Application of Novel Quantitative Measurement of Iris Angiography in Diabetic Retinopathy

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Research Article

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Abstract

Introduction: The purpose of this study was to explore the application of a novel quantitative measurement of iris angiography (IA) in diabetic retinopathy (DR).

Methods: This was a single-center, cross-sectional prospective study. Totally 168 consecutive patients with diabetic mellitus (DM) were involved into this study from May 2016 to December 2020, 168 subjects were devided into 3 groups, 32 non-retinopathy patients,96 non-proliferative diabetic retinopathy patients(NPDR), and 40 proliferative diabetic retinopathy (PDR) patients. All patients underwent ophthalmologic examination, such as best corrected visual acuity, intraocular pressure, slit-lamp microscopy, gonioscopy, ultra-wide-field fundus photography, fluorescein angiography of the iris (IFA), and indocyanine green angiography of the iris (IIGA). The starting of iris vascular leakage time(LT) was recorded, and the range of pupil fluorescein leakage was measured by a novel measurement.

Results: LT of iris in non-retinopathy, non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR) groups were 33.14 ± 3.03s, 32.45 ± 5.17s, and 25.67 ± 5.03s, respectively. The LT in the PDR group was significantly faster than those in the NPDR and non-retinopathy groups (P=0.000). The iris pupil leakage range (LR) of the non-retinopathy, NPDR, and PDR groups were 21.21 ± 30.06, 62.48 ± 42.17, and 141.31 ± 73.61°, and LR in PDR groups were largest among all groups (P=0.000). Neovascularization of the iris (NVI) was detected in 8 eyes (26.7%) in the PDR group. No NVI was found in non-retinopathy and NPDR groups.

Conclusions: The novel quantitative measurement of IA can be used to evaluate the severity of diabetic iridopathy (DI) accurately and detect invisible NVI as early as possible to provide a basis for the formulation of personalized treatment for DR patients.

Introduction

The main complications of diabetic retinopathy were diabetic iridopathy (DI) and diabetic retinopathy (DR) [1]. Iris angiography (IA), which primarily includes iris fluorescein angiography (IFA) and iris indocyanine green angiography (IICGA), has been used for the evaluation of DI [2]. However, there is currently no precise quantitative evaluation of the severity of DI. To assess the advantages and sensitivity of quantitative measurement of IA in DR progress, a group of patients with DR were recruited and the IA results were reported.

Method

Study design and participants

This is a single-center, cross-sectional prospective study. This study is approved by the Ethics Committee of Tianjin Medical University Eye Hospital (2016KY-04). Informed consent is obtained from parents and participants 18 years or older.

168 patients (168 eyes) with DR who underwent IA at Tianjin Medical University Eye Hospital are recruited from May 2016 to December 2020. All patients underwent standard ophthalmologic examination, including best corrected visual acuity, intraocular pressure, slit-lamp microscopy, gonioscopy, ultra-wide-field fundus photography and IA. The baseline characteristics of the patients are shown in Table 1. Enrollment criteria are as follows : (1) confirmed diagnosis of type 1 or type 2 diabetes, (2) fundus examinations of patients confirmed for non-retinopathy fundus, NPDR or PDR with vitreous hemorrhage or preretinal hemorrhage according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification. The exclusion criteria are as follows: (1) combination with retinal vein occlusion or neovascular glaucoma (NVG); (2) significant dioptric media opacities (such as severe keratopathy, cataract and vitreous hemorrhage), which interfered with IA imaging; and (3) any history of retinal photocoagulation, intravitreal anti-vascular endothelial growth factor (VEGF) injections, vitrectomy or other ocular surgeries. Diagnostic criteria of neovascularization of the iris (NVI): visible newly formed blood vessels on the iris surface or pupil margin.

