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Original Article

Analysis of efficacy of pulse dye laser on infantile hemangioma and related factors affecting the prognosis

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Abstract: Objective: To investigate the effects of pulse dye laser surgery on inflammatory factors, skin lesions and prognosis of infantile hemangioma. Methods: A total of 120 infants with hemangioma who received treatment in Hainan Women and Children's Medical Center were elected as study subjects and divided into two groups. The control group (CG) was given timolol eye drops for external application (60 cases), and the observation group (OG) received treatment of pulsed dye laser (60 cases) in addition to the CG. The skin lesions and incidence of adverse events of the two groups were compared before and after intervention. The expression of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β1) and basic fibroblast growth factor (bFGF) in the two groups were detected by ELISA and RT-PCT before and after treatment. Independent risk factors affecting curative effects of infantile hemangioma were analyzed using Cox regression analysis. Results: The skin lesions in the OG were better than those in the CG. The total effective rate of the OG was remarkably higher than that of the CG. The incidence of adverse reactions in the OG was remarkably lower than that in the CG. After treatment, the expression of IL-2, IL-6 and mRNA and protein expression levels of VEGF, TGF-β1 and bFGF in the OG were significantly lower than those in the CG, while the level of IL-10 was significantly higher than the CG. Cox regression analysis showed that low birth weight, multiple pregnancies, maternal medication during pregnancy, family history of hemangioma, maternal occupation, high expression of IL-2, IL-6, VEGF, TGF-β1 and bFGF, and low expression of IL-10 were all independent risk factors affecting the prognosis in infants with hemangioma. Conclusion: Pulsed dye laser treatment can promote the regression of skin lesions in infantile hemangioma, reduce the levels of inflammation and improve the levels of growth factors.

Keywords: Hemangioma, pulsed dye laser, inflammatory factor, skin lesion

Introduction

Infantile hemangioma, originating from hemangioma stem cells [1], is the most common vascular tumor found in childhood; with permanent local tissue damage, ulcers, infections and even functional effects [2]. Clinically, such diseases can spontaneously subside after proliferation, often making medical providers believe that they can be cured without intervention. Due to the rapid development of the disease, however, it will eventually give rise to dysfunction or permanent disfigurement [3, 4]. Therefore, it is clinically recommended to detect and treat infantile hemangioma as early as possible. Although there are many treatments for hemangioma [5], single drug therapy is not effective, and there is an urgent need to focus on novel treatments that disrupt the process of hemangioma.

Timolol eye drops are a type of adrenergic receptor antagonist [6]. Clinically, 0.5% timolol solution was found to be suitable for small superficial hemangioma and facial hemangioma, with ease of management and minimal risk of systemic side effects. Although the treatment effect is clear, there are side effects such as sleep disorders [7], and single drug treatment is not effective in treating infantile hemangioma. Pulsed dye laser can emit high-energy yellow light beams, which can be used to treat a variety of vascular and non-vascular diseases [8], and has a good effect on various benign skin vascular lesions. Its wavelength and duration are optimized for the selective

therapy of vascular lesions [9]. Studies have shown that pulsed dye laser has been considered as the gold standard for treatment of skin vascular disease clinically [10]. Research of pulsed dye laser in infant hemangioma shows that as the pulsed dye laser has a long pulse width, it can cause heat coagulation of various blood vessels in infants, thus reducing the recurrence and proliferation of hemangioma in infants, and promoting the extinction of tumors in infants, which is considered to be a non-invasive and safe treatment [11].

At present, clinical evidence shows that a single drug treatment of infantile hemangioma effect is poor. Therefore, this study analyzed the combination of timolol eye drops and pulsed dye laser to observe the effect of the combination on inflammatory factors and skin lesions in infants, and to observe the risk factors affecting the treatment effect; so as to provide a better reference for the treatment of infants with hemangioma.

