

Correlation Between Ultrawide-Field Fluorescence Contrast Results and White Blood Cell Indexes in Diabetic Retinopathy

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Research Article

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Abstract

Background: Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes. DR involves a state of systemic inflammation, and chronic inflammation can promote microvascular and macrovascular diseases in diabetic patients and accelerate disease progression. Ultrawide-field FFA (UWFA) systems are increasingly being used to examine a wider retina. To explore the correlation between the different manifestations of retinopathy under UWFA and the systemic indicators of white blood cells in patients with diabetic retinopathy .

Methods: This retrospective study included the hospitalized DR patients in the Department of Ophthalmology and Endocrinology of the Affiliated Hospital 2 of Nantong University between January 2016 and March 2019. This study examined the correlations between the UWFA examination results and glycated hemoglobin (HbA1c), routine blood tests, blood coagulation function, liver and kidney function, and the neutrophil-to-lymphocyte ratio of patients with clinically diagnosed DR during hospitalization.

Results: A total of 115 patients with DR (53 females and 62 males) were included (199 eyes: 102 right eyes and 97 left eyes). UWFA revealed that most eyes (77.4%) had grade 4 microvascular leakage, 52.8% had grade 0 capillary non-perfusion area, 59.3% had grade 0 neovascularization, and 92.0% had grade 0 fibrous proliferative membranes. Microvascular leakage was correlated with the NLR ($r=0.186$, $P=0.027$). Capillary non-perfusion area was correlated with the monocyte ratio ($r=0.144$, $P=0.042$) and the eosinophil ratio ($r=0.123$, $P=0.044$). Neovascularization was correlated to the monocyte ratio ($r=0.324$, $P=0.018$). Finally, the fibrous proliferative membrane was correlated to the monocyte ratio ($r=0.418$, $P=0.002$). Only the eosinophil ratio was independently associated with proliferative DR (odds ratio=1.25, 95% confidence interval: 1.04-1.51, $P=0.018$).

Conclusion: The results of UWFA imaging in patients with DR are correlated with white blood cell population indexes. The eosinophil ratio was independently associated with proliferative DR.

Introduction

Chronic hyperglycemia (and other factors) can trigger biochemical and physiological changes that result in microvascular damage and retinal dysfunction [1, 2]. Diabetic retinopathy (DR) is a complication that causes blindness in diabetic patients [1, 2]. DR is the main cause of blindness in adults [1, 2]. The worldwide prevalence of DR in adults with diabetes is 34.6% [3]. The risk factors include long-duration diabetes, chronic hyperglycemia, nephropathy, hypertension, and dyslipidemia [1]. The course of the disease is difficult to reverse, and timely clinical intervention is needed [1, 2].

According to the fundus characteristics and different pathological changes, DR can be classified based on disease severity [1, 2]. Non-proliferative DR (NPDR) is characterized by retinal vascular abnormalities. Mild cases only involve microaneurysms. Moderate and severe cases involve additional vascular abnormalities. Proliferative DR (PDR) is characterized by retinal neovascularization in addition to vascular abnormalities. Diabetic macular edema is characterized by thickening of the retina near the macula.

The main tool used to distinguish between different grades of DR is fundus fluorescein angiography (FFA) [1, 2, 4]. It is a valuable diagnostic examination method. The retinal microvascular function can be observed

dynamically. The abnormal proliferation of new blood vessels and fibrous tissue can be displayed, which is used to evaluate the severity of retinopathy [1, 2, 4]. In the past, traditional angiography could only show the posterior polar retina at approximately 50°. Ultrawide-field FFA (UWFA) systems are increasingly being used to examine the peripheral retina [5]. A wider range of nonperfusion areas, microvascular abnormalities, and neovascularization have been found in the peripheral retina and are involved in most DR lesions [5, 6]. In the UWFA examination system, the image features of DR patients have been regraded and analyzed to obtain a more comprehensive understanding of DR progression [5–7].

