RESEARCH

Aqueous humor cytokine levels in patients with subretinal fibrosis in neovascular age-related macular degeneration

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Abstract

Purpose To investigate aqueous humor cytokine levels in neovascular age-related macular degeneration (nAMD) patients with subretinal fibrosis and to explore the relationship between cytokine levels and disease severity.

Methods The aqueous humor samples were collected from 16 eyes with subretinal fibrosis due to nAMD (SRFi group), 33 eyes with nAMD without subretinal fibrosis (nAMD group) and 28 eyes with cataract patients (control group). Clinical samples were analyzed for 5 cytokines, including vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), basic fibroblast growth factor (bFGF), transforming growth factor-α (TGF-α), platelet-derived growth factor-BB (PDGF-BB).

Results Aqueous humor cytokines VEGF and bFGF were significantly higher in nAMD patients than controls (all $P < 0.05$), and VEGF, bFGF and TGF-α levels were significantly higher in SRFi patients than controls (all $P < 0.05$). No significant differences in 4 cytokine levels were observed between nAMD and SRFi patients in aqueous humor. We also identified a positive correlation between the aqueous humor levels of IL-6 and VEGF in the SRFi group, while bFGF and TGF-α in the nAMD group. Moreover, VEGF levels were strongly related to BCVA, and bFGF levels were positively related to the maximum thickness of subretinal hyperreflective material (SHRM) in fibrosis due to nAMD.

Conclusion VEGF and bFGF levels in aqueous humor were elevated in macular neovascularization with and without subretinal fibrosis. TGF-α levels exclusively differed in neovascular AMD with fibrosis. Cytokines are distributed differently and play a synergistic role in different stages (angiogenesis and fibrogenesis) of nAMD. The bFGF levels could predict the negative prognosis in fibrosis due to nAMD.

Keywords Aqueous humor, Cytokine, Neovascular age-related macular degeneration, Subretinal fibrosis

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Introduction

Age-related macular degeneration (AMD) is a progressive, degenerative disease of the human retina which impacts the eye's posterior segment and damages the macula. The neovascular AMD is characterized by disruption of Bruch's membrane and formation of macular neovascularization (MNV). The formation of MNV causes damage to the photoreceptors, RPE, and choriocapillaris, results in fluid accumulation in the tissues induces a drastic aggravation of the retinal detachment and subretinal hemorrhages, which acclerates the progression of subretinal fibrosis, eventually dramatically impair the visual acuity. Vascular endothelial growth factor (VEGF) and many other cytokines are crucial mediators of inflammatory responses and play important regulatory roles in neovascularization in patients with nAMD [1, 2]. Although anti-VEGF treatment generally improves visual acuity, subretinal fibrosis can develop and has been identified as a cause of poor therapeutic outcomes in nAMD [3]. Systemic levels of aqueous humor cytokines in nAMD have also been investigated in some literatures [4–9].

Subretinal fibrosis often develops in the natural progression of nAMD. However, little is known about the intraocular cytokine differences in the late stage of subretinal fibrosis. Understanding the cytokine changes in eyes with involutional stage of age-related macular degeneration (AMD) may further elucidate the underlying pathogenic mechanisms of subretinal fibrosis. Therefore, we aimed to investigate the possible roles of cytokines involving in fibrogenesis by comparing the cytokines levels in eyes with cataracts, eyes with nAMD and eyes with subretinal fibrosis due to nAMD. Furthermore, we also evaluated correlations between cytokines and clinical manifestations in fibrosis due to nAMD.

Materials and methods

This was a cross-sectional study comparing cytokine levels of aqueous humor in patients with subretinal fibrosis due to nAMD or nAMD without subretinal fibrosis and healthy controls (age- and sex-matched cataract patients without any other ocular complication). A total number of 77 patients with 16 subretinal fibrosis due to nAMD, 28 nAMD without subretinal fibrosis and 33 cataract patients were recruited with prior informed consent. The study was approved by the ethics committee of Shaanxi Provincial People's Hospital (Xi'an, China) and adhered to the tenets of the Declaration of Helsinki.

