Int J Clin Exp Med 2026;19(1):11-19
www.ijcem.com /ISSN:1940-5901/IJCEM0165076

Original Article

Multivariate analysis of recurrence in patients with melasma after Q-switched Nd:YAG 1064-nm laser treatment: a prospective observational study

Junmo Yang[1], Ke Lu[2], Pingsong Li[2]

[1]*Yangzhou University School of Medicine, Yangzhou 225001, Jiangsu, China;* [2]*Department of Medical Cosmetology, Northern Jiangsu People's Hospital, Yangzhou 225001, Jiangsu, China*

Received April 5, 2025; Accepted August 11, 2025; Epub January 15, 2026; Published January 30, 2026

Abstract: Objectives: This study identifies multifactorial predictors integrating molecular biomarkers and clinical profiles for melasma recurrence after Q-switched Nd:YAG 1064-nm laser therapy. Methods: A prospective observational study analyzed 104 melasma patients (18-55 years) treated between January-December 2024. Serum levels of bFGF, VEGF, and TGF-β were quantified via protein microarray and ELISA. Clinical variables and serum biomarkers were assessed using Mann-Whitney U tests, univariate analysis and multivariate logistic regression. Results: Within 12 months post-treatment, 31 patients (29.81%) experienced melasma recurrence. The study revealed abnormal serum levels of growth factor proteins in the recurrence group. Notably, there were increased levels of bFGF (P=0.002) and VEGF (P=0.001), as well as decreased levels of TGF-β (P<0.001). Univariate and multivariate logistic regression analysis identified several factors as high-risk indicators for melasma recurrence, including age >30 years (OR=4.854, 95% CI=1.665-13.956), sun exposure >16 hours per month (OR=6.027, 95% CI=2.144-16.843), negative psychological state (OR=3.638, 95% CI=1.403-11.892), sleep duration <8 hours (OR=3.174, 95% CI=1.245-9.372), seasonal changes (OR=5.541, 95% CI=2.043-16.285), sensitive skin (OR=2.452, 95% CI=1.134-7.372), serum bFGF levels >32.5 ng/L (OR=6.376, 95% CI=2.242-15.154), and VEGF levels >34.7 ng/L (OR=6.345, 95% CI=1.729-16.583). Conversely, serum TGF-β levels >3713.4 ng/L were found to have a protective effect (OR=0.11, 95% CI=0.05-0.23). Conclusions: Recurrence post-laser melasma treatment involves synergistic interactions between angiogenic biomarkers (bFGF/VEGF), immunosuppressive TGF-β, and modifiable lifestyle factors. These findings emphasize dual targeting of molecular pathways and behavioral interventions to mitigate recurrence.

Keywords: Multivariate analysis, recurrence, melasma, Q-switched Nd:YAG 1064-nm laser treatment, prospective observational study

Introduction

Melasma is a prevalent chronic skin pigmentation condition, predominantly impacting women, youth, and those with darker skin tones [1]. It is distinguished by the development of irregular brown or gray patches on the face, neck, and occasionally the upper limbs [2]. The treatment strategy for melasma mainly targets slowing down the proliferation rate of melanocytes, preventing the formation of melanosomes, and accelerating their degradation process. Treatment methods usually include the use of compounds such as hydroquinone or kojic acid [3], which may also include keratolytic agents such as retinoic acid and glycolic acid [4]. Other treatment options include phototherapy, microdermabrasion, and energy-based therapies, such as laser treatment using CO_2, erbium:YAG, or Q-switched Nd:YAG lasers. The Q-switched laser at 1064 nm is commonly used in the treatment of melasma due to its deeper penetration and relative safety in darker skin types [5]. However, patients may still experience recurrences or new hyperpigmentation issues after laser treatment.

