ± 43.44	-12.6 ± 33.8	0.058
Months 2	142.27 ± 39.87	-8.9 ± 29	0.084
Months 3	164.55 ± 26.68	6.1 ± 26.2	0.577
Months 4	157.39 ± 39.11	2.1 ± 32.3	0.868
Months 5	177.39 ± 37.2	14.5 ± 28.5	0.061
Months 6	175.22 ± 34.89	12.7 ± 25.8	0.061
p-value(r)			0.001*
Total hair count (cm^2)			
Baseline	167.83 ± 31.33	Reference	
Months 1	144.21 ± 44.14	-12 ± 24.54	0.04*
Months 2	153.18 ± 45.29	-5.95 ± 32.28	0.198
Months 3	169.09 ± 28.44	4 ± 25.28	0.873
Months 4	162.61 ± 41.26	-0.37 ± 27.92	0.624
Months 5	182.61 ± 33.06	11.61 ± 23.96	0.093
Months 6	175.22 ± 34.89	5.88 ± 19.91	0.277
p-value(r)			0.019*

Values presented as mean ± SD. P-value corresponds to (t) Paired t test and (r) Repeated ANOVA test.

6.2.2 Significant increased hair shaft diameter after treated with 1550 nm Er:Glass fractional laser

At baseline hair shaft diameter mean is 42.52 ± 9.82 mm and it continued become larger in diameter. For 1st month the mean is 42.95 ± 10.02 mm (p = 0.948), 2nd month mean is 46.41 ± 11.88 mm (p = 0.209) and 47.91 ± 10.84 mm (p = 0.108) for 3rd month compared to baseline in orderly. On 4th month, hair shaft diameter became significant larger compared to baseline which mean is 50.74 ± 10.69 mm (p = 0.027). And still increased significantly on 5th month and 6th month which mean is 54.39 ± 8.85 (p = 0.001) and 64.13 ± 11.82 (p < 0.001) in order. (Figure 6.5)

For entire 6 months of study it revealed that the trend of hair shaft diameter through 6 month was significantly increased with P-value < 0.001 correspond to ANOVA test. (Table 6.3)

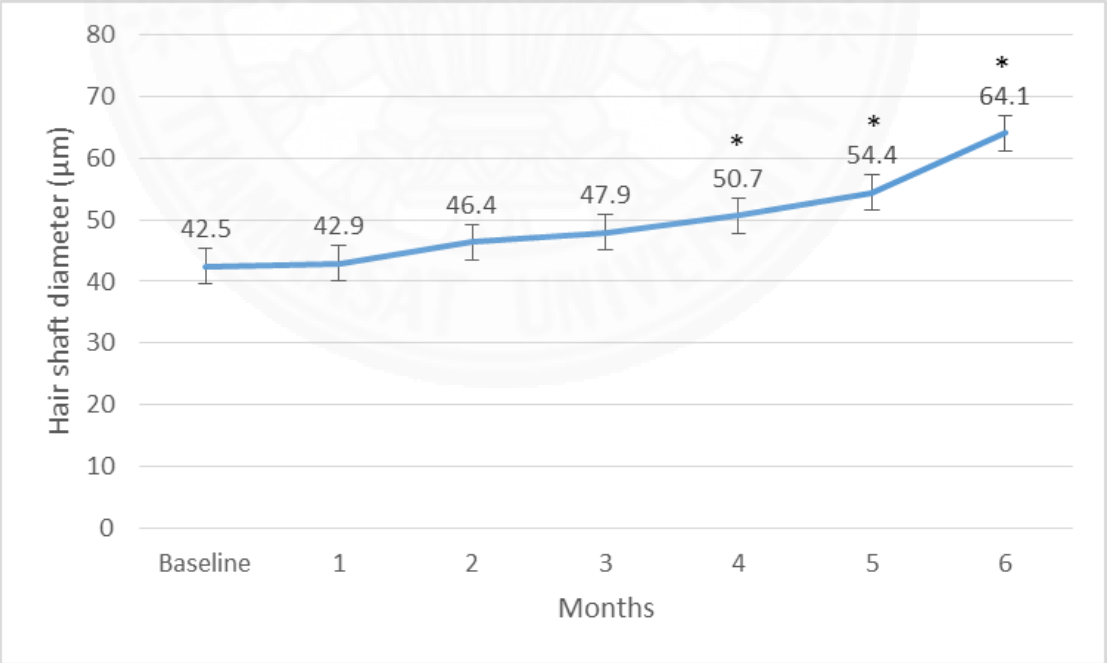

Figure 6.5 Hair shaft diameter (µm) mean ± SD, *p < 0.05 corresponds to Paired t test.

6.2.3 Significant decreased of vellus hair : non-vellus hair ratio after treated with 1550 nm Er:Glass fractional laser

At baseline the proportion of vellus hair to non-vellus hair mean is 0.06 ± 0.08. After the first treatment, the vellus hair : non-vellus hair ratio gradually decreased from baseline. On 1st month the ratio of vellus hair : non-vellus hair mean fall from baseline to 0.1 ± 0.15 (p = 0.164), on 2nd month mean is 0.07 ± 0.08 (p = 0.466), 3rd month mean is 0.03 ± 0.05 (p = 0.125). At 4th month the mean of vellus hair: non-vellus hair ratio is significantly decreased to 0.01 ± 0.1 (p = 0.008) compared to baseline. For 6th month the mean of vellus hair : non-vellus hair ratio is 0 (p = 0.003), these result showed significant decreasing of vellus hair : non-vellus hair ratio at the end of study compared to baseline. (Figure 6.6)

For over entire study of 6 months, the result showed that the total hair count trend to increase significantly toward the study with P-value < 0.001 corresponds to ANOVA test. (Table 6.3)

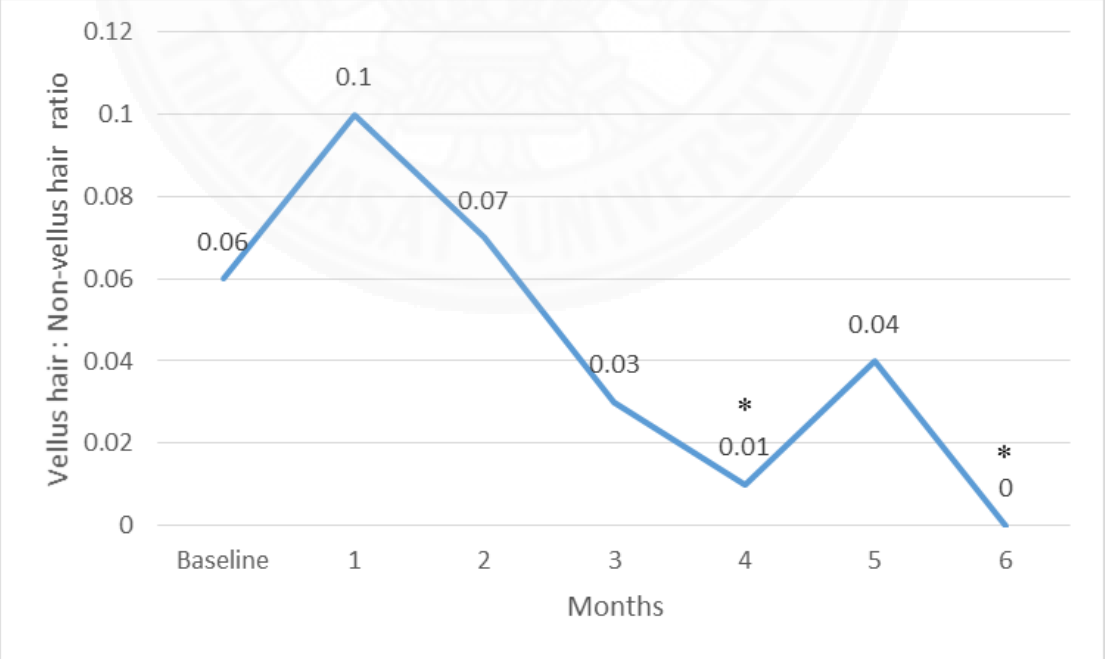

Figure 6.6 Vellus hair : Non-vellus hair ratio mean ± SD, *p < 0.05 corresponds to Paired t test.

Table 6.3 Hair thickness and vellus hair : non-vellus hair ratio through 6 months

	mean ± SD	% Change of baseline	(t)p-value (6 mo)
Hair shaft diameter (µm)			
Baseline	42.52 ± 9.82		
Months 1	42.95 ± 10.02	5.7 ± 35.6	0.948
Months 2	46.41 ± 11.88	19.9 ± 49.2	0.209
Months 3	47.91 ± 10.84	19.5 ± 38.6	0.108
Months 4	50.74 ± 10.69	27.4 ± 46.2	0.027*
Months 5	54.39 ± 8.85	35 ± 38.3	0.001*
Months 6	64.13 ± 11.82	61.1 ± 58.1	<0.001*
p-value(r)			<0.001*
Vellus hair : Non-vellus hair			
Baseline	0.06 ± 0.08	Reference	
Months 1	0.1 ± 0.15	125.46 ± 405.38	0.164
Months 2	0.07 ± 0.08	21.34 ± 126	0.466
Months 3	0.03 ± 0.05	-51.05 ± 97.76	0.125
Months 4	0.01 ± 0.02	-100 ± 0	0.008*
Months 5	0.04 ± 0.1	-40.26 ± 106.83	0.498
Months 6	0 ± 0	-	0.003*
p-value(r)			<0.001*

Values presented as mean ± SD. P-value corresponds to (t) Paired t test and (r) Repeated ANOVA test.

The result from above showed that after treated with 1550 nm Er:Glass fractional laser, hair density and hair shaft diameter significantly improved from baseline and they were correlated significant changed on the same time at 4th month of the study.

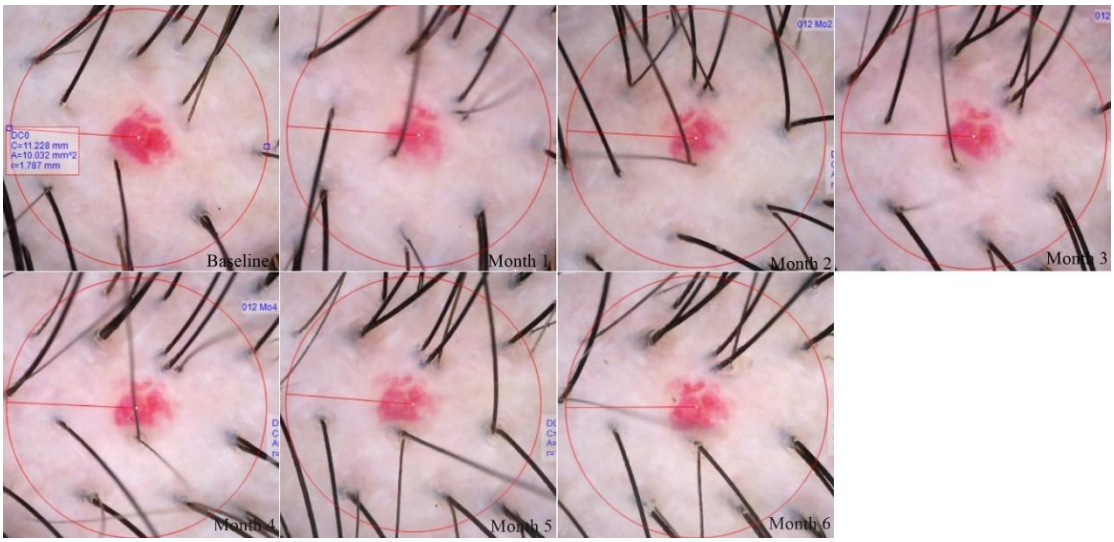

Figure 6.7 Target photograph for hair density assessment. Baseline, 1st month, 2nd month, 3rd month, 4th month, 5th month, 6th month

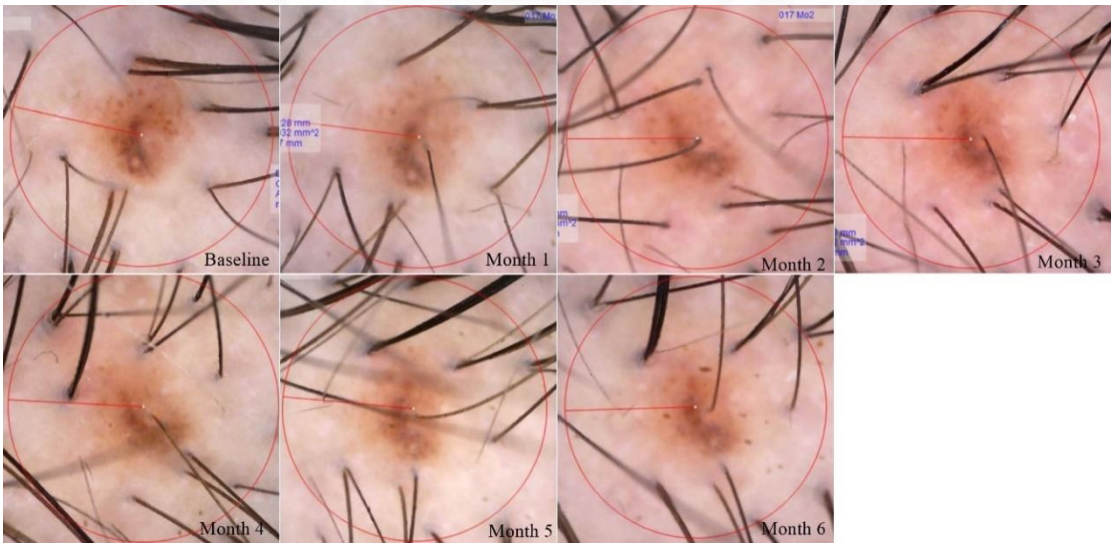

Figure 6.8 Target photograph for hair density assessment. Baseline, 1st month, 2nd month, 3rd month, 4th month, 5th month and 6th month.

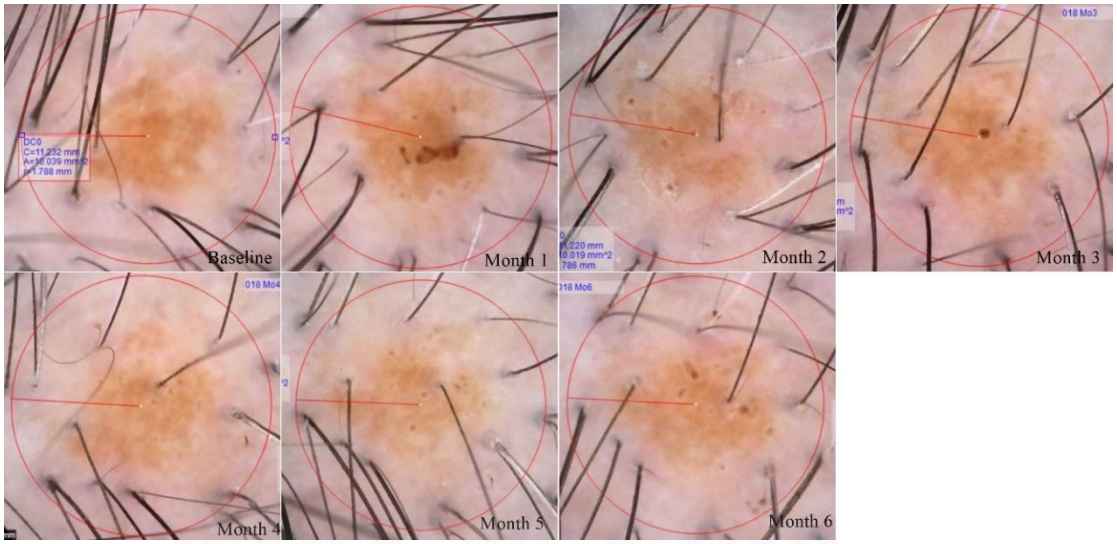

Figure 6.9 Target photograph for hair density assessment. Baseline, 1st month, 2nd month, 3rd month, 4th month, 5th month and 6th month.

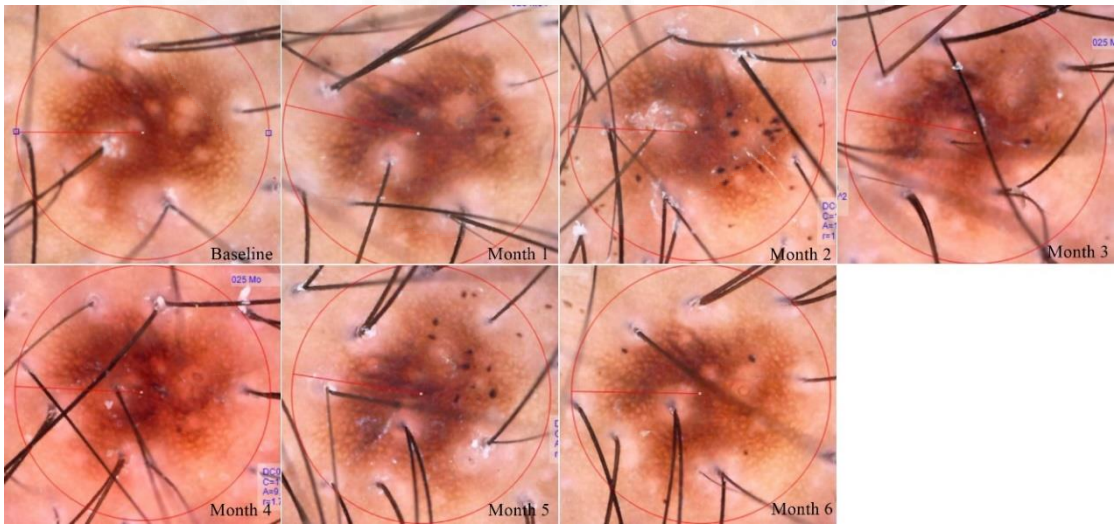

Figure 6.10 Target photograph for hair density assessment. Baseline, 1st month, 2nd month, 3rd month, 4th month, 5th month and 6th month.

6.3 Histology result

The histology of scalp revealed the number of follicular unit, total hair count included terminal hair, vellus hair, anagen hair and telogen hair at each time of tissues sacrificed consist of 1st moth, 2nd month and 3rd month compared to baseline.

6.3.1 Follicular unit

From histology result, the follicular unit number among 3 groups do not increase significant after treated with fractional laser. Only 2nd month group that follicular unit was increased from baseline after treated with laser from mean baseline 11.25 ± 2.5 to 11.4 ± 1.34 at after treatment which difference is 0.5 ± 1.91 (p = 0.638). The other groups both 1st and 3rd month, the follicular unit do not increase in number after treatment, their difference of follicular unit between base line and after treatment is -0.25 ± 2.36 (p = 0.846) for 1st month group and -0.4 ± 1.95 (p = 0.670) for 3rd group respectively. After treated with 1550 nm Er:Glass fractional laser follicular unit dose not increase in number all of the following 3 groups. There is also no significant in post treatment follicular unit number comparing between each group of time. (Table 6.4)

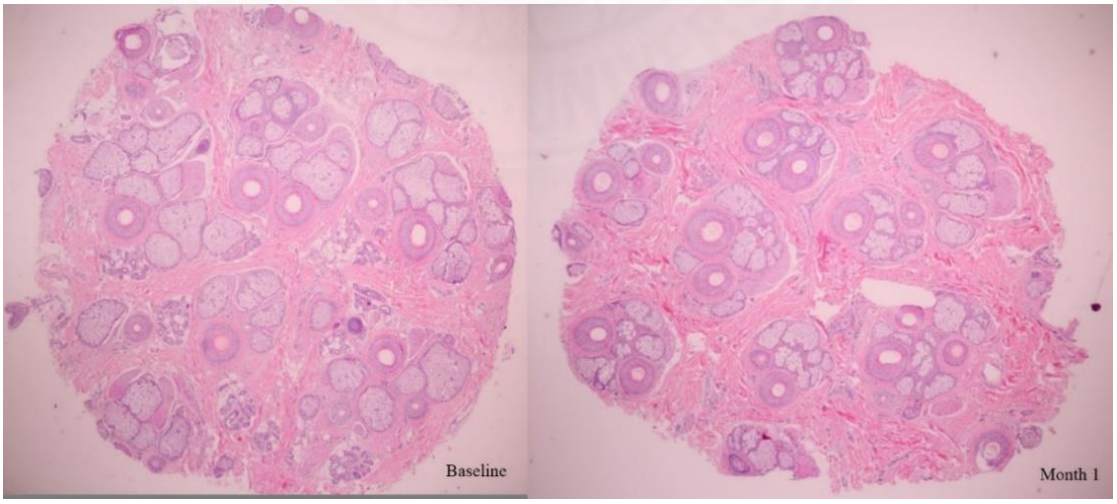

Figure 6.11 Histologic finding showed increase hair count and follicular hair unit from baseline to 1st month after treatment.

6.3.2 Hair count

The histology from 3 groups showed increase of hair count toward the time, as time pass the hair count trend to increase more than in early period.

The total hair count baseline mean of 1st month group is 22.25 ± 5.38 and after treatment mean is 22.4 ± 2.41 (p = 1.000), for 2nd month group baseline mean of total hair count is 19.5 ± 6.14 and after treatment mean is 22.4 ± 4.93 (p = 0.196) and 3rd month group total hair count baseline mean is 23.6 ± 6.62 and after treatment mean is 29.4 ± 12.24 (p = 0.118). Comparing the post treatment total hair count between each group, the 3rd month group showed more increased of total hair count than the other 2 groups. The result of post treatment hair count showed as 1st month versus 3rd month P-value is 0.273 and 2nd month versus 3rd month P-value is 0.27. (Table 6.4)

From total hair count, divided into terminal hair count and vellus hair count. The after treatment terminal hair count expressed correlated to the total hair count, more increased as the time passed. Terminal hair count of 1st month group at baseline is 13 ± 2.94 and post treatment mean is 17.4 ± 3.36 (p = 0.288), for 2nd month group baseline mean is 12.25 ± 4.03 and post treatment mean is 14.4 ± 4.62 (p = 0.335) and 3rd month group baseline mean is 15.2 ± 3.96 and post treatment mean is 21 ± 8.49 (p = 0.096). The terminal hair count at after treatment on 3rd month was more increase than other two groups. (1st month vs 3rd month p = 0.404) (2nd month vs 3rd month p = 0.165) (Table 6.4)

Although the terminal hair count was correlated with total hair count, vellus hair count seemed to be different. The result from 1st month group, vellus hair was dropped after treated with fractional laser, from baseline mean at 9.25 ± 3.2 to 5 ± 1.22 (p = 0.060). While the 2nd month group mean of vellus hair was increase from 7.25 ± 2.22 at baseline to 8 ± 2.55 at post treatment (p = 0.092). On 3rd month the mean of vellus hair count dose not changed change from 8.4 ± 3.29 at baseline to 8.4 ± 3.97 at post treatment (p = 1.000). (Table 6.4) After treatment with fractional laser, terminal hair and total hair count was increased number and highest increased on 3rd month compared with 1st and 2nd month after started the first session of laser.

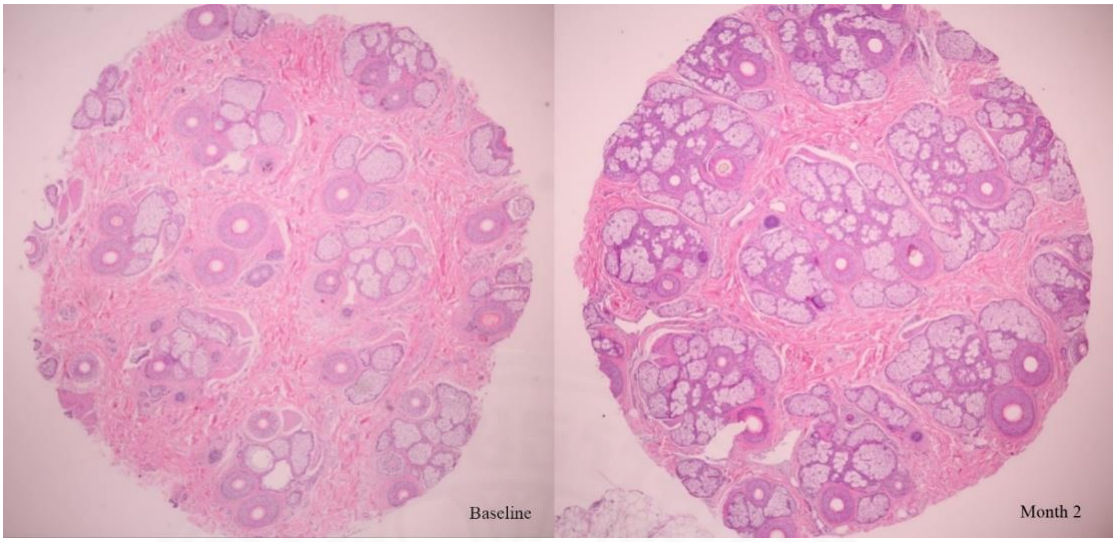

Figure 6.12 Histologic finding showed increase follicular hair unit from baseline to 2nd month after treatment.

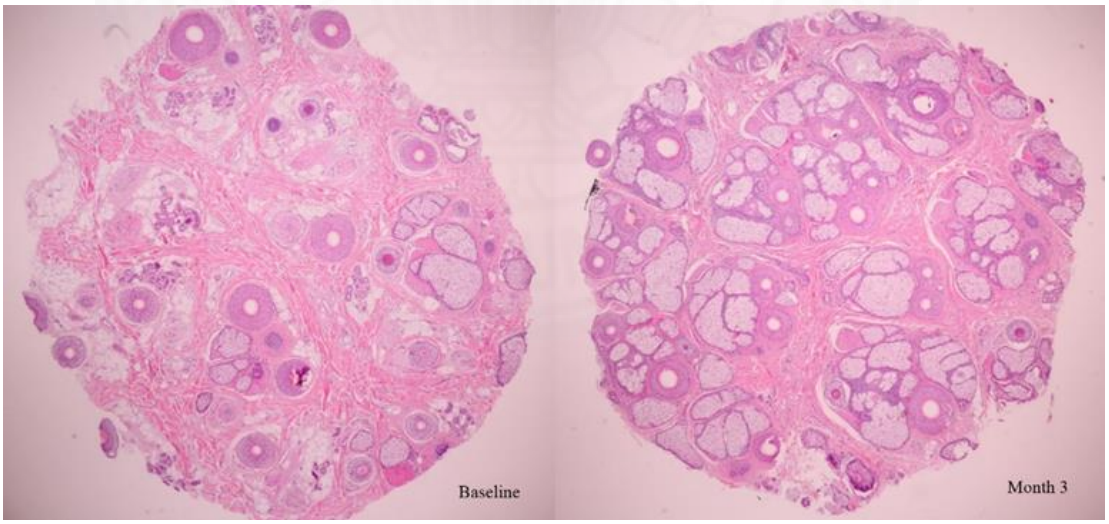

Figure 6.13 Histologic finding showed increase anagen hair count and follicular hair unit from baseline to 3rd month after treatment.

6.3.2.1 Increased anagen:telogen ratio after treated with 1550 nm Er:Glass fractional laser

As the total hair count was increased by the fractional laser, each hair phases were divided into anagen hair phase and telogen hair phase to determine the new hair growth from the effect of 1550 nm Er:glass fractional laser in androgenetic alopecia.

Telogen hair count from histology result at after treatment of each groups do not increased in number. After treated with the fractional laser at 1st month, the telogen hair count was decreased compared to baseline. The baseline of telogen hair count of 1st month group is 5 ± 1.41 and after treatment is 3 ± 1.22 (p =0.236). In the other 2 groups, the telogen hair count at baseline and post-treatment not decreased but not increased too. The 2nd month baseline mean of telogen hair count is 3.5 ± 1.91 and post treatment is 3.2 ± 1.1 (p = 1.000). The 3rd month baseline mean of telogen hair count is 5 ± 4 and post treatment is 5.6 ± 4.39 (p = 0.553). (Table 6.4)

The ratio of anagen:telogen revealed the density of new hair growth compared with the regression hair which ready to fall out. The anagen:telogen ratio after treatment was increased in all groups. For the 1st month group, anagen:telogen mean at baseline is 3.69 ± 1.53 and after treatment is 8.53 ± 6.54 (p = 0.318), 2nd month group baseline mean is 5.48 ± 2.84 and after treatment is 6.43 ± 2.01 (p = 0.694) and 3rd month group baseline mean is 5.68 ± 2.85 and after treatment is 7.57 ± 8.47 (p = 0.614) respectively. (Table 6.4)

From the result above, compared after treatment anagen:telogen ratio of each group, there was no significant difference between each group.