Table 1
General Characteristics of DR Patients

Variables	Groups			Total
	non-retinopathy	NPDR	PDR	
Age(years)	62.64±8.29	58.29±8.19	56.40±8.98	58.46±8.71
Male	12	41	17	70
Female	20	55	23	98
Right eye	15	52	20	87
Left eye	17	44	20	81

Note: DR=diabetic retinopathy; NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy.

IA

All patients underwent IFA and ICGA using a German Heidelberg confocal laser scanning fundus angiography instrument (Spectralis HRA). None of the patients had fluorescein sodium allergies, and no pupil dilatation was performed during the examination. A mixture of 10% fluorescein sodium (3 ml) and aseptic injection (3 ml) dissolving indocyanine green (25 mg) was quickly injected through the cubital vein within 5 s. Abnormal iris blood vessel fluorescein leakage within the first minute was recorded as the early phase of IFA. Then, the iris fluorescein leakage within 10 minutes was recorded as late phase of IFA.

Quantitative analysis of iris angiography (Patent Application Number: 202010476895.1)

Once the imaging of IA was completed, the circumference range of pupil margin fluorescein leakage was measured by the self-developed measurement software. The DR patients with iris new blood vessels which was not around the pupil margin was excluded to the statistic analysis. The protocol was as follows: (1) The center point of the pupil was marked (Figure 1A). (2) The area of leakage lesions around pupil was marked. The starting and ending points of all lesions were set up in the selected area where each lesion was located on the uninterrupted fluorescein leakage area around pupil margin. The degree of the angle between the starting and ending points was generated automatically. Different colors were applied to distinguish each leakage lesions automatically by the software (Figure 1B). (3) After marking all the lesions, the sum total degree of fluorescein leakage area were calculated by the software automatically (Figure 1C). The leakage ranges of all patients were repeatedly marked and measured three times.

Statistical analysis

All statistical analysis is performed using SPSS software version 22.0. All the measurement data are expressed as the mean ± standard deviation (±s), One-way ANOVA is used to compare the LT and range between groups. A P value < 0.05 is considered to be statistically significant.

Results

General characteristics

Among 168 DR patients, 73 (45%) were male and 92 (55%) were female, with an average age of 58.46 ± 8.71 years. 87 (52%) patients received a right eye examination, and 81 (48%) a left eye examination (Table 1).

Fluorescence leakage time (LT) in normal subjects and DR patients

In DR patients, the leakage cases in the non-retinopathy group, NPDR group, and PDR group were 28(87.50%), 87(90.63%), and 32(100%), respectively. The LT of three groups were 33.14 ± 3.03, 32.45 ± 5.17 and 25.67 ± 5.03 s, respectively (Table 2). There was no significant difference in LT between the NPDR group and the non-retinopathy group (P = 0.993). The LT of the PDR group was significantly different from the NPDR group and non-retinopathy group (P = 0.000, respectively) (Table 4).

Table 2. Fluorescence Leakage Time and range in Patients

Groups	Leakage Cases(%)	Total Cases	Leakage Time(s)	Leakage Range(degree)
Non-retinopathy	28(87.5)	32	33.14±3.03	21.21±30.06
NPDR	87(90.63)	96	32.45±5.17	62.48±42.17
PDR	32*	32	25.67±5.03	141.31±73.61

Note: DR=diabetic retinopathy; NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy; NA=not applicable. *8 PDR patients with iris surface neovascularization were not included in the calculation of leakage time.

Table 3. Fluorescence Leakage Time and range in NPDR groups and PDR Group

Groups	Leakage Cases	Total Cases	Leakage Time(s)	Leakage Range(degree)
Mild NPDR	29	32	33.17±2.87	44.14±20.09
Moderate NPDR	29	32	32.97±5.58	66.41±49.05
Severe NPDR	29	32	31.21±6.45	76.90±46.34
PDR	32*	32	25.67±5.03	141.31±73.61

Note: NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy. *8 PDR patients with iris neovascularization were not included in the calculation of leakage time.