Managing melasma, especially in cases of treatment resistance, remains a challenge. The recurrence mechanism of melasma involves multiple factors, including ultraviolet exposure, fluctuations in hormone levels, thyroid issues,

https://doi.org/10.62347/JIYV8739

improper skincare measures, and the use of specific drugs (e.g., phenytoin sodium) [3]. The pathology of melasma is characterized by skin inflammation with reactive oxygen species [6]. These excessive reactive oxygen species can activate tyrosinase, thereby increasing the synthesis of melanin. The formation of melasma is closely related to the disruption of the oxidation-antioxidant balance [7]. Moreover, various interleukins and cytokines can stimulate melanocyte proliferation, up-regulate melanin production, and promote the transfer of melanosomes [8-10]. Therefore, the recurrence of melasma after laser treatment is likely related to skin inflammation. However, the characteristic inflammatory factors have not yet been identified.

To date, no studies have systematically and comprehensively explored the factors influencing the recurrence of melasma after laser treatment. This study adopts a prospective design and includes patients with melasma receiving Q-switched Nd:YAG 1064-nm laser treatment. Data on daily activities, treatment processes, and prognosis are collected through hospital medical records, questionnaires, and follow-ups. Protein microarray testing is conducted on the collected serum samples to explore the related factors of recurrence and hyperpigmentation after laser skin-whitening treatment. The aim is to provide reference evidence for the clinical prevention and treatment of hyperpigmentation and to offer theoretical support for the development of effective nursing strategies. In addition, we investigated the association between inflammatory factors and the recurrence, with the hope of providing a new method for predicting the recurrence of melasma after laser treatment.

Materials and methods

Study patients

This study analyzed patients with melasma who received Q-switched Nd:YAG 1064 nm laser treatment at the Beauty Center of Northern Jiangsu Provincial People's Hospital from January to December 2024. The study subjects ranged in age from 18 to 55 years. The inclusion criteria: 1) voluntary participation and completion of follow-up; 2) provision of complete personal information. The exclusion criteria: 1) uncontrolled chronic diseases such as infectious diseases, hypertension, diabetes, chronic liver disease, or kidney disease; 2) a history of keloid or hypertrophic scars; 3) suffering from other pigmentation diseases, or recent use of pigmentation-enhancing drugs; 4) a history of corticosteroid use; 5) pregnancy; 6) having received facial chemical peels, filler injections, plastic surgery, or laser treatment in the past six months. This study received approval from the Research Ethics Committee of Northern Jiangsu Provincial People's Hospital (Approval Number: 2023120505). All participants provided written informed consent after being fully informed of the study purposes, procedures, potential risks, and benefits, and that they have the right to withdraw from the study at any time without consequence.

The sample size estimation was based on a recurrence rate of approximately 30% after laser treatment for melasma reported in previous literature [5]. With α=0.05 (two-sided) and β=0.20 (80% statistical power), according to the estimation formula [11], at least 98 patients were calculated to be included after accounting for a 10% loss to follow-up rate. This study ultimately enrolled 104 patients, which met this requirement.

The Q-switched Nd:YAG 1064-nm laser treatment

During the laser treatment process, all operations were completed by two experienced dermatologists and one assistant. The used Q-switched Nd:YAG 1064 nm laser equipment had predefined and standardized irradiation parameters, including a fixed total number of treatments and specific irradiation parameters. The specific laser parameter settings were: energy density of 4 J/cm^2, pulse duration of 0.3 ms, spot diameter of 8 mm, and a frequency of 10 Hz [6].

Serum sample collection and protein microarray detection

At the first postoperative follow-up, we collected a 3 mL blood sample from the patients and separated the serum supernatant through a centrifugation process. The serum samples were then stored at -80°C for subsequent analysis. Next, we used protein microarray technology (specifically Raybiotech's quantitative protein microarray, product number AAH-GF-1) to detect the protein expression profiles in the serum samples and investigate the differences

in growth factor protein expression between the melasma recurrence group and the non-recurrence group. Through cluster analysis, we identified proteins with specific expression from the recurrence group that may have potential indicative roles in the recurrence of melasma.

Enzyme-linked immunosorbent assay verification of growth factors

This study utilized enzyme-linked immunosorbent assay (ELISA) to assess the levels of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β) in serum (abcam, USA, catalogue numbers: ab99979, ab209882, ab100647). All ELISA experiments were conducted according to the manufacturer's instructions provided with the respective kits.

