Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°5, 1499-1503
DOI: 10.24205/03276716.2020.1147

Therapeutic effect of Astragalus Chinese herbal compound on children with thalassemia and its influence on KLF1 gene expression

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Abstract

Objective: To observe the effect of Astragalus Chinese herbal compound on children with thalassemia and its influence on KLF1 gene expression.

Methods: Using a randomized controlled double-blind clinical study, 108 patients with β-thalassemia were randomly assigned into the traditional Chinese medicine treatment group and the placebo control group. The children in the traditional Chinese medicine treatment group were given Astragalus Chinese herbal compound, and the control group was given placebo granules 12 weeks. Observe blood routine and fetal hemoglobin ratio and other hematological indicators. And real-time fluorescent quantitative PCR reaction was used to detect the mRNA level of KLF1 gene.

Results: After the 12-week course of treatment, the Hb value of the children in the Chinese medicine group was 82.25±6.10 g·L-1 and the control group was 66.20±11.50 g·L-1. The difference between the two groups was statistically significant (P<0.04). The proportion of HbF after treatment in the Chinese medicine group was also significantly higher than before treatment (61.08%±13.02% vs. 52.31%±17.16%, P <0.01). Real-time fluorescent quantitative PCR experiment results showed that traditional Chinese medicine treatment had a significant effect on the expression of KLF1 gene. After treatment, the expression level of KLF1 gene was significantly lower than before treatment (0.33±0.19 vs. 0.81±0.40, P <0.01). TCM syndromes in the treatment group The scores were lower than those of the control group (P<0.05).

Conclusion: Astragalus Chinese herbal compound has a good effect on the treatment of β-thalassemia in children, and its molecular mechanism may be related to the decreased expression of KLF1 gene.

Keywords: β-thalassemia; Astragalus Chinese medicine compound; Hb; KLF1

Introduction

Beta thalassemia is a common hereditary blood disease distributed along the Mediterranean coast, Southeast Asia and southern China. In China, a large number of children with β-thalassemia rely on blood transfusions and iron chelating agents for a long time to maintain their lives. Regardless of whether it is blood transfusion or iron removal treatment, there are side effects such as endothelial damage and exposure to infectious diseases, and the cost is also very high [1,2]. The only radical cure for thalassaemia major is hematopoietic stem cell transplantation, but it is currently difficult to popularize due to many factors such as source risk and cost [3]. Globin gene drug induction is another relatively new treatment method for β-thalassemia. It uses drugs to induce the re-expression of the γ-globin gene, which is gradually closed after the neonatal period, to synthesize fetal hemoglobin (HbF) as a substitute. Compensation for adult hemoglobin (adult hemoglobin, HbA) low caused by β-globin gene defects[4,5]. Clinical studies [6] have shown that Astragalus Chinese medicine has a good effect in treating children with β-thalassemia, which can improve their hematological indicators and increase HbF levels. Thalassemia can be diagnosed by hematology screening and genetic testing. However, severe patients have severe anemia and require life-long blood transfusion to maintain hemoglobin content, and the mortality rate is

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extremely high. Clinicians need to pay attention to its treatment. Clinical treatments for thalassemia mainly include blood transfusions, iron removers, alkylating agents, and bone marrow transplantation. However, patients often suffer from growth inhibition, osteoporosis, immune dysfunction and other complications, and the treatment costs are high [7]. Erythroid Kriippel-like factor is an important factor closely related to erythroid development. It can regulate a series of genes related to the development of red blood cells. In addition, it is also involved in regulating fetal hemoglobin (HbF) to adult hemoglobin (HbA) transformation [8]. Recent reports have shown that KLF1 directly activates the expression of P-globin gene and indirectly inhibits the expression of Y-globin by up-regulating the expression of Bdlla, thereby regulating the conversion of fetal hemoglobin to adult hemoglobin [9]. In recent years, there have been clinical reports on the application of Astragalus medicinal herbs in the treatment of thalassemia, and good clinical effects have been achieved, but reports on its influence on BCL 11A gene expression are relatively rare. Therefore, based on the randomized controlled double-blind clinical study using traditional Chinese medicine, this study also explored the changes of KLF1 in children after medication, and analyzed the in vivo mechanism of Chinese medicine from the perspective of gene expression changes.

1.Materials and Methods

1.1General Information

This study was approved by the hospital ethics committee. A total of 108 children with β-thalassemia intermediate who were treated at Zhangzhou Hospital Affiliated to Fujian Medical University from January 2018 to December 2019 were enrolled, all of which obtained the informed consent of themselves or their guardians. Inclusion criteria: (1) age 1-18 years; (2) patients with β-thalassaemia diagnosed by genetic testing of thalassaemia, meeting the criteria for intermediate β-thalassaemia in the "Diagnosis and Efficacy Standards for Blood Diseases" [12]; (3)) No blood transfusion or any anti-anemia medication has been taken in the past 12 weeks. Exclusion criteria: (1) People with immune system, cardiovascular system, liver, kidney disease and other primary diseases of blood system; (2) People with allergies; (3) People with mental illness or poor compliance with previous medications.