Subjects and clinical diagnosis

All patients received a comprehensive ophthalmological examination at baseline, including best corrected visual acuity (BCVA) testing, intraocular pressure measurement, dilated fundus examination, OCT and FFA. BCVA was measured by certified operators using a standard Snellen chart. For statistical analysis, BCVA were converted to logarithm of the minimum angle of resolution (logMAR) units. The maximum thickness of subretinal hyperreflective material (SHRM) was measured by OCT. Clinical diagnosis was made by medical retina specialists in all the study subjects recruited under this study. Diagnosis was based on optical coherence tomography (OCT), retinal angiography using fudusfluorescein (FFA) and indocyanine green (ICGA) and optical coherence tomography angiography (OCTA) features. Subretinal fibrosis due to nAMD is diagnosed, on fundus biomicroscopy, as a well-demarcated, elevated mound of yellowish-white tissue, with variable location in the macular area [10, 11]. On fundus fluorescein angiography (FFA), subretinal fibrosis criteria consist of staining, with minimal or no leakage in the late phase, giving rise to a hyperfluorescent, heterogeneous lesion with concave borders. On optical coherence tomography (OCT) fibrosis harbors an aspect of a subretinal hyperreflective lesion, of variable size and location, with possible loss of adjacent retinal pigment epithelium (RPE) and ellipsoid zone. Optical coherence tomography angiography (OCTA) of fibrosis showed almost constantly a perfused, abnormal vascular network and collateral architectural changes in the outer retina and the choriocapillaris layer [12]. Patients with neovascular AMD showed classic CNV or occult CNV with fluorescein angiography, and multimodel imagery show no evidence of fibrosis, as well as without sub-RPE scar/fibrosis/hyperreflective material. Subjects more than 50 years of age with exudative AMD with no intravitreal injection treatment for at least 1 year as the only ocular pathology were included as cases in the study. Control subjects (> 50 years of age) were patients undergoing cataract surgery with no retinal disease and having only senile cataracts. Exclusion criteria included cases of fibrosis secondary to causes other than AMD. Patients with previous performed intraocular surgery or procedures within the last 6 months were excluded, such as ocular trauma or laser treatment.

Aqueous humor collection

At the time of intravitreal injection, samples of aqueous humor (50–100uL) was collected aseptically from cases and controls under topical anesthesia and direct visualization with patients in an inclined position using an insulin syringe with attached 30-gauge needle. Samples of conrol group were also collected into sterile tubes at the time of cataract surgery. After the collection, clinical samples were immediately transferred into prelabelled sterile 1.5-mL microcentrifuge tubes and stored at -80° freezer until final analysis.

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Cytokines measurement

Aqueous humor samples were thawed at 4℃ and centrifuged at 16000 g for 4 min. The supernatant from aqueous humor were separated and transferred onto ice, and 50 uL of each sample was used for cytokine analysis in duplicate reactions. The Luminex200 platform was applied for the analysis of 5 cytokines (IL-6, VEGF, bFGF, TGF-α and PDGF-BB) in the clinical samples following the manufacturer's guidelines (Millipore Burlington, MA, USA). The Luminex plates were read with Magpix system (Luminex Corp) following the manufacturer's guidelines, and Bio-Plex manager 6.1 software (BioRad Laboratories) with a 5-parameter curve-fitting algorithm was used to analyze the data.

Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences statistical software for Windows, version 17.0 (SPSS Inc). Differences between each pair of groups including SRFi, neovascular AMD and controls were assessed by the Mann–Whitney U test for continuous variables and by the χ 2 test or Fisher exact test for categorical variables. Each pair of group differences among SRFi and nAMD patients and controls was analyzed using one-way analysis of variance or nonparametric Mann–Whitney U test, depending on normality assumptions for continuous variables. To examine correlations, Spearman's rank–order correlation coefficients were calculated.

Results

Clinical characteristics

Of 77 cases with subjects, 16 cases had subretinal fibrosis with nAMD and 33 cases had nAMD without subretinal fibrosis, and 28 cataract cases as control group (Table 1). The mean age of patients with subretinal fibrosis due to nAMD and nAMD without subretinal fibrosis was 73.69 ± 10.20 (mean ± SD) years and 74.70 ± 9.93 years respectively, and the control group was 71.04 ± 10.46 years. The best corrected visual acuity (BCVA) in subretinal fibrosis due to nAMD patients and nAMD without subretinal fibrosis patients showed significant difference with control group. The baseline characteristics of patients are in Table 1.

