Serum levels of SOCS6 are decreased in diabetic retinopathy and are related to severity of the disease

Lan Li[1,A–D], Xiaoyan Wu[2,E], Lemin He[3,E], Rong Luo[1,E], LiQiong Lou[4,F]

[1] Department of Ophthalmology, Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College, China
[2] Department of Ophthalmology, Yihuang County People's Hospital, Fuzhou, China
[3] Department of Ophthalmology, Poyang County Traditional Chinese Medicine Hospital, Shangrao, China
[4] Sterilization and Supply Center, Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(8):873–880

Address for correspondence
LiQiong Lou
E-mail: louliqiongtt@163.com

Funding sources
None declared

Conflict of interest
None declared

Received on April 20, 2022
Reviewed on August 30, 2022
Accepted on January 11, 2023

Published online on August 17, 2023

Abstract

Background. Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes mellitus (DM). A recent in vitro study found that the suppressor of cytokine signaling 6 (SOCS6) plays a protective role in DR and DM. However, to date, no clinical studies have focused on the role of SOSC6 in DR development.

Objectives. The present study aimed to investigate the expression and clinical significance of serum SOCS6 in DR.

Materials and methods. A total of 159 DR patients were enrolled in the study. Additionally, 156 type 2 DM (T2DM) patients without DR were recruited as controls. Serum levels of SOCS6, C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), vascular endothelial growth factor (VEGF), and angiopoietin-2 (ANG-2) were measured using enzyme-linked immunosorbent assay (ELISA). Demographic and clinical data were collected.

Results. Age, the course of DM, systolic blood pressure (SBP), diastolic blood pressure (DBP), and the levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) were significantly higher in proliferative DR (PDR) patients. Serum SOCS6 levels in PDR patients were remarkably lower than in non-PDR patients or non-DR T2DM patients. The Pearson's analysis showed that SOCS6 was negatively correlated with CRP, IL-6, TNF-α, IL-1β, VEGF, and ANG-2. The serum levels of CRP, IL-6, TNF-α, IL-1β, VEGF, and ANG-2 in the SOCS6 low expression group were significantly increased compared to patients with high SOCS6 levels. Receiver operating characteristic (ROC) curves showed that SOCS6 could be a potential diagnostic biomarker for DR. For logistic regression, 3 models were used. It was found that SOCS6, the course of DM, SBP and DBP in model 1, IL-1β and TNF-α in model 2, and VEGF and ANG-2 in model 3 were risk factors for DR.

Conclusions. The SOCS6 is decreased in DR patients and is related to severity and clinical outcomes, including inflammatory and angiogenic factors.

Key words: inflammatory factors, angiogenic factors, SOCS6, diabetic retinopathy

Cite as
Li L, Wu X, He L, Luo R, Lou L. Serum levels of SOCS6 are decreased in diabetic retinopathy and are related to severity of the disease. *Adv Clin Exp Med*. 2023;32(8):873–880. doi:10.17219/acem/159215

DOI
10.17219/acem/159215

Background

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia. Type 2 DM (T2DM) is currently the most common type of the disease, accounting for 95% of total DM cases.[1,2] In 2021, DM was estimated to affect 10.5% (536.6 million) of individuals aged 20–79 years old, worldwide.[3] China is the country with the greatest number of DM cases in the world. Over the past 30 years, the prevalence of DM in China has increased by about 10 times.[4,5]

Diabetic retinopathy (DR) is one of the most common microvascular complications of DM, with a global prevalence rate of about 27%.[6] Diabetic retinopathy is divided into 2 types, namely nonproliferative DR (NPDR) and proliferative DR (PDR), which are classified from low to high, according to severity.[7] Diabetic retinopathy is one of the main causes of visual impairment and blindness in adults, with studies reporting that DR accounts for 4.8% of cases of blindness around the world.[8] Many risk factors are reported to be associated with DR, including DM, increased blood pressure, elevated blood glucose levels, and impaired kidney function.[9] However, deeper insights are still needed to predict DR onset and clinical outcomes.

As a member of the suppressor of cytokine family of proteins, suppressor of cytokine signaling 6 (SOCS6) is generally considered to regulate bacterial infection-induced inflammation.[10] Studies have suggested that SOCS6 can regulate a variety of physiological and pathological processes, including inflammation, cell proliferation, apoptosis, and angiogenesis.[11–13] In the pathogenesis of DR, it is thought that neuroinflammation, oxidative stress and vascular-related risk factors play central roles.[14–16] A recent in vitro study found that SOCS6 plays a protective role in DR and DM.[12] However, to date, no clinical studies have focused on the role of SOSC6 in the development of DR.

Objectives

In this prospective observational study, we aimed to explore the expression of SOCS6 in DR patients and to examine its correlation with clinical results. The study may reveal the clinical significance of SOCS6 in patients with DR and provide novel targets for DR treatment.