At the same time, DR involves a state of systemic inflammation, and chronic inflammation can promote microvascular and macrovascular diseases in diabetic patients and accelerate disease progression [8]. There are five kinds of white blood cells in the peripheral blood: neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The neutrophils and neutrophil-to-lymphocyte ratio (NLR) can, to a certain extent, reflect the dynamic balance between inflammation and immune reactions [9]. Previous studies have shown that neutrophils, the NLR, and DR have a certain relationship with features of DR [10, 11]. Still, no studies are available for UWFA results.

Therefore, this study aimed to explore the correlation between the different manifestations of retinopathy under UWFA and the systemic indicators of white blood cells. The results could help refine the relation between retinal changes and systemic inflammation markers.

Methods

Study design and patients

This study complied with the Helsinki Declaration and was approved by the Ethics Committee of the Affiliated Hospital 2 of Nantong University (2021KT004). The requirement for informed consent was waived by the Ethics Committee of the Affiliated Hospital 2 of Nantong University because of the retrospective nature of the study.

This retrospective study included the hospitalized patients in the Department of Ophthalmology and Endocrinology of the Affiliated Hospital 2 of Nantong University between January 2016 and March 2019. The inclusion criteria were 1) patients clinically diagnosed with type 2 diabetes [12], 2) patients with complete hematological indicators (HbA1c, routine blood tests, blood coagulation function, liver and kidney function, etc.) during hospitalization, 3) patients who completed UWFA examination during hospitalization and were diagnosed with DR, and 4) no history of eye surgery or laser treatment. The exclusion criteria were 1) opacity of the refractive media (keratopathy, cataract, vitreous hemorrhage, etc.) that did not allow the effective observation of the angiographic image results, thus affecting the image classification and grading, 2) history of eye treatment, including retinal laser photocoagulation, intravitreal injection, vitrectomy, etc., 3) patients who underwent internal eye surgery for other non-DR diseases (such as anti-glaucoma, cataract extraction, retinal detachment surgery, etc.), 4) previous and new cases of uveitis, 5) patients with other forms of retinopathy revealed by angiography, or 6) patients with infectious or inflammatory lesions of other organs of the body and obvious abnormal routine blood tests caused by drugs.

UWFA

In order to exclude contraindications, all subjects were injected with 3 ml of 20% sodium fluorescein through a precubital vein, and fundus images were taken using a Heidelberg Spectralis ultrawide-angle lens (102°). The venous UWFA images of all eyes were read and reanalyzed by two physicians with more than 10 years of experience in fundus angiography diagnosis. The results of the analyses by the two doctors were compared, and the images with different judgments were re-assessed to obtain the final result.

During the venous phase of UWFA, four image features were extracted, graded, and recorded. (1) Microvascular dye leakage. Those with no leakage anywhere in the retina were classified as grade 0, and the rest were classified as grade 1-4 according to the number of quadrants with dye leakage. (2) Capillary nonperfusion area. The capillary nonperfusion area was divided into two grades. The image measurement tools of the Heidelberg fundus camera were used. Those with a nonperfusion area less than 7 DD were rated as grade 0, and those with a nonperfusion area greater than 7 DD were rated as grade 1. (3) Neovascularization. No neovascularization of the retina or optic disc was recorded as grade 0. According to the number of quadrants in which new blood vessels were present, grades 1-4 were assigned. For optic disc neovascularization, the center of the optic disc was taken as the point of origin, and the optic disc was divided into four quadrants horizontally/vertically. The neovascularization in each quadrant of the optic disc was combined with the corresponding retinal quadrant for calculation. (4) Fiber proliferative membrane. A retina or optic disc without fibrous proliferative membrane was rated as grade 0, and those with fibrous proliferative membrane were rated as grade 1 (Figure 1).

DR was classified as NPDR and PDR according to the International Clinical Classification of DR (2002) [13]. The core difference between PDR and NPDR is the formation of retinal neovascularization, in which the new blood vessels in the retina break through the inner boundary membrane.