Table 4
Multiple Comparisons of Leakage Time and Range between Groups of Normal Subjects and DR Patients

Reference Groups	Groups	P Value for Leakage Time	P Value for Leakage Range
Non-retinopathy	NPDR	0.993	0.000
	PDR	0.000	0.000
NPDR	PDR	0.000	0.000

Note: DR=diabetic retinopathy; NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy.

Patients with NPDR were further divided into mild NPDR, moderate NPDR, and severe NPDR. and the LTs were 33.17 ± 2.87, 32.97 ± 5.5 and 31.21 ± 6.45 s, respectively (Table 3), with no significant difference between these groups (P = 0.192, P = 0.145, P = 0.878, respectively). The LT of the PDR group was significantly different from each NPDR subgroups (P = 0.000, respectively) (Table 5).

Table 5
Multiple Comparisons of Leakage Time and Range between NPDR Subgroups and PDR Group

Reference Groups	Groups	P Value for Leakage Time	P Value for Leakage Range
PDR	Severe NPDR	0.000	0.000
	Moderate NPDR	0.000	0.000
	Mild NPDR	0.000	0.000
Severe NPDR	Moderate NPDR	0.192	0.955
	Mild NPDR	0.145	0.006
Moderate NPDR	Mild NPDR	0.878	0.165

Note: NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy.

Fluorescence leakage range (LR) in normal subjects and DR patients

The LR of the non-retinopathy group, NPDR group, and PDR group was 21.21 ± 30.06°, 62.48 ± 42.17°, and 141.31 ± 73.61°, respectively (Table 2), with significant differences between these groups (P = 0.000) (Table 4). The LR of the mild NPDR, moderate NPDR, and severe NPDR groups was 44.14 ± 20.09°, 66.41 ± 49.05°, and 76.90 ± 46.34°, respectively (Table 3). There was no significant difference in these groups (P = 0.955, P = 0.165, respectively) except for severe NPDR and mild NPDR (P = 0.006). The LR of the PDR group was significantly different from those of mild NPDR, moderate NPDR, and severe NPDR groups (P = 0.000, respectively) (Table 5, Fig. 4, A-E).

Other results

No visible new blood vessels were observed in any of the patients with slit lamp examination. No neovascularization in the anterior chamber angle (NVA) was observed in any of the patients using gonioscopy examination. Eight eyes in PDR patients were diagnosed with NVI, which had the neovascular leakage not around the pupillary margin. These 8 patients was excluded to the statistic data due to the different location of new blood vessels (Fig. 4.F). IFA findings revealed iris new blood vessel morphology at early stage of IFA, and the vascular morphology was quickly blurred due to persistent leakage at the late stage of IFA. While during IICGA, NVI presented as clearly neovascularization morphology no matter the phase of IICGA(Figure3). These results demonstrated that IFA could distinguish the neovascularization from the normal blood vessel leakage, but could not clearly display the morphology of neovascularization.

Discussion

With the progression of DR, retinal ischemia is gradually aggravated, which leads to NVI and even NVG [3-4]. The formation of NVI is reported as a risk factor for the occurrence of NVG [5-7]. Therefore, Early detection of NVI can predict the occurrence of NVG. There is correlation between DI and the severity of DR. Both fundus and iris angiography can be simultaneously performed to evaluate the conditions of fundus and iris blood vessel leakage in DR patients. However, when the DR patients with cataract and vitreous hemorrhage, the fundus cannot be displayed clearly due to dioptric media opacities. the severity of DI may be the new clue to evaluate the DR using IFA examination. IA imaging can provide a crucial basis for determining the severity of DR [8]. It also can predict the progress of DR, especially the proliferative DR, and provide personalized treatment for these patients.