Materials and methods

Patients

A total of 159 DR patients who were admitted to Jiangxi Provincial People's Hospital between March 2019 and December 2021 were enrolled in the study. The diagnosis of T2DM was in line with the guidelines for the prevention and treatment of T2DM in China (2017 edition),[17] including 1) typical diabetes symptoms (polydipsia, polyuria, hyperphagia, unexplained weight loss) and 2) fasting plasma glucose concentration higher than 7.0 mmol/L, plasma glucose concentration higher than 11.1 mmol/L, or oral glucose tolerance test 2-hour plasma glucose concentration higher than 11.1 mmol/L. Diabetic retinopathy was diagnosed with fundus photography with dilated pupils using a retinograph (CR-2 AF Digital Retinal Camera; Canon Inc., Tokyo, Japan), and all patients were diagnosed and classified according to the international clinical DR severity scales.[18]

The criteria for inclusion were as follows: 1) patients diagnosed with DR according to the above guidelines; and 2) patients ≥18 years old. The exclusion criteria were: 1) diagnosis of type 1 DM, gestational DM or another special type of DM; 2) presence of diabetic ketoacidosis, hyperosmolar nonketotic diabetic coma or other acute DM complications; 3) presence of cataracts, glaucoma, uveitis, retinal detachment, optic nerve disease, high myopia, and other diseases significantly affecting the fundus examination; and 4) presence of serious infection, malignancy, or severe liver, renal or cardiovascular dysfunction. All patients who met the above criteria were consecutively enrolled.

The DR patients were further divided into: 1) patients with no fundus abnormalities; 2) NPDR patients, including patients with microaneurysms, hard exudates, retinal hemorrhages, and intraretinal microvascular abnormalities; and 3) PDR patients, including patients with neovascularization, fibrous tissue or hemorrhages in the vitreous body. Additionally, 156 T2DM patients without retinopathy were recruited during the same period. The inclusion and exclusion criteria for T2DM patients were the same as the diagnostic criteria for T2DM listed above.

For sample size calculations, the following formula[2] was used[1,2] (Equation 1):

$$n = \frac{(Z_\alpha + Z_\beta)^2 \times \sigma^2}{d^2} \quad (1)$$

According to previous studies, $\delta = 2$, $\sigma = 7$ and $Z_{1-\alpha/2} = 1.96$ (δ – tolerance error; σ – standard deviation, $1-\alpha$ – confidence level), and the minimal sample size was 48. All patients signed a written informed consent form. The study was approved by the Ethical Committee of Jiangxi Provincial People's Hospital (approval No. 2021007).

Blood sampling and ELISA

Serum levels of SOCS6, C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), vascular endothelial growth factor (VEGF), and angiopoietin-2 (ANG-2) were measured using enzyme-linked immunosorbent assay (ELISA). Briefly, fasting cubital venous blood (5 mL) was collected from all patients within 24 h of admission. The blood samples were centrifuged at 2000 g for 15 min. After centrifugation, the levels of SOCS6, CRP, IL-6, TNF-α, IL-1β, VEGF,

and ANG-2 were measured using commercially available ELISA kits (SOCS6: MBS2610988 (MyBioSource, San Diego, USA), detection range 0.156–10 ng/mL; CRP: EK1316, detection range 312–20000 pg/mL; IL-6: EK0410, detection range 4.69–300 pg/mL; TNF-α: EK0525, detection range 15.6–1000 pg/mL; IL-1β: EK0392, detection range 3.9–250 pg/mL; VEGF: EK0539, detection range 31.2–2000 pg/mL; ANG-2: EK0657, detection range 156–10000 pg/mL), according to the manufacturer's (unless stated otherwise: Boster Bio-Engineering, Wuhan, China) instructions.

Patient data collection

Demographic and clinical data were collected from all patients, including age, body mass index (BMI), sex, the course of DM, systolic blood pressure (SBP), diastolic blood pressure (DBP), use of tobacco, etc. Whole blood testing was performed using an automatic biochemical analyzer (Hitachi 7600; Hitachi Corp., Tokyo, Japan), and the levels of fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were recorded.

Statistical analyses

Continuous data are presented as mean ± standard deviation (M ±SD) for normally-distributed data or median (minimum to maximum) for non-normally-distributed data (confirmed with Kolmogorov–Smirnov test). For non-normally distributed data, Mann–Whitney U test was used for comparisons between the 2 groups. For normally distributed data, comparisons between the 2 groups were conducted using Student's t-test. The χ^2 test was used to analyze the rates. The correlations among SOCS6, inflammatory factors and angiogenic factors were determined with Pearson's rank correlation analysis. Receiver operating characteristic (ROC) curves were used for the analysis of SOCS6 in DR patients. The logistic regression was conducted for DR risk factors. The Hosmer–Lemeshow test and the Nagelkerke's R^2 were used for the goodness-of-fit analysis. A value of $p < 0.05$ was considered statistically significant. The SPSS v. 18.0 software (SPSS Inc., Chicago, USA) was used for all statistical analyses.

Results

Basic patient characteristics

This study included 159 DR patients – 81 NPDR patients and 78 PDR patients. The course of DM was significantly longer, and the age, SBP, DBP, and the levels of LDL-C and TC were significantly higher in PDR patients compared to the NPDR patients ($p < 0.05$). Compared to the T2DM patients without retinopathy (n = 156), the course of DM was significantly longer, and the age, SBP, DBP, ratio of smokers, and the levels of LDL-C and TC were markedly higher in the DR patients (Table 1). No other significant differences were found.

Expression of SOCS6

Next, the expression levels of serum SOCS6 were determined. The DR patients showed significantly lower SOCS6 levels compared to the T2DM patients without retinopathy (Fig. 1). In addition, the serum SOCS6 levels in PDR patients were remarkably lower than in NPDR patients, suggesting that SOCS6 is associated with the severity of DR.