Data Collection

Patient characteristics, glycated hemoglobin (HbA1c) levels, neutrophil values, lymphocyte values, monocyte values, and the NLR 1 week before angiography were collected.

Statistical analysis

SPSS 23.0 (IBM, Armonk, NY, USA) was used for statistical analysis at the eye level. Spearman correlation analysis was conducted between peripheral blood indexes and UWFA image features. Logistics regression analysis was used to analyze the factors influencing PDR. Variables with $P < 0.10$ in the univariable analyses were included in the multivariable analysis (enter method). P -values < 0.05 were considered statistically significant.

Results

Characteristics of the patients and eyes

The study included 115 patients (53 females and 62 males). There were 199 eyes; 102 right eyes and 97 left eyes were examined. The mean age was 56.7 ± 8.9 years. The course of diabetes was 11.2 ± 7.2 years. Fifty-four

patients had a history of hypertension. There were 118 cases of NPDR in 199 eyes, including 15 mild NPDR eyes, 10 moderate NPDR eyes, and 93 severe NPDR eyes. PDR was present in 81 eyes (Table 1).

Table 1
Characteristics of the patients

Characteristics	n=115
Age (years)	56.7±8.9
Sex (male/female)	62/53
Course of diabetes (years)	10 (1, 30)
HbA1c (%)	9.6 (5.9, 15.4)
Hypertension, n (%)	54 (47.0)
Course of hypertension (years)	2.0 (0, 40)
Number of eyes, n (%)	199
NPDR	118 (59.3)
Mild	15 (12.7)
Moderate	10 (8.5)
Severe	93 (78.8)
PDR	81 (40.7)
White blood cell count ($\times 10^9/L$)	6.50 (3.30, 10.30)
Neutrophil ratio (%)	60.90 (5.00, 83.00)
Lymphocyte ratio (%)	29.10 (9.30, 48.90)
Monocyte ratio (%)	6.40 (1.40, 11.90)
Basophil ratio (%)	0.40 (0.00, 5.70)
Eosinophil ratio (%)	1.60 (0.10, 8.80)
NLR (%)	2.14 (0.78, 40.41)

HbA1c: glycated hemoglobin; NPDR: non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; NLR: neutrophil-to-lymphocyte ratio.

UWFA Examination

UWFA revealed that most eyes (77.4%) had grade 4 microvascular leakage, 52.8% had grade 0 capillary non-perfusion area, 59.3% had grade 0 neovascularization, and 92.0% had grade 0 fibrous proliferative membranes (Table 2).

Table 2
Characteristic classification of UWFA in diabetic retinopathy

Image features	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Microvascular leakage	19 (9.5)	13 (6.5)	4 (2.0)	9 (4.5)	154 (77.4)
Capillary nonperfusion area	105 (52.8)	94 (47.2)	-	-	-
Neovascularization	118 (59.3)	32 (16.1)	19 (9.5)	14 (7.0)	16 (8.0)
Fibrous proliferative membrane	183 (92.0)	16 (8.0)	-	-	-
Data are presented as n (%).					

Correlation Analysis

Microvascular leakage was correlated with the NLR ($r=0.186$, $P=0.027$). Capillary non-perfusion area was correlated with the monocyte ratio ($r=0.144$, $P=0.042$) and the eosinophil ratio ($r=0.123$, $P=0.044$). Neovascularization was correlated to the monocyte ratio ($r=0.324$, $P=0.018$). Finally, fibrous proliferative membrane was correlated to the monocyte ratio ($r=0.418$, $P=0.002$) (Table 3).