6.3.2.2 Increased terminal:vellus ratio after treated with 1550 nm Er:Glass fractional laser

The ratio of terminal:vellus hair after treatment with fractional laser also increased in number in every group. In 1st month group the baseline mean of terminal:vellus ratio is 1.51 ± 0.49 and after treatment is 3.72 ± 1.29 (p = 0.069). In 2nd month group the value terminal:vellus ratio of baseline mean is 1.7 ± 0.24 and up to 1.96 ± 0.99 after treatment with the fractional laser (p = 0.459). For the 3rd month group the terminal:vellus ratio appear to be significantly increased after treatment with fractional laser, the baseline mean of terminal:vellus in ratio in 3rd month group is 2.03 ± 0.97 and after treatment mean is 2.73 ± 0.95 (p = 0.016). (Table 6.4)

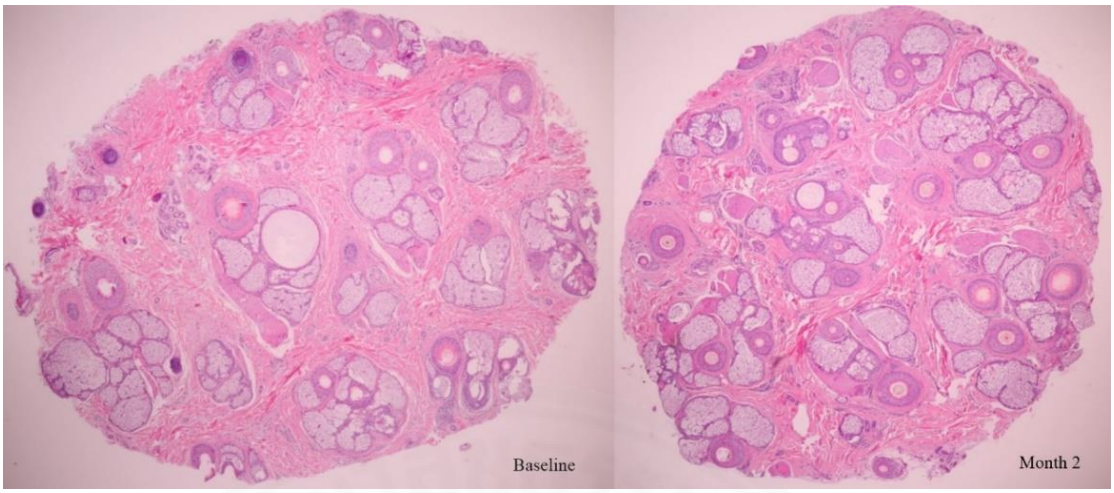

Figure 6.14 Histologic findings showed increase total hair count, anagen:telogen ratio and follicular unit on 2nd month.

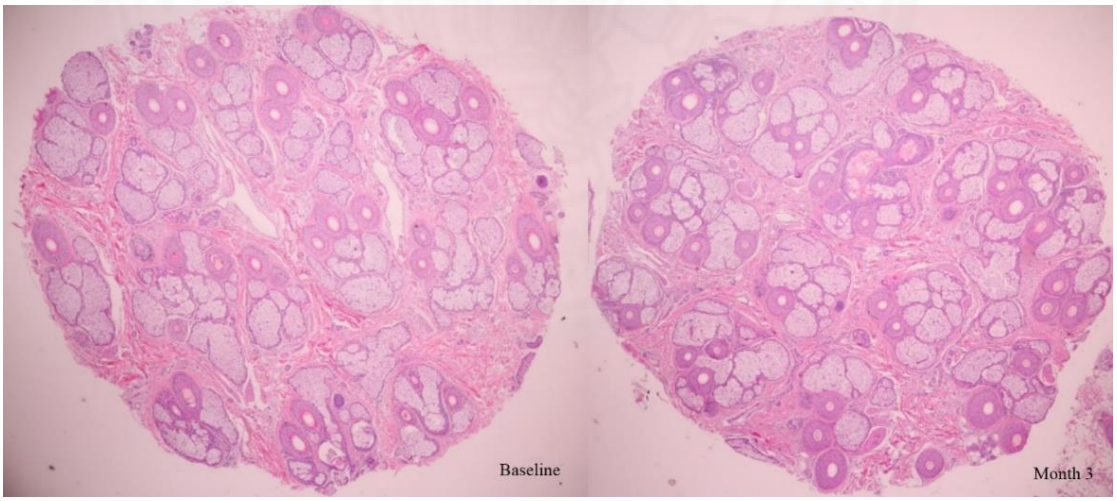

Figure 6.15 Histologic findings showed increase total hair count, anagen:telogen ratio and follicular unit on 3rd month.

Table 6.4 Histologic findings

	Group 1 Month (n=5)	Group 2 Month (n=5)	Group 3 Month (n=5)	P-value 1 month vs. 2months	P-value 1 month vs. 3months	P-value 2 month vs. 3months
Follicular unit						
Baseline	10.75 ± 0.96	11.25 ± 2.5	10.8 ± 2.95	0.722	0.975	0.815
After	10.6 ± 1.52	11.4 ± 1.34	10.4 ± 3.65	0.403	0.914	0.59
Difference	-0.25 ± 2.36	0.5 ± 1.91	-0.4 ± 1.95	0.639	0.92	0.51
p-value	0.846	0.638	0.670			
Total hair (terminal + vellus)						
Baseline	22.25 ± 5.38	19.5 ± 6.14	23.6 ± 6.62	0.525	0.752	0.373
After	22.4 ± 2.41	22.4 ± 4.93	29.4 ± 12.24	1	0.273	0.27
Difference	0 ± 7.16	4.75 ± 5.74	5.8 ± 6.53	0.341	0.245	0.808
p-value	1.000	0.196	0.118			
Terminal						
Baseline	13 ± 2.94	12.25 ± 4.03	15.2 ± 3.96	0.774	0.388	0.307
After	17.4 ± 3.36	14.4 ± 4.62	21 ± 8.49	0.274	0.404	0.165
Difference	4.25 ± 6.6	3.75 ± 6.55	5.8 ± 5.97	0.918	0.723	0.639
p-value	0.288	0.335	0.096			
Vellus						
Baseline	9.25 ± 3.2	7.25 ± 2.22	8.4 ± 3.29	0.344	0.708	0.57
After	5 ± 1.22	8 ± 2.55	8.4 ± 3.97	0.045*	0.105	0.854
Difference	-4.25 ± 2.87	1 ± 0.82	0 ± 0.71	0.031*	0.056	0.089
p-value	0.060	0.092	1.000			
Anagen						
Baseline	17.25 ± 5.74	16 ± 4.97	20.8 ± 6.18	0.753	0.407	0.249
After	19.4 ± 2.51	19.2 ± 4.92	25.6 ± 12.66	0.938	0.314	0.323
Difference	2 ± 8.04	4.75 ± 5.56	4.8 ± 7.05	0.594	0.595	0.991
p-value	0.653	0.186	0.203			
Telogen						
Baseline	5 ± 1.41	3.5 ± 1.91	5 ± 4	0.254	1	0.516
After	3 ± 1.22	3.2 ± 1.1	5.6 ± 4.39	0.792	0.238	0.27
Difference	-2 ± 2.71	0 ± 2.58	0.6 ± 2.07	0.326	0.145	0.71
p-value	0.236	1.000	0.553			
Anagen: Telogen ratio						
Before	3.69 ± 1.53	5.48 ± 2.84	5.68 ± 2.85	0.310	0.251	0.918
After	8.53 ± 6.54	6.43 ± 2.01	7.57 ± 8.47	0.512	0.845	0.778
Difference	5.31 ± 8.89	0.94 ± 4.33	1.88 ± 7.71	0.410	0.555	0.834
p-value	0.318	0.694	0.614			
Teminal: vellus ratio						
Before	1.51 ± 0.49	1.7 ± 0.24	2.03 ± 0.97	0.501	0.359	0.532
After	3.72 ± 1.29	1.96 ± 0.99	2.73 ± 0.95	0.042*	0.205	0.244
Difference	2.25 ± 1.62	0.47 ± 1.1	0.7 ± 0.4	0.119	0.075	0.669
p-value	0.069	0.459	0.016*			

Values presented as mean ± SD. P-value corresponds to Paired t test (comparison within group) and Independent t-test (comparison between groups).

6.4 Molecular result

At baseline, median of IGF-1 mRNA level from all participants is 4.39 and IGF-1 mRNA level median at 24 hours after third laser treatment on 1st month is 1.33 (p = 0.445) (Table 6.5)

While median of WNT10A mRNA level at baseline is 2.21 and at 24 hours after third laser treatment at 1st month is 0.67 (p = 0.136) (Table 6.5)

The result obtained from data revealed that there is an up regulation of both IGF-1 and WNT10A mRNA expression among all of the participants at 24 hours after 3rd laser treatment on 1st month of study.

Table 6.5 IGF-1 and WNT10A mRNA level expression

	Median (IQR)	**p-value**
IGF-1		
Before	4.39 (1.24, 6.33)	
After	1.33 (0.75, 4.32)	
Difference	**-1.7 (-4.09, 3.32)**	**0.445**
WNT10A		
Before	2.21 (1, 4.03)	
After	0.67 (0.43, 1.89)	
Difference	**-0.57 (-2.98, -0.03)**	**0.136**

Values presented as median (IQR). P-value corresponds to Wilcoxon Signed Ranks Test

6.5 Clinical improvement assessment score

6.5.1 Dermatologist assessment score on 3rd month compared to baseline

On 3rd month of study the participants were all received laser treatment for 5 session. There are 13 participants (59.1%) whose clinical presentation were shown to be improved, stabilize hair thickness for 8 participants (36.4%) and 1 participant (4.5%) got worsening compared with baseline. (Table 6.6)

6.5.2 Dermatologist assessment score on 6th month compared to baseline

On 6th month of study participants were received 14 session of laser treatment. The improvement group contained of 14 participants (60.9%), 7 participants (30.4%) for stabilize group and 2 participants (8.7%) got worsening in condition (p = 1). (Table 6.6)

Table 6.6 Dermatologist assessment of androgenic alopecia on 3rd month and 6th month

	3rd month	**6th month**	**p-value**
Improvement (+1/+2/+3)	13 (59.1%)	14 (60.9%)	**1**
Stabilization (0)	8 (36.4%)	7 (30.4%)	
Worsening(-1/-2/-3)	1 (4.5%)	2 (8.7%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon Signed Ranks Test

6.5.3 Patient satisfaction assessment score

All of the patients are satisfied with the result of 1550 nm fractional erbium-glass laser at the end of study. Their assessment score of 23 patients (100%) were are all in improvement range consist of slightly satisfy (+1) 4 patients (17.4%), moderately satisfied 15 patients (65.2%) and significantly satisfy 4 patients (17.4%). (Table 6.7)

Table 6.7 Patient satisfied assessment

	n	**Percent (%)**
Improvement (+1/+2/+3)	**23**	**100%**
+1	4	17.4%
+2	15	65.2%
+3	4	17.4%
Stabilization (0)	**0**	**0%**
Worsening(-1/-2/-3)	**0**	**0%**

The result obtained from data was presented as frequency (%) Values presented as frequency (%).

Table 6.8 Clinical improvement and correlation factors

	Improvement (n=14)	**Stabilization** (n=7)	**Worsening** (n=2)	**p-value**
Age	40 ± 6.78	40.71 ± 8.85	33.5 ± 9.19	0.490
Female (n=7)				
Ludwig 2	4 (57.1%)	3 (42.9%)	0 (0%)	0.495
Male (n=16)				
Hamilton-Norwood 3 (n=6)	3 (50%)	3 (50%)	0 (0%)	0.147
Hamilton-Norwood 3V (n=5)	4 (80%)	0 (0%)	1 (20%)	0.286
Hamilton-Norwood 4 (n=5)	3 (60%)	1 (20%)	1 (20%)	0.816
Up regulation of IGF-1				
No	11 (55%)	7 (35%)	2 (10%)	0.330
Yes	3 (100%)	0 (0%)	0 (0%)	
Up regulation of WNT10A				
No	10 (52.6%)	7 (36.8%)	2 (10.6%)	0.211
Yes	4 (100%)	0 (0%)	0 (0%)	

Values presented as mean ± SD. and frequency (%). P-value corresponds to ANOVA test and Chi-square test.

6.6 Clinical improvement and correlation factors

From patients characteristics, age, sex and severity may not directly associated with the 1550 nm Er:Glass fractional laser response. These factors cannot be used as clinical response predictor. Interestingly, among variety of response there are certain factors which can improve the clinical after treated with 1550 nm Er:Glass fractional laser. Patients with IGF-1 and WNT10A mRNA level increased in expression compared to baseline are all have an improvement of hair thickness. (Table 6.8)

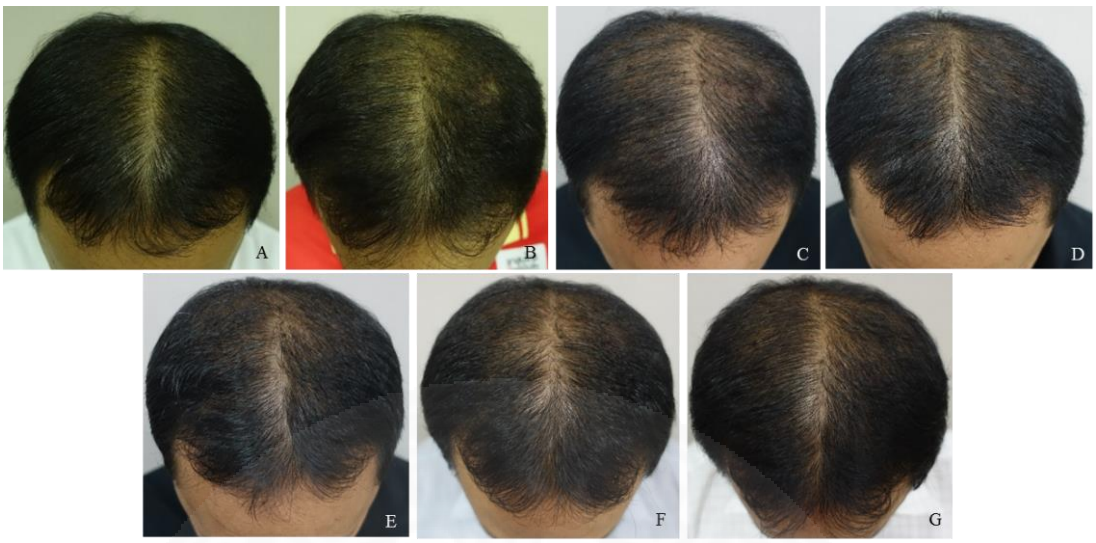

Figure 6.16 Clinical presentation on each month of patient. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

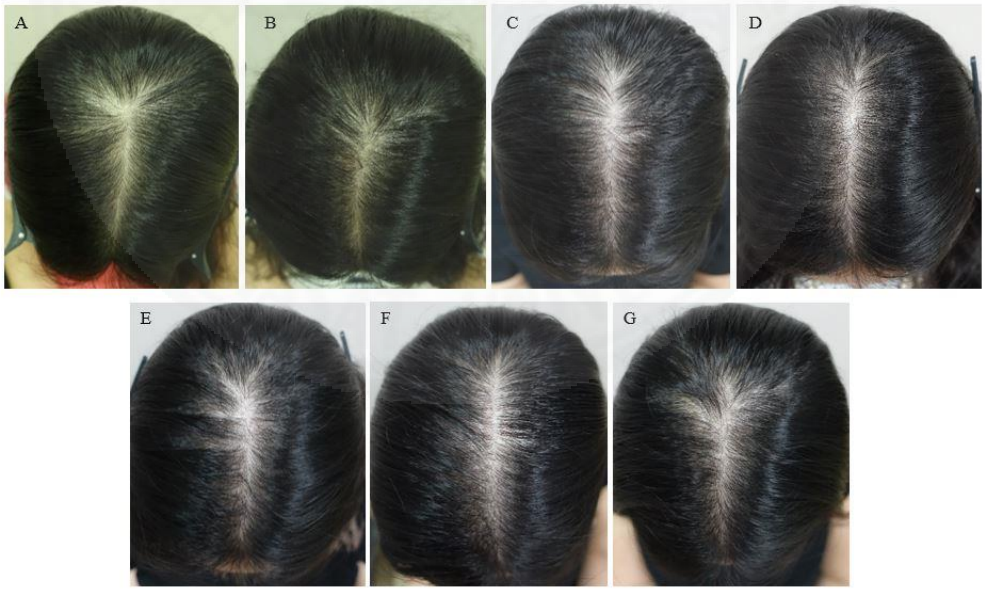

Figure 6.17 Clinical presentation on each month of patient. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

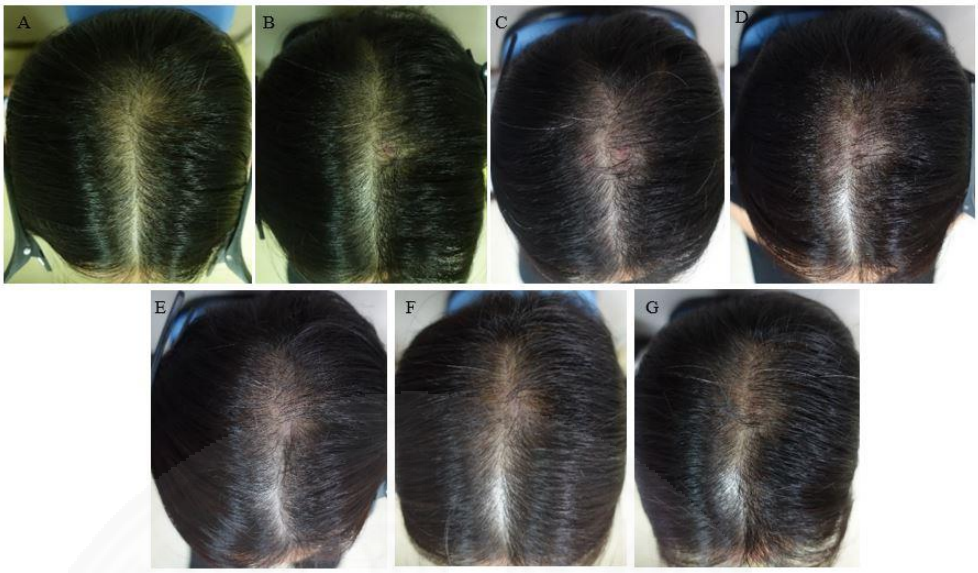

Figure 6.18 Clinical presentation on each month of patient. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

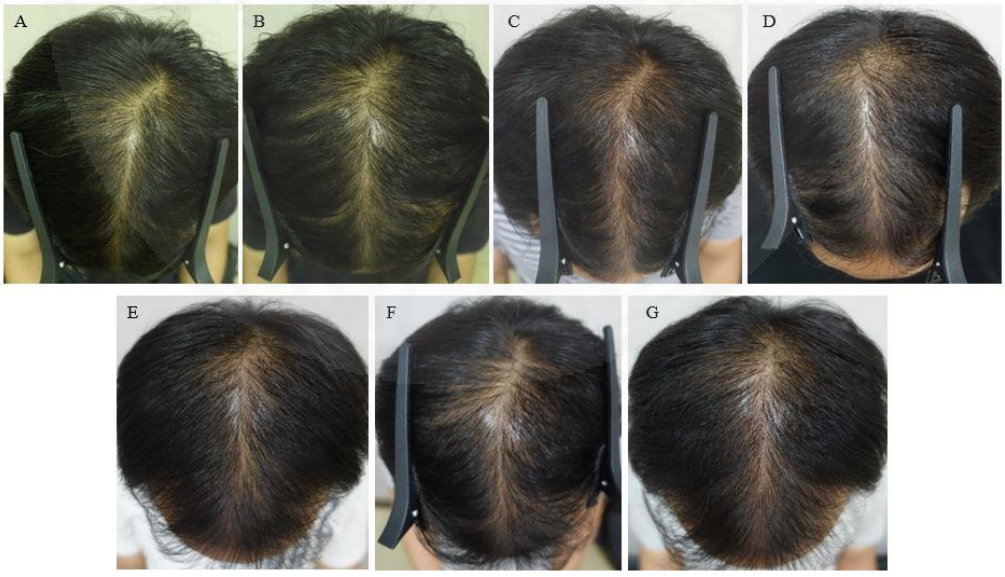

Figure 6.19 Clinical presentation on each month of patient. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

6.7 Adverse effect

All of the participants felt a little bit hurt but tolerable and itching a lot on first session of treatment, on the following time they used to it and not complained about the pain.

Some patients developed mild erythema on treated area but they all spontaneous resolved within 10 minutes without visible wound or scar left. Other side effect of laser treatment had not noted such as hair shaft damaged, infection, bleeding or scar.

CHAPTER 7

DISCUSSION

7.1 Significant increase in hair density and hair shaft diameter since 4th month by the effect of 1550 nm Er:Glass fractional laser

The result from our study showed that both hair density and hair shaft diameter are significantly improved after treated with 1550 nm Er:Glass fractional laser for 14 sessions (6 months). Divided from hair density, terminal hair count per cm^2 and intermediate hair count per cm^2were collected (non-vellus hair), both type of hair count were increased continuously. After 8 sessions of treatment, terminal hair count significantly increased in number since the 4th month compare to baseline. (Table 6.2) Also on hair shaft diameter, the hair shaft thickness started to change significantly, larger in diameter on 4th month compare to baseline. (Table 6.2) The proportion of vellus hair to non-vellus hair also appeared to be significantly reduced after treatment with fractional laser for entire 6 months of study. These result showed that fractional laser can stimulate the new hair growth which is not a vellus hair. (Table 6.3)

7.1.1 Decreased in hair density on 6th month of study

Although the trend of hair density was significantly increased but on 6th month, hair density mean was dropped in number. (Figure 6.1, 6.2), (Table 6.2) May these event resulted from normal hair cycle and their own hair and scalp condition. Normally, there are four stages of hair cycle included anagen, catagen, telogen and exogen. In androgenetic alopecia the anagen phase hair is prematurely turned in to telogen phase, so the proportion of telogen hair phase is increased while anagen is decreased (46, 48). Telogen hair phase is known as a regression phase of hair, it last on scalp only about 3 months long and then it fall out (48). In our study, the patients are all diagnosed androgenetic alopecia which their scalp contained a large proportion of telogen hair which ready to fall out. These may resulted in hair density dropped on 6th month of the study. Even the telogen effluvium condition may induced these hair fall,

like from the stressed at biopsied time on baseline and first month which may inducted prior telogen hair turning on participants scalp.

7.2 Efficacy of 1550 nm Er:Glass fractional laser on histopathology of androgenic alopecia

The study result showed that there are changes in histological finding among 3 groups of sacrificed time consist of baseline – 1st month group, baseline – 2nd month group and baseline – 3rd month. The number of follicular unit, total hair count and anagen hair were increased. The hair density result from histology at each time after treatment was correlated to the hair count from target photography. The proportion of terminal to vellus hair ratio after treatment was all increased after treatment with fractional laser in every group. These refer that 1550 nm Er:Glass fractional laser can induce the new terminal hair growth not only the vellus hair.

7.2.1 Decreases hair density on 1st month of treatment

On 1st month after treatment with laser, hair density from target photography was reduced compared to baseline. (Table 6.2) This result related to histology result on 1st month after treatment. Focused on histology hair count, at 1st month the telogen hair count was decreased from baseline and vellus hair also decreased too while the anagen hair was increased after treatment with fractional laser on 1st month. (Table 6.3) These indicated that the reduction of hair count on 1st month was occurred from the fallen of telogen which known to be the regression hair phase of the scalp. For the coincidence of telogen fall out on 1st month after treatment this may because of it was from their normal hair cycle. Secondly, the 1550 nm fractional laser induce the new hair growth by shortening the telogen to anagen period which like previous papers were discussed (4-6), this resulted in the hair cycle accelerated for prepare the new hair forming and push the telogen to fall out(77).

After the 1st month of treatment, the hair count from both clinical and histology trended to increase in number. The anagen hair count was increased after treatment compared to baseline in every group, 1st month, 2nd month and 3rd month.

When compared anagen hair and telogen hair of pateints in every group, the anagen:telogen ratio after treatment also increased too. Even the result from histology is not significant increase in number of hair density but it correlated with the target photograph at the same time of result. This result proved the effective of the fractional laser in stimulating the new hair growth in androgenetic alopecia.

Interestingly, our study showed that after treated with 1550 nm fractional laser, vellus hair was found to be decreased on 1st month group and for the 2nd and 3rd group vellus hair seem not to change in their number. This finding quite different from the previous study on 2011, Seoul Korea – the period of tissue sacrificed from 5 patients was on 1st month after first time treated, they found that mostly new formed hair follicle of their study were vellus hair without complete pilosebaceous unit composed (4). From our study the new follicular unit which is the complete hair follicle consist of pilosebaceous unit and pilo-erector muscle trend to be increased in number through the time. This may inferred that 1550 nm fractional laser treatment can promote the complete new hair growth not only the vellus hair.

7.3 Efficacy of 1550 nm Er:Glass fractional laser on IGF-1 and WNT10A mRNA expression in androgenetic alopecia

In a current modern trend of treatment, the specific molecule or mechanism of that disease has been focused for developed such a targeted therapy to maximize the effect of treatment together with least to none side effect from the treatment. Nowadays the standard treatment that was approved by FDA still be a systemic 5α-reductase inhibitor and topical minoxidil for androgenetic alopecia. Other treatment both drugs and physical therapy are all off-label used. In these few years many new modalities were introduced included fractional laser for treat the hair loss condition (78, 79). Since the wound healing process has been shown involving the new hair growth by it inflammatory process, many cytokines were pooled in the area of healing (8-10). The fractional laser seemed to be the good tools for stimulating the new hair growth by creating the controlled regular microscopic thermal wound which penetrated depth into dermis. The wound healing process from fractional laser induce the blood flow and

cytokines for promoting the healing which included growth factors such as FGF family, EGF, IGFs, HGF, TGF-β, VEGF, NGF and interleukins(12, 13). The dermal papilla may directly altered by those cytokines, proliferation was occurred on this site included hair stem cells that resided in the dermal papilla.

Both IGF-1 and Wnt/β-catenin are the promoting factors of telogen transit to anagen. Abundant expression of IGF-1 can improved the wound healing and stimulated new hair follicle formation in the mice (11). IGF-1 also known as anagen maintenance, without IGF-1 could lead premature catagen phase turning (30). IGF-1 are found to be down regulated in androgenetic alopecia dermal papilla (29). In addition, IGF-1 was found to be up regulated in androgenetic alopecia patient who received finasteride with clinical improvement (31). The signal from Wnt/β-catenin pathway can initiate hair follicle formation and stimulate the new hair forming (80-83). The recent study show that topical methyl vanilate which has Wnt/β catenin as an active ingredient promote hair mass index in female pattern hair loss (22). Further than this in murine model study of fractional laser induce hair regrowth, WNT/β catenin were found up regulated after treatment (15, 16).

WNT/β catenin is essential for initiating stem cell proliferation, hair stem cell also one of a kind. Even stem cell is not required for the hair follicle stem cell maintenance in resting phase, but in differentiation of terminal hair Wnt is criticized for this step (84). In hair regeneration, outer root sheath layer expressed abundant of Wnt10b and also at dermal papillae whereas Wnt5a expression was found at upper outer root sheath. After a regeneration process Wnt10a expressed continuously at the bottom of hair follicle. Through the process of hair regeneration β catenin level was found to be up regulation too (85). Outer root sheath forming failed without β-catenin expression that means terminal hair forming failure (86).

7.3.1 Optimal time of WNT10A and IGF-1 increased in expression

The result from our study revealed an increasing expression of IGF-1 and WNT10A mRNA but not significant among all participants. This quite different from previous pilot study in human which WNT10A was increased in highest level at 24 hours after treatment (4). The time that tissue was sacrificed for mRNA level extraction may not be the proper time for the expression of WNT10A and IGF-1 mRNA for human.

7.3.2 Laser parameter affected on WNT10A and IGF expression

The energy or parameter that had been used in this study may not proper to stimulate the dermal papilla. In murine model, the parameters of 1550 nm Er:Glass fractional laser which can enhanced the anagen entry on should not least than 10mJ with 1500 MTZ/cm^2 in densities, less energy and densities cannot promote reentry of anagen. Increase in pulse energy, the required densities were reduced. The anagen reentry was observed since day 9 to day 11 in mice (16). In this study, the 1550 nm Er:Glass fractional laser with energy 6mJ, 300 densities spot per cm^2 were used when compared to the study in murine model, our study used lower energy than the parameter that they had suggested.

The lower laser energy, the lower side effect. The high energy of laser parameter as well as the high density of laser beam might be painful comparing with lower parameter. Higher in pulse energy can cause ulcer that could lead to the scar formation and permanent hair loss (16).

Interestingly even the lower energy was used, the clinical results showed the good outcome, hair density and hair shaft diameter were significant improved on 4^{th} month even the long time had taken but it improved. This may refer that at the low level parameter the anagen reentry could be promoted with multiple sessions of treatment, regularity and a period of time that may take for few months. The thermal that caused by the laser may alter dermal papilla directly and induce the hair stem cell regeneration (68, 69).

7.3.3 1550 nm Er:Glass fractional laser induce hair growth through inflammation cascade

The Wnt/β catenin pathway and IGF-1 may not be the only key factor of new hair growth. Other cytokines and pathways may involve in these part. The inflammation that cause by laser beam may induce the hair regrowth. In mice that were irradiant with fractional laser the inflammatory cytokines such as TNF-α, IL-β, and IL-6 were increased in expression. Moderate intensity with subclinical inflammation can induce the new hair growth in mice (16).

In human photorejuvenation treatment with 1550 nm Er:Glass fractional laser, pro-inflammatory were found up regulation at after treatment included IL-1 and TGF-β which induced cutaneous regeneration, proliferation of fibroblasts, as well as collagen synthesis (72-75).

For those participants whose IGF-1 and WNT10A mRNA level was increased at 24 hours after 3rd treatment at 1st month. Their clinical seemed to be correlated with the IGF-1 and WNT10A mRNA level, among these patients the clinical improvement is 100%. (Table 6.7) From the methyl vanilate with active ingredient of Wnt/β catenin topical application in androgenetic alopecia, the WNT10B mRNA expression was significantly increased expression on 6th month from baseline as well as the clinical that also significantly improved (22).