Data and methods

General data

A total of 120 cases of infants with hemangioma in the neonatology department of Hainan Women and Children's Medical Center were elected as study subjects. They were divided into two groups in accordance with the treatment plan, the control group (CG) was treated with external application of timolol eye drops (60 cases), and the observation group (OG) received treatment of pulsed dye laser based on the external application of timolol eye drops (60 cases). There were 33 males and 45 females in the OG, aged (3.53±0.49) months, with the tumor area of (2.07±0.15) cm^2. There were 35 males and 25 females in the CG, aged (3.48±0.54) months, with the tumor area of (2.12±0.14) cm^2. These experiments were submitted to the Hainan Women and Children's Medical Center ethics committee for review and implemented after obtaining the approval. Both subjects and their guardians have signed the full informed consent. Inclusion criteria were as follows: Patients whose hemangioma was confirmed by imaging [12]. Patients did not receive treatment of chemotherapy or radiotherapy. Patients were diagnosed for the first time. Patients did not have major organ dysfunction. Patients whose clinicopathological data were complete. Exclusion criteria were as follows: Patients who were combined with other immune functional diseases, organ failure, or drug allergies. Patients who were lost to follow up.

Therapies

In the CG, the infants were given timolol eye drops for external application: it was evenly applied on the external surface of the hemangioma of the infants, and covered with cotton balls that had been soaked with drugs, and then sealed with plastic wrap and fixed for 1 h. The infants were treated twice a day for 3-6 months according to their conditions. Infants in the OG were treated with pulsed dye laser treatment in addtion to CG: the sunfleck was adjusted to 7 mm and less than 20% when overlapped sunfleck was observed, the pulse width was adjusted to 1.5 ms, and the energy density was adjusted for 10.5-12.5 J/cm^2. The energy was adjusted according to the skin purpura, lesions and conditions after treatment of infants, to guarantee maximum effectiveness for each infant and minimizes skin damage. After the treatment, the infant was given an ice pack for external application. When the treatment was repeated, it was performed at the position where the scab fell off. The time between pulse dye laser treatment would be 4 to 6 weeks depending on the condition of the infant.

Outcome measures

(1) Skin color integral (integral score of 0-3, infants with good recovery had lower scores) and skin lesion integral (integral score of 0-3, infants with good recovery had lower scores) of infants in the two groups during treatment were compared.

(2) Clinical effect: marked effect: the skin color was normal, the tumor area was reduced and >75%, the function of the infant recovered, without recurrence. Effective: the appearance of the skin was close to the normal color, or with a little pigmentation, and the tumor reduced by more than 50%. Ineffective: the tumor, pigment and clinical symptoms were not improved after treatment. Total effective rate = (marked effect + effective)/total cases × 100%.

(3) Adverse safety hazards of the two groups of infants in the process of treatment were observed and recorded.

Detection methods

(1) Determination of expression of serum IL-2, IL-6, and IL-10 and protein expression levels of bFGF, VEGF, TGF-β1: 5 mL of venous blood was collected 1 d before treatment and early in the morning after treatment, centrifuged 1500 × g at 4°C for 10 min and stored in -70°C freezer for future use. Expression level in serum was detected using ELISA, and the procedures were carried out according to the instructions for IL-2, IL-6, IL-10, VEGF, TGF-β1, and bFGF (Shanghai Hengfei Biotechnology Co., Ltd., Art. No.: bs-4586R-2, bs-0782R-2, SEA056Gu, bs-0279R-1, 130-095-066, bs-0217R-1).

(2) Detection of mRNA expression of VEGF, TGF-β1 and bFGF in peripheral blood: Total RNA extraction kit (Shanghai Hengfei Biotechnology Co., Ltd., Art. No.: R1200) was used to extract total RNA in peripheral blood, and then reverse transcription kit (Shanghai Hengfei Biotechnology Co., Ltd., Art. No.: 76405-69506-59395) was used to reverse-transcribe RNA into cDNA, followed by polymerase chain reaction. PCR amplification conditions were as follows: predenaturation at 95°C for 5 min, denaturation at 95°C for 30 s, denaturation at 58°C for 30 s, and denaturation at 72°C for 20 s. GAPDH, VEGF, TGF-β1, and bFGF were subjected to 25, 26, 32, and 32 cycles, respectively, and extended at 72°C for 10 minutes. The resulting mRNA was observed using a gel imaging system. Target gene optical density/β-action optical density = mRNA expression.