Table 3
Correlation between peripheral blood indexes and wide-angle FFA image features

White blood cell count (r, P)	Neutrophil ratio (r, P)	Lymphocyte ratio (r, P)	Monocyte ratio (r, P)	Basophil ratio (r, P)	Eosinophil ratio (r, P)	NLR (r, P)	
Microvascular leakage	0.049, 0.488	0.150, 0.048	0.051, 0.476	0.105, 0.039	0.002, 0.983	-0.036, 0.610	0.186, 0.027
Capillary nonperfusion area	0.067, 0.346	0.029, 0.686	0.023, 0.747	0.144, 0.042	-0.092, 0.196	0.123, 0.044	-0.028, 0.693
Neovascularization	0.048, 0.500	0.068, 0.339	-0.024, 0.732	0.324, 0.018	-0.079, 0.269	0.097, 0.174	0.023, 0.742
Fibrous proliferative membrane	0.005, 0.946	0.027, 0.701	0.009, 0.900	0.418, 0.002	-0.055, 0.440	-0.007, 0.923	-0.045, 0.529
NLR: neutrophil-to-lymphocyte ratio.							

Factors Influencing PDR

The univariable analyses showed that sex ($P=0.085$), the monocyte ratio ($P=0.032$), and the eosinophil ratio ($P=0.040$) could be entered into the multivariable analysis. Only the eosinophil ratio was independently associated with PDR ($OR=1.25$, 95%CI: 1.04-1.51, $P=0.018$) (Table 4).

Table 4
Influencing factors of PDR

Variable	Univariable analyses			Multivariable analysis		
	OR	95%CI	P	OR	95%CI	P
Age	0.997	0.965-1.029	0.833			
Sex	1.658	0.933-2.919	0.085	1.662	0.914-3.023	0.096
Course of diabetes	1.026	0.983-1.071	0.240			
HbA1c	0.985	0.850-1.142	0.845			
Hypertension	1.197	0.679-2.109	0.535			
Course of hypertension	1.009	0.969-1.051	0.672			
White blood cell count	1.076	0.900-1.286	0.423			
Neutrophil ratio	1.018	0.987-1.050	0.255			
Lymphocyte ratio	0.999	0.963-1.036	0.941			
Monocyte ratio	0.872	1.748-2.018	0.032	0.854	0.724-1.008	0.062
Eosinophil ratio	1.169	1.081-1.392	0.040	1.250	1.039-1.505	0.018
Basophil ratio	1.281	0.634-2.587	0.491			
NLR	0.971	0.874-1.079	0.585			

OR: odds ratio; CI: confidence interval; HbA1c: glycated hemoglobin; NLR: neutrophil-to-lymphocyte ratio.

Discussion

Previous studies have shown that neutrophils, the NLR, and DR have a certain relationship with features of DR [10, 11]. Still, no studies are available for UWFA results. Therefore, this study aimed to explore the correlation between the different manifestations of retinopathy under UWFA and the systemic indicators of white blood cells. This study retrospectively analyzed the different features of UWFA images in DR patients and observed retinal microvascular lesions more extensively and deeply, reflecting the process of disease progression. Each image feature was graded and compared with the peripheral blood leukocyte system. The results suggest that the features of UWFA imaging in patients with DR are correlated with white blood cell population indexes. The study results further confirmed the inflammatory injury mechanism of DR and provided some insight for us to predict the degree of DR microvascular disease from the level of circulating white blood cells.

Many DR lesions are related to the peripheral retina, and a broader observation of the peripheral retina is very important for the screening, diagnosis, treatment, and prognosis of the disease [14]. However, conventional angiography can only show 30°-50° of the retinal surface in a single view. For patients with DR, UWFA provides a better view of the peripheral retina than conventional fundus angiography [15]. A retrospective study showed that UWFA could display an angiographic sign associated with DR known as peripheral vascular leakage (PVL), which is a late leakage of dye in the retinal arteries and veins, reflecting the breakdown of the blood-retinal

barrier (BRB) in active retinopathy. In this hospital and during the study period, a Heidelberg Spectralis ultrawide field lens (102°) [16] was used for UWFA to observe a wider range of peripheral retinal changes and obtain a broader understanding of DR. On this basis, the four main imaging features (microvascular leakage, nonperfusion, neovascularization, and proliferative membranes) were analyzed. The dye leakage of retinal arteries and veins suggested the destruction of the BRB. When the nonperfusion of the retina is greater than 7 DD, new blood vessels can be generated and are proportional to the size of the nonperfusion area [17]. Therefore, in this study, 7 DD was used as the basis for classifying nonperfusion areas in UWFA images. The progression of DR involves a long period of nonperfusion and hypoxia that induces new blood vessels and fibroproliferative membranes.