Response time to 1550 nm Er:Glass fractional laser treatment of both molecular and clinical presentation might be individualized. The variability of each person such as ages, severity of diseases, their health status and nutrition might affected wound healing process.

The global photograph was use as the clinical assessment for blinded dermatologist. The mainly enrolled patients in this study were the pattern hair loss on vertex so the patients with mainly anterior hair loss or temporal site may not be perceive on that area well.

In conclusion, the 1550 nm Er:Glass fractional laser can improve hair density and hair shaft diameter statistically significant since 4th month. The histology results revealed the new formation of follicular unit and anagen hair. In molecular part, at 24 hours after 3rd treatment with fractional laser, Wnt/β catenin pathway and IGF-1

did not always increase in level at this time. This result may depend on individualized health status, nutritional condition, ages and severity of disease. Wnt/β catenin pathway and IGF-1 may not be the only key factor of new hair growth, inflammatory cytokine may take or other growth factor may involve in initiate the new hair growth. The lower pulse energy of laser or the higher pulse energy affected the outcome. The lower pulse energy may not appropriate on directly stimulating the dermal papillae, while the higher energy could cause the permanent damage than the treatment effect such as scarring or fibrosis.

7.4 Recommendations

7.4.1 Further studies using various parameter of laser should be conducted for evaluating treatment effects, adverse effect as well as the optimized parameter for treatment.

7.4.2 Further studies for determine mRNA level of Wnt/β catenin pathway and IGF-1 at a different time and other factors such as inflammatory cytokines.

7.4.3 Long term follow up should be recorded, after stop the treatment.

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APPENDICES

APPENDIX A
PATIENT RECORD FORM

แบบฟอร์มบันทึกข้อมูล

ลำดับที่.....

ชื่อ-สกุล..... โทร.....

ข้อมูลทั่วไป

1. เพศ ชาย หญิง อายุ.....ปี เชื้อชาติ..... สัญชาติ.....

2. น้ำหนัก.....กิโลกรัม ส่วนสูง.....เซนติเมตร

3. โรคประจำตัว.....

ยาที่ใช้อยู่ในขณะนี้ หรือใช้เป็นประจำ.....

ประวัติแพ้ยา.....

ข้อมูลเกี่ยวกับโรคผมบางแบบพันธุกรรม (Androgenic alopecia)

1. อายุที่เริ่มมีอาการผมบาง.....
2. ระดับความรุนแรงของโรค (Staging).....

เคยได้รับการรักษามาก่อนหรือไม่ เคย ไม่เคย

ยา หรือผลิตภัณฑ์ที่ใช้เพื่อกระตุ้นการงอกใหม่ของผม.....

หยุดการรักษาเป็นเวลา.....

3. ประวัติคนในครอบครัวที่มีภาวะผมบางแบบพันธุกรรม……
4. ลักษณะหย่อมผมบาง

การรักษาด้วย 1,550 nm Er:glass fractional laser

ครั้งที่	วันที่	พลังงาน	หมายเหตุ
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

ข้อมูลสำหรับสิ่งส่งตรวจ

1. Code……

2. Scalp biopsy 2 mm.

Baseline 24 hours

3. Scalp biopsy 4 mm.

Baseline 3 month

ข้อมูลสำหรับวิเคราะห์

	Hair density	Hair shaft diameter	Hair count/cm^2
Baseline			
1			
2			
3			
4			
5			
6			

	Wnt/β-catenine mRNA expression	IGF-1 mRNA expression
Baseline		
24 hours		

APPENDIX B

PATIENT CHARACTERISTIC DATA

Table B1 Patient characteristics data

Patient No.	Age	Stage	Gender
No.1	44	Hamilton-Norwood 3V	Male
No. 2	43	Hamilton-Norwood 3	Male
No.3	51	Hamilton-Norwood 3V	Male
No.4	49	Hamilton-Norwood 3V	Male
No.5	40	Ludwig 2	Female
No.6	33	Ludwig 2	female
No.7	37	Ludwig 2	female
No.8	36	Hamilton-Norwood 3	Male
No.9	40	Hamilton-Norwood 4	Male
No.10	29	Hamilton-Norwood 3	Male
No.11	36	Ludwig 2	female
No.12	38	Ludwig 2	female
No.13	38	Ludwig 2	female
No.14	48	Hamilton-Norwood 4	Male
No.15	27	Hamilton-Norwood 4	Male
No.16	40	Hamilton-Norwood 3	Male
No.17	27	Hamilton-Norwood 3V	Male
No.18	35	Hamilton-Norwood 3	Male
No.19	32	Hamilton-Norwood 4	Male
No.20	49	Hamilton-Norwood 4	Male
No.21	54	Ludwig 2	female
No.22	41	Hamilton-Norwood 3	Male
No.23	45	Hamilton-Norwood 3V	male

APPENDIX C
GLOBAL PHOTOGRAPH OF PATIENTS

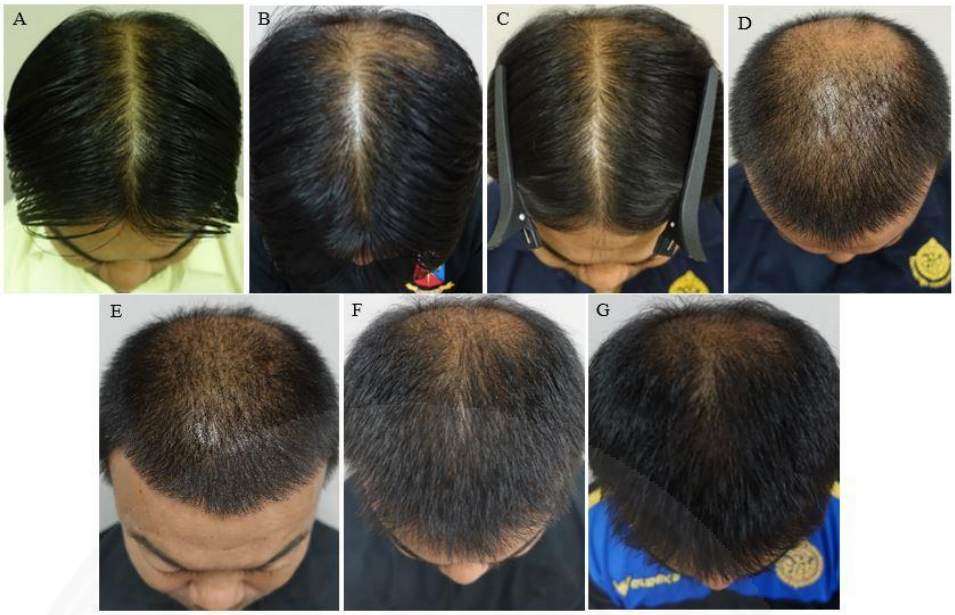

Figure C1 Global photograph of patient No.1 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

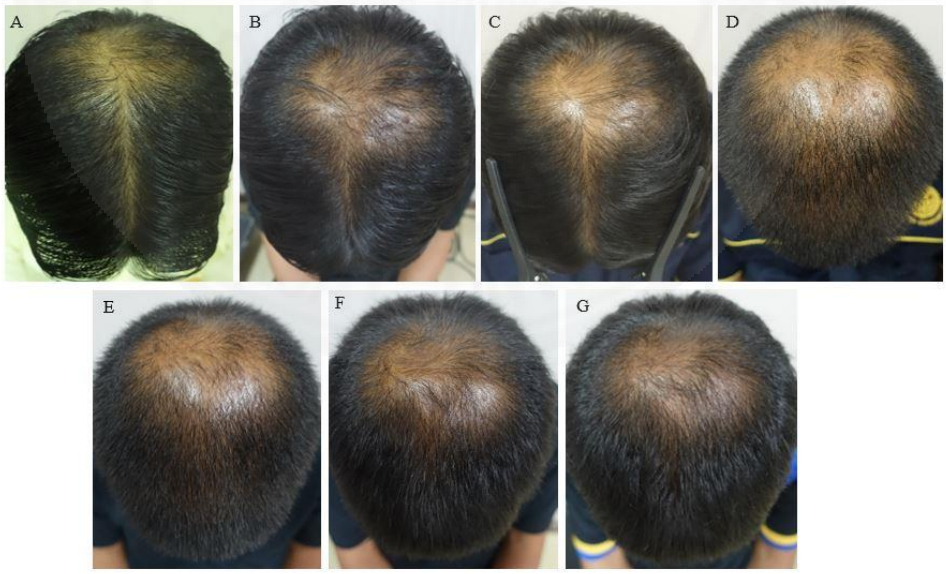

Figure C2 Global photograph of patient No.1 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

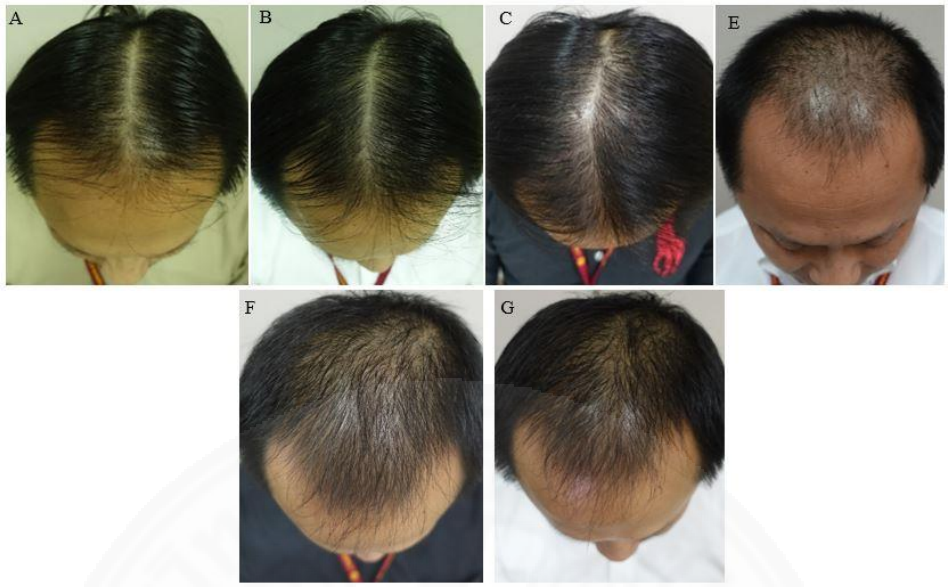

Figure C3 Global photograph of patient No.2 in 45º. Baseline (A), 1st month (B), 2nd month (C), 4th month (E), 5th month (F), 6th month (G).

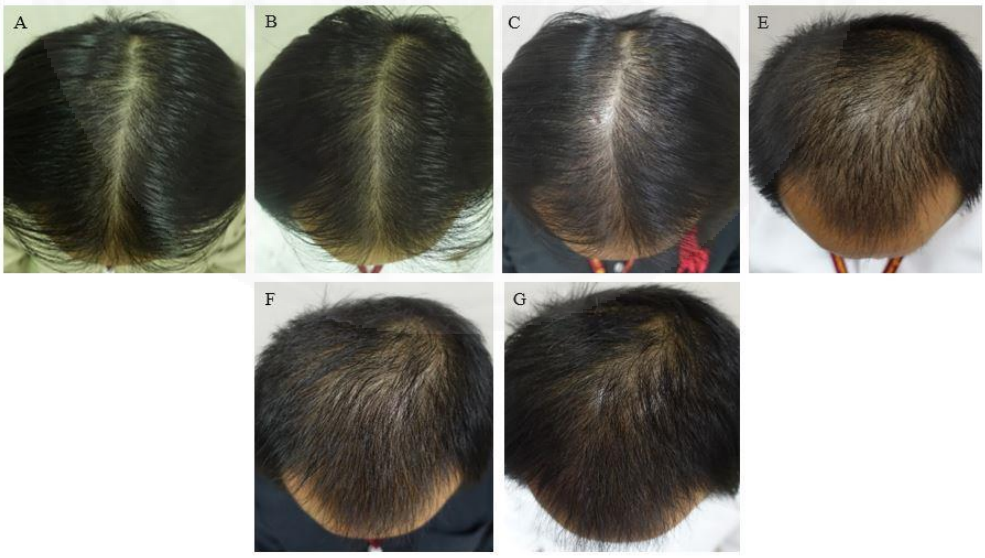

Figure C4 Global photograph of patient No.2 in 90º. Baseline (A), 1st month (B), 2nd month (C), 4th month (E), 5th month (F), 6th month (G).

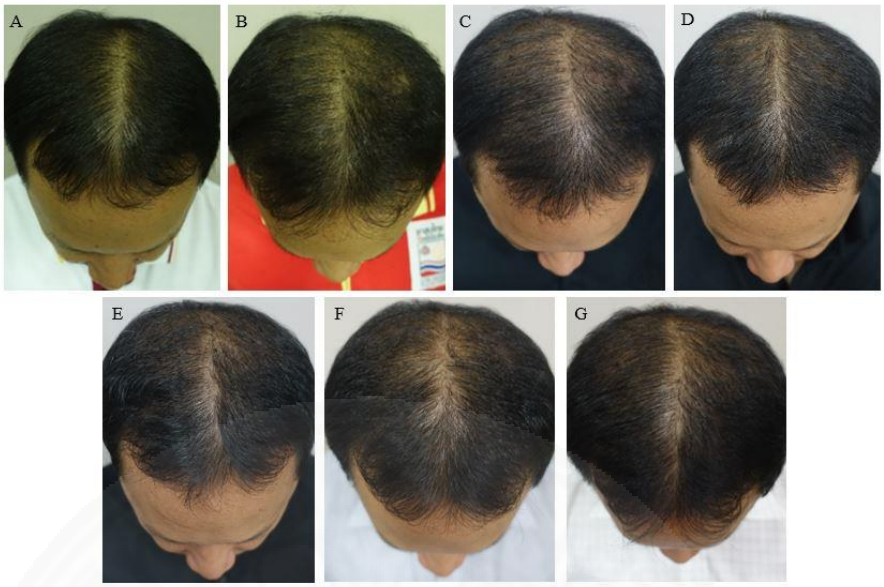

Figure C5 Global photograph of patient No.3 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

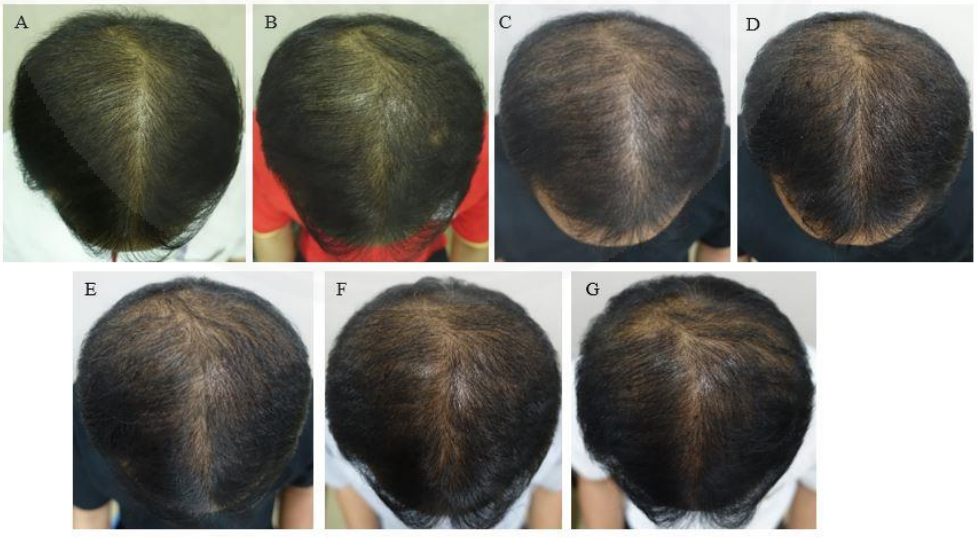

Figure C6 Global photograph of patient No.3 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

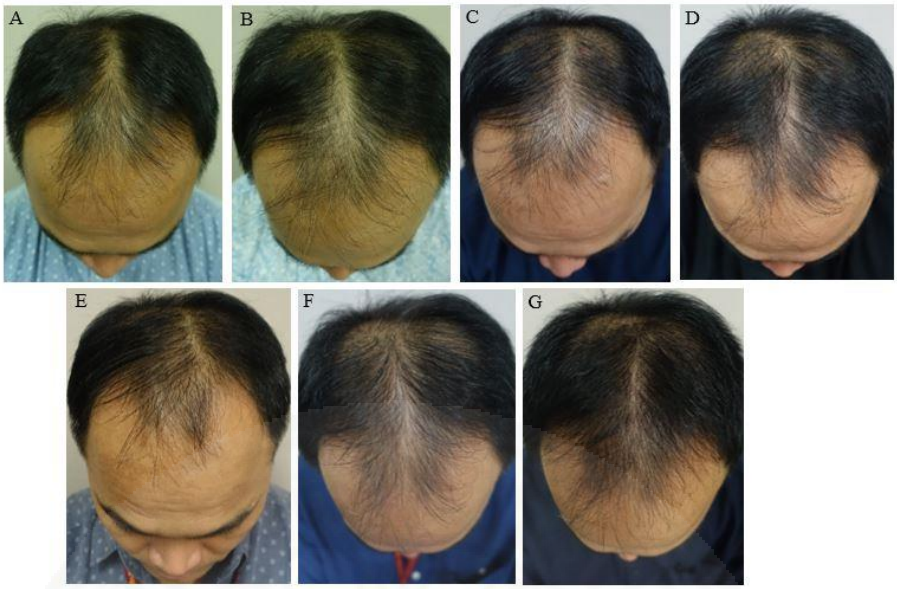

Figure C7 Global photograph of patient No.4 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

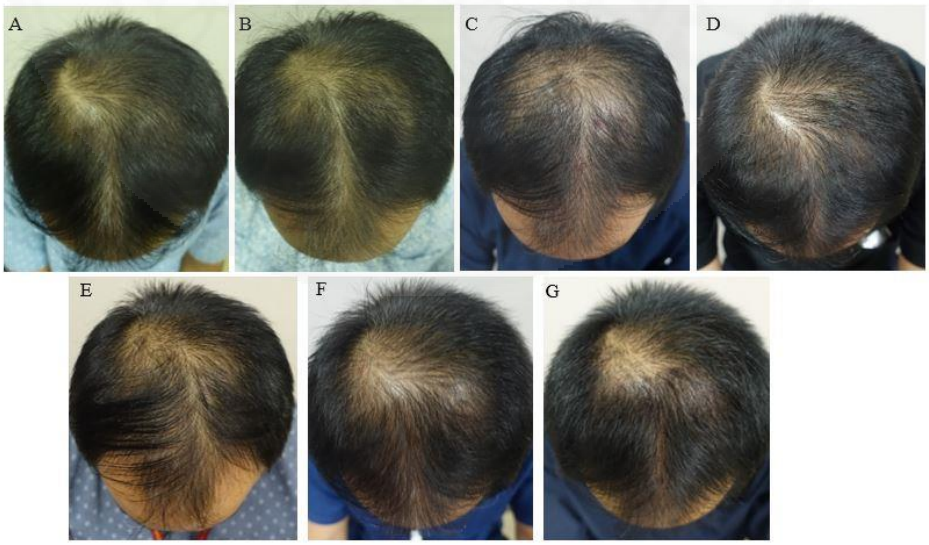

Figure C8 Global photograph of patient No.4 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

Figure C9 Global photograph of patient No.5 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

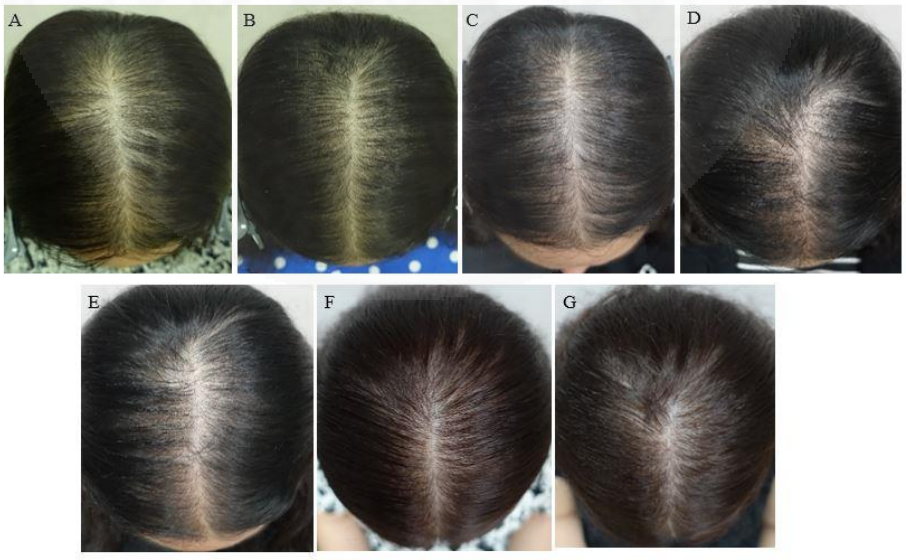

Figure C10 Global photograph of patient No.5 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

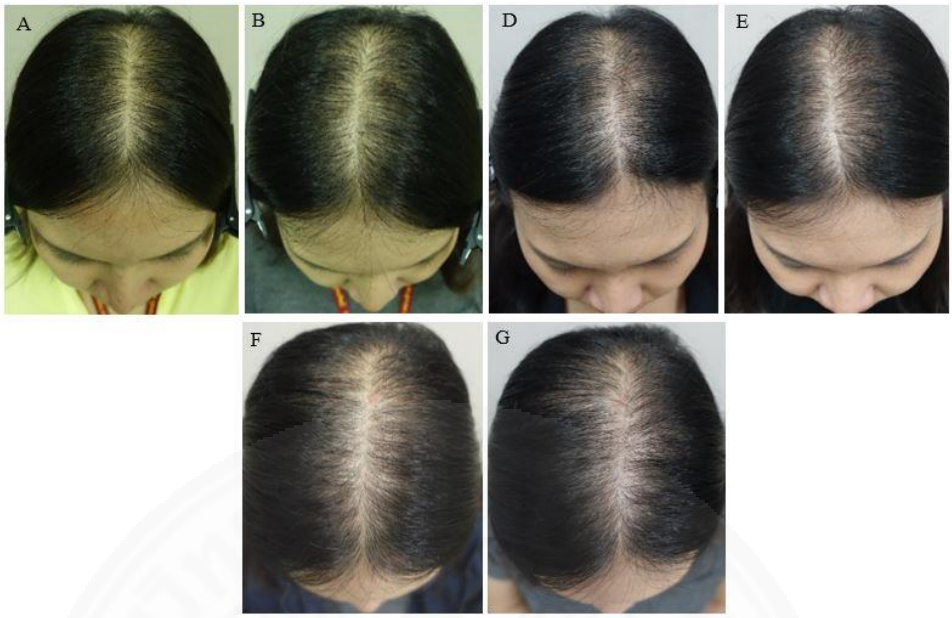

Figure C11 Global photograph of patient No.6 in 45º. Baseline (A), 1st month (B), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

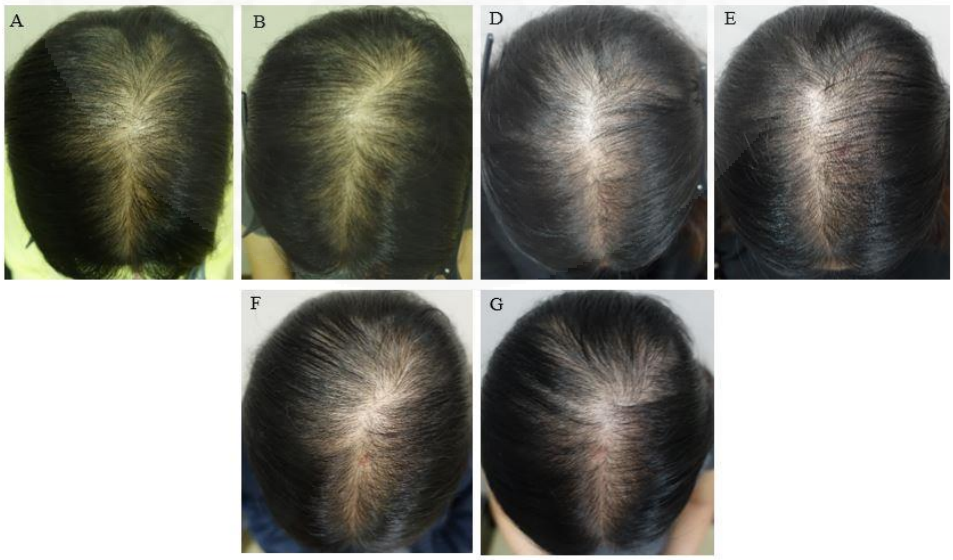

Figure C12 Global photograph of patient No.6 in 90º. Baseline (A), 1st month (B), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

Figure C13 Global photograph of patient No.7 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

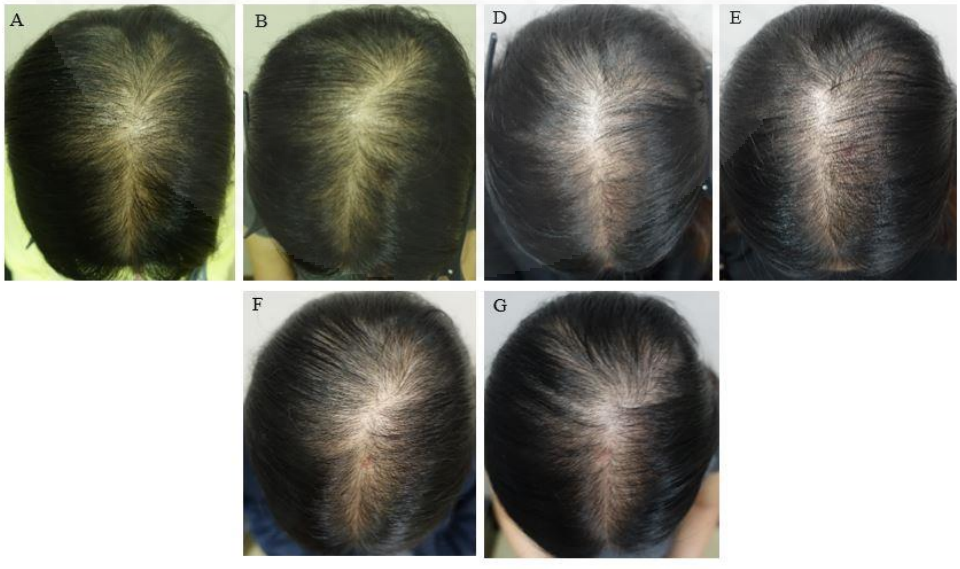

Figure C14 Global photograph of patient No.7 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

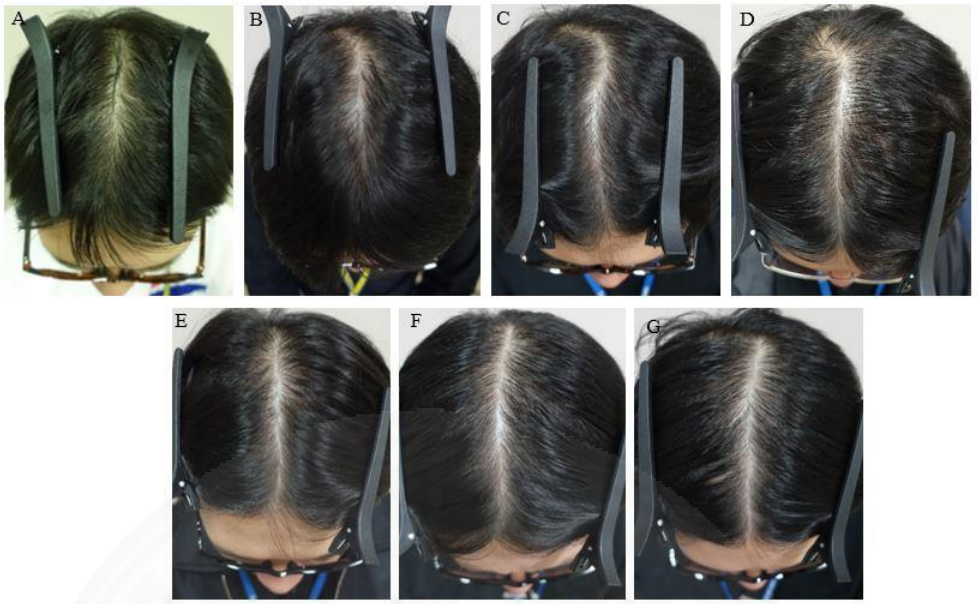

Figure C15 Global photograph of patient No.8 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