Data processing and statistical analysis

In the analysis of protein microarray data, we normalized the raw data using R.page to maintain consistency and comparability of the results. Subsequently, we performed moderated t-tests using the limma package to assess protein expression levels. To address multiple comparisons in protein microarray analysis, the Benjamini-Hochberg (BH) method was applied to control the false discovery rate (FDR). The criteria for screening differentially expressed proteins were the BH-corrected adjusted *P*-values and logFC (i.e., the logarithm of the fold change in expression levels). Then, we identified differentially regulated proteins through clustering analysis.

For the analysis of clinical pathological characteristics and serum proteins, we used SPSS 26.0 software (IBM, Armonk, NY, USA). Qualitative variables were reported in terms of numbers and percentages, and their differences were tested using the chi-square test. For quantitatively distributed data that did not conform to a normal distribution, we used the median of the interquartile range (IQR) to describe and compare the data between groups, and Mann-Whitney U tests were applied. To adjust for potential confounding factors, we conducted multivariate logistic regression analysis to evaluate the effectiveness of different predictive tools in predicting disease recurrence. The cut-off values for serum proteins bFGF, VEGF, and TGF-β were determined based on the median values of all melasma patients. All *P*-values were two-tailed, with $P<0.05$ serving as the threshold for statistical significance.

Results

Incidence of recurrence and hyperpigmentation after Q-switched Nd:YAG 1064-nm laser treatment for melisma

This study included a total of 104 patients with melasma who underwent laser treatment. Follow-up results at 12 months post-treatment showed that there were 31 patients with recurrence and hyperpigmentation, with a recurrence rate of 29.81% (31/104).

Serum levels of bFGF, VEGF and TGF-β were increased in patients with melasma recurrence after Q-switched Nd:YAG 1064-nm laser treatment

The microarray analysis of serum growth factors indicates that compared to the non-recurrence group, the recurrence group shows differences in the expression of 50 proteins, with 22 proteins upregulated and 28 proteins downregulated (**Figure 1A**). Notably, the expression of bFGF and VEGF is the most significantly upregulated (FDR-adjusted $P<0.05$), while the expression of TGF-β is the most significantly downregulated (FDR-adjusted $P<0.05$) (**Figure 1B**). ELISA analysis further validated these results, showing that the levels of bFGF and VEGF in the serum of patients with recurrence were significantly higher than those in non-recurrent patients (**Figure 1C** and **1D**, both $P<0.05$), whereas the level of TGF-β was significantly lower in patients with recurrence (**Figure 1E**, $P<0.05$). These data suggest that the levels of serum bFGF, VEGF, and TGF-β are associated with the recurrence of melasma after Q-switched Nd:YAG 1064-nm laser treatment. Therefore, we chose these three proteins for further study.

Univariate analysis of recurrence and hyperpigmentation after Q-switched Nd:YAG 1064-nm laser treatment for melisma

Univariate analysis did not identify any significant differences between the recurrence and non-recurrence groups in gender, alcohol history, smoking history, the frequency of facial cleansing, and liver function ($P>0.05$, **Table 1**). However, significant differences were observed between the two groups in age, family history,