After tissue migration and activation of peripheral blood neutrophils under pathological conditions, cytokines, chemokines, matrix metalloproteinases, and other substances can be secreted, leading to tissue damage and immune cell infiltration [18]. Some phenotypes also inhibit lymphocytes [19], which can promote the occurrence of chronic inflammation. To a certain extent, the NLR can reflect the dynamic balance between inflammation and the immune response [9, 11]. Previous studies have suggested that neutrophils impair the integrity of the retinal pigment epithelial barrier [20]. In this study, microvascular dye leakage in UWFA images suggested BRB destruction and vascular endothelial function damage. The vascular endothelium performs important functions, including managing microvascular permeability, coagulation, inflammation, vascular tension, and neovascularization [21]. Diabetes mellitus, hypercholesterolemia, and hypertension lead to a dysregulation of vascular endothelial L-arginine/nitric oxide synthase (eNOS) and resulting in vascular endothelial dysfunction [22]. HbA1c is strongly associated with increased levels of circulating adhesion molecules (ICAM, VCAM, etc.), which are considered to be indicators of endothelial cell injury and are correlated with the severity of DR [23]. This study showed that microvascular dye leakage was correlated with the proportion of neutrophils, monocytes, and the NLR in peripheral blood leukocytes. The results were therefore consistent with previous studies.

Damaged capillary endothelial cells, reduced tissue perfusion and oxygenation, and aggravated vascular damage occlusion resulted in no perfusion on UWFA. Schroder et al. [24] proposed for the first time in 1991 the concept that activated white blood cells lead to capillary obstruction and proved that granulocytes and monocytes were trapped in retinal capillaries. Compared with normal cells, white blood cells in diabetic patients, especially those in capillaries, release more reactive oxygen species, damage endothelial cells, and pericytes, and lead to retinopathy through oxidative stress [25, 26]. Elevated levels of inflammatory mediators caused by the accumulation of advanced glycation end products can also lead to persistent chronic inflammation of the retina, resulting in the activation of white blood cells, adherence to vascular endothelium, and extravasation [27, 28]. In addition, there is evidence that elevated white blood cell count within the normal range is associated with the occurrence of microvascular and macrovascular complications of diabetes [29]. The results showed that the degree of capillary occlusion was correlated with the proportion of monocytes and eosinophils in peripheral blood. These conditions indicate that the retina progresses from vascular endothelial cell damage in the early stage to capillary occlusion in the later stage, and the ratio of eosinophils and monocytes in peripheral blood might reflect the course of the disease.

Monocytes are one of the major leukocyte subtypes and are also considered markers of inflammation [30]. Animal studies have shown that when many monocytes enter the retinal tissue, the retinal pigment epithelium

acts as a channel for monocytes to transport to the retina [31, 32]. Studies have shown that plasma levels of monocyte chemotactic protein-1 (MCP-1), which regulates monocyte chemotactic and inflammatory processes, are significantly increased in patients with DR [33]. There is also an association between peripheral blood monocyte levels and the prevalence of DR [34]. In this study, the range of retinal neovascularization and the presence of fibroproliferative membrane in UWFA images were significantly correlated with the proportion of peripheral blood mononuclear cells. Studies have shown that monocytes and macrophages might be involved in the process of angiogenesis in atherosclerosis [35, 36]. Monocytes in circulating blood are considered precursors to tissue macrophages, whose function is to transform into a variety of tissue-sensing macrophages during normal homeostasis and inflammation. After tissue injury, inflammation and macrophage aggregation are induced, and macrophage proliferation *in situ* and synthesis of vascular endothelial growth factors (VEGFs) are promoted. Subsequently, it promotes the growth of new blood vessels and activates fibroblasts to produce collagen [37]. During DR progression, chronic inflammation leads to microvascular injury, which goes through the damage repair process involving macrophages. This process explains the results of this study well. The ratio of monocytes in peripheral blood is correlated with the degree of retinal neovascularization and proliferative fibrous membrane.