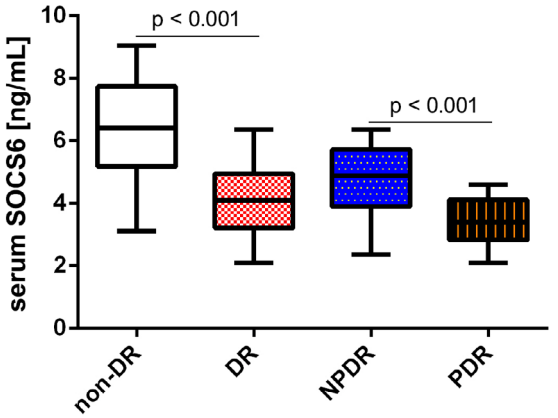

Fig. 1. Serum levels of suppressor of cytokine signaling 6 (SOCS6) in all patients. In the boxplots, the data are expressed as medians (minimum to maximum). The continuous data were compared using Student's t-test. All data are normally distributed

DR – diabetic retinopathy; NPDR – nonproliferative DR; PDR – proliferative DR.

Correlations between SOCS6 and angiogenic and inflammatory factors

The IL-6, TNF-α, IL-1β, VEGF, and ANG-2 levels in PDR patients were remarkably higher than in NPDR patients (Fig. 2). Comparisons with T2DM patients without retinopathy showed that the CRP, IL-6, TNF-α, IL-1β, VEGF, and ANG-2 levels in DR patients were also pronouncedly higher. The Pearson's analyses showed that SOCS6 levels correlated negatively with CRP, IL-6, TNF-α, IL-1β, VEGF, and ANG-2 (Table 2).

Correlations between serum SOCS6 levels and T2DM patients' clinical outcomes

Next, all patients were divided into SOCS6 high-expression and low-expression groups according to the mean value (4.15 ng/mL), and the clinical characteristics between these groups were compared. As summarized

Table 1. Basic clinical characteristics of all patients

Variables	Non-retinopathy T2DM (n = 156)	All DR (n = 165)	NPDR (n = 87)	PDR (n = 78)	U/χ^2	p_1	U/χ^2	p_2
Age [years]	58 (44–74)	62 (46–75)	62 (46–75)	62 (51–73)	10228.00	0.010	3296.00	0.751
Female, n (%)	70 (44.87)	72 (43.63)	38 (43.68)	34 (43.59)	0.031	0.999	<0.001	0.999
BMI	25.24 (21.02–29.32)	25.12 (20.95–29.23)	25.39 (20.95–29.21)	24.42 (20.96–29.23)	12485.00	0.643	2885.00	0.097
Current smoker, n (%)	31 (19.87)	70 (42.42)	37 (42.53)	33 (42.31)	13.816	0.010	0.001	0.999
Course of DM [years]	8 (1–15)	15 (7–22)	14 (7–20)	16 (10–22)	3958.00	<0.001	2029.00	<0.001
Complications								
Serum uric acid, n (%)	18 (11.54)	21 (12.74)	9 (10.34)	13 (16.67)	0.068	0.999	1.715	0.214
Diabetic nephropathy, n (%)	35 (22.44)	52 (31.52)	24 (27.59)	28 (35.90)	2.092	0.151	1.594	0.289
Coronary disease, n (%)	41 (26.28)	57 (35.55)	27 (31.03)	30 (38.46)	2.012	0.219	1.217	0.372
SBP [mm Hg]	127.45 (107.12–144.96)	139.15 (117.88–157.90)	135.12 (117.88–148.47)	143.86 (124.61–157.90)	5616.00	<0.001	1583.00	<0.001
DBP [mm Hg]	83.86 (71.41–94.21)	95.43 (82.98–103.45)	91.66 (82.98–99.69)	97.52 (89.74–103.45)	2549.00	<0.001	1236.00	<0.001
FBG [mmol/L]	8.31 (7.23–9.33)	8.28 (7.22–9.33)	8.38 (7.23–9.33)	8.23 (7.22–9.33)	12541.50	0.693	3259.00	0.662
TC [mmol/L]	4.86 (3.36–6.09)	5.13 (3.34–7.65)	4.63 (3.34–6.09)	5.83 (3.49–7.65)	10425.50	0.030	1473.50	<0.001
TG [mmol/L]	1.49 (0.68–2.35)	1.62 (0.71–2.36)	1.57 (0.71–2.35)	1.74 (0.78–2.36)	11159.50	0.400	2938.50	0.138
HDL-C [mmol/L]	1.02 (0.48–1.52)	1.00 (0.50–1.52)	1.03 (0.50–1.52)	0.99 (0.50–1.52)	12694.00	0.832	3207.50	0.545
LDL-C [mmol/L]	3.00 (1.89–3.75)	3.30 (1.85–4.98)	2.93 (1.85–3.75)	3.90 (2.53–4.98)	9227.00	<0.001	897.50	<0.001

DR – diabetic retinopathy; NPDR – nonproliferative DR; PDR – proliferative DR; BMI – body mass index; T2DM – type 2 diabetes mellitus; SBP – systolic blood pressure; DBP – diastolic blood pressure; FBG – fasting plasma glucose; TC – total cholesterol; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; p_1 – comparison between non-retinopathy T2DM patients and all DR patients; p_2 – comparison between NPDR patients and PDR patients. All continuous data were non-normally distributed (age, BMI, course of DM, SBP, DBP, FBG, TC, TG, HDL-C, and LDL-C), and were expressed as median (minimum to maximum). The comparisons between the 2 groups were analyzed using the Mann–Whitney U test. The χ^2 test was used for comparing rates (sex, current smoker, serum uric acid, diabetic nephropathy, coronary artery disease (CAD)). U/χ^2 – levels of U in Mann–Whitney U test or χ^2 in χ^2 test.