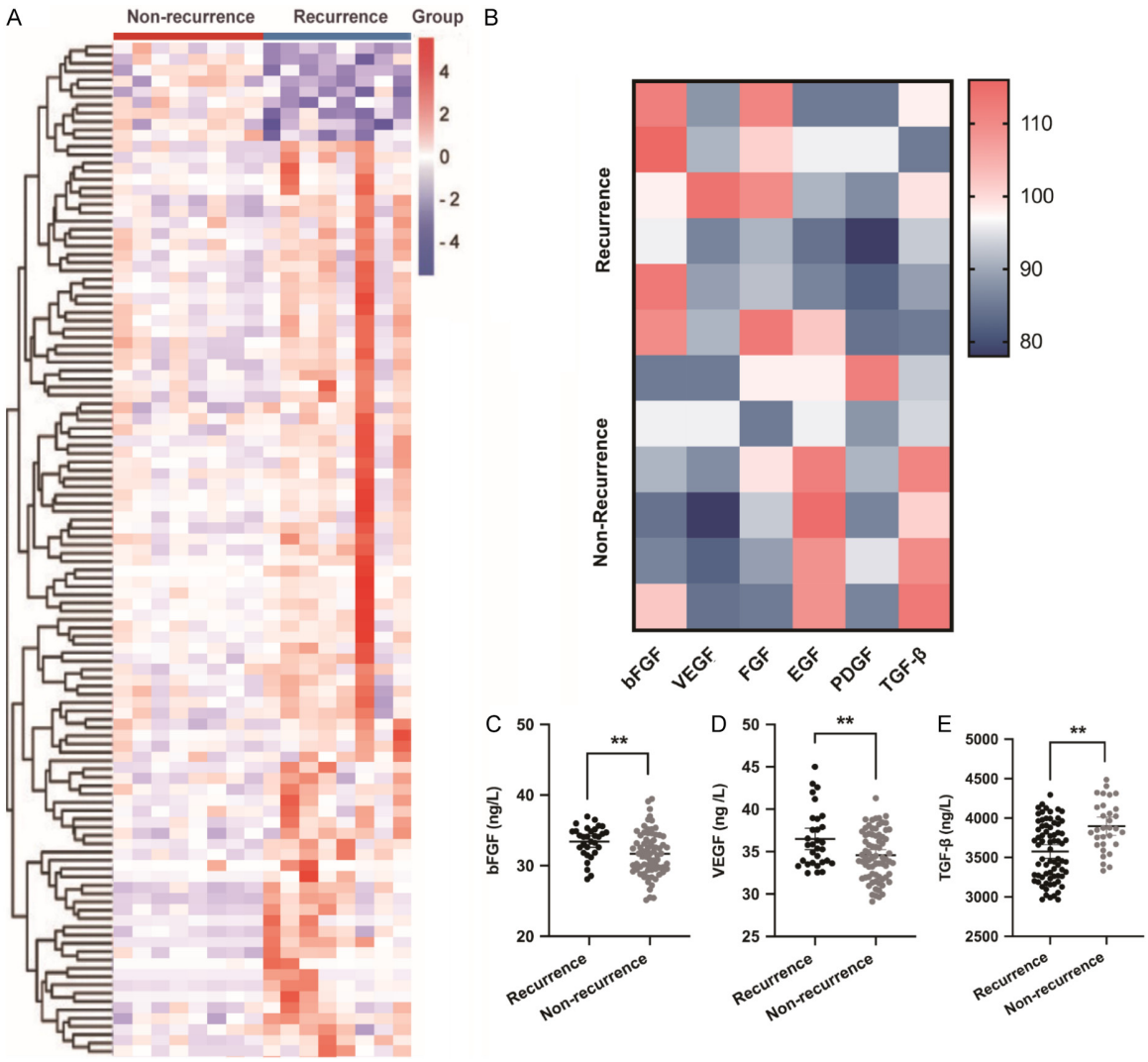

Figure 1. Growth factor analysis of recurrence in patients with melasma after the laser treatment. (A) The soluble growth factors in the human antibody array analysis were employed. The normalized array data for all proteins were analyzed, and the relative concentrations of these factors were represented as "heatmaps". (B) The 3 proteins with the highest upregulation (bFGF, VEGF, and FGF) and the three proteins with the highest downregulation (TGF-β, PDGF, and EGF) were represented as "heatmaps". All reported *P*-values are FDR-adjusted. (C-E) ELISA was used to compare the levels of serum bFGF (C), VEGF (D), and TGF-β (E) between recurrent patients and non-recurrent patients. *P<0.05, **P<0.01.

frequency of sun exposure, usage of skin-colored retinoic acid, psychological emotional state, sleep duration, seasonal changes, sensitive skin, and serum levels of bFGF, VEGF, and TGF-β (P<0.05, **Table 1**).

