Effects of compound glycyrrhizin on serum IFN-γ, IL-10 and immunological index in patients with alopecia areata

**Aims:** To investigate the effect of compound glycyrrhizin in the treatment of alopecia areata and its effect on serum Interferon-γ (IFN-γ), Interleukin-10 (IL-10) and other immunological indexes.

**Methods:** 150 patients with alopecia areata were randomly divided into study group and control group, 75 cases in each group. The cases in the control group were treated with LingDan tablets. The cases in the study group were treated with the compound glycyrrhizin tablets based on control group’s treatment. The two groups were treated for 3 months. The severity of alopecia tool (SALT) score, IFN-γ and IL-10 levels, immunological indicators were recorded before and after treatment.

**Results:** The total effective rate of the study group and the control group were 93.3%, 77.3% after the treatment (P<0.05). The SALT scores were decreased in both groups, and the SALT scores of the study group was lower than that of the control group (P<0.05). Compared with the control group, the levels of IFN-γ were decreased and IL-10 were higher in the study group. The levels of CD3+, CD4+, CD4+/CD8+, IgA, IgG and IgM were both increased, which was significantly higher than those in the control group (P<0.05), indicating that the improvement of the study group was significantly better than that of the control group. The incidence of adverse reactions in the study group and the control group was 2.7%, 4%, respectively. (P>0.05)

**Conclusion:** On the basis of LingDan tablets and minoxidil tincture at the skin, adding compound glycyrrhizin tablets in the treatment of alopecia areata has a high clinical total effective rate, which can improve the condition and immunization of alopecia areata and regulate the level of Th1/Th2 cytokines. Our study provides a new sight of compound glycyrrhizin in the clinical treatment of alopecia areata and deserves further clinical application.

**Keywords:** alopecia areata • compound glycyrrhizin • minoxidil tincture • IFN-γ
specific autoimmune disease, regulated by the T lymphocyte-mediated growth and characterized as limited hair follicle damage and hair loss. Epidemiological studies have shown that the disease is a multi-gene disease, is the result of environment, genetic susceptibility and other factors. At present, the treatment and efficacy of the disease varied different. Previous studies showed that minoxidil tincture can promote hair growth, but cannot prevent the progress of alopecia areata disease. Compound glycyrrhizin is a complex compound containing glycyrrhizin, hemiglycine and glycine as the main component, functioned as anti-inflammatory, anti-allergic and immunomodulatory effects, which can regulate T cell activation, the production of γ-interferon and activation of NK cells. Compound glycyrrhizin can work as glucocorticoids, but with no side effects of glucocorticoids. In recent years, the regulation of peripheral type 1 helper T lymphocyte/type 2 helper T lymphocyte factor (Th1/Th2) in alopecia areata has attracted researcher' attention. In clinical practice, we achieved some clinical results that adding the compound glycyrrhizin tablets on the basis of oral LingDan tablets and minoxidil tincture in the skin lesions. In this study, we detected the levels of IFN-γ, IL-10 and immunological indicators in peripheral blood and evaluated its therapeutic effect.

**Data and methods**

**General data**

From July 2015 to February 2017, we selected 150 patients with alopecia areata eligible for the study in our hospital dermatology clinic.

**Inclusion criteria**

i. meet the diagnostic criteria of alopecia areata in the "clinical dermatology" (third edition);

ii. normal skin in the hair loss area, no obvious inflammatory reaction;

iii. patients can be prescribed medication on time, treatment and follow-up.

**Exclusion criteria**

i. patients received systemic hormone drug therapy within 4 weeks or accepted the local hormone therapy within 2 weeks;

ii. pseudo-alopoeia areata, scarring hair loss and other clear cause of hair loss;

iii. patients suffering from nervous, mental illness or severe endocrine diseases, other skin diseases, infectious diseases and severe systemic primary disease;

iv. patients with severe immune function loss, or the need for long-term use of corticosteroids, immunosuppressive agents, antihistamines and sedatives;

v. pregnancy, to be pregnant, postpartum within 12 months and lactating women and allergic patients.

150 patients were randomly divided into study group and control group, each group contained 75 cases. In the study group, 35 males and 40 females were aged from 18 to 64 years (mean 47.8 ± 3.2 years). The course of the disease was 7 days to 20 months with average course (mean 6.4 ± 1.3 months). 70 cases were single hair alopecia areata and 5 cases were general alopecia; 50 cases in active courses (lesions showed a positive hair test, alopecia area has been expanding or new bald area appears), 25 cases in stable courses (lesions showed a negative test, no new bald area was found within 1 month and there may be a small amount of hair grow). The control group: 36 males and 39 females were aged 17 to 65 years (mean, 48.1 ± 2.7 years). The course of the disease was 6 days to 21 months with average course (mean, 6.3 ± 1.2 months). 69 cases were single hair alopecia areata and 6 cases were general alopecia; 51 cases in active courses and 24 cases in stable courses. There is no difference in the gender composition, age distribution, duration, type and degree of disease and other information in the two groups of patients (P> 0.05). As shown in TABLE 1.