Cytokine levels in aqueous humor

Of 5 cytokines tested in each sample, 4 cytokines (IL-6, VEGF, bFGF, and TGF-α) had detection rates 98.70% in aqueous humor, and the level of PDGF-BB was below detection limits of the assay (< 4.225 pg/mL), the detection rate was only 3.9%. Table 2 shows the concentrations of the 4 cytokines in the aqueous humor. Concentrations of aqueous humor cytokines VEGF and bFGF were significantly higher in the nAMD cohort than controls (VEGF $P = 0.005$; bFGF $P = 0.025$). Concentrations of aqueous humor cytokines levels VEGF, bFGF and TGF-α were significantly higher in SRFi subjects compared with controls (VEGF $P = 0.02$; bFGF $P = 0.036$; TGF-α $P = 0.031$). However, none of the 4 cytokine levels were different between nAMD and SRFi subjects. Of note, there were no significant differences in TGF-α levels between nAMD group and controls ($P > 0.05$). There were no significant differences in IL-6 concentrations among the groups ($P > 0.05$). Table 2 shows the concentrations of the 4 cytokines in aqueous humor.

Correlations between aqueous cytokines in nAMD and SRFi groups

Table 3 showed the correlation between aqueous humor cytokines in nAMD and SRFi patients. However, although the sample size was small, there was a limited, but apparent positive correlation between the aqueous humor levels of IL-6 and VEGF in SRFi patients ($r = 0.647$, $P = 0.012$). The aqueous humor levels of bFGF were positively correlated with the levels of TGF-α in nAMD patients ($r = 0.443$, $P = 0.030$).

Table 1 Demographic and clinical characteristics among 3 groups

	SRFi group	nAMD group	control	F/×2	P
Age (year)	73.69 ± 10.20	74.70 ± 9.93	71.04 ± 10.46	1.007	0.370
Sex (male/female)	16 (9/7)	33 (16/17)	28 (9/19)	2.839	0.242
Eye (right/left)	16 (9/7)	33 (16/17)	28 (13/15)	0.410	0.815
Smokern (%)	4 (25.0%)	8 (24.2%)	5 (17.9%)	0.459	0.795
Hypertension n (%)	9 (56.3%)	23 (69.7%)	13 (46.4%)	3.417	0.181
Diabetes n (%)	3 (18.8%)	3 (9.1%)	1 (3.6%)	2.737	0.254
IOP (mmHg)	13.00 ± 2.34	13.27 ± 2.73	12.75 ± 2.68	0.299	0.742
BCVA	1.25 ± 0.54	0.93 ± 0.56	0.76 ± 0.73	3.180	0.047*

IOP Intraocular pressur, *BCVA* Best corrected visual acuity

* $P < 0.05$

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Table 2 Levels of 4 Cytokines in aqueous humor among 3 groups

Analytes	SRFi (pg/ml)	nAMD (pg/ml)	Control (pg/ml)	P1 (SRFi vs nAMD)	P2 (SRFi vs Control)	P3 (nAMD vs Control)
IL-6	7.31 ± 11.16	11.09 ± 16.32	8.77 ± 8.19	0.147	0.188	0.965
VEGF	59.40 ± 24.65	61.78 ± 25.72	42.29 ± 13.78	0.88	0.02*	0.005*
bFGF	9.28 ± 8.87	12.34 ± 17.15	4.94 ± 2.39	0.785	0.036*	0.025*
TGF-α	0.77 ± 0.31	1.05 ± 1.46	0.49 ± 0.41	0.927	0.031*	0.052

Levels are expressed as the mean ± SD pg/ml

IL-6 Interleukin-6, *VEGF* Vascular endothelial growth, *bFGF* Basic fibroblast growth factor, *TGF-α* Transforming growth factor-α

* $P < 0.05$

Table 3 Correlations among aqueous humor factors in SRFi group and nAMD group

	SRFi group				nAMD group			
	IL-6 (r) p	VEGF (r) p	bFGF (r) p	TGF-α (r) p	IL-6 (r) p	VEGF (r) p	bFGF (r) p	TGF-α (r) p
IL-6	-	(0.647) 0.012*	(0.438) 0.117	(0.348) 0.222	-	(-0.017) 0.936	(0.037) 0.865	(0.145) 0.498
VEGF		-	(0.262) 0.366	(0.031) 0.915		-	(0.040) 0.853	(-0.198) 0.353
bFGF			-	(0.002) 0.994			-	(0.443) 0.030*
TGF-α				-				-

Correlation coefficient (r) and *P* values are calculated by Spearman's correlation

IL-6 Interleukin-6, *VEGF* Vascular endothelial growth, *bFGF* Basic fibroblast growth factor, *TGF-α* Transforming growth factor-α

* $P < 0.05$

Correlations between aqueous cytokines and clinical parameters

The maximum thickness of subretinal hyperreflective material (SHRM) in the SRFi group was 307 ± 104.31 μm. The central location of SHRM and off-central location of SHRM ratios were 14:2 in subretinal fibrosis due to nAMD patients. The subjects with subretinal fluid and subjects without subretinal fluid ratios were 13:3.