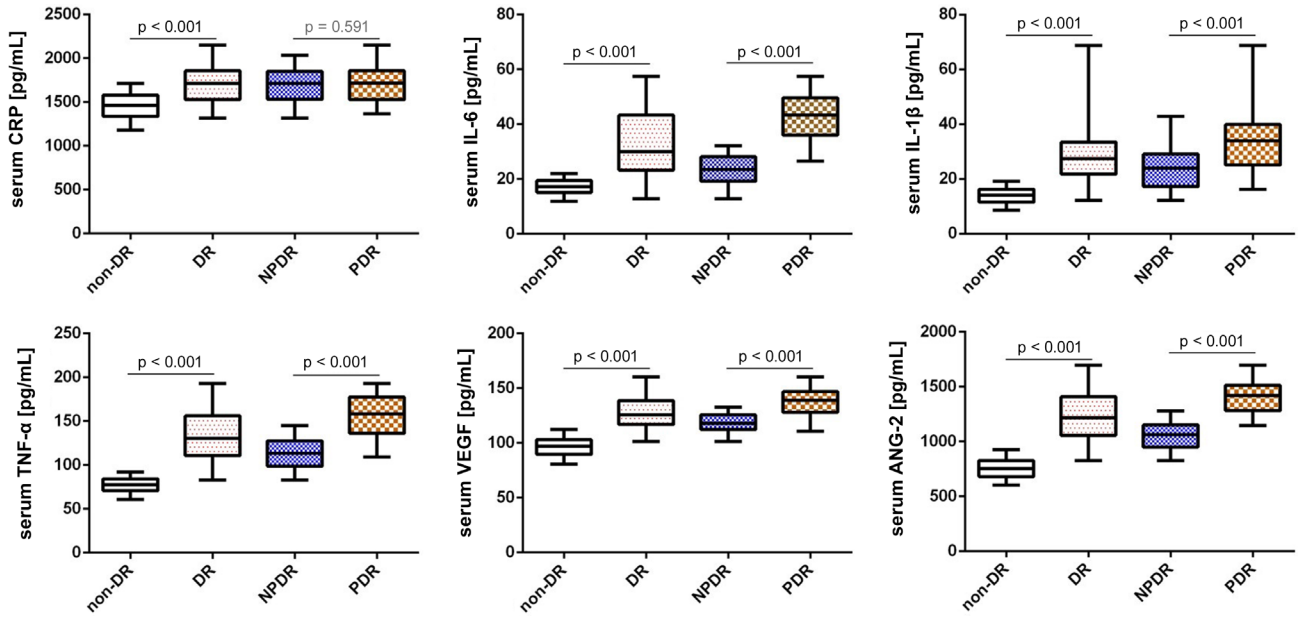

Fig. 2. Serum levels of C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), vascular endothelial growth factor (VEGF), and angiopoietin-2 (ANG-2) in all patients. In the boxplots, data are expressed as medians (minimum to maximum). The continuous data were compared using Student's t-test. All data are normally distributed

DR – diabetic retinopathy; NPDR – nonproliferative DR; PDR – proliferative DR.

Table 2. Correlation analysis of SOCS6, angiogenic factors and inflammatory factors

Variables and Pearson's correlation		SOCS6	CRP	IL-6	TNF-α	IL-1β	VEGF	ANG-2
SOCS6	Pearson's correlation	1	–0.373	–0.592	–0.627	–0.561	–0.597	–0.646
	p-value	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CRP	Pearson's correlation	–0.373	1	0.378	0.454	0.401	0.491	0.459
	p-value	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.001
IL-6	Pearson's correlation	–0.592	0.378	1	0.781	0.682	0.754	0.820
	p-value	<0.001	<0.001	–	<0.001	<0.001	<0.001	<0.001
TNF-α	Pearson's correlation	–0.627	0.454	0.781	1	0.729	0.758	0.818
	p-value	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001
IL-1β	Pearson's correlation	–0.561	0.401	0.682	0.729	1	0.658	0.749
	p-value	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001
VEGF	Pearson's correlation	–0.597	0.491	0.754	0.758	0.658	1	0.794
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	–	<0.001
ANG-2	Pearson's correlation	–0.646	0.459	0.820	0.818	0.749	0.794	1
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–

SOCS6 – suppressor of cytokine signaling 6; CRP – C-reactive protein; IL-6 – interleukin-6; TNF-α – tumor necrosis factor alpha; IL-1β – interleukin-1β; VEGF – vascular endothelial growth factor; ANG-2 – angiopoietin-2.

Table 3. Comparison of clinical outcomes between patients with high and low expression of SOCS6