**Treatment**

**Treatment in control group**

On the basis of healthy education of alopecia areata, including notice of alopecia areata, prevention of hair loss, prohibition of spicy food, limitation of adverse mental stimulation, adequate sleep, oral self-made Chinese medicine LingDan tablets, 3 times a day, every time 3 tablets. Minoxidil Tincture (MenDi, ZheJiang Wanma Pharmaceutical Co.Ltd. Specifications:60 mL/bottle, with 5% minoxidil) should be smeared in the local skin lesions outside twice a day, 1 mL/times for 3 months. A brief massage on the local line should be applied after treatment.
**Treatment in study group**

On the basis of treatment of control group, adding compound glycyrrhizin tablets (ShuaiNeng, Henan new ShuaiKe Pharmaceutical Co.Ltd. Specifications:25 mg/tablets) in the treatment, 3 times a day, every time 2 tablets for 3 months.

**Observational indexes**

The following indicators were observed before and after treatment:

i. clinical manifestations, including the new growth situation, hair thickness and color, the new distribution of intensity, pull hair test were observed according to the clinical manifestations of efficacy evaluation;

ii. SALT score: The degree of hair loss before and after treatment was assessed according to the international general SALT (Severity of Alopecia Tool) scoring criteria;

iii. The expression of Th1 / Th2 cytokines in serum mononuclear cells (PBMC) was detected by ELISA. Th1 cytokines were detected by IFN-γ and Th2 cytokines were detected by IL-10. The kit was purchased from ShenZhen JingMei Biological Engineering Co.Ltd;

iv. The levels of T lymphocyte subsets (CD3 +, CD4 +, CD8 +, CD4 + / CD8 +) and immunoglobulin (IgA, IgG, IgM) in peripheral blood were detected using flow cytometry and immune scatter turbidimetric assay, respectively;

v. Adverse reactions, including blood, urine, liver and kidney function, electrolyte examination and clinical symptoms and signs of abnormal performance were observed.

**Judgement standard**

**Efficacy standard:** According to Deshpande D [9] criteria, the efficacy standard were divided into: ① Recovery: New hair growth could be found in the Alopecia areata area with intensively distribution and normal hair thickness and color. Hair test was negative; ② excellence: Hair growth could be generally found in the Alopecia areata area with no loss of hair and new hair grows 50% to 90%. Hair test was negative; ③ Improved: Hair growth could be generally found in the Alopecia areata area and new hair grows 10% to 49%. Hair test was negative or positive; ④ Invalid: No new hair growth or new growth <10% in the alopecia areata area, or accompany with loss of hair. Total effective rate = (number of recovered cases + conspicuous cases)/total number of cases × 100%.

**SALT scoring:** The scalp of the patient is divided into four regions: the left and right temporal regions, the occipital region and the former area. Then, each region are subdivided into four regions according to the horizontal and vertical lines. ① left and right temporal area: occupied 18% of the total scalp area, the score of subdivided area were 5 points, 4 points, 4 points, 5 points from left to right, top to down order and a total of score of each area was 18 points; ② occipital area: occupied 24% of the total scalp area, the score of subdivided area were 6 points and a total of score of occipital area was 24 points; ③ former area: occupied 40% of the total scalp area, the score of subdivided area were 10 points and a total of score of former area was 40 points. The scores for all regions are superimposed as the patient’s SALT score.

**Statistical methods**

Quantitative data are expressed as the means ± SD. The values were compared using a student’s t-test. Categorical data and rate comparison using X2 test. All statistical analyses were performed using SPSS 21.0 software. A p-value of <0.05 was considered statistically significant.

**Results**

**Comparison of efficacy**

The total effective rate was 93.3% in the study group, which was significantly higher than that in the control group, 77.3% (P <0.05) (TABLE 2).
Comparison of SALT scoring
There was no significant difference in SALT score between the two groups (P > 0.05). After treatment, the SALT scores of the two groups were significantly lower (P < 0.05), but SALT scores of the study group was significantly lower than that in the control group (P < 0.05) (TABLE 3).

Levels of IFN-γ and IL-10
There were no significant differences in serum IFN-γ and IL-10 levels between the two groups before treatment (P > 0.05). After treatment, the level of IFN-γ was decreased and IL-10 was increased in the study group (P < 0.05). The difference of serum IFN-γ and IL-10 between the two groups was statistically significant (P < 0.05) (TABLE 4).