Multivariate logistic regression analysis of recurrence and hyperpigmentation after Q-switched Nd:YAG 1064-nm laser treatment for melisma

After univariate analysis, 11 factors related to recurrence were identified and assigned values, as detailed in **Table 2**. The cut-off values for serum proteins bFGF, VEGF, and TGF-β were determined based on the median values of all melasma patients. Further multivariate logistic regression analysis revealed that factors such as age over 30 years, average monthly sun exposure of 16 to 30 hours, seasonal changes, sleep duration less than 8 hours, non-use of skin-colored retinoic acid, negative psychological emotions, sensitive skin, serum bFGF levels above 32.5 ng/L, serum VEGF levels above 34.7 ng/L were the risk factors for recurrence and hyperpigmentation after laser treatment

Table 1. Comparative analysis of clinicopathological features between the melasma recurrence group and non-recurrence group

	N	Recurrence group (n=31)	Non-recurrence group (n=73)	*p* value
Age (year)				0.005
>30	52	22 (71.0)	30 (41.1)	
≤30	52	9 (29.0)	43 (58.9)	
Gender				0.290
Man	12	2 (6.5)	10 (13.7)	
Female	92	29 (93.5)	63 (86.3)	
Family history				0.007
Present	31	15 (48.4)	16 (21.9)	
Absent	73	16 (51.6)	57 (78.1)	
Drinking habit				0.997
Present	47	14 (45.2)	33 (45.2)	
Absent	57	17 (54.8)	40 (54.8)	
Smoking habit				0.664
Present	47	13 (41.9)	34 (46.6)	
Absent	57	18 (58.1)	39 (53.4)	
Frequency of sun exposure				0.004
Often	48	21 (67.7)	27 (37)	
Occasionally	56	10 (32.3)	46 (63)	
The frequency of facial cleansing				0.518
Often	42	14	28	
Occasionally	62	17	45	
Usage of skin-colored retinoic acid				0.024
Yes	61	13	48	
No	43	18	25	
Psychology				0.004
Positive	53	9 (29.0)	44 (60.3)	
Negative	51	22 (71.0)	29 (39.7)	
Sleep duration				0.004
>8 h	59	11 (35.5)	48 (65.8)	
≤8 h	45	20 (64.5)	25 (34.2)	
Seasonal variation				0.005
Present	42	19 (61.3)	23 (31.5)	
Absent	62	12 (38.7)	50 (68.5)	
Sensitive skin				0.007
Present	40	18 (58.1)	22 (30.1)	
Absent	64	13 (41.9)	51 (69.9)	
Liver function				0.578
Normal	46	15 (48.4)	31 (42.5)	
Abnormal	58	16 (51.6)	42 (57.5)	
bFGF (ng/L)		33.42±2.24	31.68±3.09	0.0055
VEGF (ng/L)		36.50±3.42	34.57±2.87	0.0038
TGF-β (ng/L)		3576±364.95	3896±313.45	<0.0001

(P<0.05, **Table 3**). Additionally, serum TGF-β levels above 3713.4 ng/L were found to be a protective factor against the recurrence of melasma.

Discussion

The formation of melasma is primarily related to the activation of melanocytes, which are

Table 2. Assigned values for the factors identified from the univariate analysis

Variable	Value assignment
Age	≤30=0, >30=1
Family history	Absent =0, Present =1
Frequency of sun exposure	Occasionally =0, Often =1
Usage of skin-colored retinoic acid	Yes =1, No =0
Psychology	Negative =0, Positive =1
Sleep duration	>8 h =0, ≤8 h =1
Seasonal variation	Absent =0, Present =1
Sensitive skin	Absent =0, Present =1
Serum bFGF	<32.5 ng/L =0, >32.5 ng/L =1
Serum VEGF	<34.7 ng/L =0, >34.7 ng/L =1
Serum TGF-β	<3713.4 ng/L =0, >3713.4 ng/L =1

cells in the skin responsible for producing melanin. Their activity is regulated by various factors [12]. The formation mechanism of melasma involves the release of various bioactive mediators induced by inflammation and neuropeptides, such as heparin, bradykinin, serotonin, thromboxane, prostaglandins, and leukotrienes [6].