Variables	High SOCS6 (n = 79)	Low SOCS6 (n = 86)	U/t/χ^2	p-value
Age [years]	62 (46–75)	62 (48–75)	3272.50	0.684
Female, n (%)	31 (39.24)	41 (47.67)	1.436	0.254
Course of DM [years]	14 (7–22)	15 (7–22)	2608.00	0.010
Current smoker, n (%)	35 (40.70)	35 (44.30)	0.270	0.775
BMI	25.72 (20.95–29.21)	24.48 (20.96–29.23)	2852.50	0.076
FBG [mmol/L]	8.29 (7.22–9.33)	8.26 (7.23–9.33)	3218.00	0.559
SBP [mm Hg]	136.30 (117.88–154.50)	142.66 (120.08–157.90)	2436.00	0.002
DBP [mm Hg]	93.62 (82.98–102.99)	96.06 (84.63–103.45)	2333.50	0.001
TC [mmol/L]	4.90 (3.34–7.55)	5.48 (3.38–7.65)	2500.50	0.003
TG [mmol/L]	1.60 (0.71–2.35)	1.65 (0.78–2.36)	3299.50	0.750
HDL-C [mmol/L]	1.03 (0.52–1.52)	0.99 (0.50–1.52)	3028.50	0.229
LDL-C [mmol/L]	3.11 (1.85–4.85)	3.60 (1.85–4.98)	2206.00	<0.001
CRP [pg/mL]	1692.76 ±193.27	1707.83 ±212.66	0.475	0.635
IL-6 [pg/mL]	27.96 ±10.32	37.11 ±11.51	5.382	<0.001
TNF-α [pg/mL]	123.34 ±28.82	142.69 ±27.76	4.391	<0.001
IL-1β [pg/mL]	25.19 ±8.53	30.86 ±9.22	4.094	<0.001
VEGF [pg/mL]	120.84 ±12.03	132.88 ±13.86	5.968	<0.001
ANG-2 [pg/mL]	1117.45 ±210.79	1319.58 ±195.02	6.398	<0.001

SOCS6 – suppressor of cytokine signaling 6; BMI – body mass index; DM – diabetes mellitus; SBP – systolic blood pressure; DBP – diastolic blood pressure; FBG – fasting plasma glucose; TC – total cholesterol; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; CRP – C-reactive protein; IL-6 – interleukin-6; TNF-α – tumor necrosis factor alpha; IL-1β – interleukin-1β; VEGF – vascular endothelial growth factor; ANG-2 – angiopoietin-2. The comparison between high and low SOCS6 groups is expressed by p-values. Continuous data that were non-normally distributed (age, BMI, course of DM, SBP, DBP, FBG, TC, TG, HDL-C, and LDL-C) are expressed as median (minimum to maximum) and analyzed with Mann–Whitney U test. Continuous data that were normally distributed (CRP, IL-6, TNF-α, IL-1β, VEGF, and ANG-2) are expressed as mean ± standard deviation (M ±SD) and analyzed using Student's t-test. The χ^2 test was used for rates (gender and current smoker). U/χ^2 – levels of U in Mann–Whitney U test or χ^2 in χ^2 test.

in Table 3, the serum levels of IL-6, TNF-α, IL-1β, VEGF, and ANG-2 in the SOCS6 low-expression group were significantly higher compared to those in the SOCS6 high-expression group ($p < 0.05$). In addition, compared to the SOCS6 high-expression group, the course of DM was significantly prolonged, and the age SBP, DBP, as well as the levels of LDL-C and TC were significantly increased in the SOCS6 low-expression group. These results suggest

that SOCS6 is associated with clinical outcomes and severity in T2DM patients.

Diagnostic value of SOCS6 and angiogenic and inflammatory factors for DR

The ROC curves were constructed to evaluate the diagnostic value of SOCS6 in DR patients. The results showed that SOCS6 could be a potential diagnostic biomarker for DR (Fig. 3). The area under the curve (AUC) for SOCS6 was 0.871 and the cutoff value was 4.61 ng/mL, with a sensitivity of 85% and a specificity of 69%. The SOCS6 could also be used as a biomarker for NPDR or PDR patients. As shown in Fig. 3, the AUC for SOCS6 was 0.825, with a cutoff value of 3.66 ng/mL, a sensitivity of 79%, and a specificity of 64%.

Logistic regression for risk factors for DR

Finally, we used the logistic regression to identify the risk factors for DR. For logistic regression, we used 3 models for the entry method. In model 1 (age, BMI, course of DM, SBP, DBP, TC, TG, HDL-C, LDL-C, and SOCS6), the results of the Hosmer–Lemeshow test (p = 0.139) and

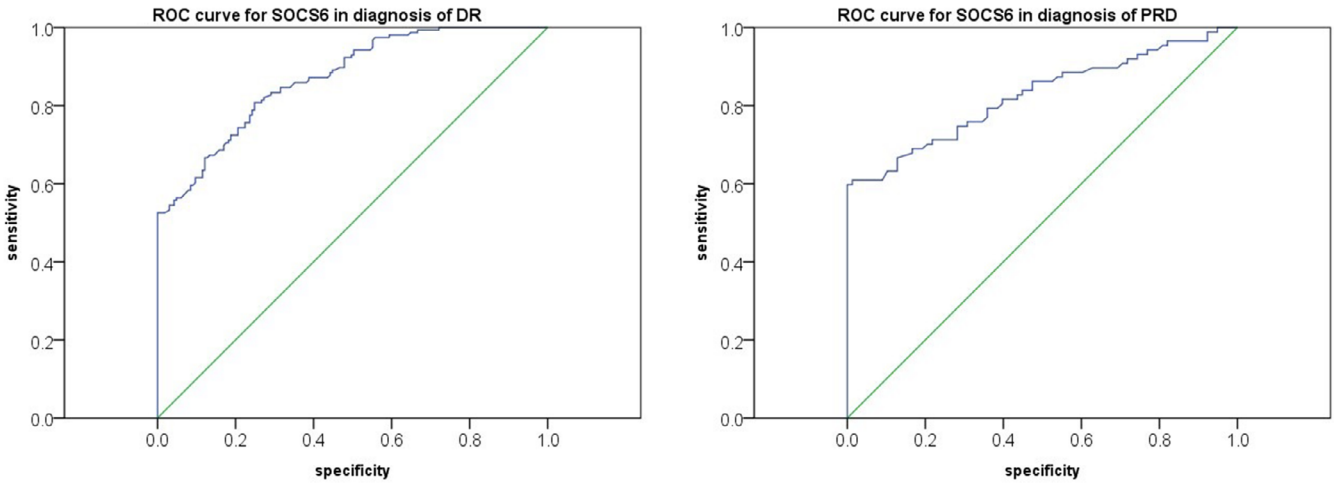

Fig. 3. Receiver operating characteristic (ROC) curves for the diagnostic value of suppressor of cytokine signaling 6 (SOCS6) for diabetic retinopathy (DR) or proliferative DR (PDR)

NPDR – nonproliferative DR.