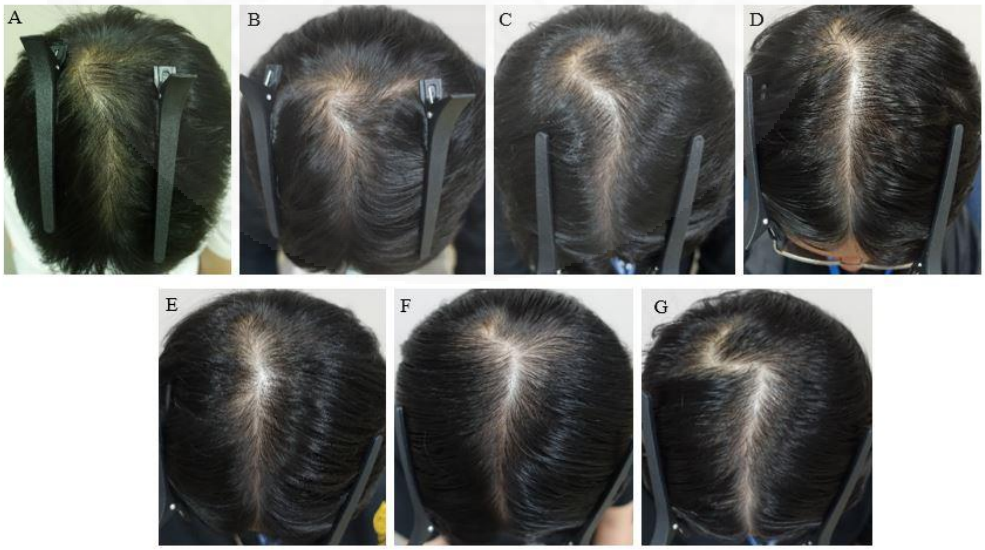

Figure C16 Global photograph of patient No.8 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

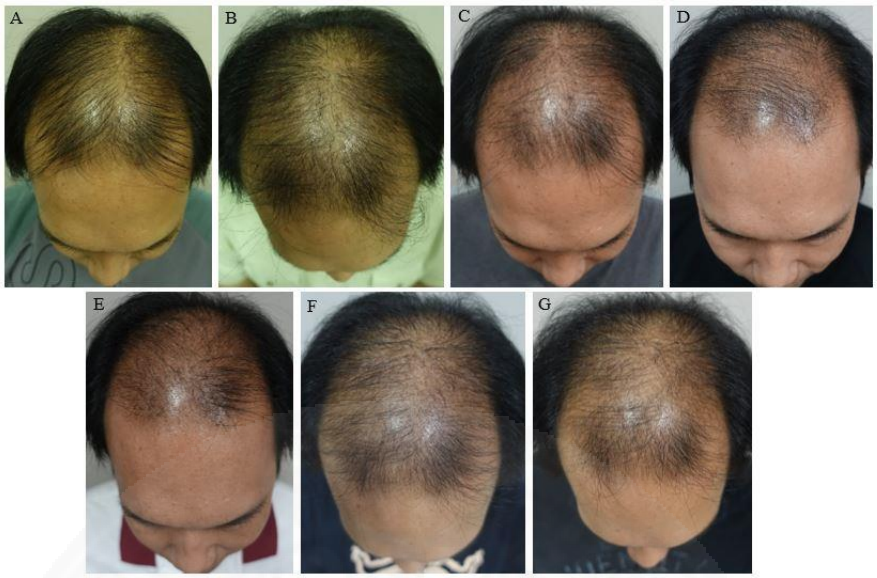

Figure C17 Global photograph of patient No.9 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

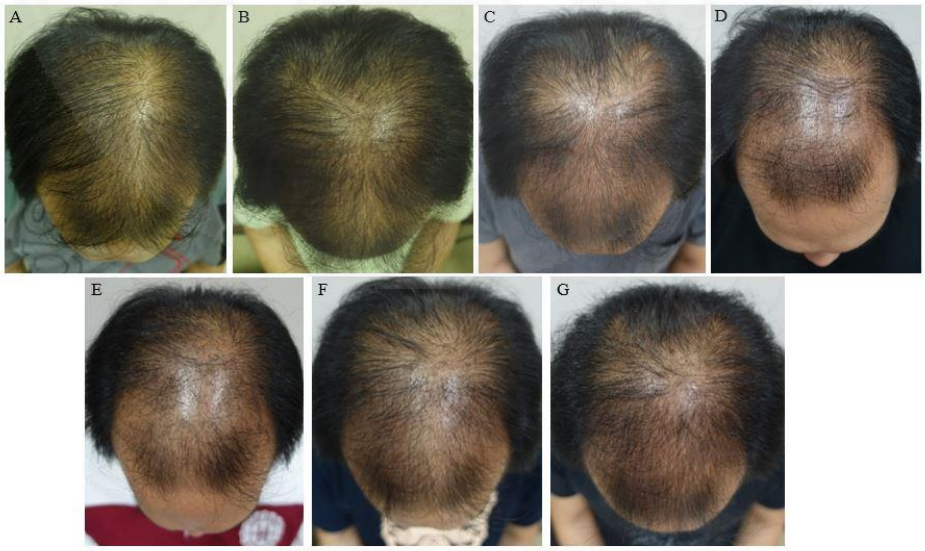

Figure C18 Global photograph of patient No.9 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

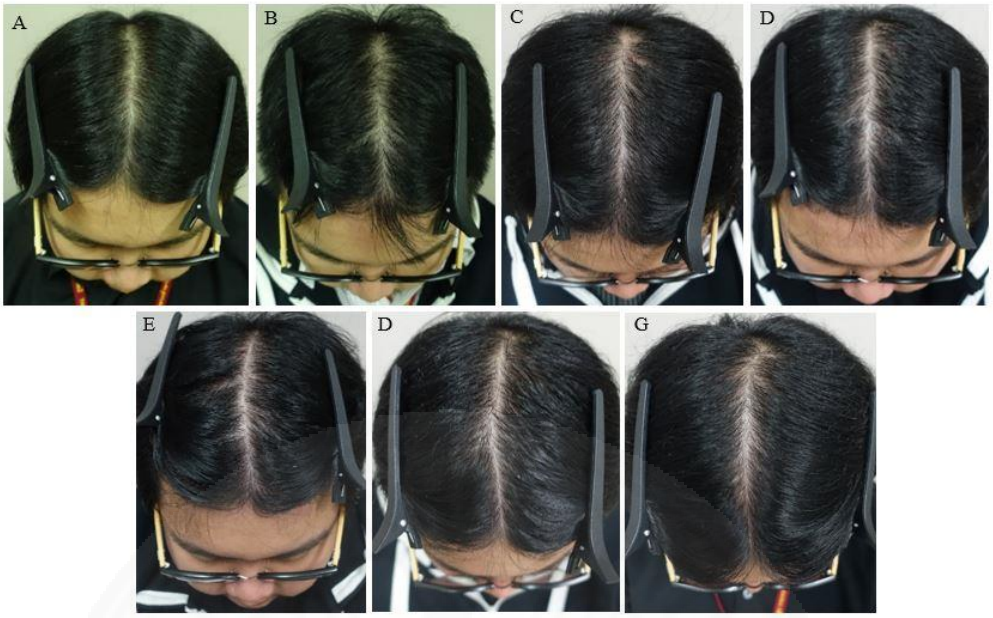

Figure C19 Global photograph of patient No.10 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

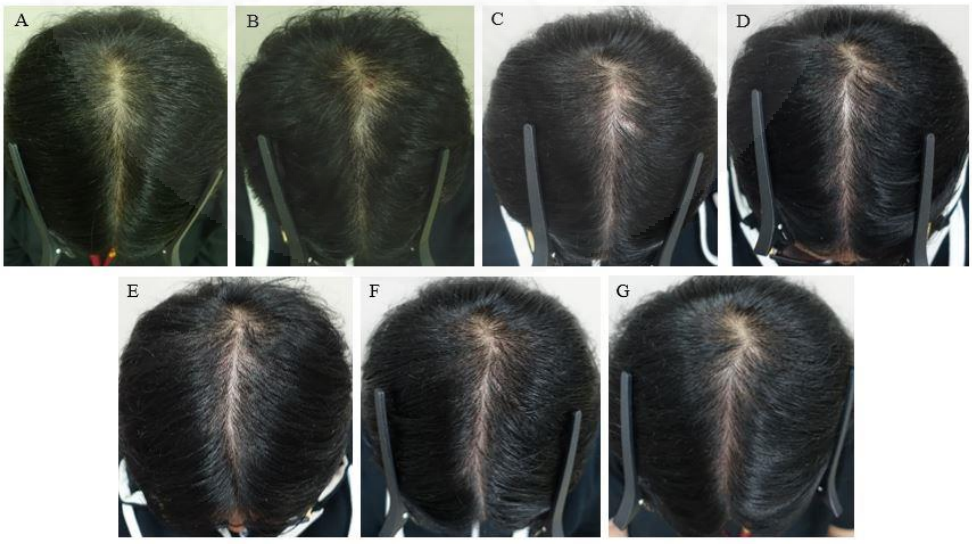

Figure C20 Global photograph of patient No.10 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

Figure C21 Global photograph of patient No.11 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

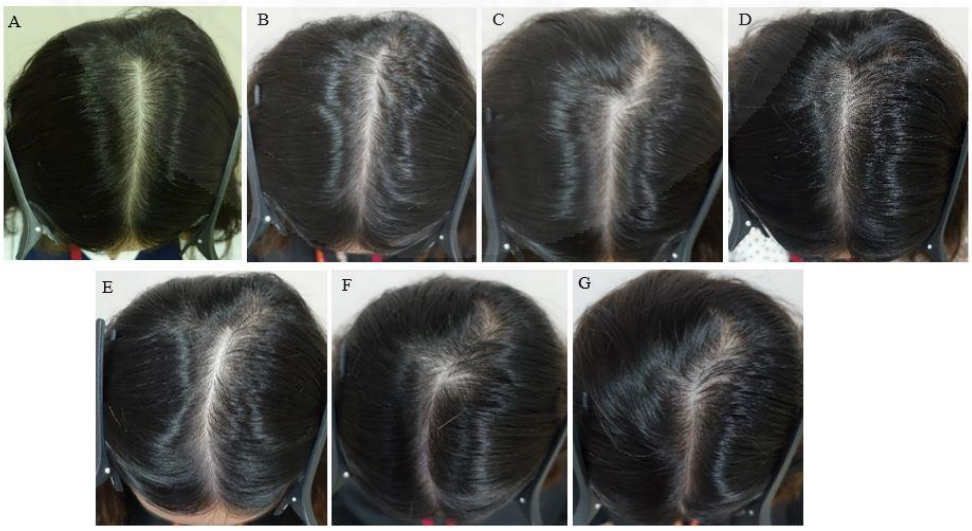

Figure C22 Global photograph of patient No.11 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

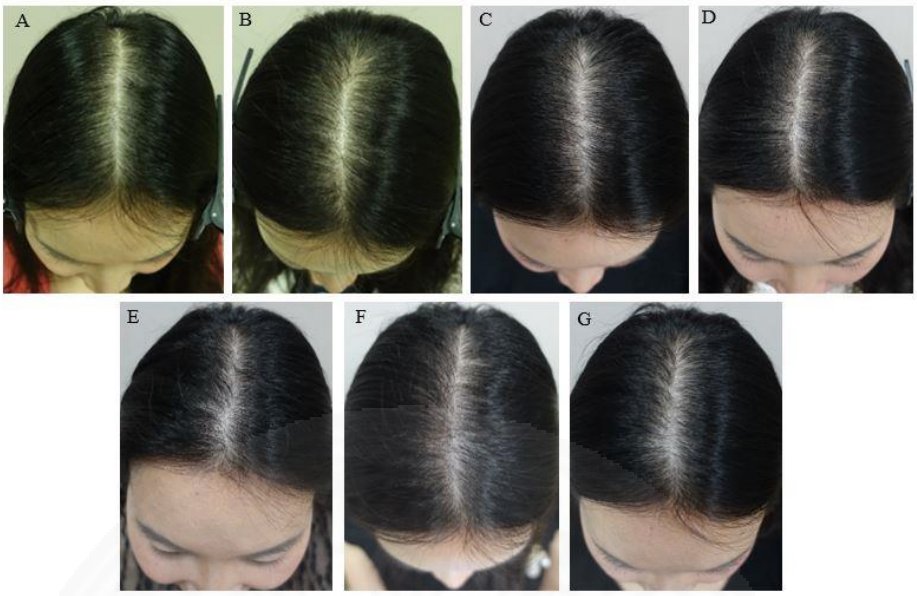

Figure C23 Global photograph of patient No.12 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

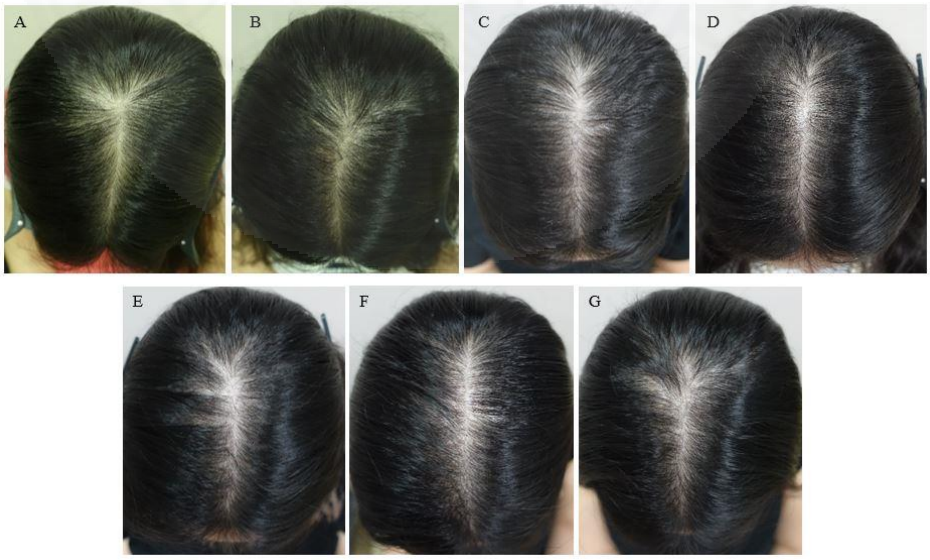

Figure C24 Global photograph of patient No.12 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

Figure C25 Global photograph of patient No.13 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

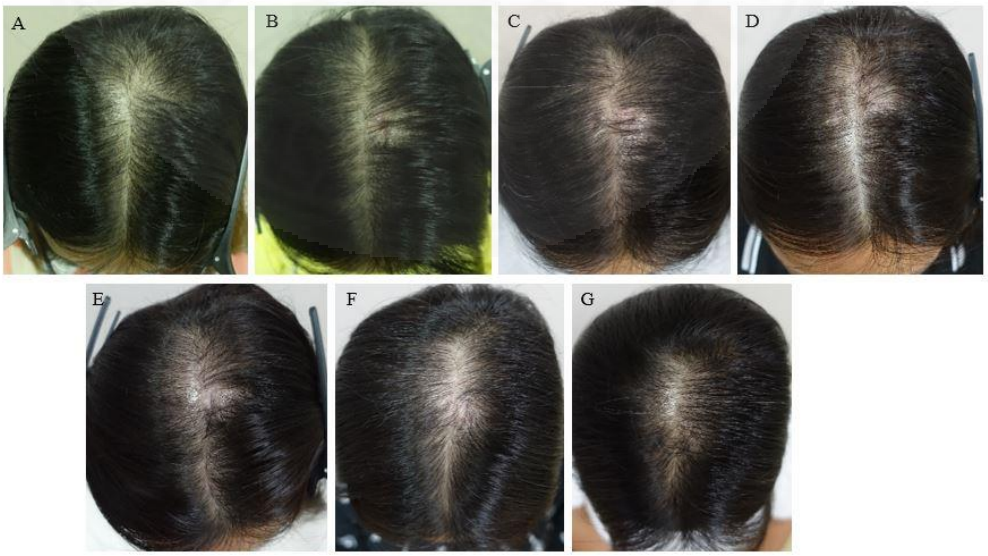

Figure C26 Global photograph of patient No.13 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

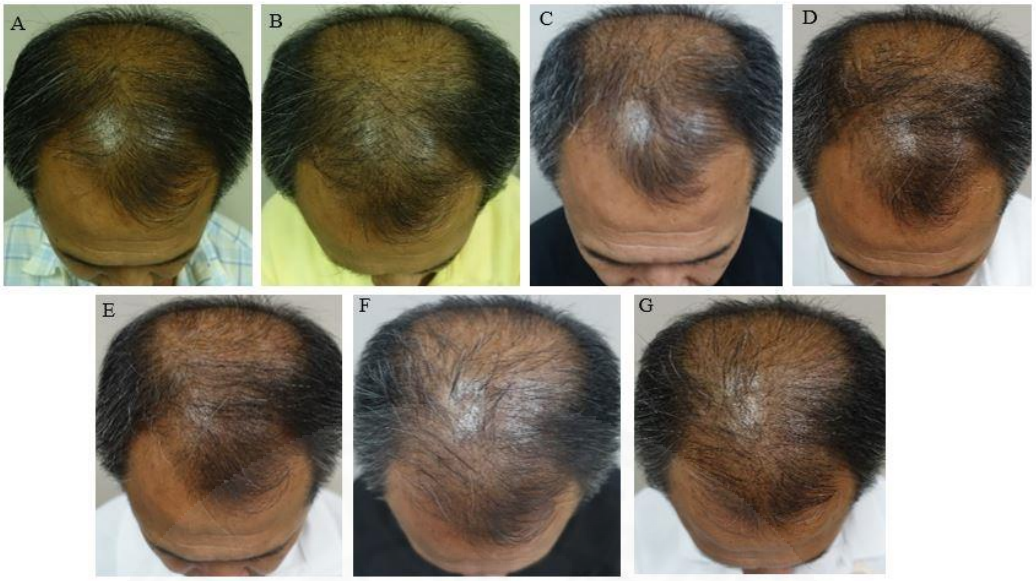

Figure C27 Global photograph of patient No.14 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

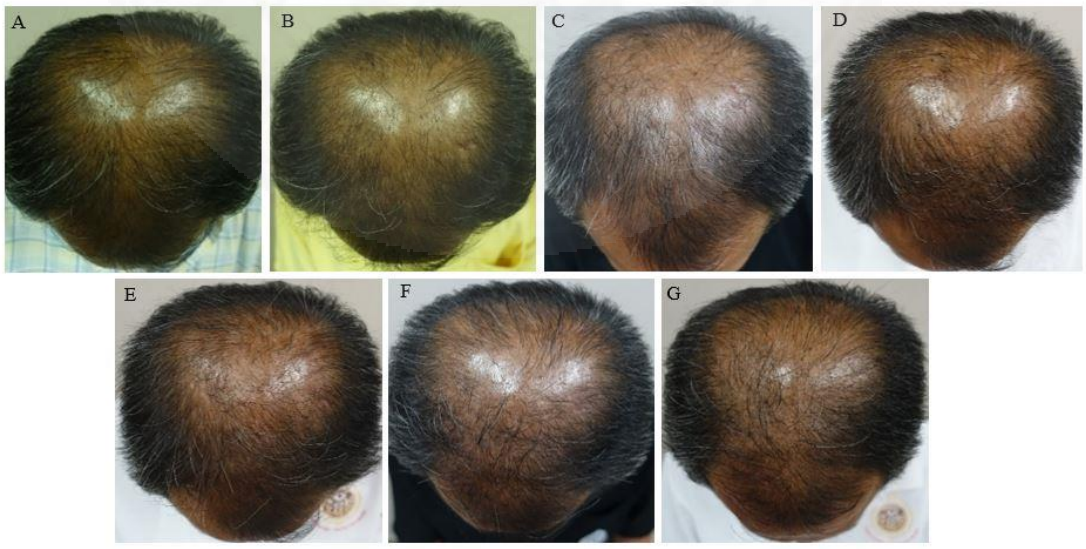

Figure C28 Global photograph of patient No.14 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

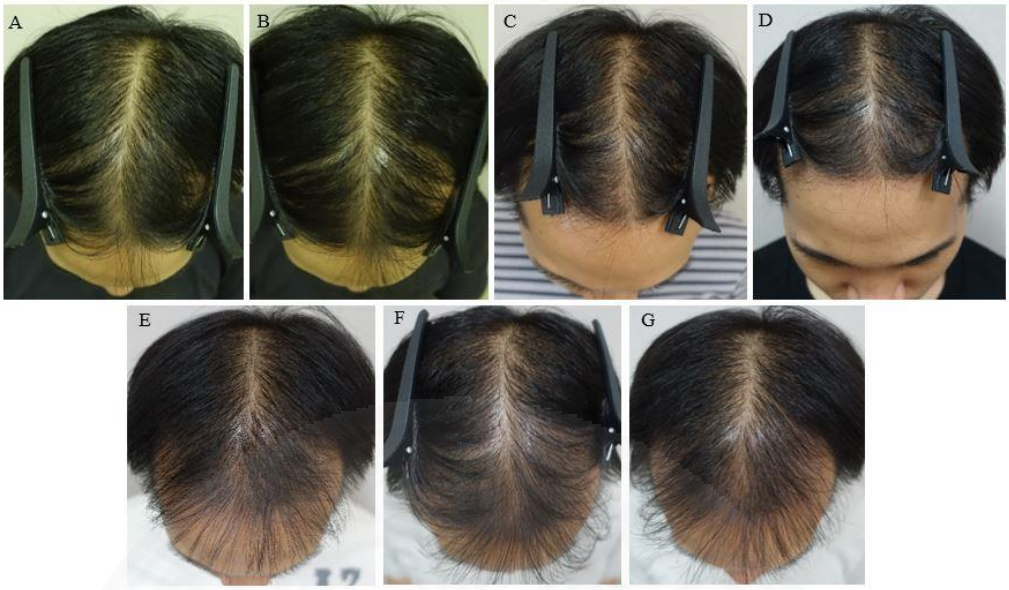

Figure C29 Global photograph of patient No.15 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

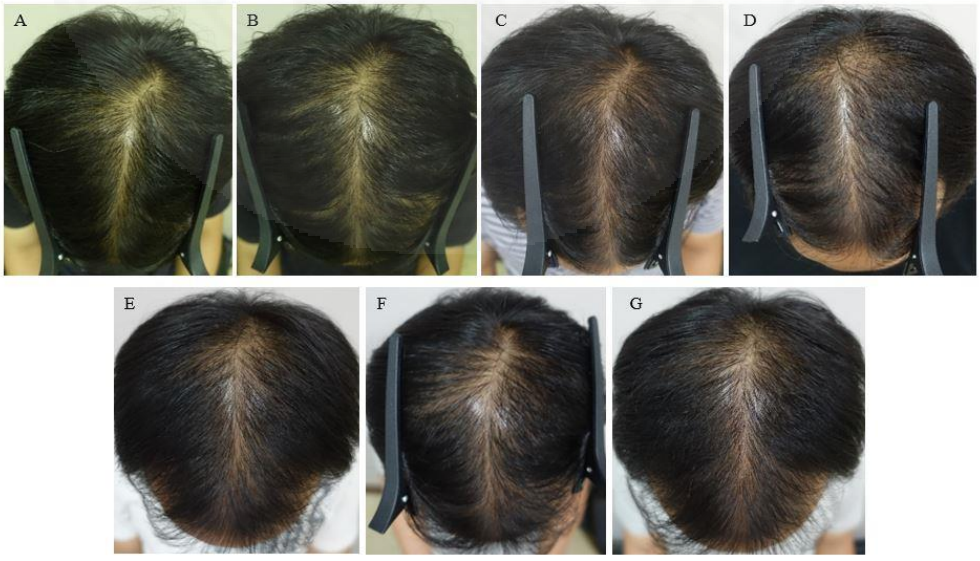

Figure C30 Global photograph of patient No.15 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

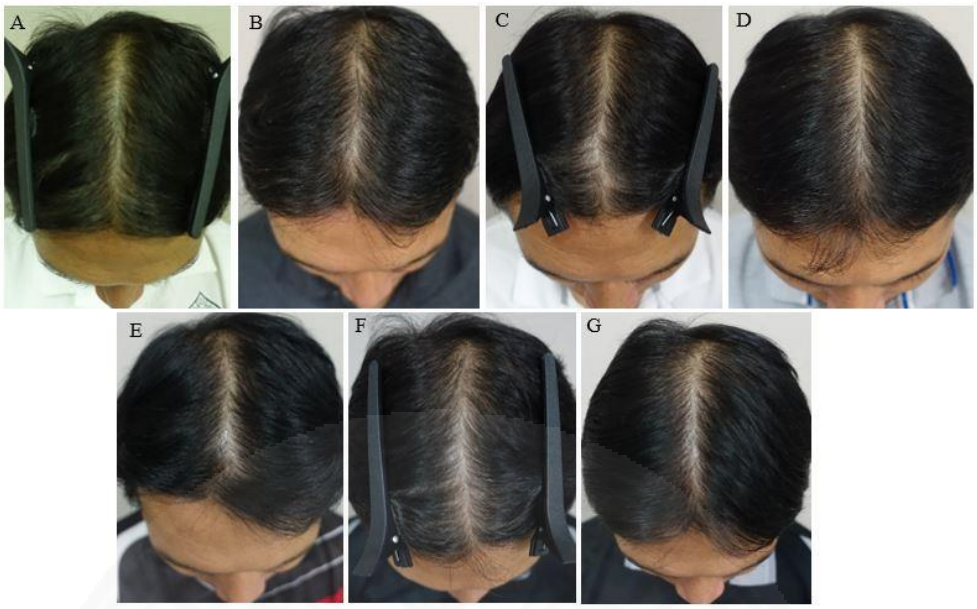

Figure C31 Global photograph of patient No.16 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

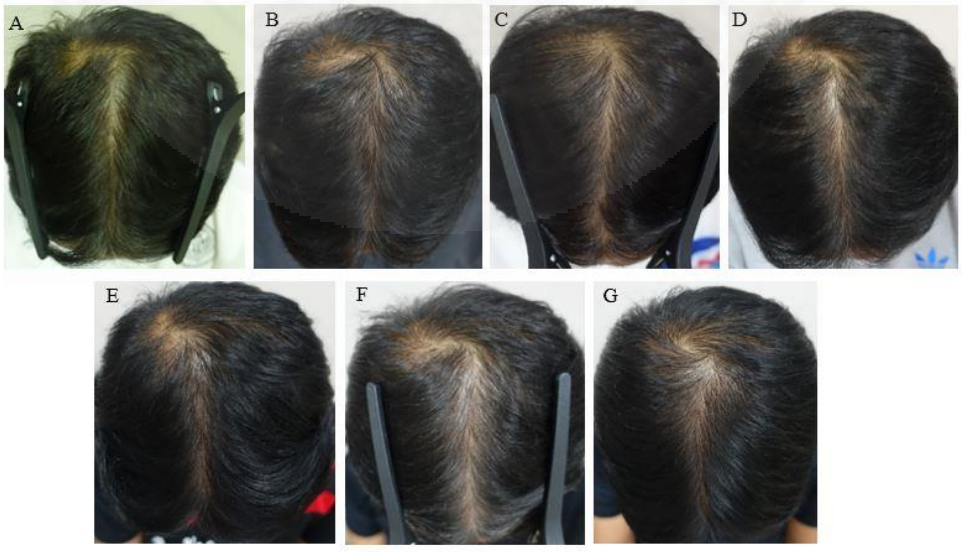

Figure C32 Global photograph of patient No.16 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

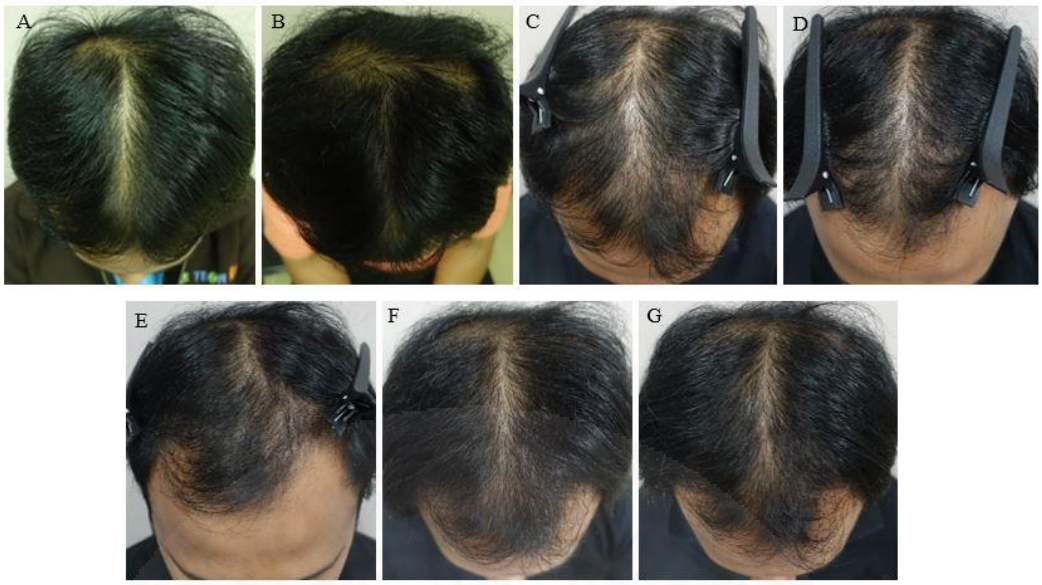

Figure C33 Global photograph of patient No.17 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