We aimed to identify potential cytokine markers associated with clinical parameters including BCVA, the maximum thickness of SHRM and the SHRM location, as well as the presence of subretinal fluid in nAMD with fibrosis. Interestingly, as shown in Table 4, the VEGF ($r = 0.724$, $P = 0.001$) and IL-6 ($r = 0.057$, $P = 0.026$) levels were positively related to BCVA, and the bFGF levels were positively related to the maximum thickness of SHRM ($r = 0.828$, $P = 0.000$). The TGF−α did not correlate significantly with BCVA, the maximum thickness

Table 4 The correlation between aqueous humor 4 cytokines and BCVA and relative OCT characteristics in SRFi group

	IL-6 (r) p	VEGF (r) p	bFGF (r) p	TGF-α (r) p
BCVA	(0.057) 0.026*	(0.724) 0.001*	(0.338) 0.185	(0.065) 0.804
maximum thickness of SHRM	(0.245) 0.344	(0.252) 0.329	(0.828) 0.000*	(0.204) 0.432
SHRM location	(-0.180) 0.490	(-0.076) 0.773	(0.277) 0.282	(-0.435) 0.081
the presence of subretinal fluid	(-0.067) 0.797	(-1.185) 0.478	(-0.158) 0.544	(0.081) 0.759

Correlation coefficient (r) and *P* values are calculated by Spearman's correlation

BCVA Best corrected visual acuity, *SHRM* Subretinal hyperreflective material, *IL-6* Interleukin-6, *VEGF* Vascular endothelial growth, *bFGF* Basic fibroblast growth factor, *TGF-α* Transforming growth factor-α

* $P < 0.05$

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of SHRM and SHRM location, as well as the presence of subretinal fluid.

Discussion

It is widely recognized that the activation of proinflammatory cytokines and subsequent upregulation of angiogenic factors in aqueous humor played an important role in the development of nAMD. VEGF, IL-6, PDGF, bFGF have been previously linked to CNV formation and tissue remodeling. Many reports have described the aqueous humor cytokines levels in AMD [4, 13–16]. However, little is known about the cytokine changes in the specific late stage of fibrosis in nAMD. In the present study, we evaluated 5 cytokines in aqueous hum [14]or from nAMD patients with or without fibrosis. It was demonstrated that significant differences existed in aqueous humor cytokine concentrations in patients with nAMD (VEGF and bFGF) compared with controls. Among patients with fibrosis due to nAMD, VEGF, bFGF and TGF-α were significantly higher compared with controls. Our results support the theory that cytokine mediated angiogenesis and fibrogenesis contributes to the development and progression of nAMD.

The VEGF is reported to be a key molecules for angiogenesis. Besides, VEGF regulates vascular permeability, monocyte infiltration, and scar-associated macrophages function [17]. Some investigators reported that the aqueous humor VEGF levels were elevated in neovascular AMD [5, 6, 9]. As angiogenesis contributes to the initiation and progression of nAMD, fibrosis is the natural involutional stage of CNV, we speculated that it would have lower levels of VEGF in the SRFi patients. Interestingly, our study reported that both SRFi and nAMD group had higher levels of VEGF compared to control eyes, indicating that sustained production of VEGF may actually accelerate fibrosis in CNV. Meanwhile, we were able to show that an upregulation of VEGF was not specific for active neovascular AMD. Previous studies have revealed a fibrogenic effect of VEGF through multiple mechanisms, including promotion of inflammation, release of fibrosis-enhancing molecules from VEGF-activated endothelial cells, and direct effects of VEGF on HSC [18, 19]. In addition, a study from Wang found that sustained production of VEGF is potentially a key inducer of subretinal fibrosis in laser-induced primate CNV model [20]. Little et al. demonstrated that anti-VEGF may inhibit the prevalence of macular scar in eyes and could be a potential treatment to subretinal fibrosis [21], although there were some contradictory results [22]. These findings support the theory that VEGF inhibition might also have beneficial effects on fibrosis resolution. Previous study indicated that there was no correlation between the VEGF and other cytokines in aqueous humor [23]. However, we found that there was a positive correlation between the VEGF and IL-6 in SRFi patients, suggesting that inflammation promotes the