Table 4. Logistic regression for risk factors of DR

Model	Variables	H–L test	Nagelkerke R^2	Wald	OR	95% CI	p-value
Model 1	age	0.139	0.844	2.926	1.051	0.993–1.113	0.087
	BMI			0.372	1.066	0.869–1.308	0.542
	course of DM			23.433	1.387	1.215–1.584	<0.001
	SBP			12.756	1.098	1.043–1.156	<0.001
	DBP			27.846	1.316	1.189–1.458	<0.001
	TC			5.416	0.508	0.287–0.899	<0.001
	TG			1.645	1.983	0.696–5.645	0.200
	LDL-C			0.123	1.155	0.516–2.586	0.726
	HDL-C			0.005	0.945	0.179–4.971	0.945
	SOCS6			19.253	0.410	0.275–0.610	<0.001
Model 2	CRP	0.999	0.981	1.714	1.008	0.996–1.102	0.190
	IL-6			2.721	1.479	0.929–2.355	0.099
	TNF-α			5.216	1.548	1.064–2.253	0.022
	IL-1β			5.003	2.238	1.105–4.532	0.025
Model 3	VEGF	1.000	0.981	8.112	1.725	1.185–2.510	0.004
	ANG-2			7.438	1.049	1.014–1.086	0.006

DR – diabetic retinopathy; H–L – Hosmer–Lemeshow test; OR – odds ratio; 95% CI – 95% confidence interval; BMI – body mass index; DM – diabetes mellitus; SBP – systolic blood pressure; DBP – diastolic blood pressure; TC – total cholesterol; TG – triglycerides; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; SOCS6 – suppressor of cytokine signaling 6; CRP – C-reactive protein; IL-6 – interleukin-6; TNF-α – tumor necrosis factor alpha; IL-1β – interleukin-1β; VEGF – vascular endothelial growth factor; ANG-2 – angiopoietin-2.

the Nagelkerke's R^2 (0.844) showed adequate goodness-of-fit, with SOCS6, the course of DM, SBP, and DBP identified as risk factors for DR (Table 4). In model 2 (inflammatory factors CRP, IL-6, TNF-α, and IL-1β), the Hosmer–Lemeshow test (p = 0.999) and the Nagelkerke's R^2 (0.981) showed adequate goodness-of-fit, and IL-1β and TNF-α were identified as risk factors for DR. In model 3 (angiogenesis factors VEGF and ANG-2), the Hosmer–Lemeshow test (p = 1.000) and the Nagelkerke's R^2 (0.981) showed adequate goodness-of-fit, with both VEGF and ANG-2 identified as risk factors for DR.

Discussion

Globally, it is estimated that the number of DR cases will grow to 191 million by 2030, with vision-threatening DR patients numbering over 56 million.[19] Despite the development of DR diagnostic and treatment strategies, DR is still the leading cause of blindness in working-age population. However, at present, DR is diagnosed using fundus photography with dilated pupils[20] and lacks specific biomarkers. Thus, it is urgent to develop new biomarkers and comprehensive approaches to reduce the risk of vision loss by prompt diagnosis and early treatment of DR. In the present study, we showed that serum SOCS6 levels were decreased in DR patients and were associated with clinical outcomes and severity.

A number of studies have indicated that cytokines, angiogenic markers and some clinical biomarker changes may cause DR. For example, a prospective study by Muni et al. found that serum high-sensitivity CRP is significantly correlated with the risk for DR.[21] In addition, a case-control study by Churchill et al. confirmed that VEGF is associated with the severity of DR.[22] In the current study, we found that the inflammatory factors CRP, IL-6, IL-1β, and TNF-α were elevated in DR patients. These results are consistent with several previous studies.[23–25] Changes in several clinical biomarkers are also associated with DR. For example, a 3-year prospective study in Taiwan showed that serum uric acid levels are associated with DR.[9] Gao et al. reported that there may be a significant correlation between serum thyroglobulin antibody levels and the severity of DR.[26] However, at present, there are no specific biomarkers for the diagnosis of DR.