Levels of Immunological index
There were no significant differences in the levels of serum CD3+, CD4+, CD8+, CD4+/CD8+, IgA, IgG and IgM between the two groups before treatment (P > 0.05). After treatment, CD3+, CD4+, CD8+, CD4+/CD8+, IgA, IgG and IgM were significantly increased and CD8+ was obviously decreased. The difference was statistically significant (P < 0.05) and the improvement of the study group was better compared with the control group (P < 0.05) (TABLE 5).

Adverse effects
During the course of treatment, the incidence of adverse reactions in the study group was 2.7% (2/75), that is, 2 cases of local irritant dermatitis; the incidence of adverse reactions in the control group was 4% (3/75), that is, 2 cases of mild facial edema, 1 case of mild edema of the lower limbs. There was no significant difference in the incidence of adverse reactions between the two groups (X² = 1.046, P > 0.05).

The adverse reactions in two groups were relieved after symptomatic treatment, which did not affect the continued treatment. No obvious changes were detected in the blood, urine, liver and kidney function in the two groups of patients before and after treatment and no other obvious adverse reactions were observed.

Discussion
Alopecia areata, commonly known as “ghost shaving head”, “ghost lick head” and “round hair removal”, is a sudden occurrence of localized patchy hair loss syndrome. Patient may have no other symptoms. The disease can occur at any age and any part of the scalp, a common disease of a skin-affiliated organ with a common age of 20 to 40 years old. Alopecia areata often characterized as sudden occurrence, round or oval plaque hair loss with different size, neat edges, normal hair loss skin, smooth scalp, no inflammation.

Table 2. Comparison of efficacy (cases)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Recovery</th>
<th>Excellence</th>
<th>Effective</th>
<th>Invalid</th>
<th>Total effective rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>75</td>
<td>51</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>93.3</td>
</tr>
<tr>
<td>Control group</td>
<td>75</td>
<td>22</td>
<td>20</td>
<td>16</td>
<td>17</td>
<td>77.3</td>
</tr>
<tr>
<td>X²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3. Comparison of SALT scoring

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Before</th>
<th>After</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>75</td>
<td>54.67 ± 7.05</td>
<td>12.85 ± 2.32</td>
<td>7.148</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>75</td>
<td>55.13 ± 6.82</td>
<td>24.91 ± 3.44</td>
<td>6.026</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>0.715</td>
<td>6.643</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Comparison of Levels of IFN-γ and IL-10 (pg/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IFN-γ</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>t</td>
</tr>
<tr>
<td>Study group</td>
<td>38.72 ± 4.51</td>
<td>28.54 ± 2.17</td>
<td>4.592</td>
</tr>
<tr>
<td>Control group</td>
<td>39.03 ± 3.94</td>
<td>34.16 ± 3.02</td>
<td>3.874</td>
</tr>
<tr>
<td>t</td>
<td>0.952</td>
<td>6.002</td>
<td>0.816</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
Effects of compound glycyrrhizin on serum IFN-γ, IL-10 and immunological index in patients with alopecia areata

Research Article

The effects of compound glycyrrhizin on serum IFN-γ, IL-10 and immunological index in patients with alopecia areata were studied. The results show that the serum levels of IFN-γ and IL-10 were significantly decreased in the study group compared to the control group after the treatment (P <0.05). The SALT score of the study group was lower than that of the control group (P <0.05). This suggests that the combination of these drugs can significantly improve the skin lesions of alopecia areata and the effect is more accurate, safe and reliable.

Table 5. Comparison of Levels of Immunological index

<table>
<thead>
<tr>
<th>Items</th>
<th>n</th>
<th>time</th>
<th>CD3+(%)</th>
<th>CD4+(%)</th>
<th>CD8+(%)</th>
<th>CD4+/CD8+</th>
<th>IgA(g/L)</th>
<th>IgM(g/L)</th>
<th>IgG(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>75</td>
<td>Before</td>
<td>57.94 ± 3.73</td>
<td>35.12 ± 3.04</td>
<td>29.69 ± 3.44</td>
<td>1.42 ± 0.8</td>
<td>1.78 ± 0.24</td>
<td>1.39 ± 0.24</td>
<td>10.07 ± 2.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>63.47 ± 3.98</td>
<td>42.81 ± 4.16</td>
<td>23.87 ± 3.12</td>
<td>1.65 ± 0.43</td>
<td>2.42 ± 0.58</td>
<td>2.27 ± 0.52</td>
<td>17.86 ± 3.19</td>
</tr>
<tr>
<td>control group</td>
<td>75</td>
<td>Before</td>
<td>57.62 ± 4.65</td>
<td>36.57 ± 2.15</td>
<td>21.06 ± 2.23</td>
<td>1.43 ± 0.31</td>
<td>1.76 ± 0.31</td>
<td>1.41 ± 0.14</td>
<td>10.05 ± 2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>59.81 ± 2.08</td>
<td>39.62 ± 4.53</td>
<td>28.07 ± 3.12</td>
<td>1.49 ± 0.52</td>
<td>2.76 ± 0.49</td>
<td>1.78 ± 0.46</td>
<td>14.98 ± 2.03</td>
</tr>
</tbody>
</table>