the formation of macular neovascularization (MNV), accelerating the progression of fibrosis resolution. In addition, previous study reported that IL-6 activates signal transducers and activators of transcription protein 3 (STAT3) signaling pathways, which promote endothelial cell migration and the progression of angiogenesis together with VEGF [24]. We also found that VEGF levels positively correlated with BCVA in fibrosis due to nAMD.

Interleukin (IL)-6 is a pleiotrophic cytokine that plays a pivotal role in biological processes such as immune response, inflammation, wound healing response and angiogenesis. In ocular fibrotic responses, IL-6 has been previously detected in the subretinal fluid and the vitreous of patients with proliferative vitreoretinopathy (PVR) [25]. In the mouse model of subretinal fibrosis, IL-6 derived from macrophages, is a crucial mediator that promotes subretinal fibrosis, and IL-6 knockdown and neutralization of IL-6 signaling pathway reduced subretinal fibrosis [26]. Although increased plasma and aqueous humor IL-6 levels were reported in a number of studies involving nAMD patients [5, 27], similar studies did not show a significant difference in aqueous humor IL-6 levels in nAMD patients [4, 6, 7, 28], that have yielded consistent results with our study. However, The controversial findings among those studies maybe not only due to the limited participants in each group, but also since patients undergoing cataract surgery do not represent a perfect control group, since intraocular inflammation as well as lifestyle factors and retinal co-morbidities associated with chronic low-grade inflammation are the strong risk factors of cataract patients. Further studies need to validate the aqueous humor IL-6 concentrations and the function of IL-6 as a molecular target in the treatment of nAMD-related subretinal fibrosis.

Basic FGF is reported to be involved in the pathophysiology of both angiogenesis and fibrosis in a variety of tissue and organ systems [29, 30]. Earlier studies suggested that FGF2 are localized in epiretinal and CNV membranes [31]. Moreover, elevated aqueous humor bFGF levels were recently shown in PVR patients and PCV patients [28, 32]. Importantly, a previous report demonstrated the anti-FGF2 treatment blocks both CNV and subretinal fibrosis in animal models of retinal disease, which was similar to that of anti-VEGF therapy [33]. Furthermore, the dual blockade of both VEGF and FGF-2 would be more efficient than the anti-VEGF monotherapy in CNV-induced animal model. The observed presence of elevated intraocular bFGF in our study indicated the functional role in the neovascularization process and fibrosis resolution of nAMD. In patients with nAMD, SHRM has been identified as a significant risk factor for scar formation associated with poor visual acuity [34, 35]. Most importantly, Willoughby and colleagues reported

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that there was a negatively correlation between SHRM dimensions with the BCVA [36]. We also attempted to explore whether increased cytokines in fibrosis of CNV correlate with disease severity. Our study found there was a positive correlation between bFGF levels and the maximum thickness of SHRM in SRFi patients. In addition, considering the effect of anti-bFGF treatment in laser-induced experimental CNV, we speculate the increasing endogenous (aqueous humor) bFGF levels might be an exacerbation factor for fibrotic scar formation in nAMD. If validated in future studies, bFGF could be a potential additional therapeutic target for preventing fibrotic scar formation in nAMD.