Statistical analysis

Analysis was performed using SPSS 21.0 statistical software (EASYBIO, China). Intra-group enumeration data were represented by number of cases/percentage [n (%)]. Comparison of enumeration data between groups was qualified by chi-square test. When the theoretical frequency in chi-square test was less than 5, the continuous correction chi-square test was used. Measurement data were represented by mean ± standard deviation (x ± sd). Independent t-test was applied for comparison of measurement data between groups, paired t-test was applied for comparison within groups. Repeated measures was used for observation and comparison at multiple time points, and Bonferroni method was used for pairwise comparison at different time points in the group. P< 0.05 was considered statistically significant.

Results

General data

There was no notable difference between the two groups in gender, age, tumor area, location, type of hemangioma, mode of production, low birth weight, multiple pregnancy, maternal medication during pregnancy, family history of hemangioma, parental smoking history, parental drinking history and other clinical baseline data (P>0.05). As shown **Table 1**.

Skin lesions

Before intervention, there was no difference in skin color integral and skin damage range integral between the two groups (P>0.05). However, the two were improved after the intervention, and the score of the OG was significantly lower than that of CG (P<0.05). As shown in **Table 2**.

Total effective rate

After intervention, the total effective rate of the OG was 93.33%, and that of the CG was 80.00%. The total effective rate after intervention was higher in the OG than in the CG (P<0.05). As shown in **Table 3**.

Adverse reactions

During the treatment of intervention, both groups of infants had adverse reactions such as local blisters and ulcer. The total adverse reactions of the two groups were significantly different, with 8.33% in the OG and 21.67% in the CG. The reactions were lower in the OG than the CG (P<0.05). As shown in **Table 4**.

Inflammatory factors

There was no significant difference in the expression levels of IL-2, IL-6 and IL-10 between the two groups before the intervention (P>0.05). After intervention, serum IL-2 and IL-6 levels in the OG were significantly lower than the CG, while serum IL-10 level was notably higher than the CG (P<0.05). As shown in **Figure 1**.

Growth factor

Before intervention, there was no difference in mRNA and protein expression of VEGF, TGF-β1, and bFGF in the two groups of infants (P>0.05). While mRNA and protein expression levels of growth factors were improved after the inter-

Table 1. General data between the two groups [n (%)] ($\bar{x} \pm sd$)

Classification	Observation group (n=60)	Control group (n=60)	t/χ^2 value	*P* value
Gender			0.136	0.713
Male	33 (55.00)	35 (58.33)		
Female	45 (45.00)	25 (41.67)		
Age (months)	3.53±0.49	3.48±0.54	0.531	0.596
Tumor area (cm^2)	2.07±0.15	2.12±0.14	1.888	0.062
Location			0.711	0.701
Head and neck	33 (55.00)	36 (60.00)		
Trunk	17 (28.33)	13 (21.67)		
Limbs	10 (16.67)	11 (18.33)		
Type of hemangioma			0.407	0.939
Strawberry Hemangioma	27 (45.00)	25 (41.67)		
Mixed hemangioma	15 (25.00)	16 (26.67)		
Cavernous hemangioma	11 (18.33)	10 (16.67)		
Hemangioma racemosum	7 (11.67)	9 (15.00)		
Mode of producing			0.300	0.584
Eutocia	28 (46.67)	31 (51.67)		
Cesarean	32 (53.33)	29 (48.33)		
Low birth weight			0.586	0.444
Yes	37 (61.67)	41 (68.33)		
No	23 (38.33)	19 (31.67)		
Multiple pregnancies			0.304	0.581
Yes	35 (58.33)	32 (53.33)		
No	25 (41.67)	28 (46.67)		
Maternal medication during pregnancy			0.135	0.714
Yes	28 (46.67)	26 (43.33)		
No	32 (53.33)	34 (56.67)		
Family history of hemangioma			0.564	0.453
Yes	25 (41.67)	21 (35.00)		
No	35 (58.33)	39 (65.00)		
Parental smoking history			2.344	0.126
With	35 (58.33)	43 (71.67)		
Without	25 (41.67)	17 (28.33)		
Parental drinking history			0.141	0.707
With	38 (63.33)	36 (60.00)		
Without	22 (36.67)	24 (40.00)		

