The Evaluation of Retinal Non-perfusion with Multicolor Imaging in Diabetic Retinopathy

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ABSTRACT

Purpose: This study was conducted to evaluate the ability of multicolor (MC)–green reflectance (GR) imaging in detecting retinal non-perfused areas (RNPAs) of eyes with diabetic retinopathy (DR).

Materials and Methods: Fifty-five degrees fundus fluorescein angiography (FA) and MC imaging were performed in eyes with DR. Images were divided into four fields as macular, nasal, superior temporal, and inferior temporal. To determine the presence and size of RNPAs, FA images were examined by a retina specialist and MC–GR images were examined independently by two masked retina specialists. The compliance of RNPAs in FA and MC–GR images and the agreement of retina specialists were analyzed.

Results: The FA and MC–GR images of the 178 eyes were analyzed. When GR images were compared to FA images in terms of RNPAs, sensitivity was 87%; specificity was 89%; the positive predictive value was 92%; the negative predictive value was 83%; and the accuracy was 87%. A substantial agreement was observed between two retina specialists in all retinal fields.

Conclusion: This study showed that 55º MC–GR imaging could be an alternative to FA in the evaluation of RNPAs. This study is the first known to explore this issue and needs to be supported and developed with new investigations.

Keywords: Diabetic retinopathy, Green reflectance imaging, Multicolor imaging, Retinal ischemia, Retinal non-perfusion.

INTRODUCTION

Diabetic retinopathy (DR) is one of the most important complications of diabetes mellitus (DM). DR is an important cause of preventable or treatable visual loss in the working-age population.[1] The main causes of visual impairment in patients with DR are diabetic maculopathy and sequelaes due to ischemia-induced neovascularization.[2] Maculopathy includes two different entities, such as macular edema and macular ischemia, and accounts for 80% of the visual loss in the non-proliferative stage of DR.[3]

Retinal examination by direct and indirect ophthalmoscopy is the most sensitive method in the diagnosis of DR. Thirty-degree color fundus photographs and red-free photographs centered on the macula can be used in the diagnosis of DR. Fundus fluorescein angiography (FA) is the gold standard method for detection of the macular edema and also for the evaluation of retinal non-perfusion.[4] FA has some disadvantages such as being invasive and time-consuming, and requiring a contrast agent.

Multicolor (MC) imaging is a modality obtained using a confocal scanner laser ophthalmoscope (cSLO) by the Heidelberg Spectralis spectral domain–optic coherence tomography (SPECTRALISSD-OCT, Heidelberg Engineering, Heidelberg, Germany). Compared to standard color fundus photograph, images produced by the cSLO have a higher resolution and higher contrast due to suppression of light scatter.[5]

MC imaging scans the retina with three different wavelengths. The infrared (815 nm), green (518 nm), and blue (486 nm) wavelengths penetrate into the retinal tissue, each at different depths. A combined MC image is computed using infrared reflectance (IR), green reflectance (GR), and blue reflectance (BR) images. The infrared laser penetrates into the deepest retinal layers and allows for

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examination of the choroid, retinal pigment epithelium, and photoreceptors. The green laser penetrates into the mid-retinal layers and provides for evaluation of blood vessels, hemorrhages, and exudates. The blue laser penetrates into the superficial layers of the retina and provides detailed images of the retinal nerve fiber layer, ganglion cells, macular pigment, and epiretinal formations.[6]

In this study, we aimed to evaluate the ability of MC–especially GR imaging–in detecting retinal non-perfused areas (RNPAs) of eyes with DR.

MATERIALS AND METHODS

This study was conducted at the Ondokuz Mayis University (OMU) Hospital in Samsun, Turkey. It was approved by the OMU Clinical Research Ethics Committee and was carried out according to principles outlined in the Declaration of Helsinki.