Transforming growth factor (TGF) family is closely associated with fibrosis. TGF-α belongs to the epidermal growth factor (EGF) family. It is well established that that TGF-α/β is a major cytokine that mediate epithelial, mesenchymal, and immune cell transformation to induce tissue remodeling and fibrosis [37, 38]. TGF-α has been implicated in fibrosis and oxidative stress. Prior studies demonstrated that TGF-α is increased during hyperoxia and fibrosis in neonatal rabbits [39]. TGF-α has the pleiotropic effects of fibrogenic and anti-fibrogenic depending on the activity of the RAS-ERK and the p38 pathways for the hepatic fibrosis process [40]. A number of experimental studies support a role for TGF-α in transient fibroproliferative processes [41]. TGF-α knock-out mice displayed eye abnormalities, including retinal dysplasia [42]. Previous studies have been reported that TGF-α is involved in the retinal response to injury [43]. Therefore, we select TGF-α for a representative marker in the development and progression of fibrosis in nAMD. Interestingly, aqueous humor TGF-α levels in eyes with SRFi group showed statistically different from those in control eyes in the present study. Of note, TGF-α also increased in nAMD with non-fibrotic eyes, but it only showed difference in fibrotic eyes, indicating a significant role of TGF-α in the progression of nAMD although the nAMD group and the control group did not vary significantly. Our results suggested the aqueous humor levels of TGF-α were positively correlated with bFGF in nAMD patients, suggesting the two growth factors act synergistically in the progression of inflammatory processes, neovascularization and damage repair in nAMD disease. Therefore, we predict that TGF-α could be a new molecular target in the detection of ocular fibrotic disease in the future.

PDGF-BB, one of the isoforms of PDGF, is a proinlflammatory and proangiogenic cytokine. Moreover, PDGF is involved in EMT and the fibrotic process, including cell proliferation, migration and ECM remodeling [44]. PDGFR-β preferentially binds PDGF-BB, previous studies have reported that blockade of PDGFR-β signaling suppresses the formation of CNV and subretinal fibrosis

in laser-induced CNV mice [45]. Recent studies reported antagonism of PDGF-BB suppressed subretinal NV and enhanced the effects when combined with the treatment of blocking VEGF-A [46, 47]. However, maybe due to limitations of our assay system or the limited sample size, the level of PDGF-BB was below detection limits of the assay in the current study, and only 3 samples were detected effectively, which was not suitable for statistical analysis and could not reflect the true level of PDGF-BB in aqueous humor. In addition, prior studies reported the aqueous humor PDGF-BB did not reach detection threshold in neovascular AMD or healthy controls with cataract [9, 28], which is in agreement with the current research. In contrary, marked expression of plasma PDGF-BB was observed in nAMD patients, which might suggest a stronger association between the PDGF-BB in nAMD [48]. Further studies are necessary to elucidate the aqueous humor PDGF-BB levels in neovascular AMD.

The present study has several limitations. First, the sample size was relatively small. True difference in cytokine levels may not be fully appreciated, and an expanded sample size is needed to further investigate in the future. In addition, to elucidate the pathogenesis of subretinal fibrosis in nAMD in detail, further studies with a broader cytokine spectrum are needed. Second, although our study identified fibrosis-related factors in nAMD patients, we did not reveal any potential mechanisms. Further animal experiments and in vitro experiments are required to explore the mechanism. Third, the period of our study was short, and the alteration of cytokines after intravitreal injection treatment need further investigation in the future.

In conclusion, VEGF and bFGF levels in aqueous humor were elevated in macular neovascularization with and without subretinal fibrosis. TGF-α levels deserve further attention as possible markers as they exclusively differed in neovascular AMD with fibrosis. Cytokines are distributed differently and play a synergistic role in different stages (angiogenesis and fibrogenesis) of nAMD. Moreover, bFGF levels were associated with the maximum thickness of SHRM in fibrosis due to nAMD. Thus, the presence of bFGF could predict the negative prognosis and may contribute to provide potential therapeutic targets in fibrosis due to nAMD.

Abbreviations

bFGF	Basic fibroblast growth factor
BCVA	Best corrected visual acuity
CNV	Choroidal neovascularization
FFA	Fundus fluorescein angiography
ICGA	Indocyanine green angiography
IL-6	Interleukin-6
MNV	Macular neovascularization
nAMD	neovascular age-related macular degeneration
OCT	Optic coherence tomography

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OCTA	Optical coherence tomography angiography
PDGF-BB	Platelet-derived growth factor-BB
SD	Standard deviation
SHRM	Subretinal hyperreflective material
SRFi	Subretinal fibrosis
TGF-α	Transforming growth factor-α
VEGF	Vascular endothelial growth factor

Availability of data and materials
Image data were extracted from the clinical PACS of Shaanxi Provincial People's Hospital. All the data supporting our findings are contained within the manuscript. Table1-4 data requests can be made to corresponding author via this email: lix-www@163.com.

Declarations

Ethics approval and consent to participate
All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Shaanxi Provincial People's Hospital (Xi'an, China). Written informed content was obtained from all patients and controls before enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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