Table 2. Skin lesions of two groups of infants ($\bar{x} \pm sd$)

Groups	Skin color integral		Skin damage range integral	
	Before intervention	After intervention	Before intervention	After intervention
Observation group (n=60)	2.38±0.25	0.56±0.12	2.51±0.56	0.21±0.01
Control group (n=60)	2.34±0.21	1.56±0.19	2.52±0.57	1.45±0.25
t	0.949	34.470	0.097	38.390
P	0.345	<0.001	0.923	<0.001

vention, and mRNA and protein expression levels of the infants in the OG were significantly lower than those in the CG (P<0.05). As shown in **Figure 2**.

Table 3. Comparison of total effective rate between the two groups after intervention [n (%)]

Class	n (%)	Markedly effect	Effective	Ineffective	Total effective rate
Observation group	60	40 (66.67)	16 (26.67)	4 (6.67)	56 (93.33)
Control group	60	28 (46.67)	20 (33.33)	12 (20.00)	48 (80.00)
χ^2 value	-	-	-	-	4.615
P value	-	-	-	-	0.032

Table 4. Comparison of total adverse reaction rate between the two groups after intervention [n (%)]

Class	Local blisters	Ulcer	Nausea and vomiting	Loss of appetite	Total adverse reaction rate
Observation group (n=60)	1 (1.67)	1 (1.67)	2 (3.33)	1 (1.67)	5 (8.33)
Control group (n=60)	3 (5.00)	3 (5.00)	5 (8.33)	2 (3.33)	13 (21.67)
χ^2 value	1.034	1.034	1.365	0.342	4.183
P value	0.309	0.309	0.243	0.559	0.041

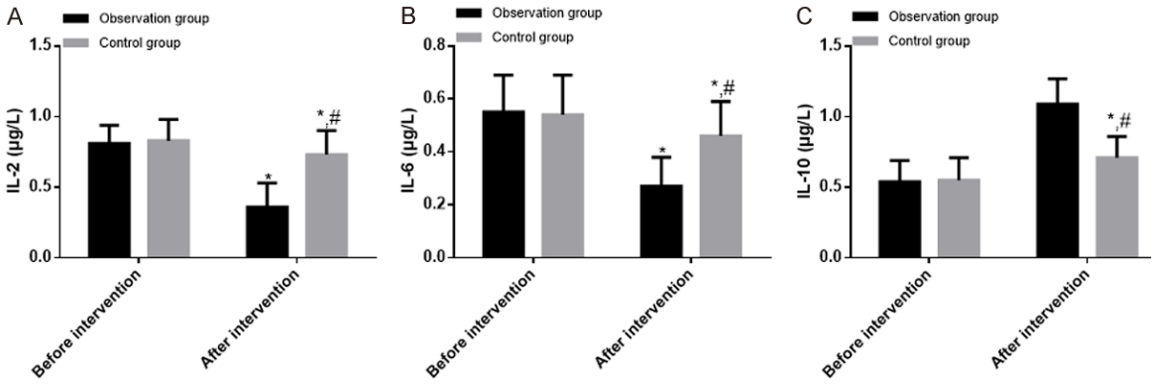

Figure 1. Comparison of inflammatory factors before and after intervention between the two groups. A. The IL-2 level in the OG was significantly lower than that in the CG after intervention. B. The level of IL-6 in the OG was significantly lower than that in the CG after intervention. C. The level of IL-10 in the OG was significantly lower than that in the CG after intervention. Note: Compared with before intervention, * <0.05. Compared with the CG after intervention, # <0.05.

Analysis of risk factors influencing curative effect

Infants were divided into an effective group and an ineffective group according to the curative effect. Among them, 104 cases were effective after treatment and 16 cases were ineffective. The univariate analysis of general factors was performed, and it was found that there was no notable difference in gender, age, tumor area, site of occurrence, type of hemangioma, and mode of production (P>0.05). While there were differences in low birth weight, multiple pregnancy, maternal medication during pregnancy, family history of hemangioma, maternal occupation, IL-2, IL-6, IL-10, VEGF, TGF-β1 and bFGF (P<0.05). As shown in **Table 5**. Significant indicators in univariate analysis were incorporated into Cox proportional hazard regression, and it was found that low birth weight, multiple pregnancy, maternal medication during pregnancy, family history of hemangioma, maternal occupation, high expression of IL-2, IL-6, VEGF, TGF-β1 and bFGF, and low expression of IL-10 were all independent prognostic factors for infants with hemangioma (P<0.05). As shown in **Table 6**.