To evaluate the severity of DI even more effectively, we creatively use novel measurement software to accurately measure the range of pupil margin fluorescein leakage. The findings demonstrate that the starting time of iris blood vessel leakage in PDR patients was significantly earlier than in severe NPDR patients, and the range of iris vascular leakage in PDR patients was also significantly larger than in severe NPDR patients, indicating that the iris neovascularization revealed the progress of DR. These results demonstrated that DI had correlated with the development of DR, the severity of retinal ischemia and hypoxia gradually increased, and a large amount of neovascular growth factor was released, such as VEGF. The VEGF released into the vitreous cavity gradually spread to the anterior chamber, which eventually led to the formation of NVI [9]. Therefore, the novel method for the quantitative measurement of IA provides an approach for the application of the quantitative evaluation of DI in IFA.

According to the result of IA, we also find IFA can distinguish physiological iris leakage from NVI. Iris capillary leakage on IFA presented as mild fluorescein leakage and disappear quickly at late stage, while NVI presented as moderate or severe leakage and enhanced leakage at late stage. Numerous studies [10-11] have found that IFA is easier to find NVI than IICGA. Animal experiments found IICGA displayed better details of whole iris vessels than IFA. However, it cannot reveal the leakage of iris new blood vessels [12]. The results of our investigation also suggested that NVI showed rapid leakage in early phase and gradually increased leakage in the late phase, IFA distinguished the neovascularization of iris better than IICGA. Therefore, we think that IFA can effectively diagnose NVI and help to distinguish it from physiological iris leakage. Additionally, Since the iris new blood vessels in the early stage of DR are mostly in the iris stoma and covered by iris pigments in the Asian population, it is difficult to detect the lesions with slit-lamp microscope. While IA can help to detect early-stage neovascularization [13], providing a new approach to evaluate iris new blood vessels. In a follow-up study, we will continue to accumulate cases and compare iris leakage between PDR patients with and without NVI, hoping to obtain reliable indicators for early warning of the occurrence of NVG.

Conclusions

In summary, the novel quantitative measurement of IA can evaluate the severity of diabetic iridopathy (DI), monitor the progress of DR, and detect invisible NVI as early as possible to provide a basis for the formulation of personalized treatment for patients with DR.

Declarations

Funding

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Longli Zhang, Yue Chai and Kaiwen Hei. The first draft of the manuscript was written by Luyuan Zhang and Xiaorong Li, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tianjin Medical University Eye Hospital (2016KY-04)

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirmed that human research participants provided informed consent for publication of the images in Figures.