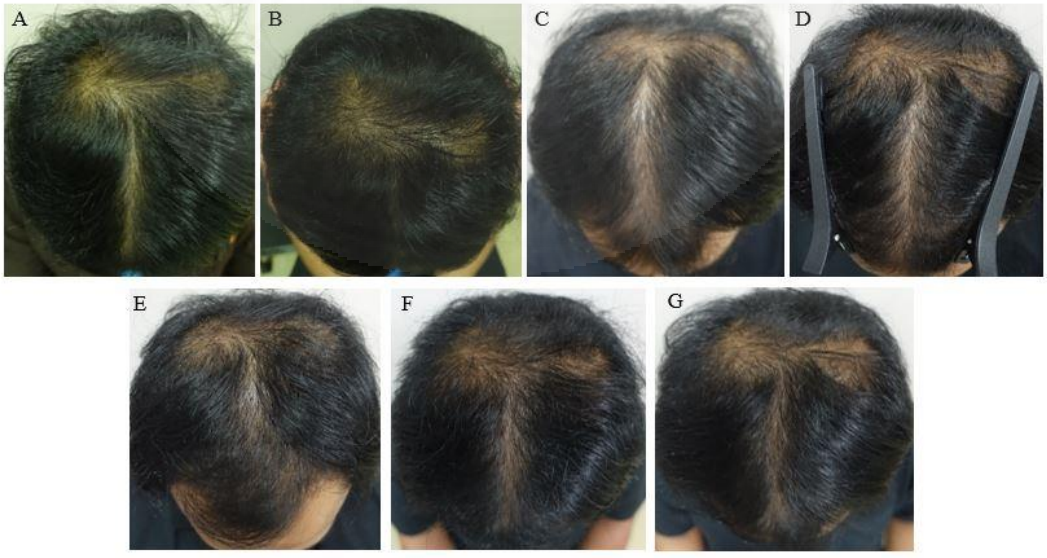

Figure C34 Global photograph of patient No.17 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

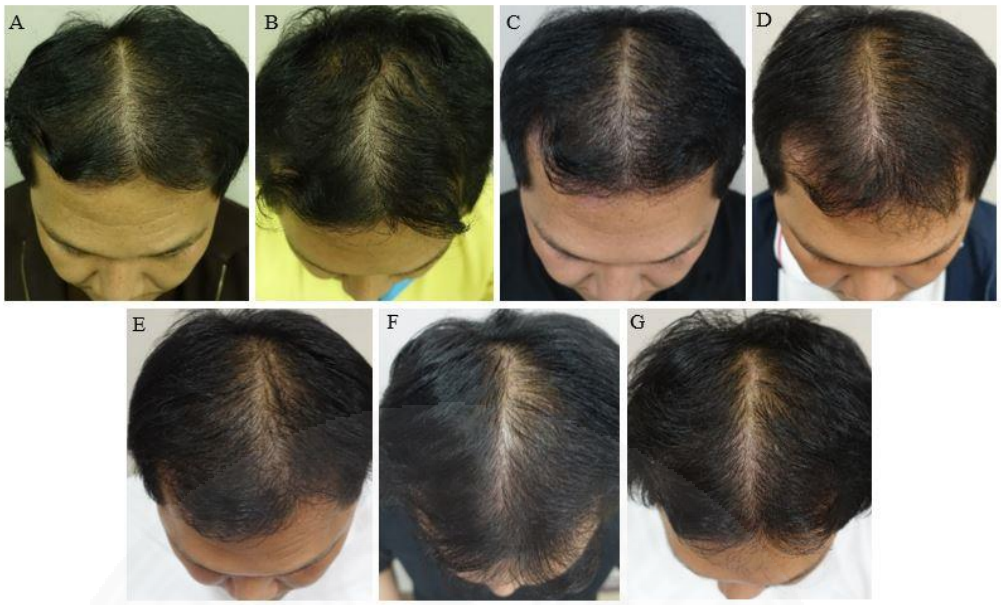

Figure C35 Global photograph of patient No.18 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

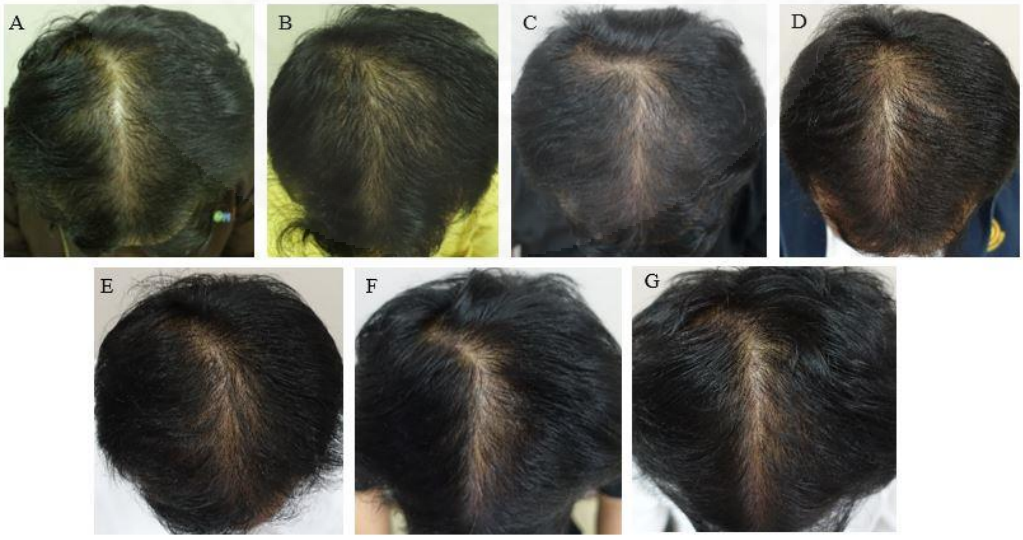

Figure C36 Global photograph of patient No.18 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

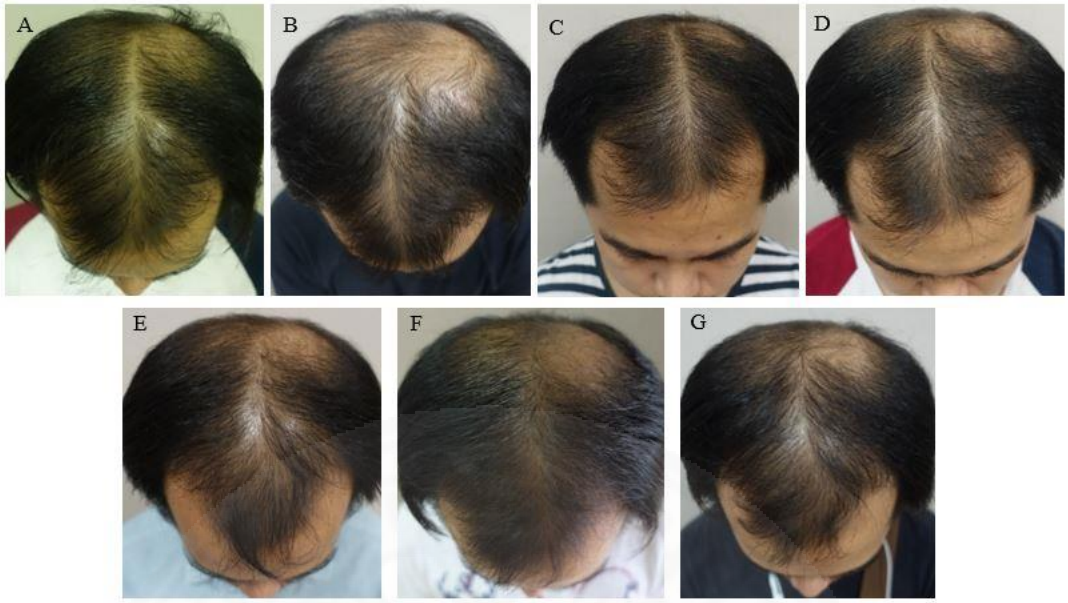

Figure C37 Global photograph of patient No.19 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

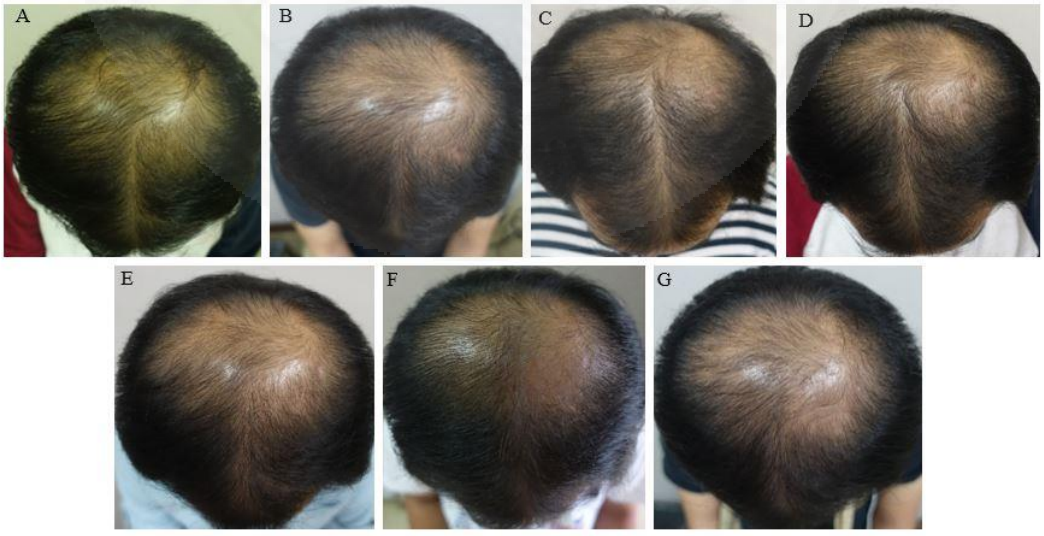

Figure C38 Global photograph of patient No.19 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

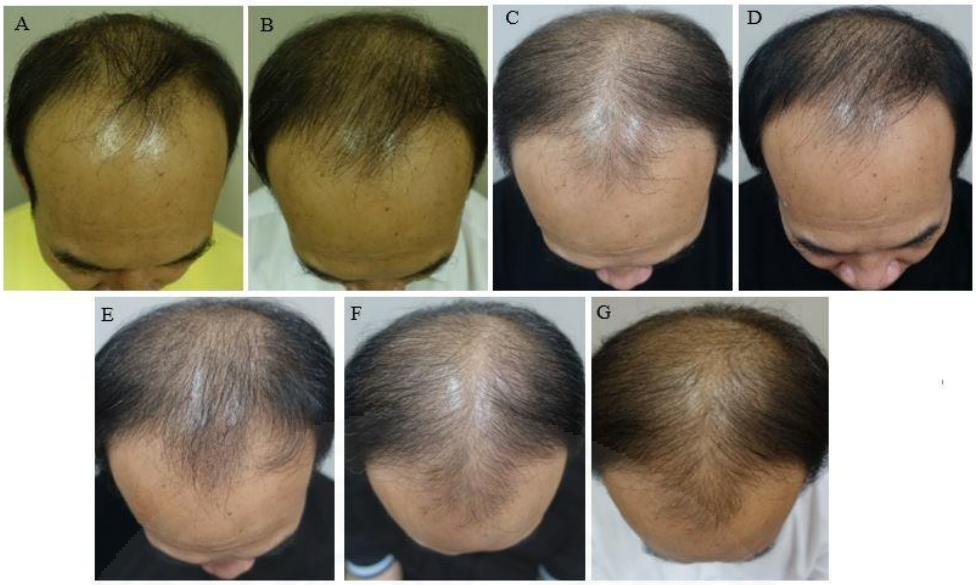

Figure C39 Global photograph of patient No.20 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

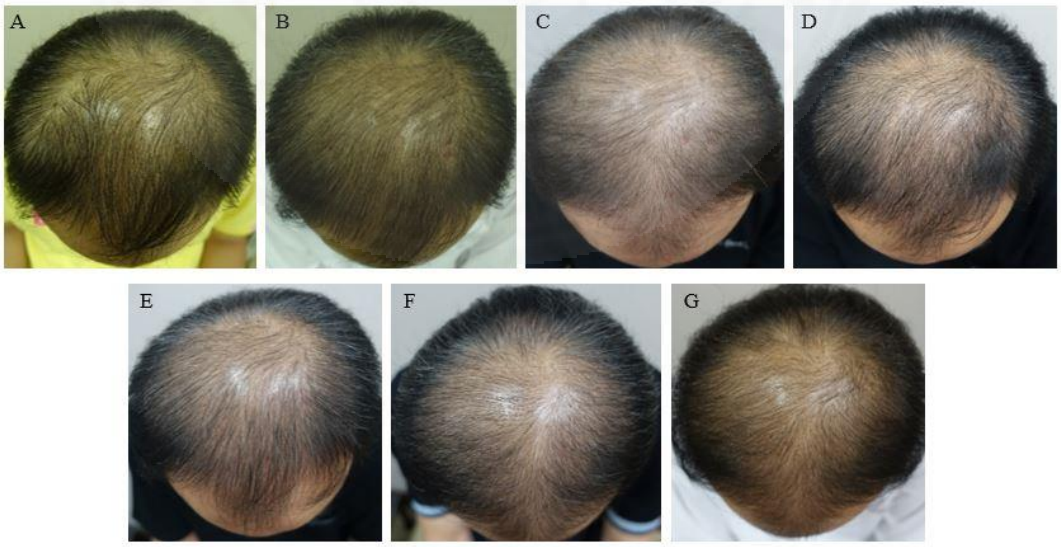

Figure C40 Global photograph of patient No.20 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

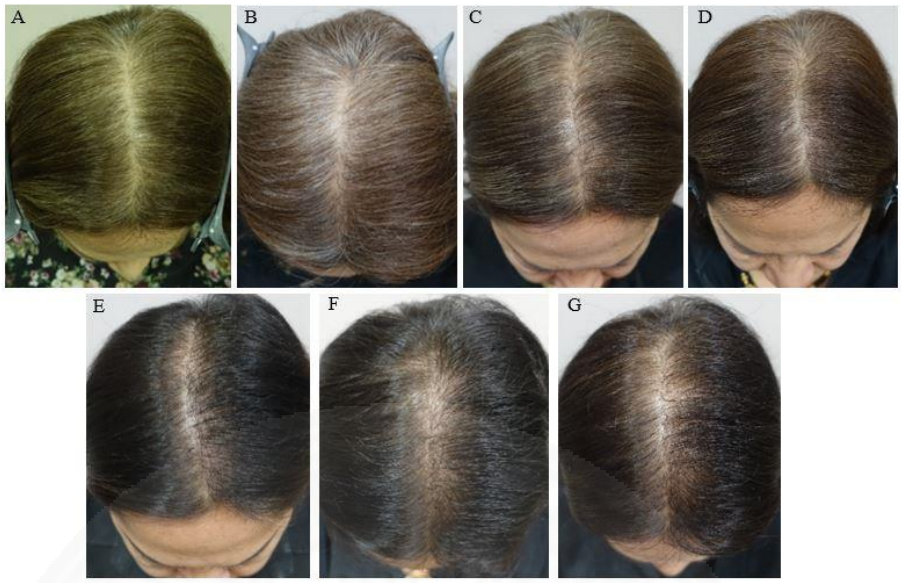

Figure C41 Global photograph of patient No.21 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

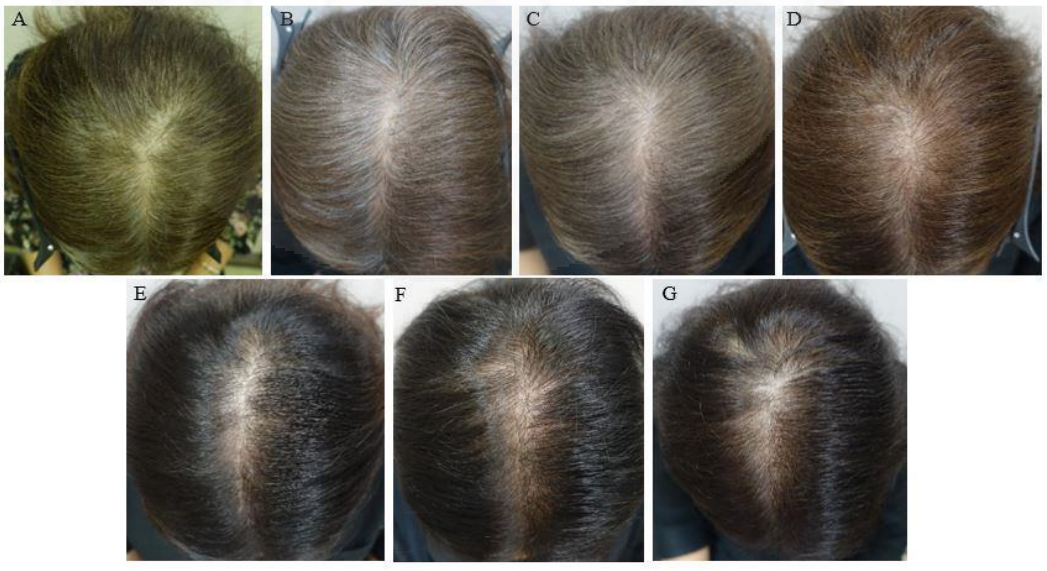

Figure C42 Global photograph of patient No.21 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

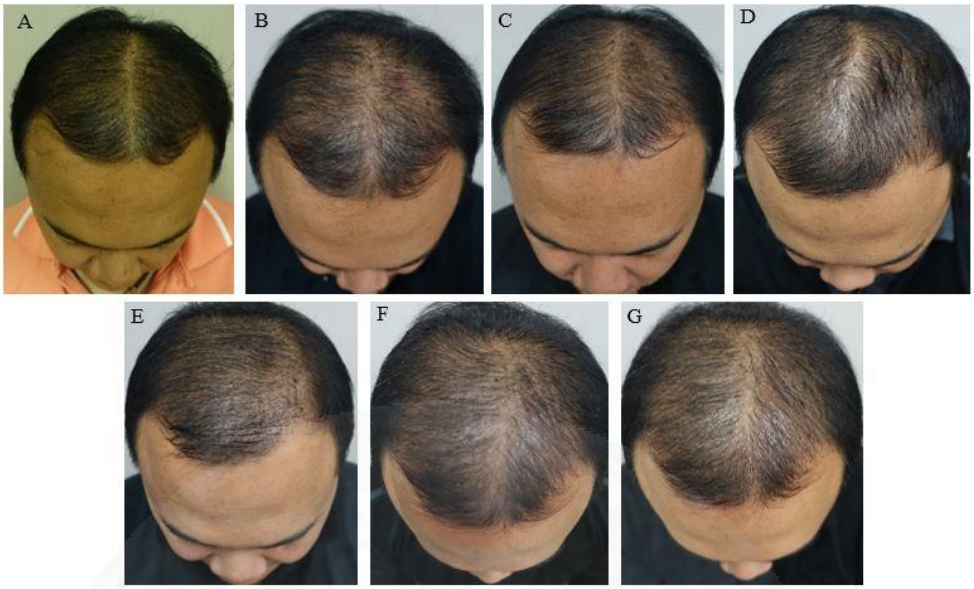

Figure C43 Global photograph of patient No.22 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

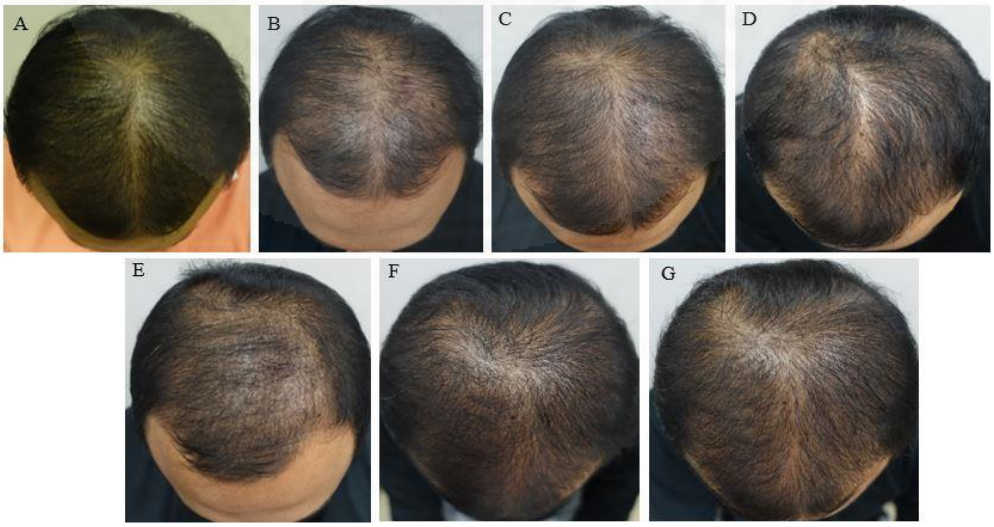

Figure C44 Global photograph of patient No.22 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

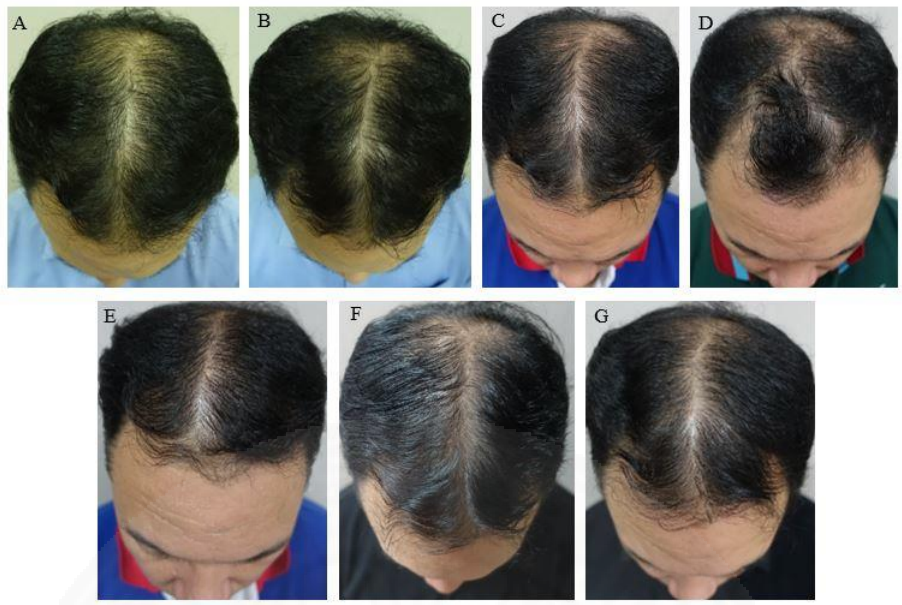

Figure C45 Global photograph of patient No.23 in 45º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

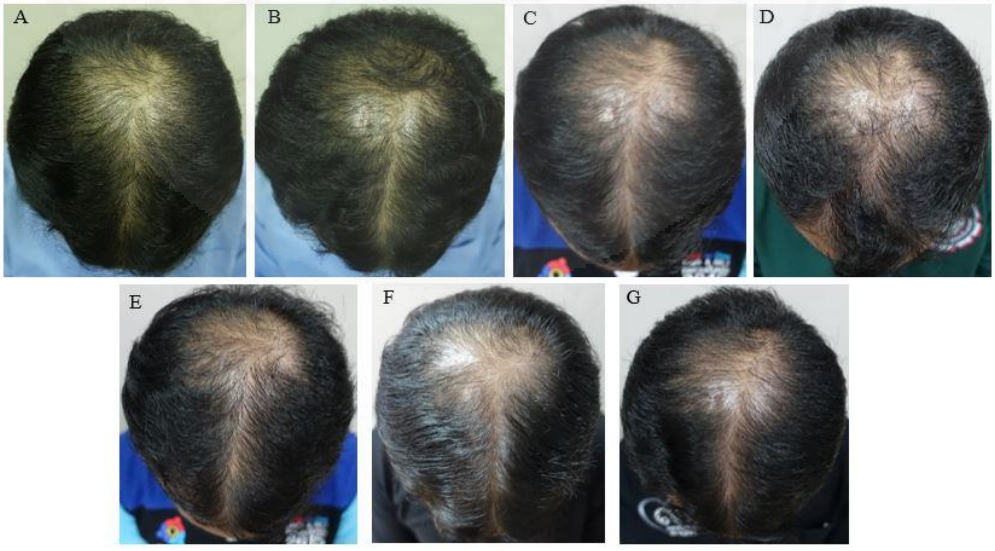

Figure C46 Global photograph of patient No.23 in 90º. Baseline (A), 1st month (B), 2nd month (C), 3rd month (D), 4th month (E), 5th month (F), 6th month (G).

APPENDIX D

CLINICAL IMPROVEMENT ASSESSMENT SCORE

Table D1 Dermatologist clinical improvement assessment score compare baseline to 3rd month and baseline to 6th month after treated with 1550 nm Er:Glass fractional laser.

Patient Number	Month 3		Month 6	
	Dermatologist No.1	Dermatologist No.2	Dermatologist No.1	Dermatologist No.2
No.1	1	-1	2	-1
No. 2	N/A	N/A	0	0
No.3	1	-1	2	0
No.4	2	1	2	2
No.5	-1	1	1	1
No.6	0	0	1	-1
No.7	2	1	3	1
No.8	1	1	2	1
No.9	0	0	0	-1
No.10	1	0	0	0
No.11	1	1	0	0
No.12	2	1	1	0
No.13	0	1	1	1
No.14	0	1	1	0
No.15	1	0	0	1
No.16	1	1	1	1
No.17	-1	-1	-1	0
No.18	0	0	3	1
No.19	0	1	0	1
No.20	1	-1	0	0
No.21	1	0	-1	1
No.22	1	0	1	-1
No.23	0	0	2	1

Table D2 Patients satisfied assessment score compared baseline to 6th month after treated with 1550 nm Er:Glass fractional laser

Patient Number	Score
No.1	3
No. 2	2
No.3	3
No.4	2
No.5	1
No.6	2
No.7	2
No.8	2
No.9	3
No.10	2
No.11	1
No.12	2
No.13	2
No.14	2
No.15	1
No.16	1
No.17	3
No.18	2
No.19	2
No.20	2
No.21	2
No.22	2
No.23	2

APPENDIX E
TARGET PHOTOGRAPH OF PATIENTS

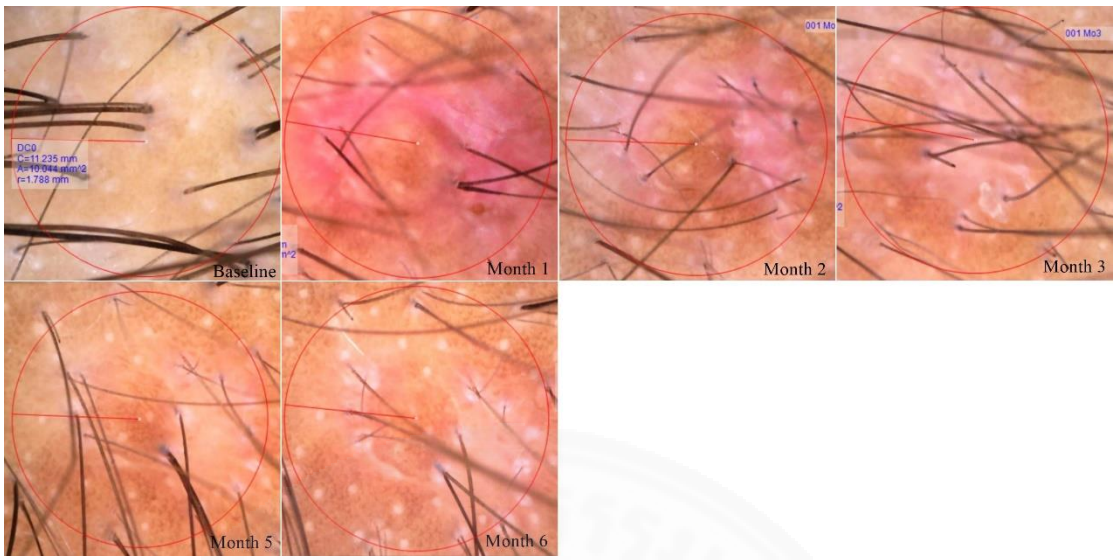

Figure E1 Target photograph for hair density assessment of patient No.1.

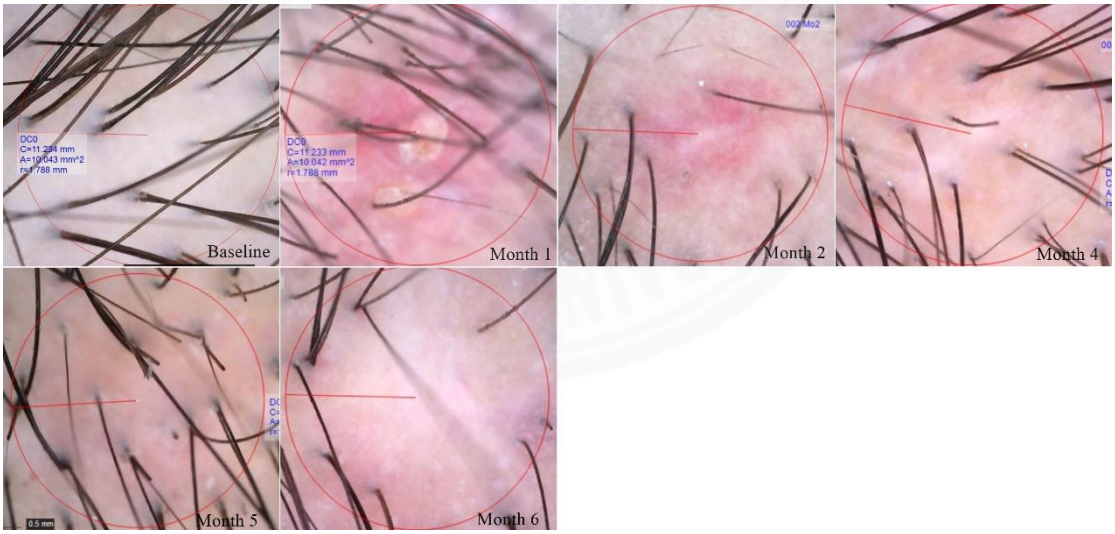

Figure E2 Target photograph for hair density assessment of patient No.2.