1.2Treatment Plan

The traditional Chinese medicine group oral Chinese medicine formula granules: Astragalus, Codonopsis, and tortoise shell are 1, 3, and 0.7 g per bag respectively, which are water extracts of 10 g of their respective decoction pieces. According to the previous clinical research of this research group [9-10], the proposed usage and dosage are as follows: 2 to 6 years old, 6 + to 12 years old, 12 + to 18 years old, respectively, take 1 bag each and 2 bags each of the above 3 kinds of Chinese medicine granules daily ((Equivalent to 20 g each of Astragalus, Codonopsis, and tortoise shell), 3 bags each (equivalent to 30 g each of Astragalus, Codonopsis, and tortoise shell), take it with warm water on an empty stomach before meals. The course of treatment is 12 weeks. The control group was given a placebo orally, prepared with dextrin according to the placebo preparation specifications, and its appearance was similar to traditional Chinese medicine granules. The treatment course of usage and dosage refers to the traditional Chinese medicine group.

1.3 Detection of dematological indicators

Every 6 weeks before and after the treatment (until 12 weeks), the blood from the fingertips of the child was taken with an automatic blood cell analyzer to test the blood routine, mainly observing hemoglobin (Hb) and red blood cell (RBC). Before treatment and after 12 weeks of treatment, the venous blood of the children was taken to detect the fetal hemoglobin (HbF) ratio by alkaline hemoglobin electrophoresis.

1.4 QPCR to detect the expression of KLF1 before and after treatment

KLF1 was detected before treatment and after 12 weeks of treatment. Peripheral blood was drawn from the two groups of children. After total RNA was extracted, real-time fluorescent quantitative PCR was used to detect the level of KLF1 gene mRNA. Firstly, the KLF1 gene sequence was found from Genbank and the primers were designed and synthesized. The primer sequence is as follows: Forward Primer: 5'-CACCCAGCACAATGAAGATCAAGAT-3'Reverse Primer: 5'-CCAGTTTTTAAATCCTGAGTCAAGC-3'. Use Trizol to extract total RNA from a single nuclear cell, use M-MLV reverse transcriptase for reverse transcription reaction, and use dye method for real-time fluorescent quantitative PCR reaction. According to the amplification curve, standard curve and Ct value, the amount of nucleic acid corresponding to the KLF1 gene of each sample is obtained. Use the respective internal reference GAPDH gene nucleic acid amount to correct, and

calculate the relative copy number of KLF1 gene mRNA level. The PCR experiment for each sample in each group was repeated 3 times.

1.5 Statistical processing methods

The original data was entered into SPSS13.0 software, and the indicators were compared between groups and before and after treatment. P<0.05 indicates that the difference is statistically significant. The difference of blood routine indicators before treatment and between 6 weeks and 12 weeks after treatment, and the relative copy number of KLF1 gene mRNA measured three times were all analyzed by repeated measurement data analysis of variance.

2 Results

2.1 General Information

A comparison of 108 patients with β-thalassemia intermedia was randomly assigned to 58 cases in the traditional Chinese medicine group and 50 cases in the control group. There was no statistically significant difference between the children in the traditional Chinese medicine group and the control group in age and gender (P>0.05), which was comparable. (Table 1).

Table 1. **The age and gender of children with β-thalassemia**

Group	Number of cases	Age	male	Female
Astragalus Chinese Medicine Group	58	6.26±5.04	26	32
Control group	50	7.92±5.22	28	22

2.2 Routine blood indicators of the two groups before and after treatment

The analysis of variance of the repeated measurement data of Hb changes in the two groups before and after treatment showed that the difference in Hb in the traditional Chinese medicine group at each time point was statistically significant (P<0.01), and the 6 weeks and 12 weeks after treatment were significantly higher than those before the treatment; The difference in Hb of the control group at each time point was not statistically significant; the difference in Hb indicators between the two groups after the end of the 12-week course of treatment was statistically significant (P=0.006), and the Chinese medicine group was significantly higher than the control group. (Table 2).

Table 2. **The Hb changes over time in children with β-thalassemia**

Group	N	Hb/g·L^{-1}		
		Before therapy	6 weeks after treatment	12 weeks after treatment
Astragalus Chinese Medicine Group	58	67.02±12.11	76.30±7.80	82.25±6.10
Control group	50	66.64±9.20	67.24±8.44	66.20±11.50
P value		0.258	0.202	0.006

2.3 HbF ratio before and after treatment

The HbF ratio of the two groups before and after treatment was changed by covariance analysis with the pre-treatment level as a covariate, and the HbF ratio of the Chinese medicine group increased significantly after treatment (P<0.001); (Table 3).