The SOCS6 is an anti-inflammatory factor in many diseases, including DM. It has been previously reported that the inhibition of SOCS6 causes a significant increase in cell permeability and inflammation, and that overexpressing SOCS6 reverses cell permeability and the inflammatory response.[27] Chen et al. found that the upregulation of lncRNA FGD5-AS1 could protect against periodontitis through enhancing the levels of SOCS6.[11] Meng et al. showed that the knockdown of miR-16-5p could suppress cell proliferation and accelerate the apoptosis of fibroblast-like synoviocytes through targeting SOCS6.[28] In addition, Xue et al. reported that miR-494-3p facilitated high glucose-induced renal fibrosis through the promotion of cell apoptosis and epithelial–mesenchymal transformation via targeting SOCS6.[29] The SOCS6 is also correlated with angiogenic markers. Yuan et al. confirmed that the upregulation of SOCS6 inhibits angiogenesis and tumor xenograft growth, and is significantly associated with the prognosis of human prostate cancer.[30]

The SOCS proteins improve glucose metabolism, reduce the deleterious effects of inflammation and promote neuroprotection. Most of the known SOCS proteins are involved in the regulation of insulin resistance, β-cell failure and the eventual development of DM.[31] Several studies have also focused on the molecular mechanistic effects of SOCS6 in DM. Luo et al. confirmed that SOCS6 could regulate inflammation, oxidative stress and apoptosis in retinal epithelial cells, and attenuate DR in a rat model.[32] In addition, Liu et al. suggested that the constitutive expression of the SOCS6 protein in retinal neurons improves glucose metabolism in vivo and in vitro.[33] Xiao et al. confirmed that MEG3 targets the miR-19b/SOCS6 axis in order to inhibit high glucose-induced human retinal microvascular endothelial cells apoptosis, while increasing the expression of SOCS6 and benefiting DR.[12]

As inflammation and vascular risk factors play important roles in the pathogenesis of DR, we inferred that SOCS6 may also influence DR development through the regulation of inflammation and angiogenesis. However, there is a lack of relevant reports regarding the role of SOCS6 in DR. In the present study, we found that the expression of serum SOCS6 is decreased in DR and DM patients, and is correlated with the severity of multiple clinical features, including inflammatory and angiogenic factors.

Limitations

The present study has some limitations. First, we only included a small sample of the study population. In addition, we examined a relatively small number of inflammatory and angiogenic factors. Finally, the molecular mechanisms of SOCS6 that influence DR development were not examined.

Conclusions

This study showed that the serum levels of SOCS6 are decreased in DR patients. Serum SOCS6 levels were also related to DR severity and clinical outcomes, including inflammatory and angiogenic factors. Thus, this study provides more evidence for the role of SOCS6 in DR.

Data availability

All data can be obtained from the corresponding author by reasonable request.

ORCID iDs

Lan Li https://orcid.org/0000-0002-9842-2601
Xiaoyan Wu https://orcid.org/0000-0001-7405-3082
Lemin He https://orcid.org/0000-0001-6194-7554
Rong Luo https://orcid.org/0000-0001-9151-9905
LiQiong Lou https://orcid.org/0000-0002-4639-2105