Cellular immunity, T cells involved, is an important part of the body’s immune system, not only directly mediated cellular immunity, but also plays an important role in regulating the immune response. The resting CD4+ T cells in the human body can differentiate into different subtypes of Th1 and Th2 under the stimulation of certain antigens. Th1 subtypes secrete IL-2, IL-12, IFN-γ, TNF-α and other cytokines, mainly mediated cellular immunity; Th2 subtype secrete IL-4, IL-5, IL-6, IL-9 and IL-10 and other cytokines, mainly mediated humoral immunity. The main part of Th1 and Th2 subtypes were IFN-γ, IL-10 respectively. In this study, serum IFN-γ and IL-10 were used as indicators to evaluate the level of Th1 and Th2. Previous studies [15,16] have shown that the imbalance of Th1/Th2 cytokine interaction is a key regulation of immune response, leading to immune disorders, which cannot effectively carry out cellular immune response, and contribute to the beginning, development and prognosis of a variety of diseases. In organ-specific autoimmune diseases, the beginning of disease can be caused by Th1 subtypes of cytokines dominantly, but dominant Th2 subtype cytokines can prevent the disease. The results of this study show that serum or scar within a night or a few days. The hair loss area can be integrated into each other. Some patients often accompanied by poor feelings, dizziness, sleepy dreams, insomnia, back pain, nocturnal emission, impotence, dry mouth and other symptoms [10,11]. The pathology of alopecia areata is not fully clear yet. Previous studies revealed that alopecia areata can be caused by certain factors such as stress, endocrine disorders, mental factors, genetic factors, subcortical and autonomic nervous system, and autoimmune dysfunction may also contribute the disease [12,13].

Compound glycyrrhizin is a complex compound containing glycyrrhizin, hemiglycine and glycine as the main component, functioned as anti-inflammatory, anti-allergic and immunomodulatory effects. Compound glycyrrhizin has the effect of glucocorticoids, but with no side effects of glucocorticoids [14]. LingDan tablets are red sugar-coated tablets with blood stasis and nourishing hair, mainly composed of salvia, chuanxiong and ganoderma lucidum. Minoxidil tincture is an agonist of prostaglandin epoxide synthase, a peripheral vasodilator. In this study, we investigated the effect of compound glycyrrhizin in the treatment of alopecia areata on the basis of oral LingDan tablet and minoxidil tincture. The total effective rate of the study group and the control group were 93.3%, 77.3% after the treatment (P <0.05). The SALT scores were decreased in both groups, and the SALT score of the study group was lower than that of the control group (P <0.05). This suggests that the combination of these drugs can significantly improve the skin lesions of alopecia areata and the effect is more accurate, safe and reliable.
Research Article Xue

IFN-γ levels increased and IL-10 levels decreased in patients with alopecia areata, suggesting that the hyper responsiveness of Th1 subtypes of cytokines. The results showed that serum IFN-γ levels decreased and IL-10 levels increased and a good trend of balance of Th1/Th2 after adding compound glycyrrhizin in the treatment of alopecia areata on the basis of oral LingDan tablet and minoxidil tincture.

CD3+, CD4+ and CD8+ are important immune cells. The normal immune response process depends on the mutual cooperation or restraint of these immune cytokines. After treatment, CD3+, CD4+, CD8+, CD4+/CD8+, IgA, IgG and IgM were significantly increased. The difference was statistically significant (P<0.05) and the improvement of the study group was better compared with the control group (P<0.05). Adding compound glycyrrhizin in the treatment of alopecia areata effectively improve the cell immune abnormalities.

The mechanism of combination of drugs

i. direct stimulation of proliferation and differentiation of follicle epithelial cells;

ii. improve the immunological indicators, immune function and the balance of Th1/Th2, promote angiogenesis, increase local blood flow, improve microcirculation around the follicle;

iii. promote the hypertrophy of original hair follicle and inhibition of T cell infiltration, inducing permanent hair changes;

iv. transform the follicles from the rest period to the active period, promote the regeneration of hair, extend the hair growth period.

In summary, On the basis of LingDan tablets and minoxidil tincture at the skin, adding compound glycyrrhizin tablets and minoxidil tincture at the skin, adding compound glycyrrhizin in the treatment of alopecia areata has a high clinical total effective rate, which can improve the condition and immunization of alopecia areata and regulate the level of Th1/Th2 cytokines. Our study provides a new sight of compound glycyrrhizin in the clinical treatment of alopecia areata and deserves further clinical application.

References