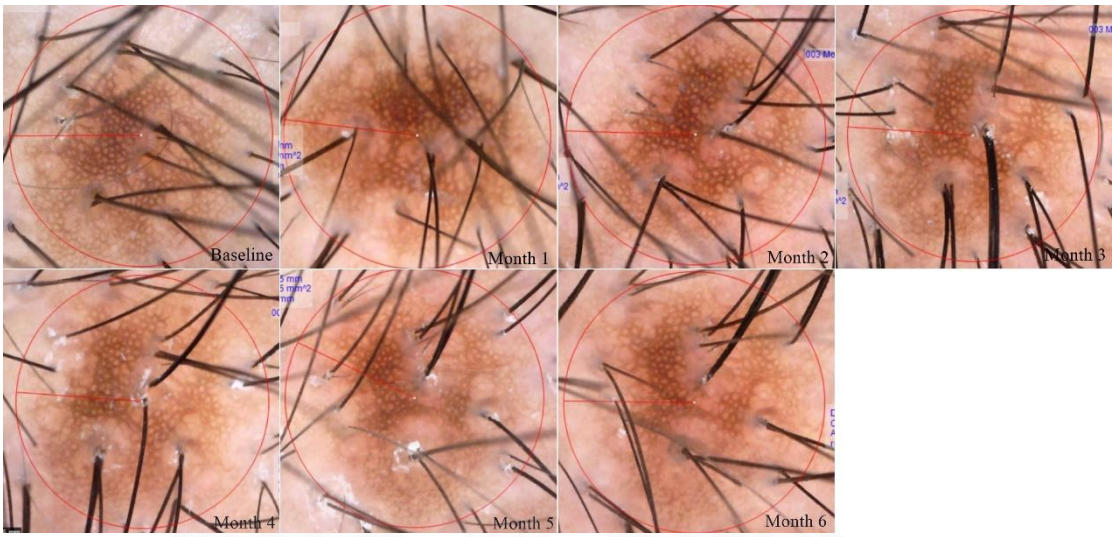

Figure E3 Target photograph for hair density assessment of patient No.3.

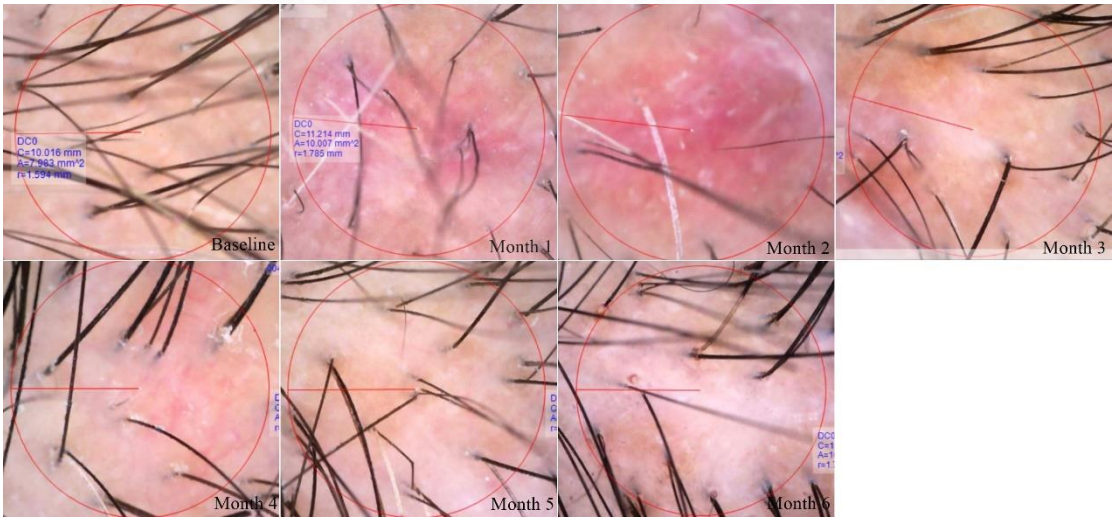

Figure E4 Target photograph for hair density assessment of patient No.4.

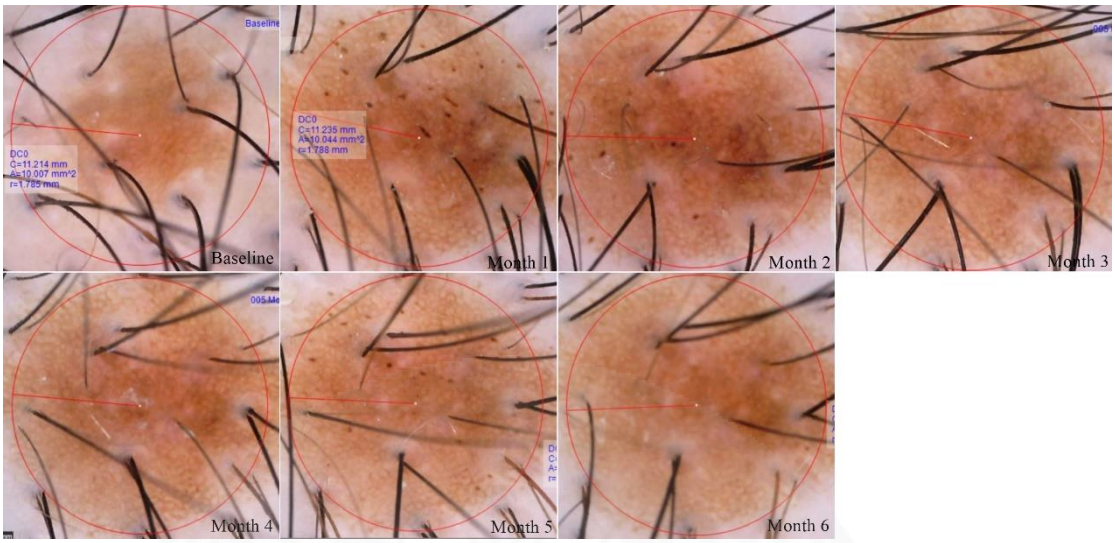

Figure E5 Target photograph for hair density assessment of patient No.5.

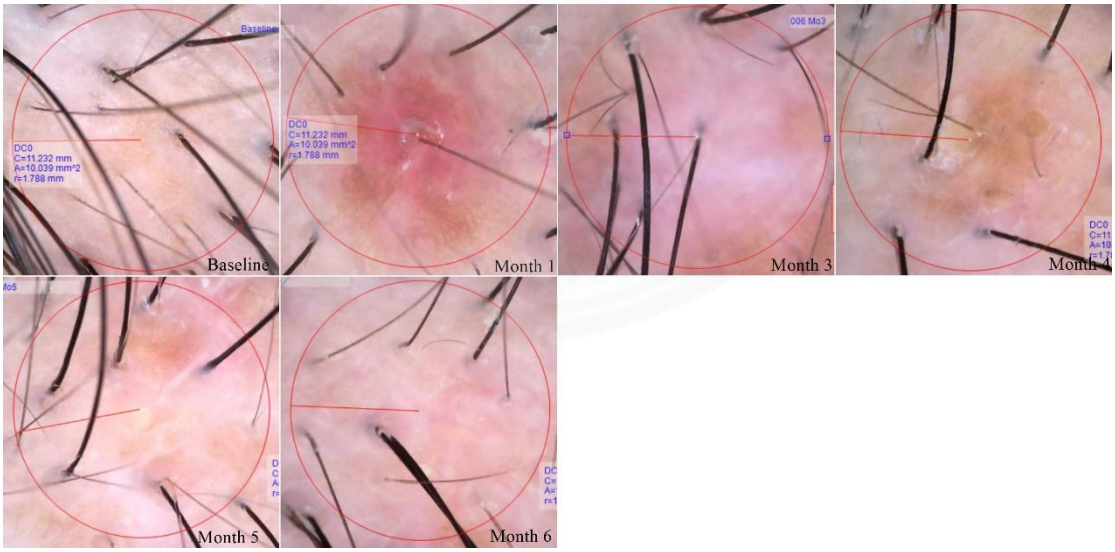

Figure E6 Target photograph for hair density assessment of patient No.6.

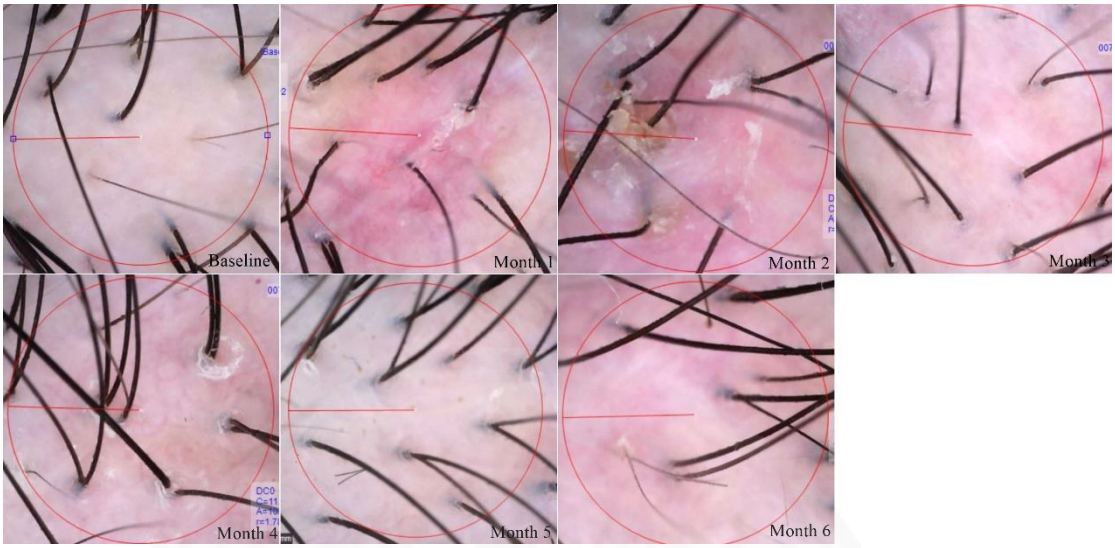

Figure E7 Target photograph for hair density assessment of patient No.7.

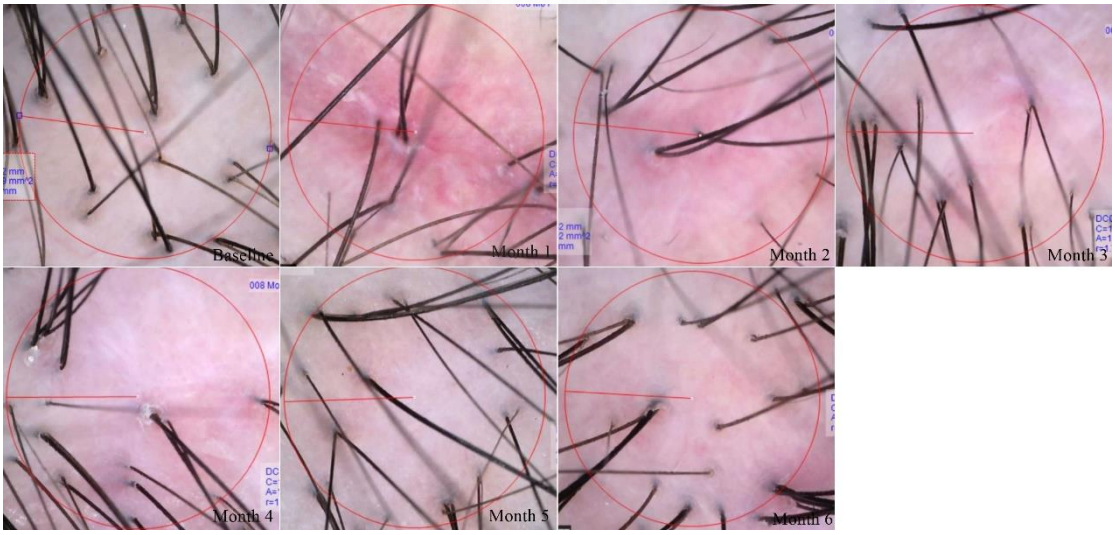

Figure E8 Target photograph for hair density assessment of patient No.8.

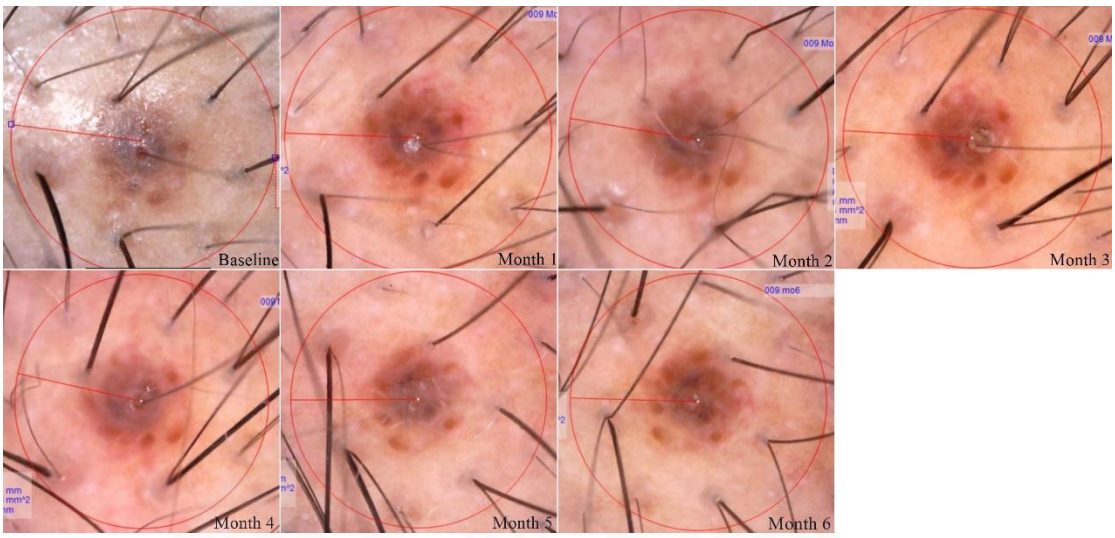

Figure E9 Target photograph for hair density assessment of patient No.9.

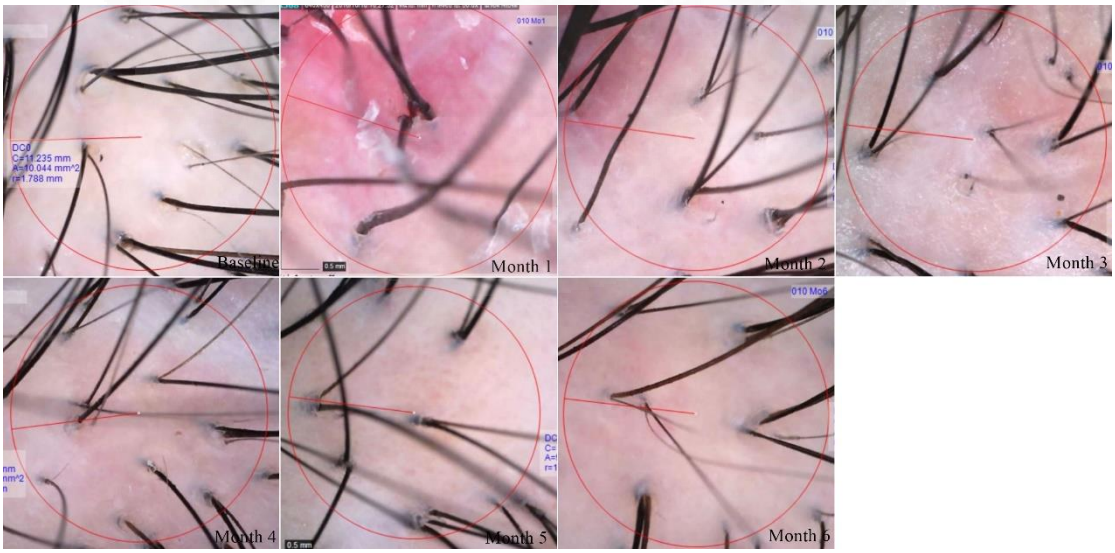

Figure E10 Target photograph for hair density assessment of patient No.10.

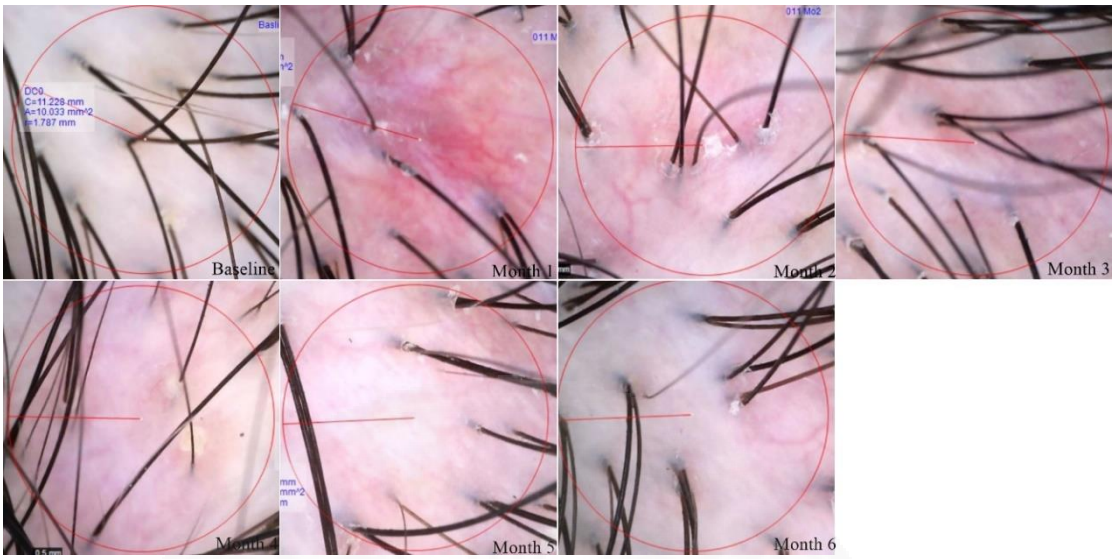

Figure E11 Target photograph for hair density assessment of patient No.11.

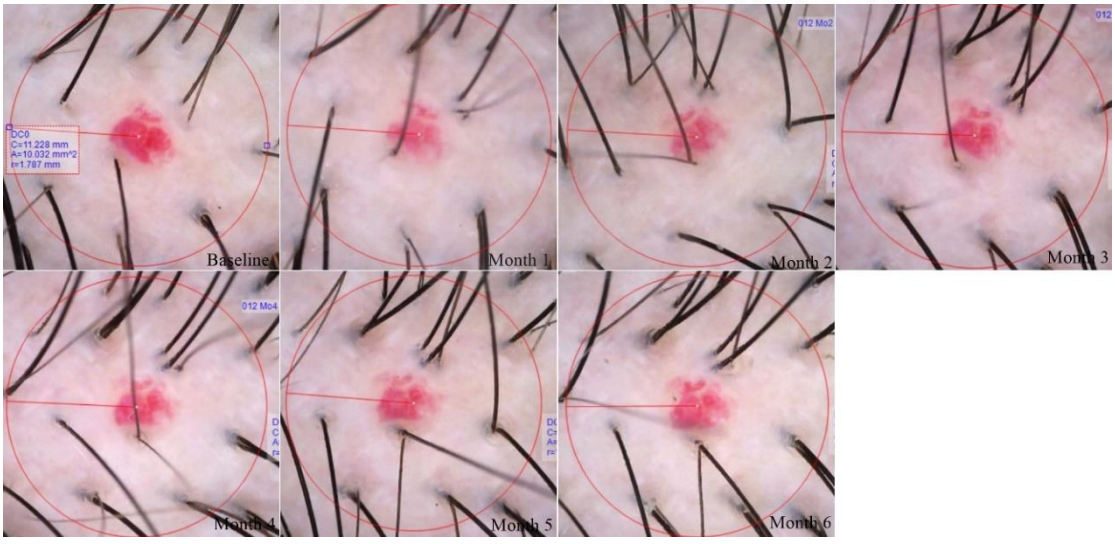

Figure E12 Target photograph for hair density assessment of patient No.12.

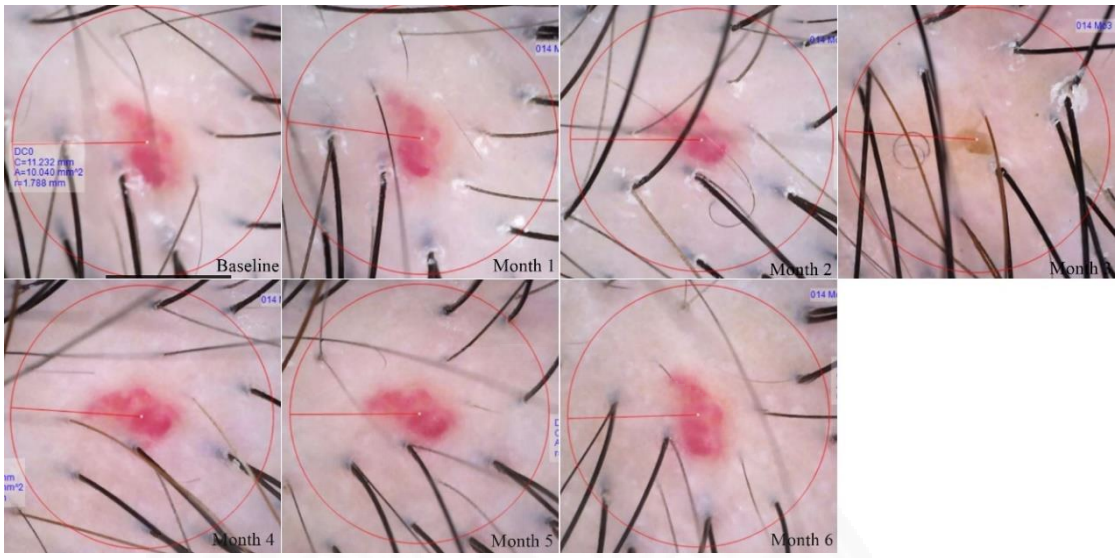

Figure E13 Target photograph for hair density assessment of patient No.13.

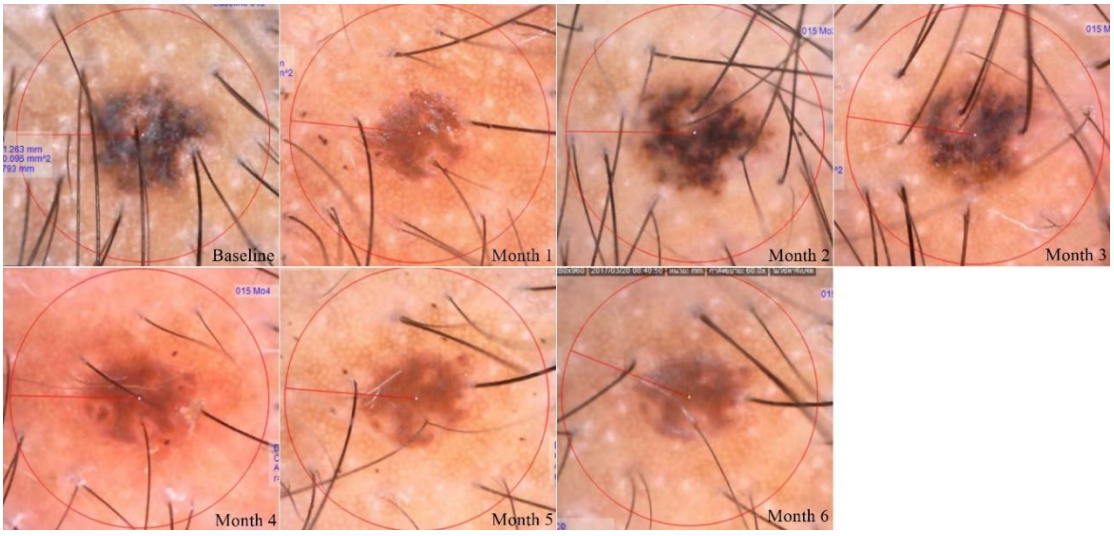

Figure E14 Target photograph for hair density assessment of patient No.14.

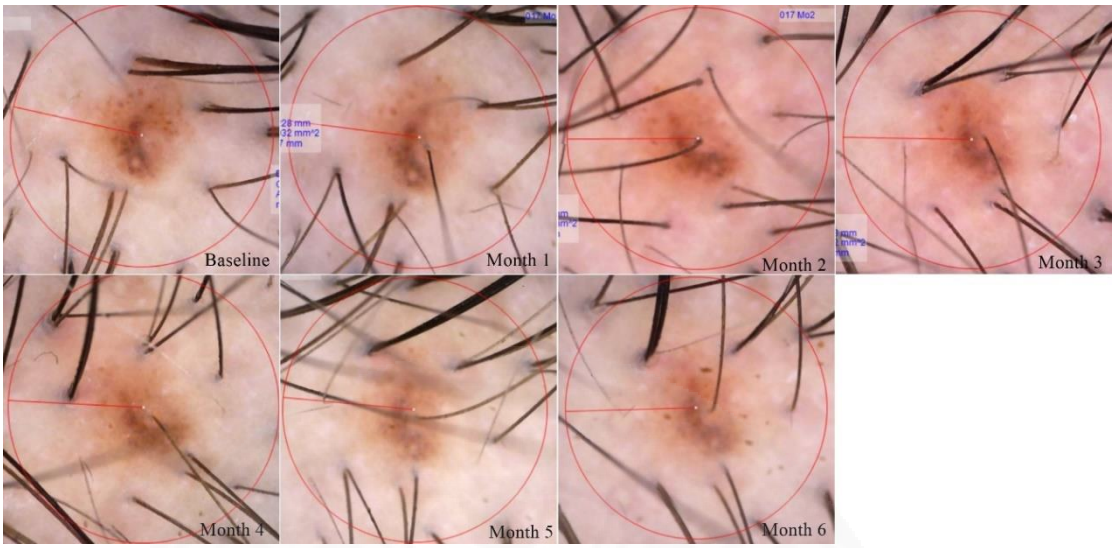

Figure E15 Target photograph for hair density assessment of patient No.15.

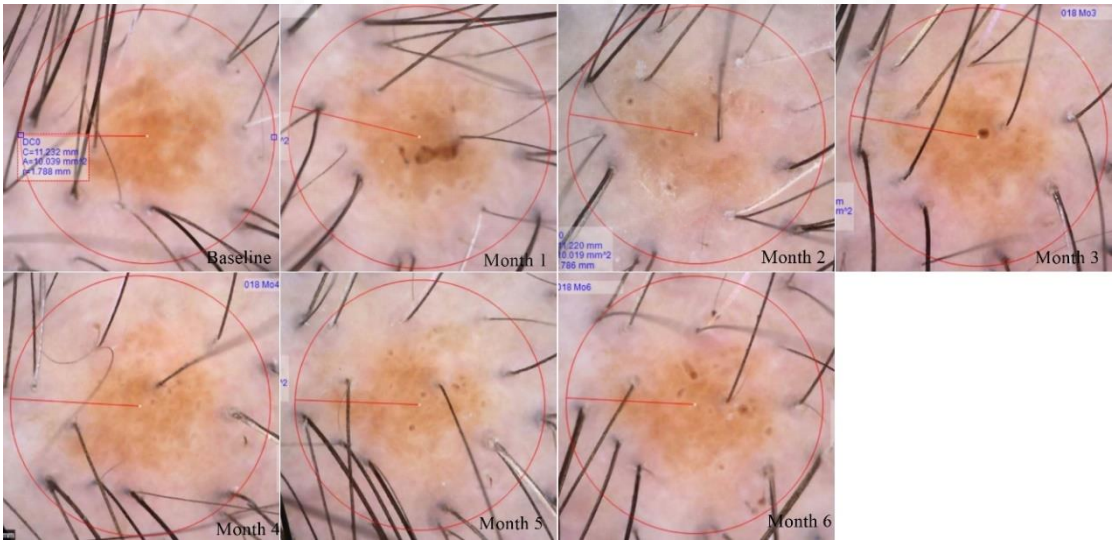

Figure E16 Target photograph for hair density assessment of patient No.16.

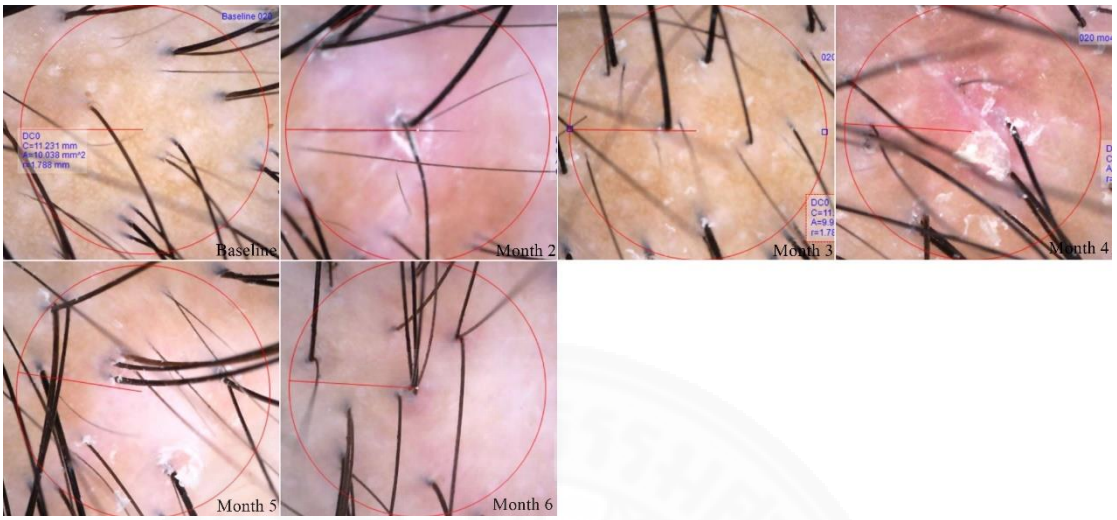

Figure E17 Target photograph for hair density assessment of patient No.17.