References

1. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
2. American Diabetes Association. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes 2021. *Diabetes Care*. 2021;44(Suppl 1):S15–S33. doi:10.2337/dc21-S002
3. Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119. doi:10.1016/j.diabres.2021.109119
4. Xu Y, Wang L, He J, et al. Prevalence and control of diabetes in Chinese adults. *JAMA*. 2013;310(9):948. doi:10.1001/jama.2013.168118
5. Weng JP, Bi Y. Epidemiological status of chronic diabetic complications in China. *Chin Med J (Engl)*. 2015;128(24):3267–3269. doi:10.4103/0366-6999.171350
6. Thomas RL, Halim S, Gurudas S, Sivaprasad S, Owens DR. IDF Diabetes Atlas: A review of studies utilising retinal photography on the global prevalence of diabetes related retinopathy between 2015 and 2018. *Diabetes Res Clin Pract*. 2019;157:107840. doi:10.1016/j.diabres.2019.107840
7. Hartnett ME, Baehr W, Le YZ. Diabetic retinopathy, an overview. *Vision Res*. 2017;139:1–6. doi:10.1016/j.visres.2017.07.006
8. Al-Turki YA. Blood sugar control, ophthalmology referral and creatinine level among adult diabetic patients in primary health care, Riyadh, Saudi Arabia. *Saudi Med J*. 2002;23(11):1332–1334. PMID:12506290.
9. Lee JJ, Yang IH, Kuo HK, et al. Serum uric acid concentration is associated with worsening in severity of diabetic retinopathy among type 2 diabetic patients in Taiwan: A 3-year prospective study. *Diabetes Res Clin Pract*. 2014;106(2):366–372. doi:10.1016/j.diabres.2014.07.027
10. Duncan SA, Baganizi DR, Sahu R, Singh SR, Dennis VA. SOCS proteins as regulators of inflammatory responses induced by bacterial infections: A review. *Front Microbiol*. 2017;8:2431. doi:10.3389/fmicb.2017.02431
11. Chen H, Lan Z, Li Q, Li Y. Abnormal expression of long noncoding RNA FGD5-AS1 affects the development of periodontitis through regulating miR-142-3p/SOCS6/NF-κB pathway. *Artif Cells Nanomed Biotechnol*. 2019;47(1):2098–2106. doi:10.1080/21691401.2019.1620256
12. Xiao F, Li L, Fu JS, Hu YX, Luo R. Regulation of the miR-19b-mediated SOCS6-JAK2/STAT3 pathway by lncRNA MEG3 is involved in high glucose-induced apoptosis in hRMECs. *Biosci Rep*. 2020;40(7):BSR20194370. doi:10.1042/BSR20194370
13. Yuan D, Wang W, Su J, et al. SOCS6 functions as a tumor suppressor by inducing apoptosis and inhibiting angiogenesis in human prostate cancer. *Curr Cancer Drug Targets*. 2018;18(9):894–904. doi:10.2174/1568009618666180102101442
14. Spijkerman AMW, Gall MA, Tarnow L, et al. Endothelial dysfunction and low-grade inflammation and the progression of retinopathy in type 2 diabetes: Original article. *Diabetes Med*. 2007;24(9):969–976. doi:10.1111/j.1464-5491.2007.02217.x
15. Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, et al. The effect of ubiquinone and combined antioxidant therapy on oxidative stress markers in non-proliferative diabetic retinopathy: A phase IIa, randomized, double-blind, and placebo-controlled study. *Redox Rep*. 2016;21(4):155–163. doi:10.1179/1351000215Y.0000000040
16. Aljundi W, Suffo S, Munteanu C, Langenbucher A, Seitz B, Abdin A. Intravitreal injection for diabetic macular edema as adjunctive therapy for proliferative diabetic retinopathy: A retrospective study. *Clin Ophthalmol*. 2022;16:135–143. doi:10.2147/OPTH.S346065
17. Chinese Elderly Type 2 Diabetes Prevention and Treatment of Clinical Guidelines Writing Group, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Society, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Health Care Society, Geriatric Professional Committee of Beijing Medical Award Foundation, National Clinical Medical Research Center for Geriatric Diseases (PLA General Hospital). Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition) [in Chinese]. *Zhonghua Nei Ke Za Zhi*. 2022;61(1):12–50. doi:10.3760/cma.j.cn112138-20211027-00751
18. Wilkinson CP, Ferris FL, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110(9):1677–1682. doi:10.1016/S0161-6420(03)00475-5
19. Zheng Y, He M, Congdon N. The worldwide epidemic of diabetic retinopathy. *Indian J Ophthalmol*. 2012;60(5):428. doi:10.4103/0301-4738.100542
20. Mookiah MRK, Acharya UR, Chua CK, Lim CM, Ng EYK, Laude A. Computer-aided diagnosis of diabetic retinopathy: A review. *Comput Biol Med*. 2013;43(12):2136–2155. doi:10.1016/j.compbiomed.2013.10.007
21. Muni RH, Kohly RP, Lee EQ, Manson JE, Semba RD, Schaumberg DA. Prospective study of inflammatory biomarkers and risk of diabetic retinopathy in the diabetes control and complications trial. *JAMA Ophthalmol*. 2013;131(4):514. doi:10.1001/jamaophthalmol.2013.2299
22. Churchill AJ, Carter JG, Ramsden C, et al. VEGF polymorphisms are associated with severity of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2008;49(8):3611. doi:10.1167/iovs.07-1383
23. Nowak M, Wielkoszyński T, Marek B, et al. Antioxidant potential, paraoxonase 1, ceruloplasmin activity and C-reactive protein concentration in diabetic retinopathy. *Clin Exp Med*. 2010;10(3):185–192. doi:10.1007/s10238-009-0084-7
24. Gustavsson C, Agardh E, Bengtsson B, Agardh CD. TNF-α is an independent serum marker for proliferative retinopathy in type 1 diabetic patients. *J Diabetes Complications*. 2008;22(5):309–316. doi:10.1016/j.jdiacomp.2007.03.001
25. Khalaf N, Helmy H, Labib H, Fahmy I, Abd El Hamid M, Moemen L. Role of angiopoietins and Tie-2 in diabetic retinopathy. *Electron Physician*. 2017;9(8):5031–5035. doi:10.19082/5031
26. Gao X, Wang X, Zhong Y, Liu L, Teng W, Shan Z. Serum antithyroglobulin antibody levels are associated with diabetic retinopathy among euthyroid type 2 diabetes patients: A hospital-based, retrospective study. *J Diabetes Res*. 2022;2022:2552186. doi:10.1155/2022/2552186
27. Ma J, Xu LY, Sun QH, Wan XY, Li B. Inhibition of miR-1298-5p attenuates sepsis lung injury by targeting SOCS6. *Mol Cell Biochem*. 2021;476(10):3745–3756. doi:10.1007/s11010-021-04170-w
28. Meng D, Pan W, Li J. MicroRNA-16-5p promoted fibroblast-like synoviocyte proliferation and suppressed apoptosis via targeting suppressor of cytokine signaling 6 (SOCS6) in human rheumatoid arthritis. *J Biomater Tissue Eng*. 2021;11(9):1744–1751. doi:10.1166/jbt.2021.2600
29. Xue X, Liu M, Wang Y, et al. MicroRNA-494-3p exacerbates renal epithelial cell dysfunction by targeting SOCS6 under high glucose treatment. *Kidney Blood Press Res*. 2022;47(4):247–255. doi:10.1159/000521647
30. Yuan D, Wang W, Su J, et al. SOCS6 functions as a tumor suppressor by inducing apoptosis and inhibiting angiogenesis in human prostate cancer. *Curr Cancer Drug Targets*. 2018;18(9):894–904. doi:10.2174/1568009618666180102101442
31. Suchy D, Łabuzek K, Machnik G, Kozłowski M, Okopień B. SOCS and diabetes: Ups and downs of a turbulent relationship. *Cell Biochem Funct*. 2013;31(3):181–195. doi:10.1002/cbf.2940
32. Luo R, Li L, Xiao F, Fu J. LncRNA FLG-AS1 mitigates diabetic retinopathy by regulating retinal epithelial cell inflammation, oxidative stress, and apoptosis via miR-380-3p/SOCS6 axis. *Inflammation*. 2022;45(5):1936–1949. doi:10.1007/s10753-022-01665-6
33. Liu X, Mameza MG, Lee YS, et al. Suppressors of cytokine-signaling proteins induce insulin resistance in the retina and promote survival of retinal cells. *Diabetes*. 2008;57(6):1651–1658. doi:10.2337/db07-1761