Discussion

Infant hemangioma is a common benign hemangioma with a unique life cycle. It proliferates during the first few months of life, and then has a invagination period that lasts for several years [13]. It can occur anywhere on the skin and mucosal surface of infants, and about 50%

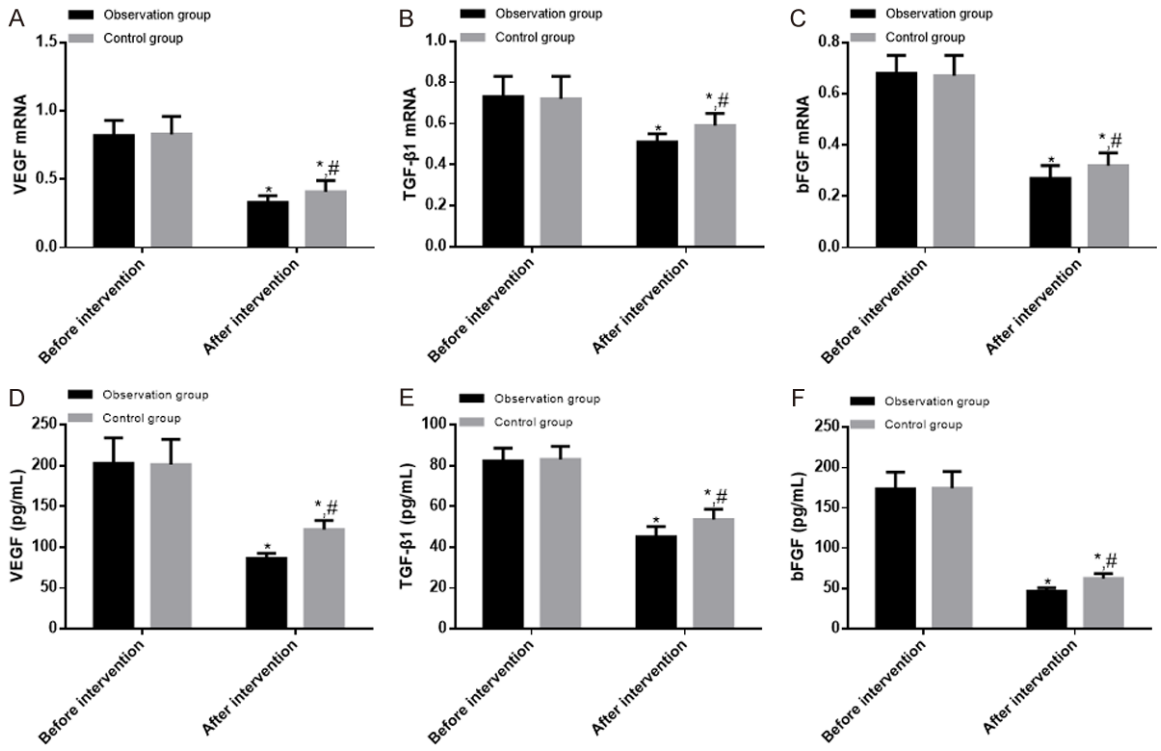

Figure 2. Determination of growth factor level in peripheral blood before and after intervention in the two groups. A. VEGF mRNA level in the OG was significantly lower than that in the CG after intervention. B. TGF-β1 mRNA level in the OG was significantly lower than that in the CG after intervention. C. bFGF mRNA level in the OG was significantly lower than that in the CG after intervention. D. The expression level of VEGF protein in the OG was significantly lower than that in the CG after intervention. E. TGF-β1 protein expression level was significantly lower in the OG than in the CG after intervention. F. The expression level of bFGF protein in the OG was significantly lower than that in the CG after intervention. Note: Compared with before intervention, * <0.05. Compared with the CG after intervention, # <0.05.