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Figures

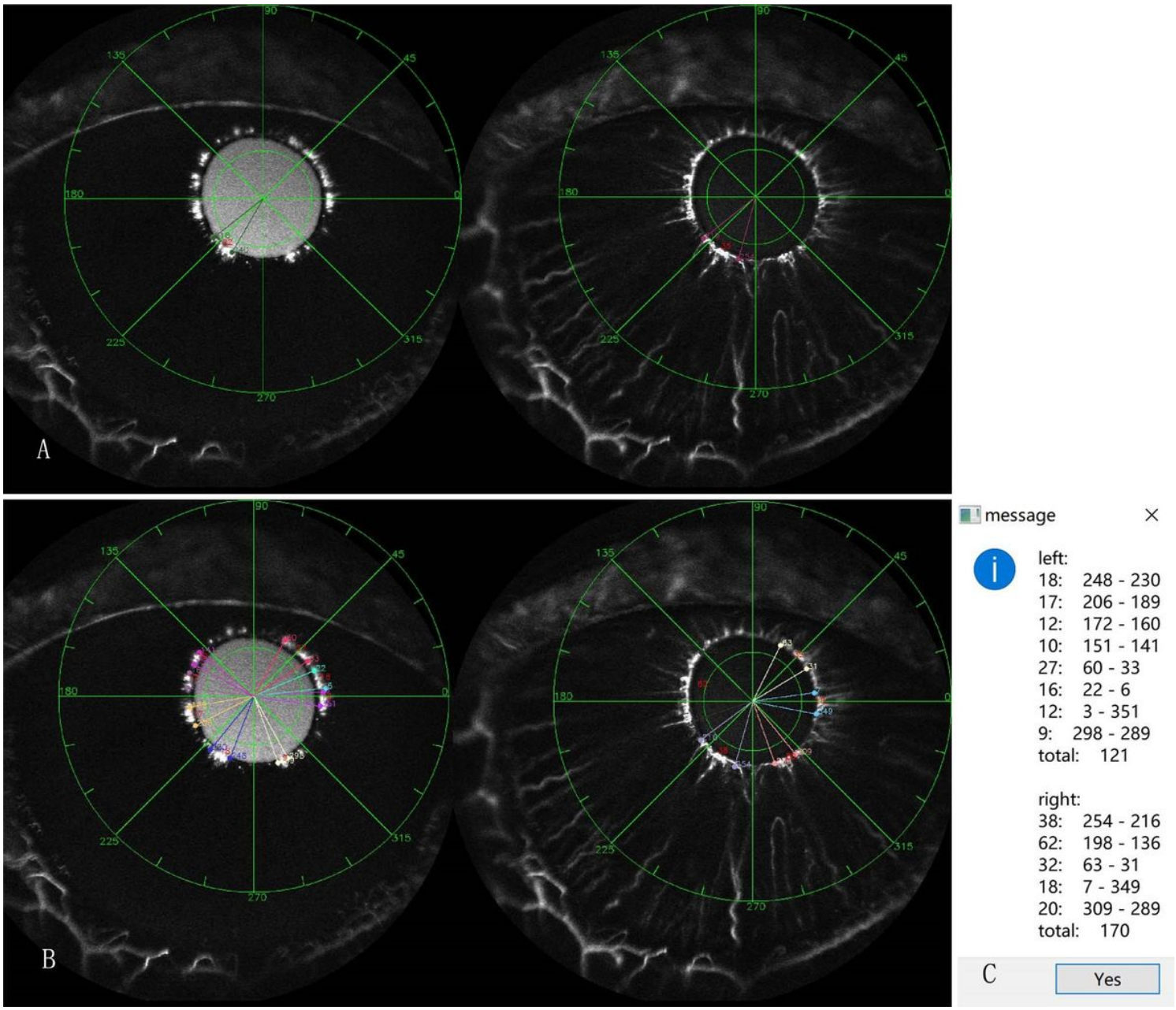

Figure 1

A.The pupil center of IFA and IICGA images was confirmed. Then, the starting and ending point of one leakage lesion was selected to determine the range of leakage lesion area. B. the measurements of all other fluorescence leakage lesion areas were completed in the same image. C. After measurement, the quantitative analysis results of the fluorescence leakage at the pupillary margin of IFA and IICGA images were summed automatically.