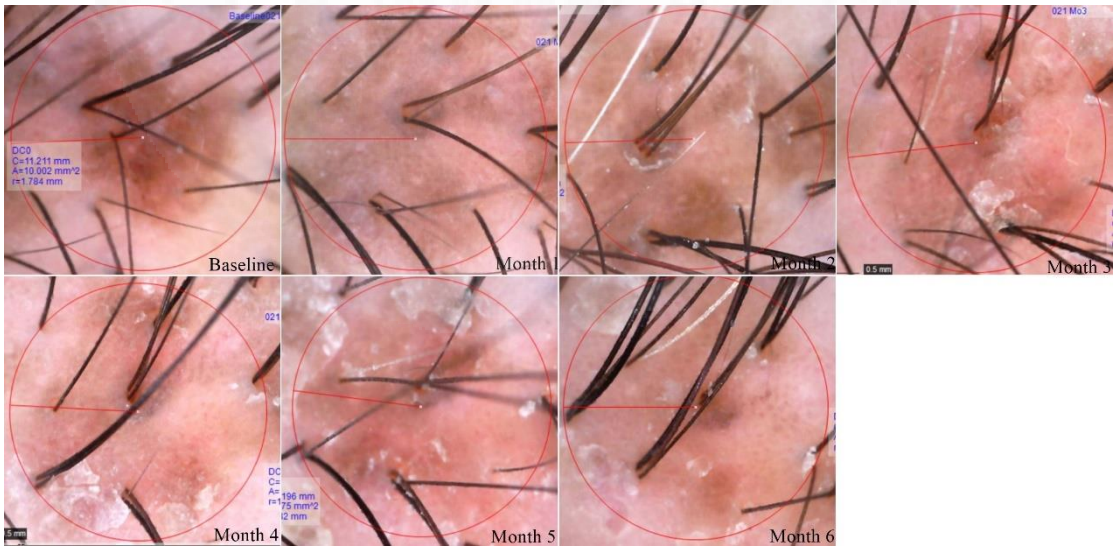

Figure E18 Target photograph for hair density assessment of patient No.18.

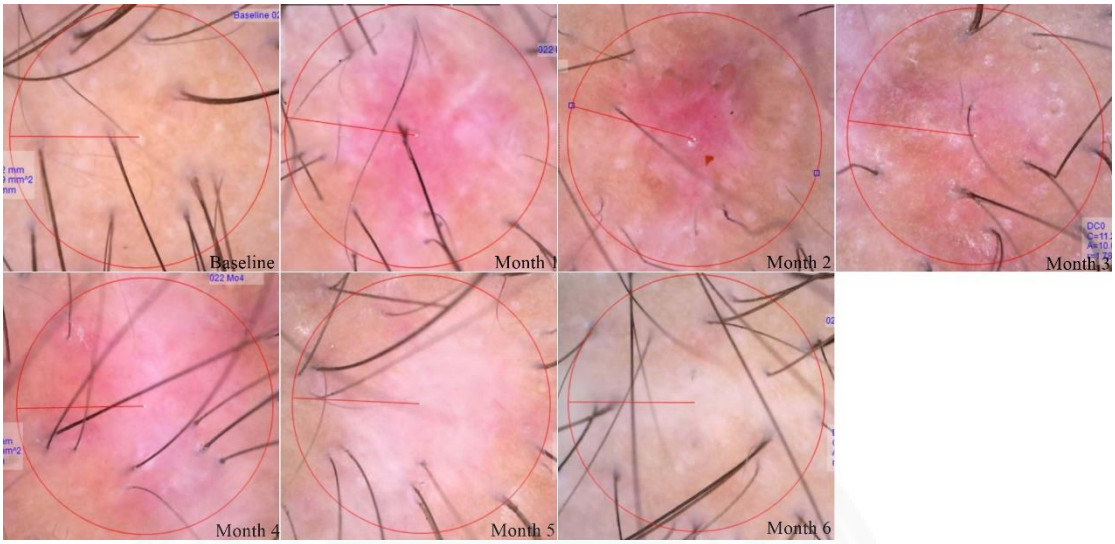

Figure E19 Target photograph for hair density assessment of patient No.19.

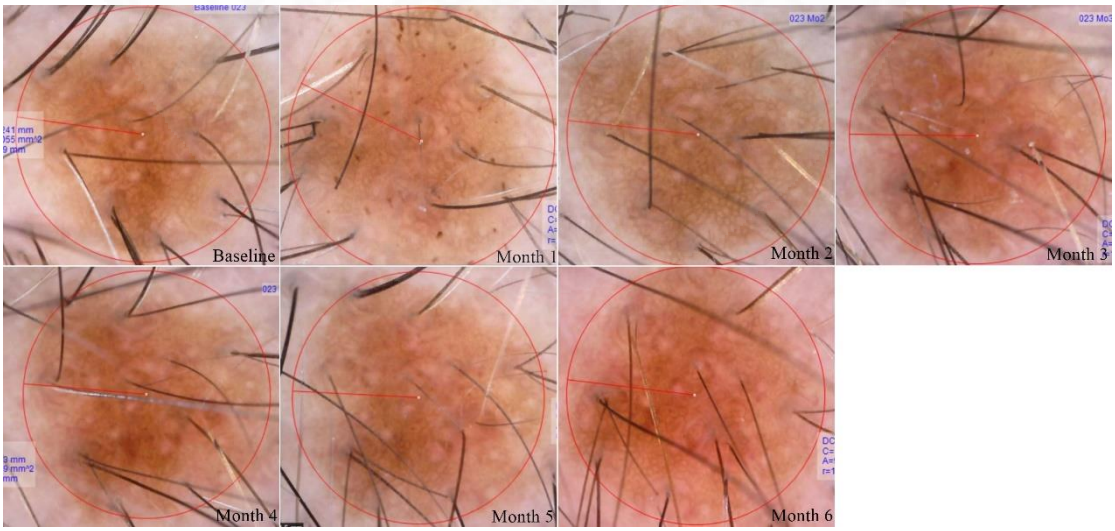

Figure E20 Target photograph for hair density assessment of patient No.20.

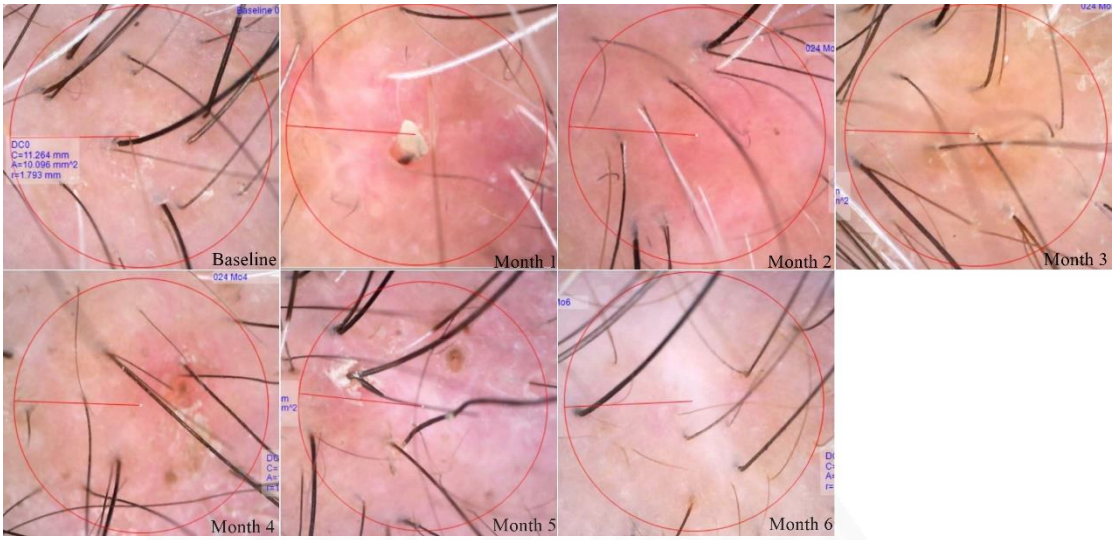

Figure E21 Target photograph for hair density assessment of patient No.21.

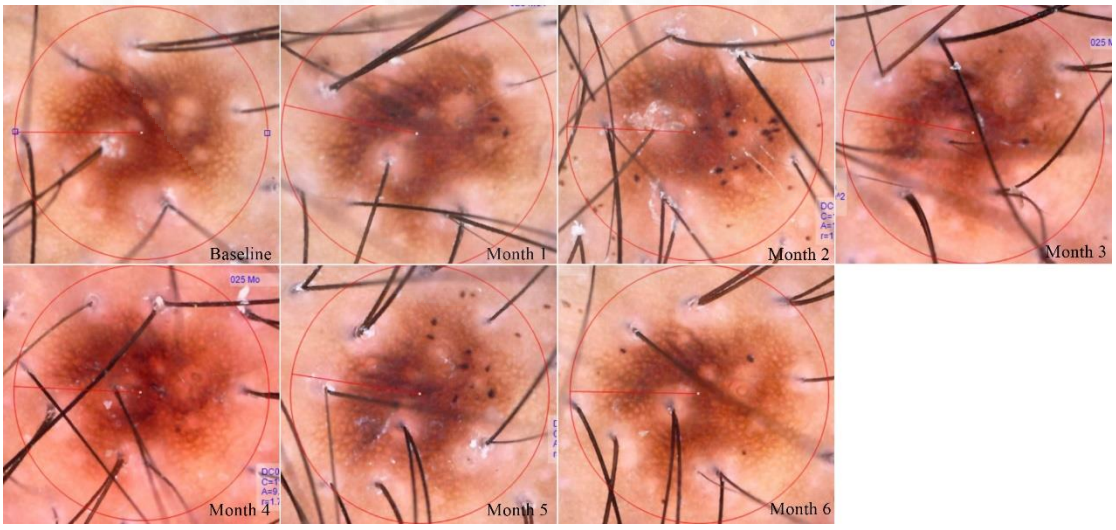

Figure E22 Target photograph for hair density assessment of patient No.22.

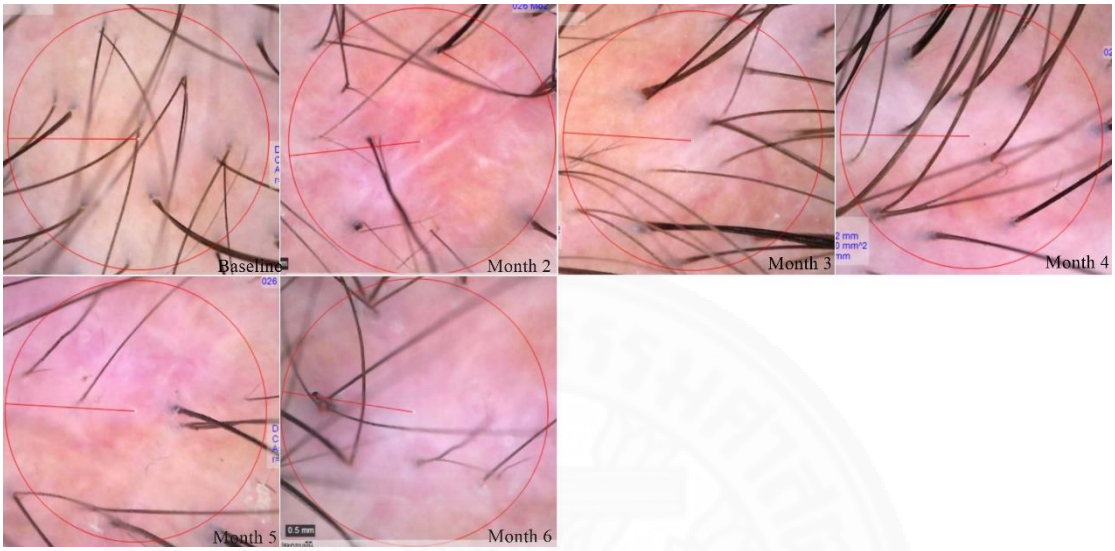

Figure E23 Target photograph for hair density assessment of patient No.23.

APPENDIX F

HAIR DENSITY AND HAIR SHAFT DIAMETER

Table F1 Hair density (per cm^2) and hair shaft diameter (μm)

Patient No.	Hair count /1 cm^2					Proportion of Terminal hair : Intermidiate + vellus	Hair shaft diameter (μm)
No.1	Terminal	Intermidiate	Vellus	total	Terminal + Intermediate hair		
Baseline	100	50	10	160	150	1.43	46
Mo1	40	50	20	110	90	0.57	44
Mo2	50	130	30	210	180	0.31	32
Mo3	80	70		150	150	1.14	43
Mo4	90	110		200	200	0.82	40
Mo5	100	80	10	190	180	1.11	52
Mo6	80	110		190	190	0.73	63
No.2	Terminal	Intermidiate	Vellus	total			
Baseline	110	60		170	170	1.83	46
Mo1	100	30		130	130	3.33	35
Mo2	70	50	10	130	120	1.16	32
Mo3							
Mo4	140	100		240	240	1.4	51
Mo5	90	120		210	210	0.75	59
Mo6	80	50		130	130	1.6	60
No.3	Terminal	Intermidiate	Vellus	total			
Baseline	80	60		140	140	1.33	32
Mo1	60	80		140	140	0.75	43
Mo2	100	110	10	220	210	0.91	44
Mo3	170	70		240	240	2.43	49
Mo4	160	40		200	200	4	55
Mo5	130	90		220	220	1.44	47
Mo6	80	140		220	220	0.57	56
No.4	Terminal	Intermidiate	Vellus	total			
Baseline	60	120		180	180	0.5	37
Mo1	60	100		160	160	0.6	41

Mo2	40	50		90	90	0.8	38
Mo3	60	110	20	190	170	0.46	36
Mo4	100	50	10	160	150	1.66	54
Mo5	150	100		250	250	1.5	43
Mo6	100	110		210	210	0.91	70

No.5	Terminal	Intermidiate	Vellus	total			
Baseline	80	60		140	140	1.33	32
Mo1	80	70	10	160	150	1	32
Mo2	70	60	40	170	130	0.7	52
Mo3	80	100		180	180	0.8	41
Mo4	110	80		190	190	1.38	46
Mo5	110	80		190	190	1.375	56
Mo6	40	120		160	160	0.33	58

No.6	Terminal	Intermidiate	Vellus	total			
Baseline	70	70	10	150	140	0.875	44
Mo1	50	40		90	90	1.25	51
Mo2							
Mo3	60	70	10	140	130	0.75	71
Mo4	60	120		180	180	0.5	56
Mo5	60	120		180	180	0.5	64
Mo6	70	80		150	150	0.875	77

No.7	Terminal	Intermidiate	Vellus	total			
Baseline	90	40	10	140	130	1.8	49
Mo1	90	30	10	130	120	2.25	44
Mo2	60	30		90	90	2	50
Mo3	70	50		120	120	1.4	56
Mo4	120	50		170	170	2.4	69
Mo5	100	70	10	180	170	1.25	67
Mo6	50	80		130	130	0.625	83

No.8	Terminal	Intermidiate	Vellus	total			
Baseline	130	60		190	190	2.17	39
Mo1	70	40		110	110	1.75	52
Mo2	70	80		150	150	0.875	55
Mo3	80	100		180	180	0.8	44
Mo4	100	130		230	130	0.77	50
Mo5	120	90		210	210	1.33	53
Mo6	70	140		210	210	0.5	61

No.9	Terminal	Intermidiate	Vellus	total			
Baseline	40	80	20	140	120	0.4	32

Mo1	50	110		160	160	0.45	31
Mo2	50	60	20	130	110	0.625	35
Mo3	50	110		160	160	0.45	37
Mo4	70	80		150	150	0.875	35
Mo5	50	60	30	140	110	0.56	53
Mo6	40	90		130	130	0.44	58

No.10	Terminal	Intermidiate	Vellus	total			
Baseline	90	90	10	190	180	0.9	57
Mo1	60			60	60		16
Mo2	70	30	10	110	100	1.75	60
Mo3	70	90		160	160	0.78	45
Mo4	120	100		220	220	1.2	61
Mo5	90	30		120	120	3	62
Mo6	90	80		170	170	1.13	96

No.11	Terminal	Intermidiate	Vellus	total			
Baseline	70	130		200	200	0.54	57
Mo1	70	30	20	120	100	1.4	47
Mo2	100	20		120	120	5	54
Mo3	100	30		130	130	3.33	56
Mo4	40	70		110	110	0.57	47
Mo5	100	50		150	150	2	59
Mo6	120	50		170	170	2.4	59

No.12	Terminal	Intermidiate	Vellus	total			
Baseline	100	70		170	170	1.43	36
Mo1	100	70	10	180	170	1.25	61
Mo2	110	60		170	170	1.83	41
Mo3	130	50		180	180	2.6	54
Mo4	140	60		200	200	2.33	57
Mo5	130	50		180	180	2.6	60
Mo6	140	80		220	220	1.75	64

No.13	Terminal	Intermidiate	Vellus	total			
Baseline	70	60	10	140	130	1	66
Mo1	70	70	20	160	140	0.78	51
Mo2	70	60	20	150	130	0.88	70
Mo3	120	60	10	190	180	1.5	57
Mo4	70	70		140	140	1	65
Mo5	70	100		170	170	0.7	48
Mo6	70	100		170	170	0.7	79

No.14	Terminal	Intermidiate	Vellus	total			
Baseline	80	60		140	140	1.33	36
Mo1	50	70	20	140	120	0.56	37
Mo2	100	100	10	210	200	0.91	32
Mo3	100	90		190	190	1.11	37
Mo4	110	40		150	150	2.75	37
Mo5	100	100		200	200	1	53
Mo6	60	60		120	120	1	47

No.15	Terminal	Intermidiate	Vellus	total			
Baseline	70	100		170	170	0.7	44
Mo1	80	80		160	160	1	55
Mo2	80	50	10	140	130	1.33	52
Mo3	80	60	10	150	140	1.14	61
Mo4	100	60		160	160	1.67	45
Mo5	150	60		210	210	2.5	61
Mo6	40	120		160	160	0.33	61

No.16	Terminal	Intermidiate	Vellus	total			
Baseline	40	130	30	200	170	0.25	34
Mo1							
Mo2	120	100	20	240	220	1	40
Mo3	90	70		160	160	1.29	44
Mo4	110	60		170	170	1.83	45
Mo5	110	90		200	200	1.22	48
Mo6	80	140		220	220	0.57	40

No.17	Terminal	Intermidiate	Vellus	total			
Baseline	70	70	30	170	140	0.7	47
Mo1							
Mo2	60	50		110	110	1.2	45
Mo3	60	100		160	160	0.6	58
Mo4	60	40		100	100	1.5	69
Mo5	120	70		190	190	1.71	64
Mo6	130	90		220	220	1.44	67

No.18	Terminal	Intermidiate	Vellus	total			
Baseline	70	50		120	120	1.4	54
Mo1							
Mo2	80	60		140	140	1.33	63
Mo3	100	40		140	140	2.5	67
Mo4	90	10		100	100	9	51
Mo5	100	50		150	150	2	67
Mo6	90	50		140	140	1.8	67

No.19	Terminal	Intermidiate	Vellus	total			
Baseline	20	150	40	210	170	0.11	42
Mo1	60	160	20	240	220	0.33	45
Mo2	50	30		80	80	1.67	29
Mo3	60	90	10	160	150	0.6	34
Mo4	60	40		100	100	1.5	31
Mo5	90	60		150	150	1.5	32
Mo6	50	120		170	170	0.42	58

No.20	Terminal	Intermidiate	Vellus	total			
Baseline	50	160	20	230	210	0.28	26
Mo1	60	160	20	240	220	0.33	45
Mo2	90	90	10	190	180	0.9	34
Mo3	110	80		190	190	1.38	31
Mo4	80	80		160	160	1	37
Mo5	120	100		220	220	1.2	48
Mo6	80	130		210	210	0.62	58

No.21	Terminal	Intermidiate	Vellus	total			
Baseline	60	130	10	200	190	0.43	33
Mo1	50	30	50	130	80	0.63	41
Mo2	30	140	30	200	170	0.18	60
Mo3	70	120	30	220	190	0.47	43
Mo4	70	60		130	130	1.17	52
Mo5	90	90	30	210	180	0.75	42
Mo6	60	160		220	220	0.38	70

No.22	Terminal	Intermidiate	Vellus	total			
Baseline	20	90		110	110	0.22	48
Mo1	30	70	20	120	100	0.33	45
Mo2	90	50		140	140	1.8	43
Mo3	100	50		150	150	2	41
Mo4	70	50		120	120	1.4	49
Mo5	110	20		130	130	5.5	51
Mo6	60	80		140	140	0.75	56

No.23	Terminal	Intermidiate	Vellus	total			
Baseline	50	150		200	200	0.33	41
Mo1							
Mo2	50	110	20	180	160	0.38	60
Mo3	70	100	10	180	170	0.64	49
Mo4	90	60	10	160	150	1.29	65
Mo5	50	60	40	150	110	0.5	62
Mo6	40	130		170	170	0.31	67

APPENDIX G
HISTOPATHOLOGY

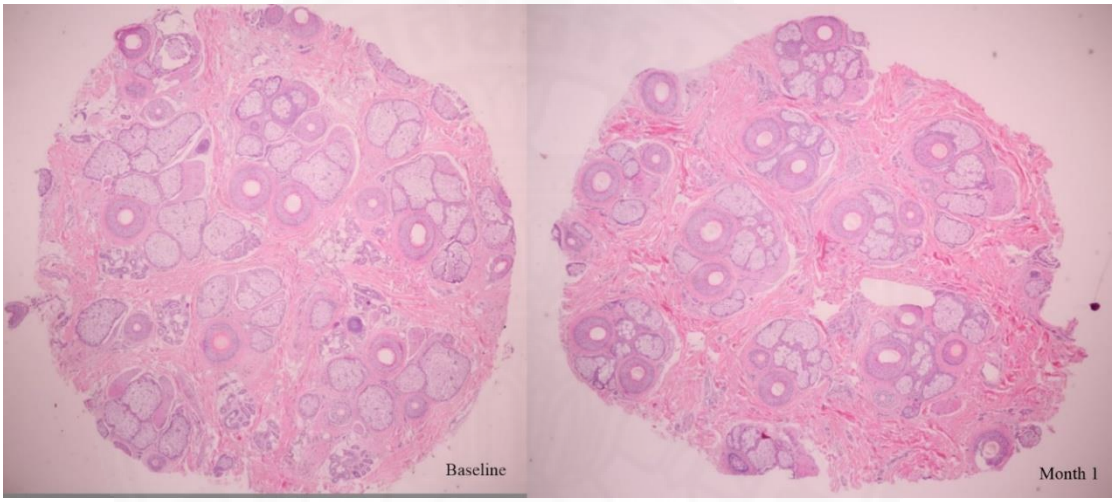

Figure G1 Histologic finding of patient No.7 compare baseline to 1st month after treatment.

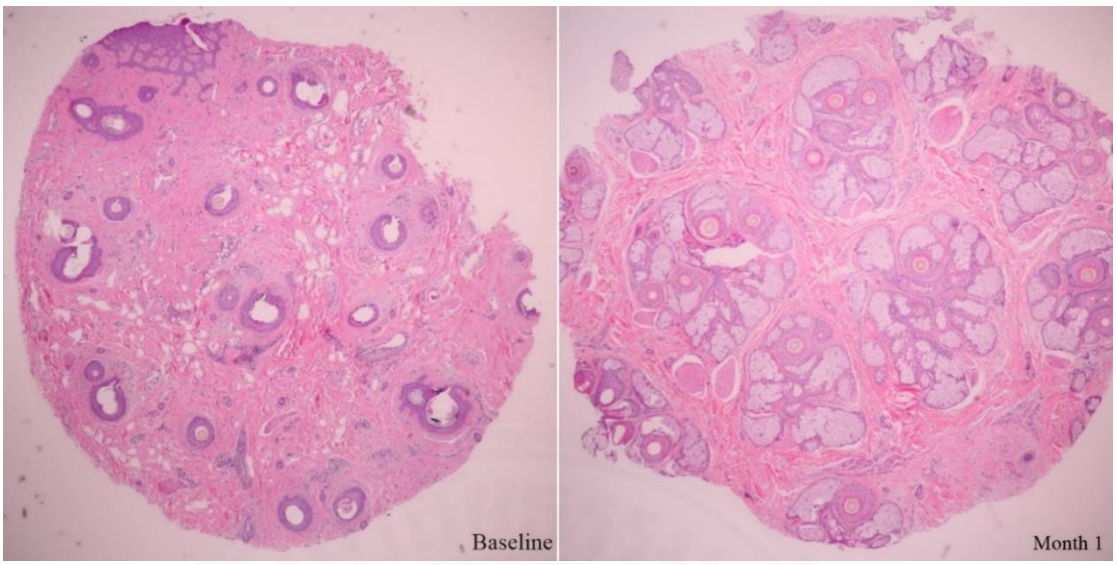

Figure G2 Histologic finding of patient No.12 compare baseline to 1st month after treatment.

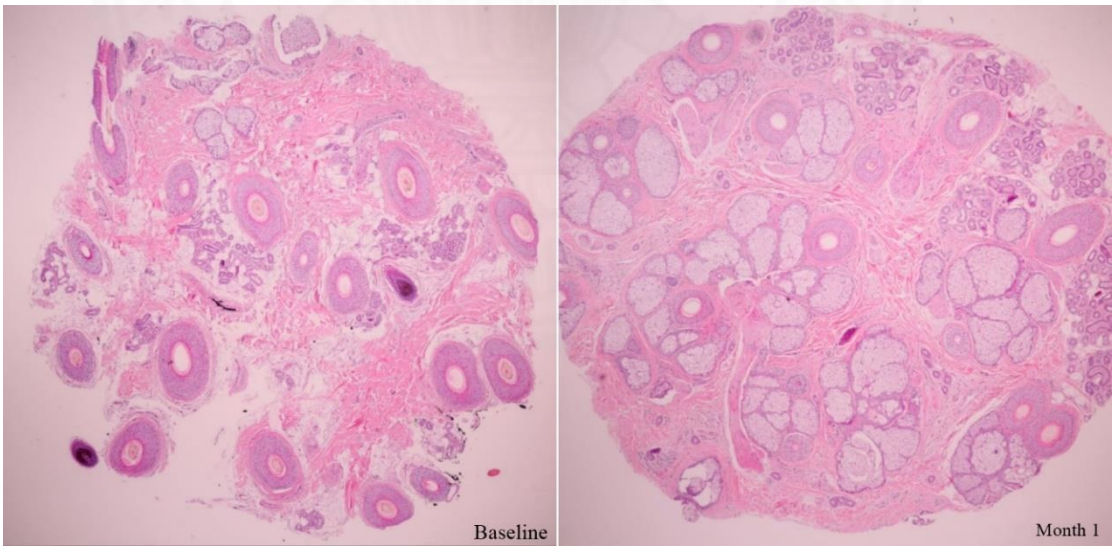

Figure G3 Histologic finding of patient No.14 compare baseline to 1st month after treatment.

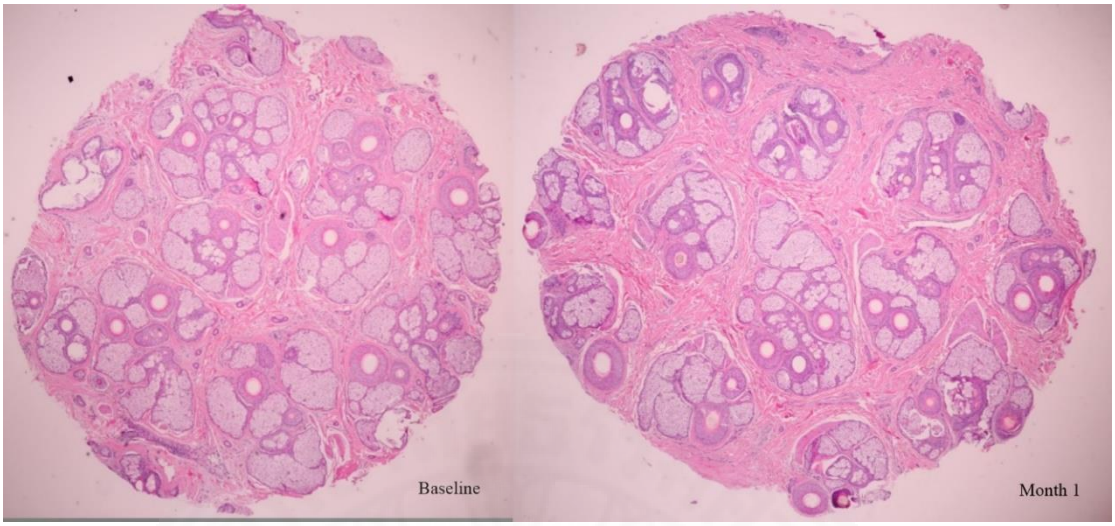

Figure G4 Histologic finding of patient No.15 compare baseline to 1st month after treatment.