Table 5. Results of univariate analysis on curative effect of infants with hemangioma

Classification	Effective group (n=104)	Ineffective group (n=16)	t/χ^2 value	*P* value
Gender			0.001	0.971
Male	59 (56.73)	9 (56.25)		
Female	45 (43.27)	7 (43.75)		
Age (years old)	3.55±0.48	3.46±0.55	0.685	0.495
Tumor area (cm^2)	2.06±0.15	2.14±0.17	1.951	0.053
Location			5.361	0.069
Head and neck	64 (61.54)	5 (31.25)		
Trunk	24 (23.08)	6 (37.50)		
Limbs	16 (15.38)	5 (31.25)		
Type of hemangioma			3.49	0.326
Strawberry Hemangioma	47 (45.29)	5 (31.25)		
Mixed hemangioma	28 (26.92)	3 (18.75)		
Cavernous hemangioma	17 (16.35)	4 (25.00)		
Hemmangioma racemosum	12 (11.53)	4 (25.00)		
Mode of producing			0.371	0.543
Eutocia	50 (48.08)	9 (56.25)		
Cesarean	54 (51.92)	7 (43.75)		
Low birth weight			4.108	0.043

Yes	64 (61.54)	14 (87.50)		
No	40 (38.46)	2 (12.50)		
Multiple pregnancies			4.836	0.028
Yes	54 (51.92)	13 (81.25)		
No	50 (48.08)	3 (18.75)		
Maternal medication during pregnancy			4.207	0.040
Yes	43 (41.35)	11 (68.75)		
No	61 (58.65)	5 (31.25)		
Family history of hemangioma			18.881	<0.001
Yes	25 (41.67)	21 (35.00)		
No	35 (58.33)	39 (65.00)		
Maternal occupation			6.358	0.012
Unemployed	67 (64.42)	5 (31.25)		
Manual work	37 (35.58)	11 (68.75)		
IL-2			5.599	0.018
High expression	45 (43.27)	12 (75.00)		
Low expression	59 (56.73)	4 (25.00)		
IL-6			4.857	0.028
High expression	41 (39.42)	11 (68.75)		
Low expression	63 (60.58)	5 (31.25)		
IL-10			5.710	0.017
High expression	71 (68.27)	6 (37.50)		
Low expression	33 (31.73)	10 (62.50)		
VEGF			8.010	0.005
High expression	45 (43.27)	13 (81.25)		
Low expression	59 (56.73)	3 (18.75)		
TGF-β1			6.713	0.010
High expression	42 (40.38)	12 (75.00)		
Low expression	62 (59.62)	4 (25.00)		
bFGF			5.571	0.018
High expression	39 (37.50)	11 (68.75)		
Low expression	65 (62.50)	5 (31.25)		

of it occurs in the head and neck [14]. With familial heritability, the rapid growth of the disease makes it impossible for clinicians to judge the occurrence of complications [15]. Therefore, early intervention in the treatment of infantile hemangioma is particularly important.

Timolol has antiangiogenic property *in vitro* and *in vivo* [16], while in infantile hemangioma, timolol may be involved in antagonistic action of the β-norepinephrine receptor, resulting in various processes, such as vasoconstriction, inhibition of angiogenesis and stimulation of cell apoptosis [17]. Studies have revealed that [18] timolol is well tolerated and has a moderate to good effect on infantile hemangioma. Laser therapy can treat a variety of skin diseases in infants [19]. Previous studies have shown [20] that clinical treatment of congenital vascular tumors is subject to other complications, and because of the specificity and calculable response, laser therapy provides the best way for local treatment and can retain the unaffected upper cortex. In this study, the skin color integral and skin damage range integral were lower in the OG than in the CG. This indicated that the OG had a clear curative effect after treatment, which could effectively improve the skin lesions of the infants, and better promote the regression of skin lesions in the infants. In the study of Asilian A et al. [21], laser therapy combined with timolol had a better effect on infantile hemangioma, with a high clearance rate for hemangioma and fewer adverse reac-

Table 6. Results of multivariate analysis on prognosis of infants with hemangioma