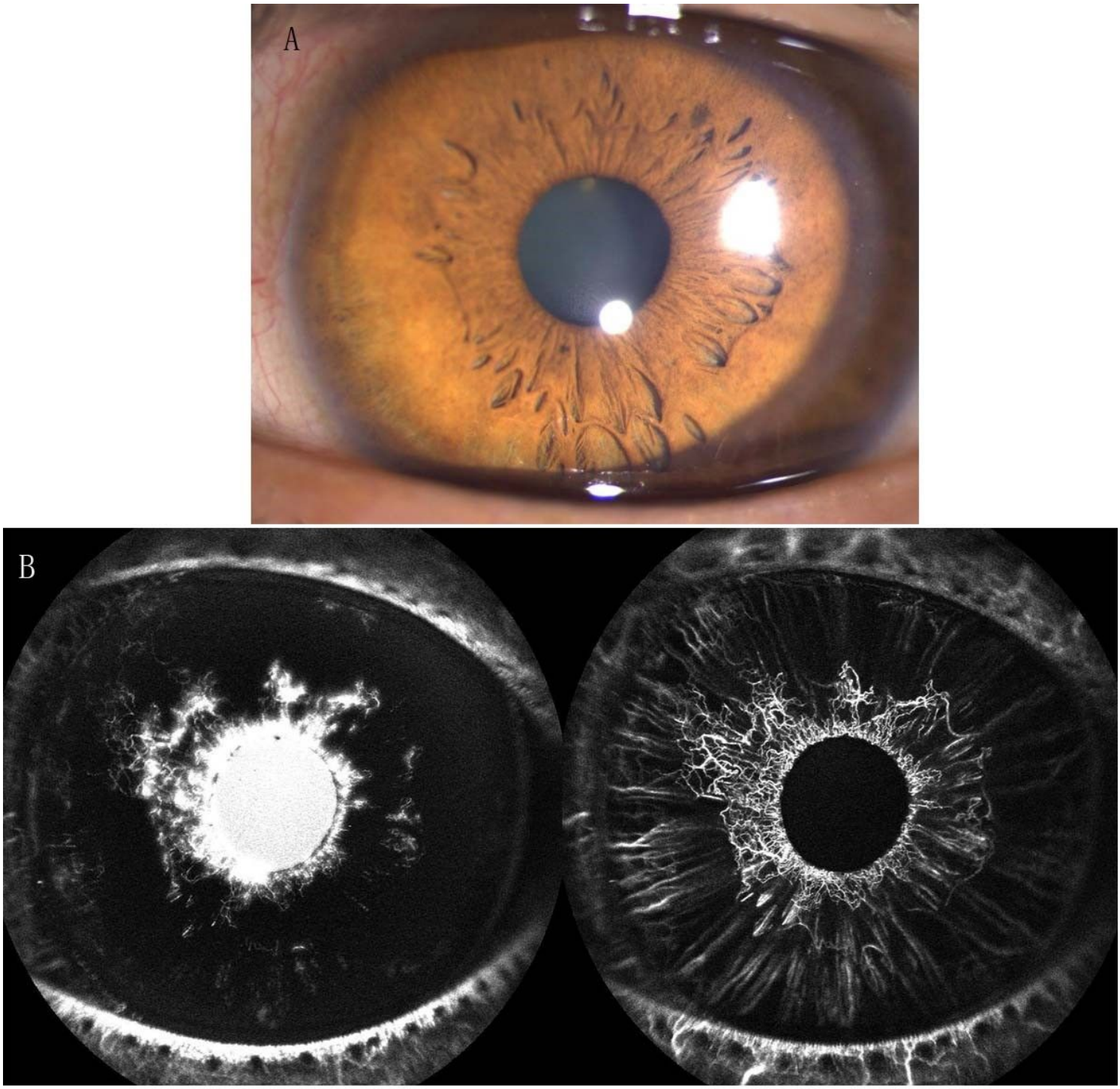

Figure 2

Slit-lamp image of PDR patient (case 1) with NVI, there is no visible new blood vessels. B. The IFA and IICGA image in PDR patient (case 1) with NVI at 36 seconds after fluorescein injection.

