Mechanism of 1550 nm Er:Glass fractional laser on insulin-like growth factor 1 and Wnt/β-catenin expression in androgenic alopecia

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Introduction

Androgenic alopecia is a common non-scarring hair loss disorder in Thailand. In 2002, the prevalence of androgenic alopecia is about 38.52% and it seems to be more frequency as the time goes by. Androgenic 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 (1). 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 (2, 3). Currently, new treatment modalities have been launched for androgenic alopecia included 1550 nm Er:Glass fractional laser. Many dermatologists are interested in 1550 nm Er:Glass fractional laser treatment in androgenic 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 androgenic alopecia treatment modalities.

Patients and Methods

Ten Thai patients both male and female who has been diagnosed as male pattern hair loss Hamilton-Norwood stage III-IV (include type III vertex) or female pattern hair loss Ludwig type II were enrolled in the study at the out patients department of Dermatology, Tobacco Monopoly hospital Thailand between September 2016 and November 2016.

All patients were provided inform consent before enrolled to this study. Patient who has a serious underlying disease or any serious infection were excluded from the study. Patients who had previous treatment for androgenic alopecia were excluded including hair transplant, systemic drug within 1 year and topical treatment within 6 months. Pregnant and lactating women were excluded from this study.

This study was designed as a single center semi-experimental before and after study and has been approved by Faculty of medicine Thammasat university review board. In this study we use a 1550 nm fractional erbium-glass laser (MOSAIC, Lutronic Co., Ltd, Seoul, South Korea). Each patient received 3 treatments at 2 weeks interval with 2x12 mm tip, power 6 mJ, spot density 300 spot/cm² static mode, 2 passes on alopecia area which involved frontovertical and also parietal region of scalp.

qRT-PCR: Scalp biopsy was done by using 2 mm punch at baseline and then 24 hours after third laser treatment (1 month after start the first laser treatment) for evaluating IGF-1 and WNT10A mRNA expression. All tissues were stored at -80 C before experiment. RNA isolation was done by using QIAGENTM (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.).

**Results**

Ten patients who enrolled the study are five males and five females. For males aged 27-49, Hamilton-norwood stage III (n=2; 40%), Hamilton-norwood stage IV (n=3; 60%). For female patients aged 33-40, Ludwig stage II (n=5; 100%) (Table 1).

Scalp biopsied tissues were performed RT-PCR at baseline mean of mRNA level IGF-1 is 4.85 ± 3.68 and mean of WNT10A mRNA is 4.54 ± 3.69. Then twenty four hours after third laser treatment (1 month) mean of IGF-1 mRNA expression is 6.14 ± 12.32 (P=0.445) and mean of WNT10A mRNA level expression is 5.29 ± 10.14 (P=0.575) (Table 2). This study showed that there is no significant increasing of both IGF-1 and WNT10A mRNA expression after laser treatment for 1 month. Only three patients that have significantly increasing of both IGF-1 and WNT10A mRNA-level expression after treatment with laser. (Figure 1, 2)

Table 1. Characteristics of 10 patients with androgenic alopecia

<table>
<thead>
<tr>
<th></th>
<th>Total (n=10)</th>
<th>Female (n=5)</th>
<th>Male (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.8 ± 6.21</td>
<td>37.2 ± 2.59</td>
<td>38.4 ± 8.91</td>
</tr>
<tr>
<td>Ludwig stage 2</td>
<td>5 (50%)</td>
<td>5 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton Norwood stage 3</td>
<td>2 (20%)</td>
<td>0</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Hamilton Norwood stage 4</td>
<td>3 (30%)</td>
<td>0</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Family history</td>
<td>10 (100%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

Table 2. IGF-1 and WNT10A mRNA level expression. Values presented as mean ± SD. P-value corresponds to Paired t test, IGF-1 = Insulin growth factor 1

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD.</th>
<th>p-value</th>
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<tbody>
<tr>
<td>IGF-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.85 ± 3.68</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>6.14 ± 12.32</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>1.29 ± 10.67</td>
<td>0.710</td>
</tr>
<tr>
<td>WNT10A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.54 ± 3.69</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>5.29 ± 10.14</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.74 ± 8.73</td>
<td>0.793</td>
</tr>
</tbody>
</table>

**Adverse Effects**

During and after laser treatment some patients develop mild erythema but just minimal and resolved within 10 minutes, no hair shaft damaged was detected, no infection was observed after laser and no topical anesthesia needed during the laser.
Discussion

Androgenic alopecia is a non scarring hair loss condition which commonly found increasing with age in Asian people (1, 4). The standard treatment is topical minoxidil and systemic 5α reductase inhibitor which are still the mainstream treatment of this condition, but limited by its low efficacy and systemic side effects (5). Since androgenic alopecia are found increasing in number nowadays, many new treatment modalities are introduced to be used as an optional choice like low-level laser therapy, hormonal therapy hair restoration and fractional photothermolysis laser (6). The core of androgenic alopecia treatment is to enhance the new hair growth, slow progression of disease with less to least systemic side effects. In this recently years, few papers showed that fractional laser has been taken a role in hair loss treatment (7-9). One of the mechanisms that resulted in hair regrowing is wound healing (10-12). Wound healing process from fractional laser induce cytokines, increased blood flow, growth factors such as FGF family, EGF, IGFs, HGF, TGF-β, VEGF, NGF as well as interleukins which may direct altered dermal papilla where the hair follicle stem cells are resided (13, 14). Over expression of IGF-1 improved wound healing and stimulate hair follicle formation in mice (15). IGF-1 is also well known as an anagen maintenance , absence of it could lead anagen phase prematurely turn to catagen phase (16). In androgenic alopecia dermal papillae expressed lower level of IGF-1 (2). In mice, after treated with fractional laser showed increasing of Wnt10b expression (Wnt/β-catenin pathway) (17). Also in pilot study that 3 patients with male pattern hair loss treated with 1550 nm Er:glass fractional laser, after treatment WNT10A mRNA level was increasing in expression and highest expression was at 24 hours after laser treatment (8).

The results from our study show that there is no significant increasing in expression of both WNT10A and IGF-1 mRNA level expression (Table1). This may be because of timing 24 hours that we choose is not the proper time of WNT10A and IGF-1 mRNA to be increasing. Also the variability of each patient like ages, sex and their health status may involved in wound healing process differently individual. But among the variability from the result of the study, both WNT10A and IGF-1 mRNA are found increase expression in 3 patients at 24 hours after treated with fractional laser (Figure1, 2). Further than this WNT10A and IGF-1 mRNA level expression trend to be in the same way, cases that were no increase expression of WNT10A mRNA level after treatment, there were no increase in IGF-1 mRNA level also. On the other hand, in 3 cases that show increasing of protein level after treated with laser, they increase in both WNT10A and IGF-1 mRNA level(Figure 1, 2). These may infer that there is some correlation between Wnt/β-catenin pathway and IGF-1 in hair cycle.
Conclusion
Our findings showed that at 24 hours after treated with 1550 nm Er:Glass laser in androgenic alopecia patients, WNT10A and IGF-1 mRNA did not always increase level of expression compared with before treatment. The mechanisms that 1550 nm Er:Glass laser induce the new hair growth may not limit to stimulate Wnt/β-catenin or IGF-1. Further studies are needed to prove the mechanism of how 1550 nm induce the new hair growth and what factors influence treatment response.

Acknowledgements

References