Item	Multivariable	
	HR (95% CI)	P
Maternal occupation	1.790 (1.016~3.153)	0.044
Low birth weight	1.895 (1.028~3.495)	0.041
multiple pregnancy	1.636 (1.053~2.541)	0.029
Maternal medication during pregnancy	2.086 (1.497~2.907)	<0.001
Family history of hemangioma	5.048 (2.412~10.563)	<0.001
IL-2	1.462 (1.041~2.677)	0.035
IL-6	1.771 (1.132~2.742)	0.012
IL-10	1.664 (1.128~2.873)	<0.001
VEGF	1.719 (1.121~3.910)	<0.001
TGF-β1	2.325 (1.146~3.969)	<0.001
bFGF	3.587 (2.167~7.012)	<0.001

tions. In addition, in the study of Sun X et al. [22], drugs combined with laser therapy were used to treat infants with hemangioma. Although symptoms such as ulcer, nausea and vomiting occurred in patients, all of them were controlled. This is similar to the consequences of this study. We compared the total effective rate of the two groups of infants after treatment and found that the total effective rate increased after treatment and intervention, and it in the OG was higher than the CG, which further proved that laser treatment with timolol could improve the treatment effect of infantile hemangioma and improve the appearance. By observing the adverse reactions in the treatment process, we found that the adverse reactions in the OG were remarkably lower than that in the CG, suggesting that the adverse reactions of laser therapy plus timolol in the treatment of infantile hemangioma were slight and safe.

Studies have illustrated that the rapid growth of infantile hemangioma in the early stage will destroy the normal tissues in the body and indicated that serum cytokine levels can be used as markers of hemangioma growth [23]. Through observing serum inflammatory factors in infants before and after treatment, it was found that the levels of inflammatory factors after intervention in the two groups were lower than before treatment, indicating that the inflammatory response in infants could be suppressed by treatment. Moreover, the levels of IL-2, IL-6 in the OG were lower than the CG, while IL-10 level in the OG was higher than the CG, suggesting that laser treatment combined with timolol could reduce proinflammatory factors in patients and increase the level of anti-inflammatory factors. The formation of hemangioma is related to the abnormal proliferation of vascular endothelial cells, and is attributed to the imbalance between angiogenic cytokines and angiogenesis inhibitors, suggesting that VEGF, bFGF and MMP-9 produce a marked effect on the formation of hemangiomas [24]. Wang F et al. [25] elaborated in their study that the expression of VEGF and bFGF were closely related to the proliferation of endothelial cells in hemangioma, indicating that both VEGF and bFGF were highly expressed in serum of infants. TGF-β1 is extremely important for wound repair in many organs, and its expression in traumatic tissues is elevated to facilitate mitosis of vascular endothelial cells and assist in body repair [26]. The results of this paper reveal that the expression of mRNA and protein of VEGF, TGF-β1 and bFGF in the OG were lower than those in the CG after intervention. Seeing the results of this study, we hypothesized that during the onset of cutaneous hemangiomas in infants, a large number of growth factors released by the lesions promoted the growth of the disease and destroyed the vascular endothelial cells, but pulse laser treatment plus timolol could interrupt this vicious link and achieve a continuous treatment effect. In the end, we performed univariate and multivariate analysis on the poor prognosis of infants with hemangioma. The results showed that low birth weight, multiple pregnancies, maternal medication during pregnancy, family history of hemangioma, maternal occupation, high expression of IL-2, IL-6, VEGF, TGF-β1, bFGF, and low expression of IL-10 were all independent risk factors affecting the curative effect infants with hemangioma.

Although this study proves that pulsed laser therapy plus timolol is a feasible method for the treatment of infants with hemangioma, there is still some room for improvement. For example, the effect on tumor formation in mice could be supplemented, and the mechanism between

inflammatory factors, growth factors and infantile hemangioma could be further verified through basic experiments, and a variety of samples for analysis could be collected to improve the results.

In summary, pulsed dye laser treatment can promote the regression of skin lesions in infants with hemangioma, reduce the levels of inflammatory and improve the level of growth factors.

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Disclosure of conflict of interest

None.

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