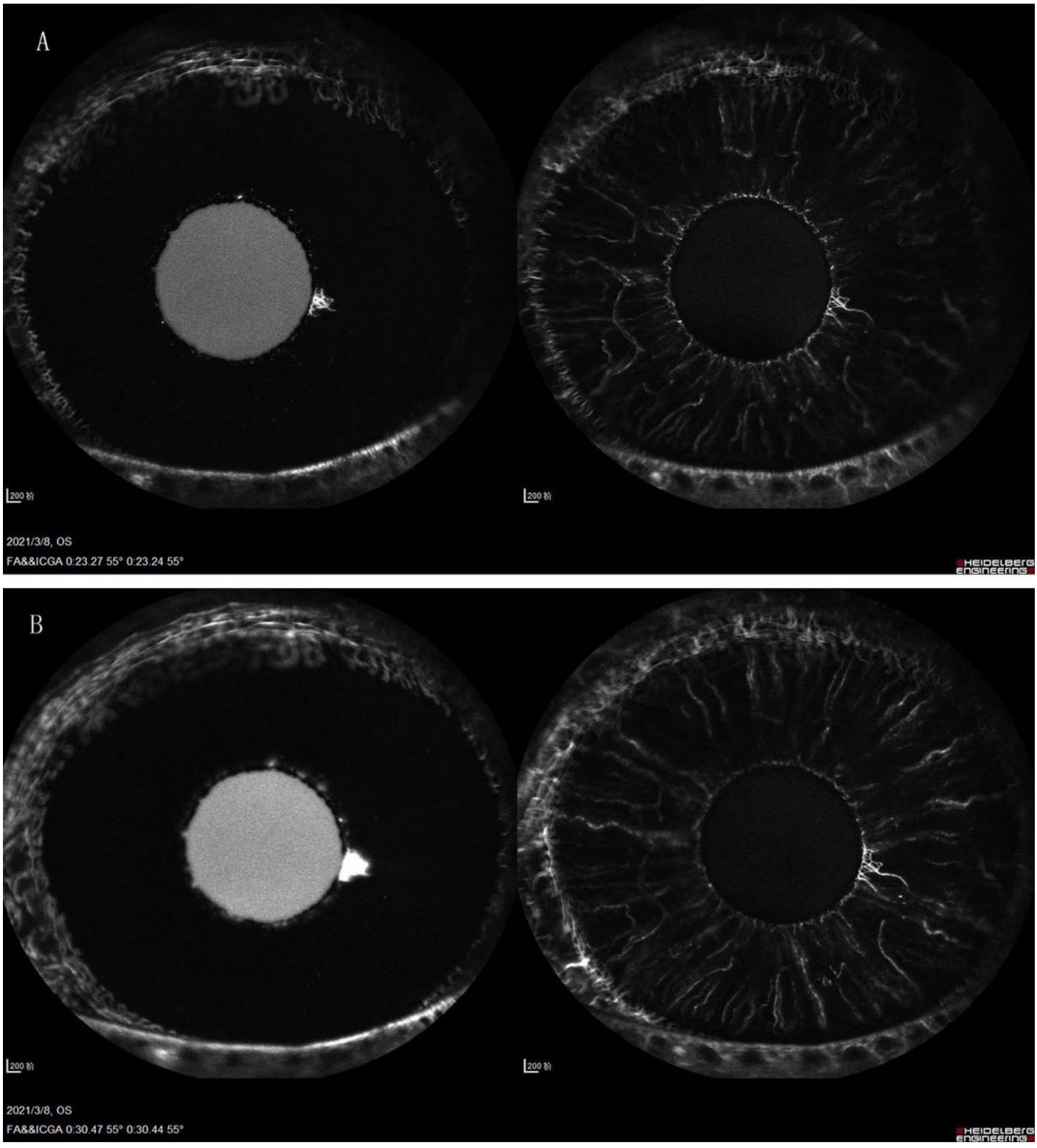

Figure 3

A. the image of IFA and IICGA in PDR patient (case 2) with NVI at 23 seconds after fluorescein injection. B. the image of IFA and IICGA in PDR patient (case 2) with NVI after 30 seconds after fluorescein injection.The novel approach for calculating the leakage range in different patients.A.DM patients with no retinopathy. B.IFA in mild nPDR patients. C. IFA in moderate nPDR patients. D: IFA in severe nPDR

patients. E: IFA in PDR patients. F: IFA in PDR patients. The new blood vessel in iris (white arrow) was not around the pupillary margin.