Currently, few studies suggest a relationship between growth factors and the development of melasma. After laser treatment, melasma patients show erythema index, transepidermal water loss, decreased melanocytes, angiogenesis, and basal membrane thickening [8]. At the same time, the expression of CD44 and bFGF decreases after treatment [8]. bFGF indicates the activity of melasma. In vitro experiments have confirmed that single or repeated ultraviolet radiation in sebaceous cell lines promotes the expression of melanogenesis and inflammatory factors, including α-MSH, EDN1, bFGF, and stem cell factor (SCF) [13]. Radiated sebaceous cells increase melanin pigmentation in melanocytes and aging markers in fibroblast cultures, leading to local skin aging and hyperpigmentation [13]. Additionally, VEGF is an important mechanism in melasma. 590 nm LED irradiation can effectively inhibit the formation of melasma, its mechanism is by reducing cell migration, tube formation, and the expression of pro-melanogenesis factors such as VEGF and SCF [14]. Decolorizing agents treated human fibroblasts and subjected to oxidative stress treatment under ultraviolet radiation or IL-1α inflammatory stress, reduced VEGF and iNOS protein synthesis in dermal fibroblasts [15]. Tranexamic acid (TA) has been proven to be an effective drug for treating melasma, TA can at least partially target VEGF receptors to inhibit angiogenesis and melanogenesis in vitro, thus treating melasma [16]. Lastly, recent studies have found a close association between TGF-β and melasma. TGF-β is a natural multifunctional polypeptide that negatively regulates melanocyte differentiation and reduces skin pigmentation [17]. In melasma and solar lentigos, TGF-β can inhibit the increased activity of key enzymes involved in melanogenesis [18]. This is consistent with our results. Multivariate regression analysis officially shows that bFGF and VEGF are risk factors for melasma, while TGF-β is a protective factor (**Table 3**).

Currently, there are almost no studies that can comprehensively explore the recurrence risk factors of melasma after laser treatment by integrating various dimensions such as basic information, medical history, nursing measures, and growth factors. This study analyzed the follow-up results of 104 patients with melasma who underwent Nd:YAG laser treatment. It was found that 31 patients experienced recurrence and hyperpigmentation, with a recurrence rate of 29.81%. Additionally, multivariate Logistic regression analysis revealed that age, high frequency of sun exposure, nonuse of skin-colored retinoic acid, seasonal changes, insufficient sleep duration, presence of negative psychological emotions, and sensitive skin were the main factors influencing recurrence and hyperpigmentation after laser melasma removal. Previous studies often only focused on the relationship between a single factor and the recurrence of melasma. Age is one of the main factors affecting postoperative recurrence and hyperpigmentation. Lee YS et al. found that patients over the age of 30 are more likely to experience recurrence and hyperpigmentation [19]. This may be due to the decline in skin's self-repair ability with age, increasing the risk of recurrence. Besides, sun exposure is also an important factor in the formation of melasma. Ultraviolet radiation can stimulate the activity of melanocytes, causing

Table 3. Multivariate logistic regression analysis for the factors identified from the univariate analysis

Variable	Regression coefficient	Standard error	*p* value	OR (95% confidence interval)
Age	1.593	0.568	0.004	4.854 (1.665-13.956)
Family history	0.793	0.524	0.101	2.323 (0.882-7.318)
Frequency of sun exposure	1.756	0.573	0.001	6.027 (2.144-16.843)
Usage of skin-colored retinoic acid	-1.328	0.482	0.069	0.976 (0.867-1.175)
Psychology	1.373	0.586	0.012	3.638 (1.403-11.892)
Sleep duration	1.254	0.501	0.020	3.174 (1.245-9.372)
Seasonal variation	1.769	0.558	0.001	5.541 (2.043-16.285)
Sensitive skin	0.968	0.425	0.004	2.452 (1.134-7.372)
Serum bFGF	2.176	0.602	0.002	6.376 (2.243-15.154)
Serum VEGF	2.654	0.638	0.001	6.345 (1.729-16.583)
Serum TGF-β	-2.142	0.528	<0.0001	0.114 (0.048-0.227)

them to produce more melanin and leading to skin pigmentation [20]. Moreover, changes in hormone levels may also lead to the occurrence of melasma, such as in pregnant women and those with endocrine disorders, who are more prone to developing melasma [20]. Patients with negative psychological emotions and poor sleep quality are more prone to recurrence. The possible reasons are the decline in sleep quality and negative psychological emotions, which may lead to endocrine imbalance and immune function decline, further affecting skin health and increasing the risk of recurrence [21]. Laser treatment is a common method for melasma removal. Lasers can selectively irradiate melanocytes and destroy their structure, thereby reducing pigmentation. The mechanism of action primarily involves the selective effect of laser energy on melanocytes and the repair and regeneration of skin tissue.