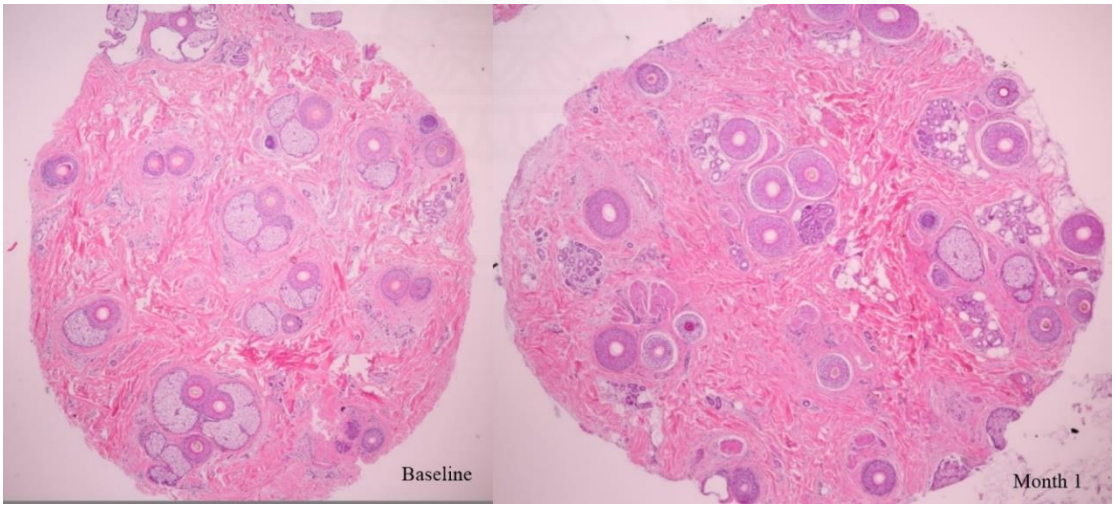

Figure G5 Histologic finding of patient No.22 compare baseline to 1st month after treatment.

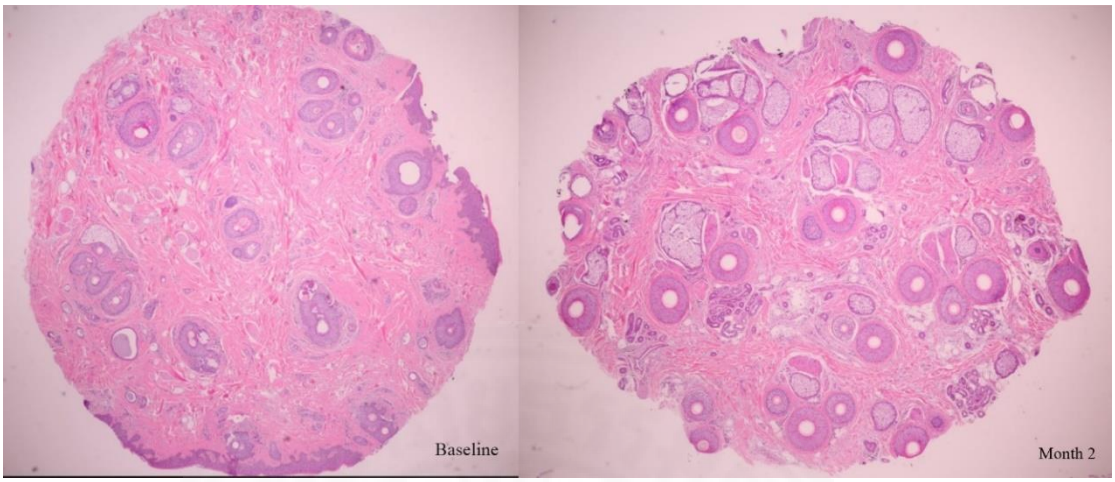

Figure G6 Histologic finding of patient No.1 compare baseline to 2nd month after treatment.

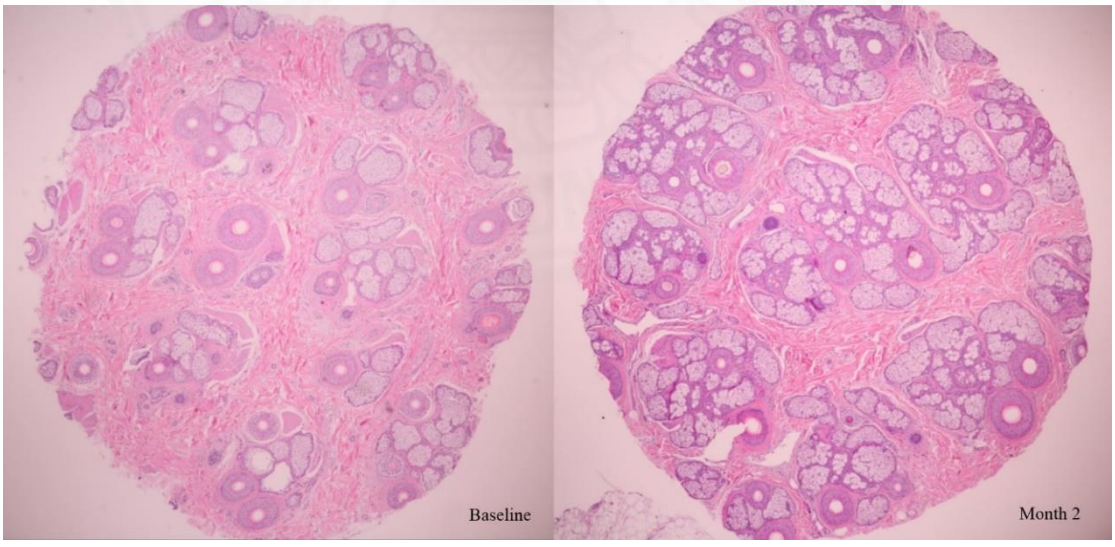

Figure G7 Histologic finding of patient No.5 compare baseline to 2nd month after treatment.

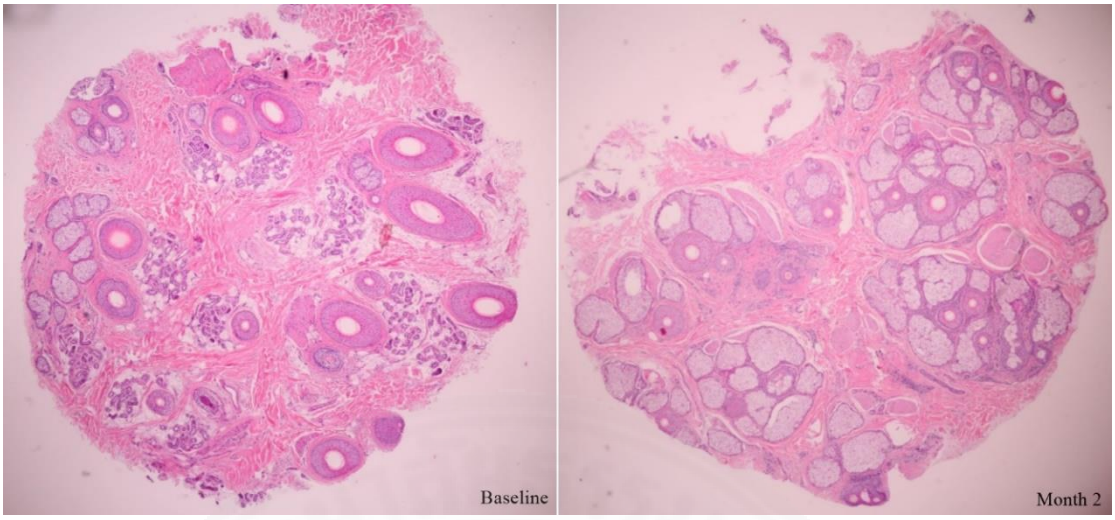

Figure G8 Histologic finding of patient No.9 compare baseline to 2nd month after treatment.

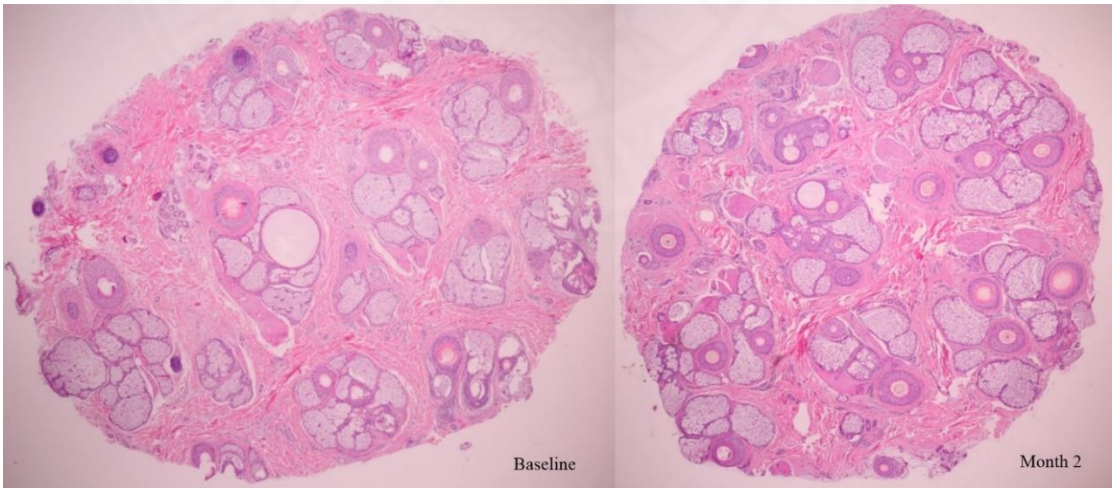

Figure G9 Histologic finding of patient No.13 compare baseline to 2nd month after treatment.

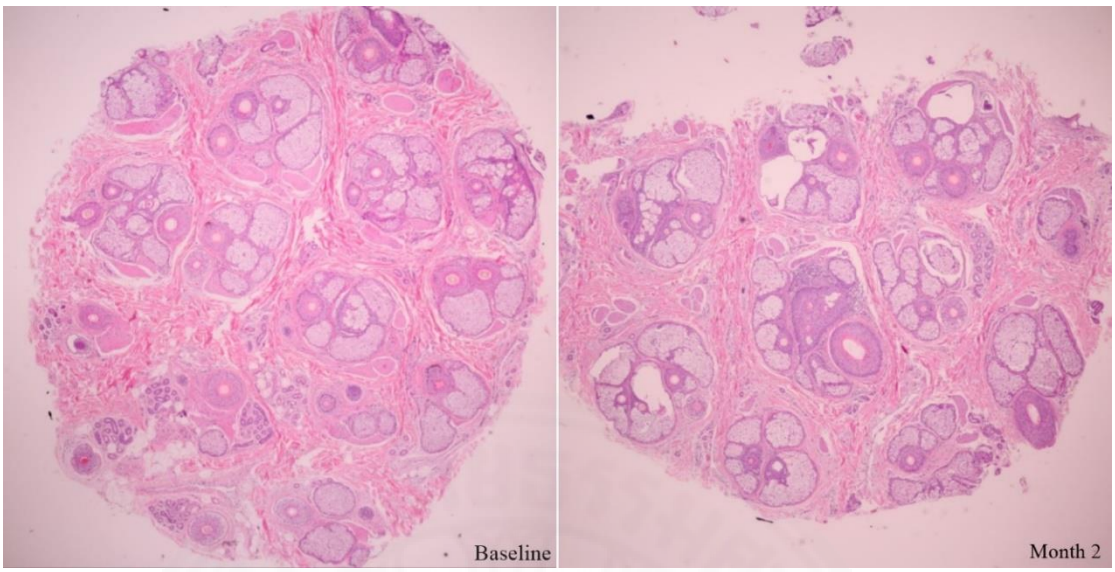

Figure G10 Histologic finding of patient No.19 compare baseline to 2nd month after treatment.

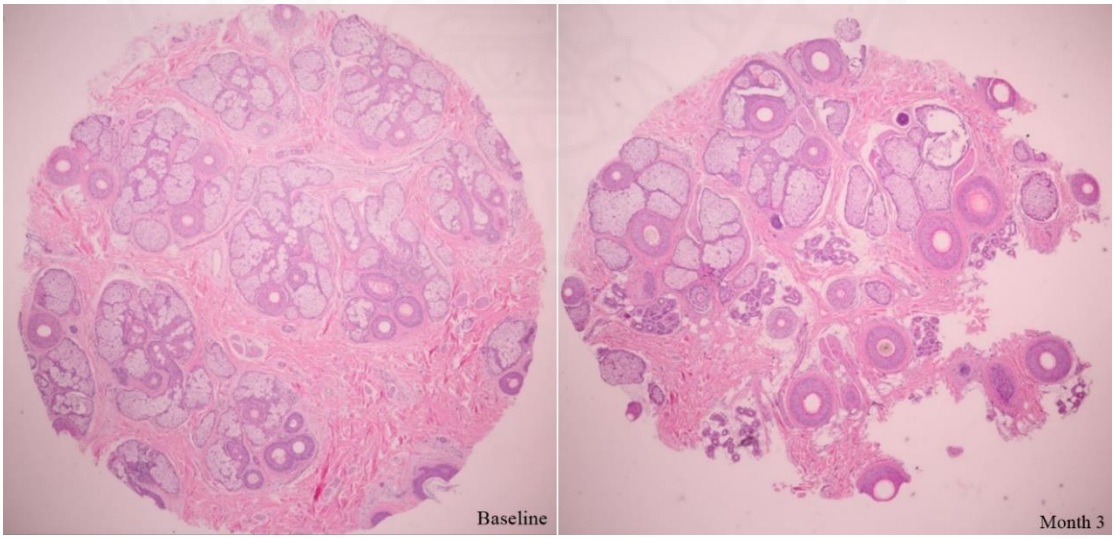

Figure G11 Histologic finding of patient No.6 compare baseline to 3rd month after treatment.

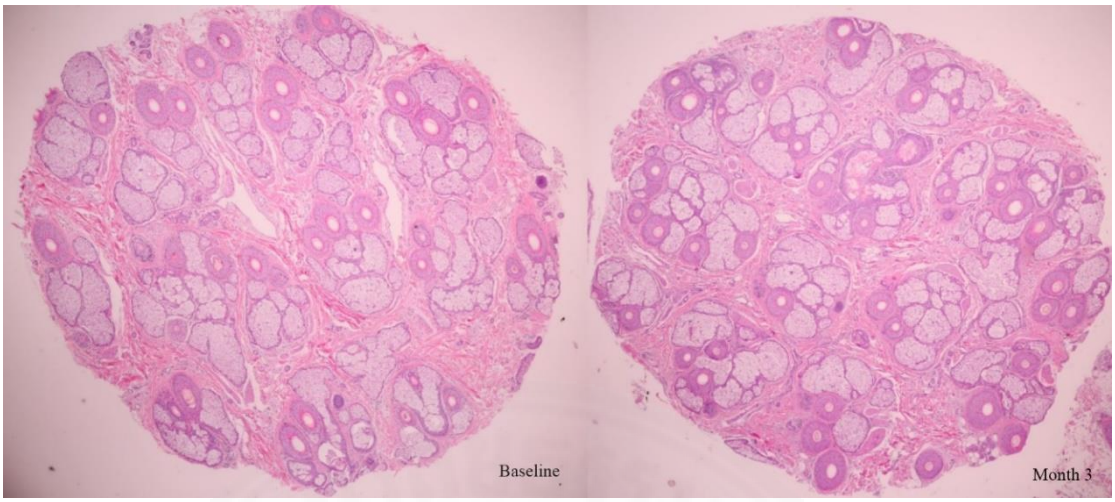

Figure G12 Histologic finding of patient No.16 compare baseline to 3rd month after treatment.

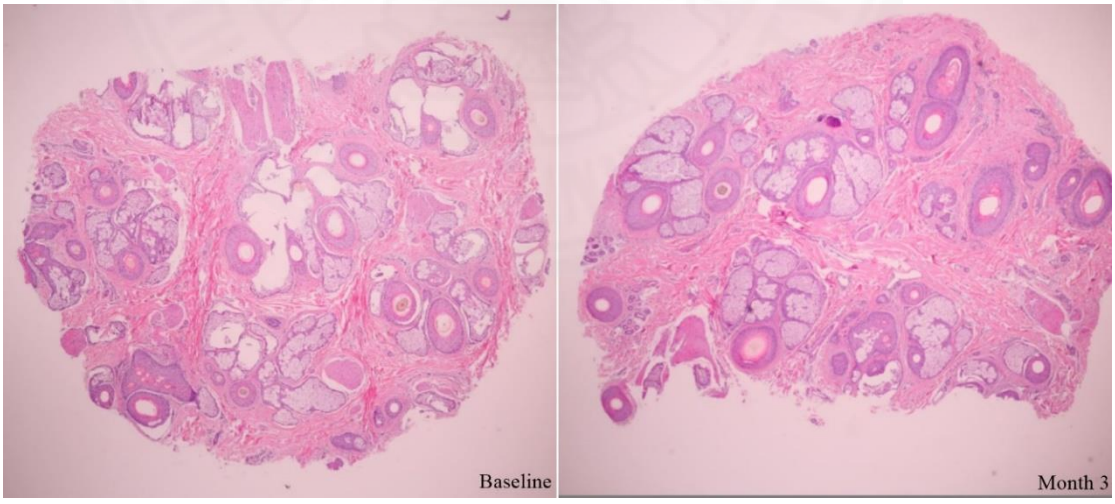

Figure G13 Histologic finding of patient No.18 compare baseline to 3rd month after treatment.

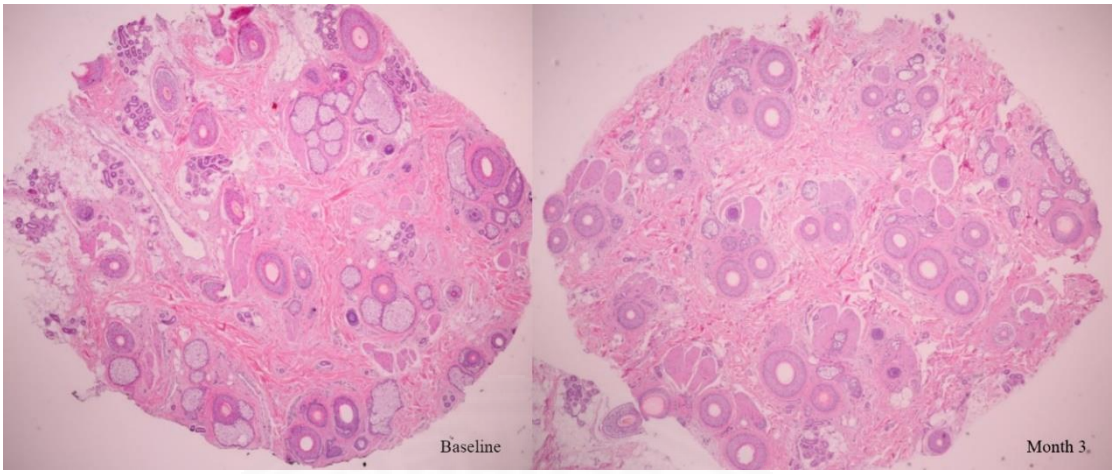

Figure G14 Histologic finding of patient No.21 compare baseline to 3rd month after treatment.

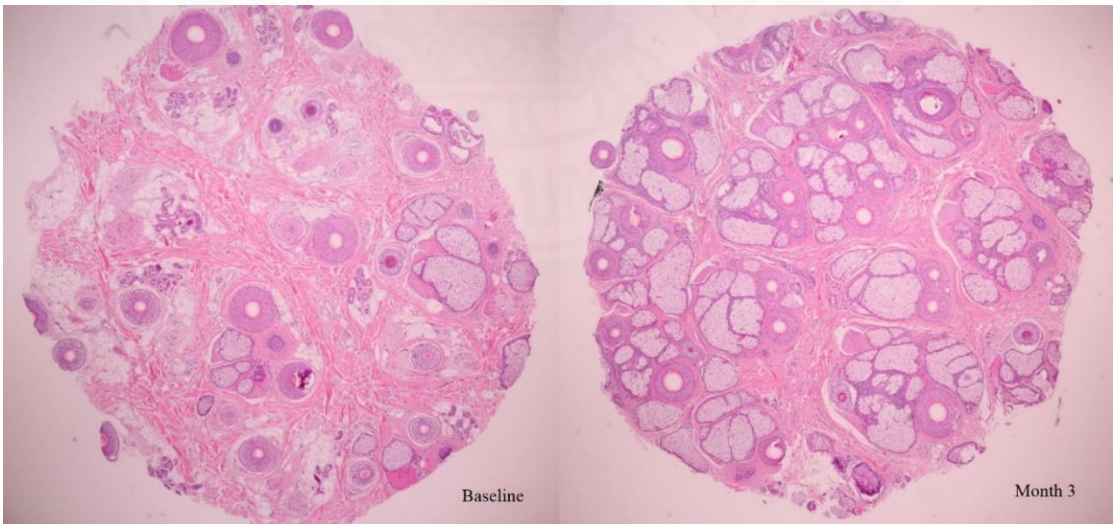

Figure G15 Histologic finding of patient No.23 compare baseline to 3rd month after treatment.

APPENDIX H

HISTOPATHOLOGY HAIR COUNT

Table H1 Histopathologic hair count

Patient No.	Visit	Follicular unit	Total	Terminal	Vellus	Anagen	Telogen	Anagen :Telogen ratio
No.1	Baseline	12	15	9	6	11	4	2.75
	Month 2	12	27	21	6	24	3	8
No.5	Baseline	11	20	12	8	18	2	9
	Month 2	13	24	15	9	21	3	7
No.6	Baseline	10	19	12	7	15	4	3.75
	Month 3	7	20	13	7	17	3	5.67
No.7	Baseline	10	17	10	7	13	4	3.25
	Month 1	10	24	19	5	21	3	7
No.9	Baseline	N/A						
	Month 2	10	15	8	7	13	2	6.5
No.12	Baseline	N/A						
	Month 1	11	23	18	5	20	3	6.67
No.13	Baseline	8	15	10	5	13	2	6.5
	Month 2	10	20	14	6	15	5	3
No.14	Baseline	12	29	17	12	24	5	4.8
	Month 1	10	19	12	7	15	4	3.75
No.15	Baseline	10	24	12	12	20	4	5
	Month 1	13	25	21	4	21	4	5.25
No.16	Baseline	15	35	22	13	31	4	7.75
	Month 3	14	47	33	14	45	2	22.5
No.18	Baseline	7	19	15	4	17	2	8.5
	Month 3 (inadequate)	6	16	13	3	12	4	3
No.19	Baseline	14	28	18	10	22	6	3.67
	Month 2 (inadequate)	12	26	14	12	23	3	7.67
No.21	Baseline	10	23	14	9	20	3	6.67
	Month 3	12	34	25	9	28	6	4.67
No.22	Baseline	11	19	13	6	12	7	1.71
	Month 1 (inadequate)	9	21	17	4	20	1	20
No.23	Baseline	12	22	13	9	21	12	1.75
	M3	13	30	21	9	26	13	2

APPENDIX I

WNT10A AND IGF-1 mRNA EXPRESSION

Table I1 WNT10A and IGF-1 mRNA level expression

Patient No.	WNT10A		IGF-1	
	Baseline	After treatment	Baseline	After treatment
No.1	1.05448	0.50532	1.04860	0.40470
No.2	1.00000	0.42905	N/A	N/A
No.3	0.30122	0.27608	N/A	N/A
No.4	0.34907	0.00000	N/A	N/A
No.5	1.40668	0.37626	1.23526	0.25812
No.6	4.02856	0.50131	4.24406	0.86632
No.7	2.82791	11.66401	4.53626	7.85144
No.8	0.02471	N/A	N/A	N/A
No.9	4.65515	0.62999	6.32867	0.75099
No.10	N/A	N/A	N/A	N/A
No.11	N/A	N/A	N/A	N/A
No.12	10.44409	32.43285	10.44409	40.53807
No.13	3.64445	0.66892	3.87381	1.44257
No.14	N/A	N/A	N/A	N/A
No.15	0.12441	N/A	N/A	N/A
No.16	N/A	N/A	N/A	N/A
No.17	11.31984	3.05755	11.08999	4.32081
No.18	2.90415	1.78130		
No.19	0.69030	2.19282	0.35016	3.74426
No.20	5.35048	0.83965	5.30325	1.20926
No.21	N/A	0.41766	N/A	N/A
No.22	N/A	0.12819	N/A	N/A
No.23	0.79568	0.97849	N/A	N/A

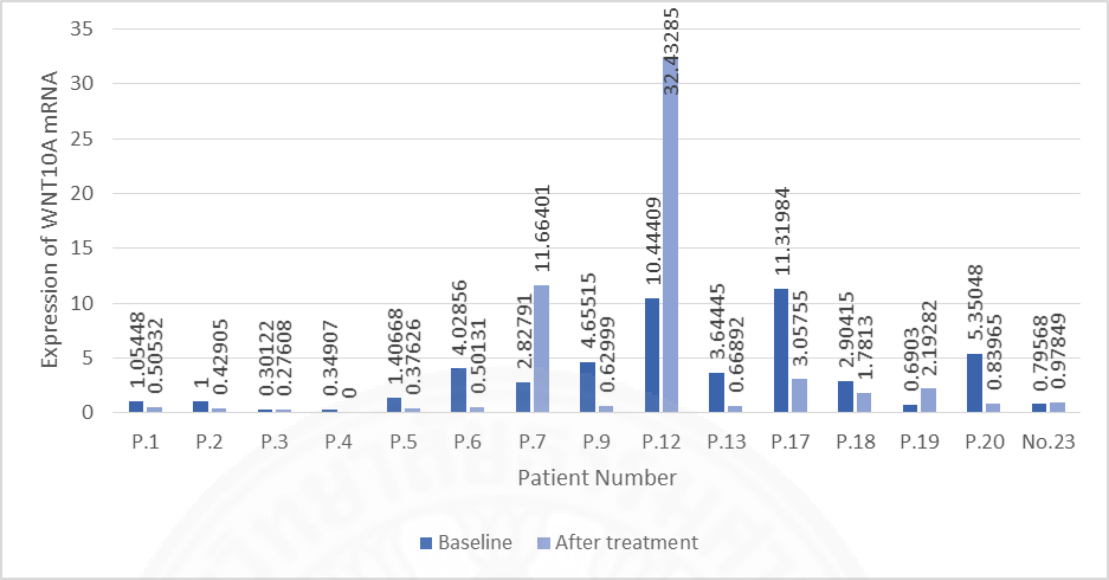

Figure I1 qRT-PCR data comparing WNT10A mRNA expression at baseline and 24 hours after third session (1st month) of treatment with 1550 nm Er:Glass fractional laser.

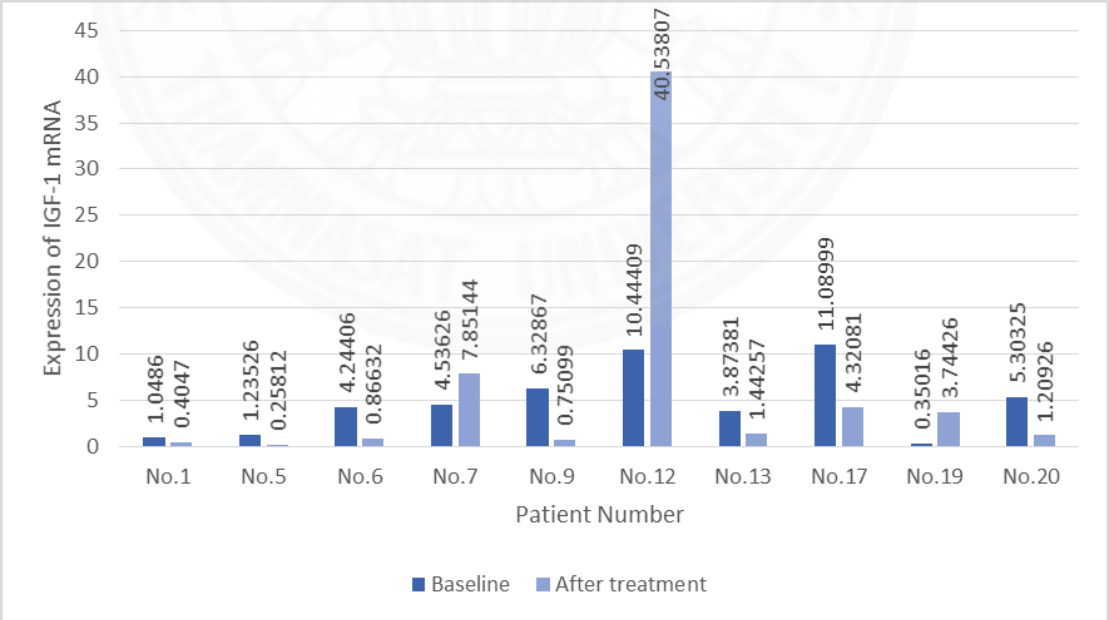

Figure I2 qRT-PCR data comparing IGF-1 mRNA expression at baseline and 24 hours after third session (1st month) of treatment with 1550 nm Er:Glass fractional laser.

BIOGRAPHY

Name	Miss Nawaporn Ungpraphakorn
Date of Birth	November 21, 1988
Educational Attainment	Academic Year 2012: Doctor of Medicine, Thammasat University, Thailand
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