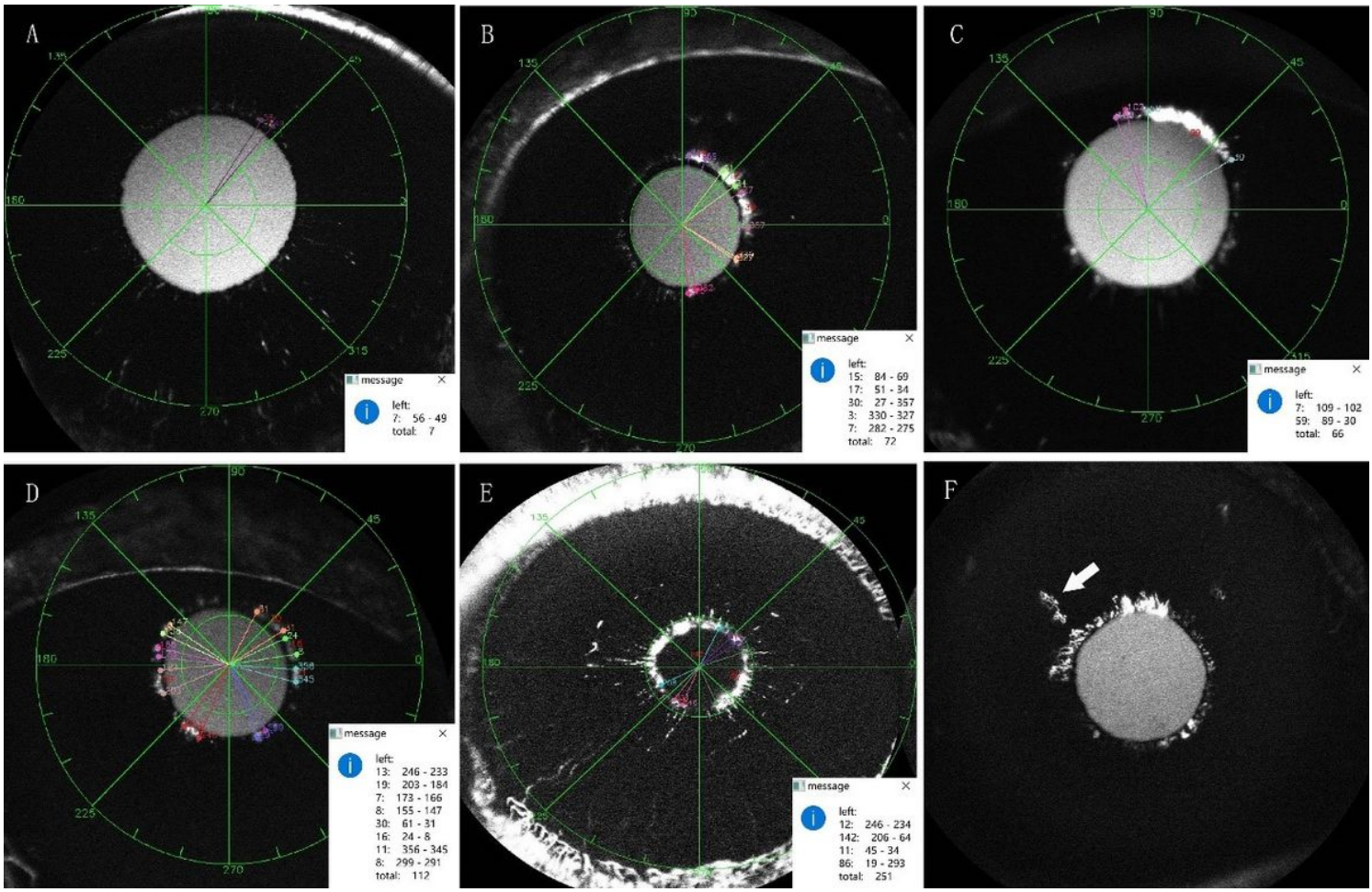

Figure 4

The novel approach for calculating the leakage range in different patients.A.DM patients with no retinopathy. B.IFA in mild nPDR patients. C. IFA in moderate nPDR patients. D: IFA in severe nPDR patients. E: IFA in PDR patients. F: IFA in PDR patients. The new blood vessel in iris (white arrow) was not around the pupillary margin.