The study has several limitations. Firstly, the sample size is relatively small (104 patients), and the number of cases in the recurrence group is also limited (31 cases), which may affect the statistical power of multivariate regression analysis, especially when adjusting for multiple factors, leading to a risk of overfitting. Due to the single-center study design (at a hospital in Yangzhou, China), the conclusions may not be fully applicable to the entire country or even other regions. In terms of time span, although a 12-month follow-up period can capture short-term recurrence status, melasma being a chronic recurrent disease, some patients may experience a rebound of pigmentation at a later time, thus limiting the assessment of long-term recurrence risk. Methodologically, variables such as psychological state and sleep quality depend on patient self-reports, which may introduce recall bias or subjective judgment errors, and potential confounding factors such as dietary patterns and air pollution exposure were not included. At the level of exploring biological mechanisms, the study focused on three growth factors in serum: bFGF, VEGF, and TGF-β. Although protein microarrays were used to screen for differentially expressed proteins, other known inflammatory factors related to pigment metabolism (such as IL-6 and TNF-α) or oxidative stress indicators were not further tested, and the lack of histological validation from local skin tissue is also a limitation.

Therefore, future studies could validate the established recurrence prediction model in a larger sample cohort and further validate its clinical utility through multi-center studies. Additionally, immunohistochemical techniques could be used to detect the expression of related factors in the dermis to determine the direct link between serum biomarkers and the microenvironment of the lesion; biological causality could be verified through cell experiments or animal models, thus providing a deeper understanding of the regulatory network of key molecules in the recurrence process of melasma.

Conclusion

This prospective study provides a multidimensional analysis of factors influencing melasma recurrence following Q-switched Nd:YAG 1064-

nm laser treatment, integrating demographic, behavioral, and molecular perspectives. Our findings underscore that recurrence is a multifactorial process, driven not only by clinical variables such as age, sun exposure, and psychological state but also by dysregulated serum growth factors. Clinically, the risk stratification model incorporating serum biomarkers and lifestyle factors offers a pragmatic framework for personalized post-treatment monitoring and preventive strategies. This study lays the groundwork for future investigations to validate these predictors in diverse populations and explore targeted interventions modulating growth factor pathways.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Pingsong Li, Department of Medical Cosmetology, Northern Jiangsu People's Hospital, 98 Nantong West Road, Yangzhou 225001, Jiangsu, China. E-mail: kexu126@126.com

References

[1] Jo JY, Chae SJ and Ryu HJ. Update on melasma treatments. Ann Dermatol 2024; 36: 125-134.

[2] Beyzaee AM, Goldust M, Rokni GR, Patil A, Mostaghiman R and Golpour M. Comparative effectiveness and safety of topical methimazole 5% monotherapy versus combination of Q-Switched Nd: YAG Laser and topical methimazole 5% in patients with refractory melasma. J Cosmet Dermatol 2023; 22: 1774-1779.

[3] Artzi O, Horovitz T, Bar-Ilan E, Shehadeh W, Koren A, Zusmanovitch L, Mehrabi JN, Salameh F, Isman Nelkenbaum G, Zur E, Sprecher E and Mashiah J. The pathogenesis of melasma and implications for treatment. J Cosmet Dermatol 2021; 20: 3432-3445.

[4] Morgado-Carrasco D, Piquero-Casals J, Granger C, Trullas C and Passeron T. Melasma: the need for tailored photoprotection to improve clinical outcomes. Photodermatol Photoimmunol Photomed 2022; 38: 515-521.