Table 3. **The HbF proportions in children with β-thalassemia before and aftertreatment**

Group	N	Before therapy HbF%	After treatment HbF%
Astragalus Chinese Medicine Group	58	52.87±18.91	61.08±13.02
Control group	50	53.16±12.36	52.31±17.16
P value		0.402	<0.001

2.4 RBC changes before and after treatment in the two groups

Repeated measurement data analysis of variance showed that 6 weeks and 12 weeks after treatment were significantly higher than before self-treatment. However, the difference of RBC in the control group at each time point was not statistically significant. The t test showed that the difference in RBC indicators between the two groups after the end of the 12-week course of treatment was statistically significant, and the Chinese medicine group was significantly higher than the control group (P=0.036). (Table 4).

Table 4. **The RBC changes over time in children with β-thalassemia**

Group	N	RBC/*10^{12}·L^{-1}		
		Before therapy	6 weeks after treatment	12 weeks after treatment
Astragalus Chinese Medicine Group	58	3.54±0.65	3.70±0.43	3.78±0.51
Control group	50	3.46±0.35	3.48±0.22	3.52±0.38
P value		0.564	0.216	0.036

2.5 Comparison of KLF1 gene expression levels and TCM syndrome scores between the two groups

The relative copy number of KLF1 gene mRNA obtained by dividing the nucleic acid amount of the target gene by the nucleic acid amount of the internal reference gene is shown in Table 5. Repeated measurement data analysis of variance results showed that after treatment, the expression of KLF1 in the treatment group decreased and the disease was alleviated. The mRNA levels before and after treatment were repeated 3 times, and the difference between the two groups was statistically significant. Whether in the traditional Chinese medicine group or the control group, the difference between different time levels before and after treatment was also statistically significant (P<0.05). (Figure 5).

Table 5. **The KLF1 gene mRNA levels in children with β-thalassemia before and after treatment**

Group	N	KLF1 gene (Copy number)	TCM syndrome score
Astragalus Chinese Medicine Group	58	0.37±0.03	79.68±6.34
Control group	50	0.18±0.05	64.93±9.23
P value		<0.01	0.046

3. Discussion

Thalassemia is a hereditary chronic hemolytic anemia disease in which the synthesis of globin chains is impaired due to defects in autosomal genes. At present, the population of southern China has a very high rate of gene carrying thalassaemia. Taking Beijing as an example, the incidence of β-thalassaemia among children is as high as 11.26%, which is significantly higher than that of other parts of the country [10]. Children with severe and some intermediate β-thalassemias need to rely on long-term regular or intermittent blood transfusion and iron removal treatment, and the poor prognosis brings a heavy burden to the family and society. With the in-depth exploration of the pathophysiology and molecular basis of β-thalassemia, researchers have found that certain drugs such as hydroxyurea can induce high expression of γ-globin gene and re-synthesize HbF to compensate for β-globin synthesis obstacles. The reduction of HbA can also be combined with excess alpha chains to reduce the damage of alpha inclusion bodies to red blood cells, thereby achieving the purpose of treatment [1, 4]. However, various western medicines that induce gamma globin have obvious shortcomings, or cause immunosuppression, potential carcinogenesis, or short-lived efficacy and high price, which severely restrict their clinical application [11]. Compared with chemical western medicine, traditional Chinese medicine has less side effects. At present, the treatment of β-thalassemia with traditional Chinese medicine is still based on case reports and general clinical observations. In the previous series of studies, astragalus, dangshen and other traditional Chinese medicines such as spleen, nourishing qi and blood, and turtle shells were screened, and a series of in vitro experimental studies and in vivo clinical studies were carried out [12,13]. The traditional Chinese medicine for producing blood and essence has a good effect on up regulating the expression of gamma globin gene, inducing HbF synthesis, and improving anemia in children with beta thalassemia.

The results of the study show that traditional Chinese medicine can significantly increase the Hb level of children with β-thalassemia intermediate and can also improve the RBC of the children to a certain extent. Further alkaline hemoglobin electrophoresis test results showed that the proportion of HbF in children also increased significantly after treatment, suggesting that the increase in Hb levels was mainly due to the induced synthesis of HbF. KLF1 is a type of factor that has an inhibitory effect on the expression of γ-mRNA. Some scholars have pointed out that the low level of KLF1 can relieve the inhibition of γ-mRNA expression [14]. The results of this study showed that after treatment, the expression level of KLF1 in the treatment group decreased, while the level of

γ-mRNA increased, confirming the correctness of the above results. The reason for this result may be related to the treatment of the treatment group using the method of invigorating the kidney and marrow, but the specific pharmacological mechanism of this result needs further study.

In this study, the blood routine, γ-mRNA and KLF1 expression changes of thalassemia patients were compared and analyzed, and it was confirmed that the method of nourishing the kidney and marrow can effectively reduce the expression of KLF1 and increase the levels of Hb, WBC, Ret and γ-mRNA in thalassemia patients. It has a good clinical effect but has no obvious effect on the level of β-mRNA. In the next step of the study, the influence mechanism of the method of tonifying the kidney and marrow on the gene level of thalassemia patients will be further studied to explore its pharmacological mechanism and make a reasonable explanation for the results of this study.

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