PDR is the final stage in the progression of DR and will lead to blindness [1, 2]. In the present study, only the eosinophil ratio was independently associated with PDR. Diabetes involves a dysregulated immunity, including eosinophils [38]. Elevated eosinophil counts have been observed in both men and women with PDR [39]. PDR might involve eosinophil-specific chemokines that play important roles in the inflammation observed in DR [40].

This study has some limitations. The sample size was small, and the patients were from a single center, leading to the possibility of regional differences. In this study, leukocyte-related indicators were studied, but other inflammatory markers in peripheral blood were not studied. In addition, the white blood cells could not be used to evaluate the damage of other diabetic target organs completely. The traditional direct evaluation of intraocular fluid is more valuable, but detecting inflammatory factors in intraocular fluid such as aqueous humor was not done for comparison. The extraction of intraocular fluid for detection is invasive, and a more safe, economical, and valuable detection method is necessary.

Conclusion

In the UWFA of DR, microvascular leakage was correlated with the NLR, the capillary non-perfusion area was correlated with the monocyte ratio and the eosinophil ratio, neovascularization was correlated to the monocyte ratio, and the fibrous proliferative membrane was correlated to the monocyte ratio. Therefore, the neutrophil ratio, monocyte ratio, eosinophil ratio, and NLR could be used to assess the retinal damage in patients with T2DM using a routine blood test. In addition, the eosinophil ratio was associated with PDR. Further studies are necessary to determine their predictive and diagnostic values.

Abbreviations

DR

Diabetic retinopathy

FFA

fluorescein angiography

UWFA

Ultrawide-field FFA

NPDR

Non-proliferative DR

PDR

proliferative DR

NLR

neutrophil-to-lymphocyte ratio

Declarations

Acknowledgments

Not Applicable.

Authors' contributions

Lili Huang and Lele Li contributed equally to this work. Lili Huang designed this study. Min Wang and Dongmei Zhang collected and double checked the data. Lele Li analyzed the data. Lili Huang and Yu Song wrote the paper. Lele Li provided critical revision to the article. All authors participated in revision and approved the final version for submission. All authors read and approved the final manuscript.

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Availability of data and materials

All data are available under request. Yu Song should be contacted if someone wants to request the data.

Ethics approval and consent to participate

The ethics approval was obtained from Ethics committee of The Affiliated Hospital 2 of Nantong University (2021KT004). The requirement for informed consent was waived by the Ethics Committee of the Affiliated Hospital 2 of Nantong University because of the retrospective nature of the study.

Consent for publication

Yes

Competing interests

The authors declare that they have no competing financial interests.

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Figures

Figure 1

Female, 52 years old, visited the hospital for 1 week of binocular vision loss (February 22, 2019), 10 years of diabetes history, insulin control, fasting glucose 14.4 mmol/ L. (A) Fundus fluorescein angiography (FFA) of the venous phase with Heidelberg Spectralis ultrawide angle lens (102°). Dye leakage was found in microvessels in all quadrants, which was recorded as grade 4. The yellow line of the contrast instrument is the nonperfusion area of the retina, which is significantly larger than 7 DD, and it was recorded as grade 1. The optic disc and the retinal surface of each quadrant showed strong fluorescence, indicating neovascularization, recorded as grade 4. No fibrous proliferative membrane was found, grade 0. (B) FFA of the venous phase with Heidelberg 55° lens. The peripheral retina was limited. (C) Fundus photography showing the presence and exudation of the retinal surface.