[5] Ko D, Wang RF, Ozog D, Lim HW and Mohammad TF. Disorders of hyperpigmentation. Part II. Review of management and treatment options for hyperpigmentation. J Am Acad Dermatol 2023; 88: 291-320.

[6] Esposito ACC, Cassiano DP, da Silva CN, Lima PB, Dias JAF, Hassun K, Bagatin E, Miot LDB and Miot HA. Update on melasma-part I: pathogenesis. Dermatol Ther (Heidelb) 2022; 12: 1967-1988.

[7] Chen T, Xue J and Wang Q. Tranexamic acid for the treatment of hyperpigmentation and telangiectatic disorders other than melasma: an update. Clin Cosmet Investig Dermatol 2024; 17: 2151-2163.

[8] Park BJ, Jung YJ, Ro YS, Chang SE and Kim JE. Therapeutic effects of new pulsed-type microneedling radiofrequency for refractory facial pigmentary disorders. Dermatol Surg 2022; 48: 327-333.

[9] Zhang C, Wu T and Shen N. Effect of platelet-rich plasma combined with tranexamic acid in the treatment of melasma and its effect on the serum levels of vascular endothelial growth factor, endothelin-1 and melatonin. Pak J Med Sci 2022; 38: 2163-2168.

[10] Tuknayat A, Bhalla M and Thami GP. Platelet-rich plasma is a promising therapy for melasma. J Cosmet Dermatol 2021; 20: 2431-2436.

[11] Ranganathan P, Deo V and Pramesh CS. Sample size calculation in clinical research. Perspect Clin Res 2024; 15: 155-159.

[12] Fu C, Chen J, Lu J, Yi L, Tong X, Kang L, Pei S, Ouyang Y, Jiang L, Ding Y, Zhao X, Li S, Yang Y, Huang J and Zeng Q. Roles of inflammation factors in melanogenesis (Review). Mol Med Rep 2020; 21: 1421-1430.

[13] Flori E, Mastrofrancesco A, Mosca S, Ottaviani M, Briganti S, Cardinali G, Filoni A, Cameli N, Zaccarini M, Zouboulis CC and Picardo M. Sebocytes contribute to melasma onset. iScience 2022; 25: 103871.

[14] Dai X, Jin S, Xuan Y, Yang Y, Lu X, Wang C, Chen L, Xiang L and Zhang C. 590 nm LED irradiation improved erythema through inhibiting angiogenesis of human microvascular endothelial cells and ameliorated pigmentation in melasma. Cells 2022; 11: 3949.

[15] Pereira AFC, Igarashi MH, Mercuri M, Pereira AF, Pinheiro ALTA, da Silva MS, Facchini G and Eberlin S. Whitening effects of cosmetic formulation in the vascular component of skin pigmentation. J Cosmet Dermatol 2020; 19: 154-160.

[16] Zhu JW, Ni YJ, Tong XY, Guo X, Wu XP and Lu ZF. Tranexamic acid inhibits angiogenesis and melanogenesis in vitro by targeting VEGF receptors. Int J Med Sci 2020; 17: 903-911.

[17] Hofny ERM, Hussein MRA, Ghazally A, Ahmed AM and Abdel-Motaleb AA. Increased expression of TGF-beta protein in the lesional skins of melasma patients following treatment with platelet-rich plasma. J Cosmet Laser Ther 2019; 21: 382-389.

[18] Gao X and Xiang W. Emerging roles of dermal fibroblasts in hyperpigmentation and hypopigmentation: a review. J Cosmet Dermatol 2025; 24: e16790.

[19] Lee YS, Lee YJ, Lee JM, Han TY, Lee JH and Choi JE. The low-fluence Q-Switched Nd:YAG laser treatment for melasma: a systematic review. Medicina (Kaunas) 2022; 58: 936.
[20] Liu W, Chen Q and Xia Y. New mechanistic insights of melasma. Clin Cosmet Investig Dermatol 2023; 16: 429-442.
[21] Singh NK, Verma AK and Gupta JK. Understanding mechanisms and key factors influencing melanogenesis for the management of melasma: an updated review. Curr Pharm Biotechnol 2024; [Epub ahead of print].