Study population

The eyes of patients with DR were prospectively evaluated between October 2016 and March 2017. The eyes of adult patients with DR due to type 2 DM, which did not receive previous retinal laser photocoagulation (RLP) treatment, were enrolled in the study. Written informed consent was obtained from all participants. Exclusion criteria included the presence of other ocular diseases that may cause retinal ischemia other than DR; insufficient media clarity and pupillary dilation for adequate fundus imaging; a history of previous RLP; a history of cataract surgery; and a history of vitrectomy surgery and endolaser photocoagulation.

Examinations

All patients underwent a detailed ophthalmologic examination including the best-corrected visual acuity (BCVA) measurements (Snellen); intraocular pressure measurements (Goldman applanation tonometer); slit-lamp biomicroscopy; and dilated fundoscopy with a 90 diopter lens. Tropicamide 1% and cyclopentolate hydrochloride 1% were used for pupillary dilation. After the ophthalmologic examination macular OCT, FA and MC imaging were performed (SPECTRALIS HRA+OCT, Heidelberg Engineering, Heidelberg, Germany).

MC images composed of three simultaneously acquired reflective images were obtained by using three laser wavelengths–BR, GR, and IR images–and were taken with a macular-centered 55° angle of view and 25% MC laser power using the MC mode of SPECTRALIS HRA+OCT. FA images were taken with a 55° angle of view and a 100% FA laser power (486 nm) using the FA mode of SPECTRALIS HRA+OCT. The camera used for imaging was HRA Camera FW version 2.6.3.0.

Assessment of FA and MC imaging

FA and 55º MC–GR images were divided into four retinal fields as macular, nasal, superior temporal, and inferior temporal (Figure 1). The macular field was the retinal region within the main temporal vascular arches. The nasal field was the nasal retinal region of the vertical line drawn at the nasal border of the optic disc. The superior temporal field was the upper retinal region from the superior temporal vascular arch. The inferior temporal field was the lower retinal region from the inferior temporal vascular arch.

FA images were examined by a retina specialist to determine the presence and size of RNPAs. MC–GR images were examined independently by two masked retina specialists to determine the presence and size of RNPAs. When assessing MC images, retina specialists were unaware of both the results of the FA and the evaluation results of the other specialist. Dark gray-colored hypo-reflective areas with marked margins in the GR image were considered as RNPAs (Figure 2). The appearance of these areas in both GR and BR images was similar and compatible with each other. Soft exudates in RNPAs appear to be hyper-reflective areas (Figure 2). Because the areas of retinal hemorrhages and hyper pigmentation were also seen as hypo-reflective areas in the GR

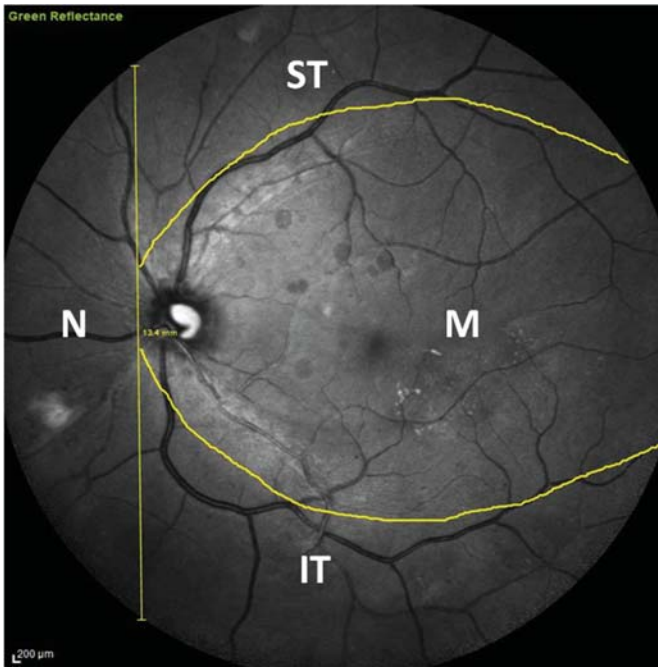

Figure 1: *A schematic representation of the division of the the 55 degree fundus image into 4 retinal fields: Macular (M), nasal (N), superior temporal (ST), and inferior temporal (IT).*

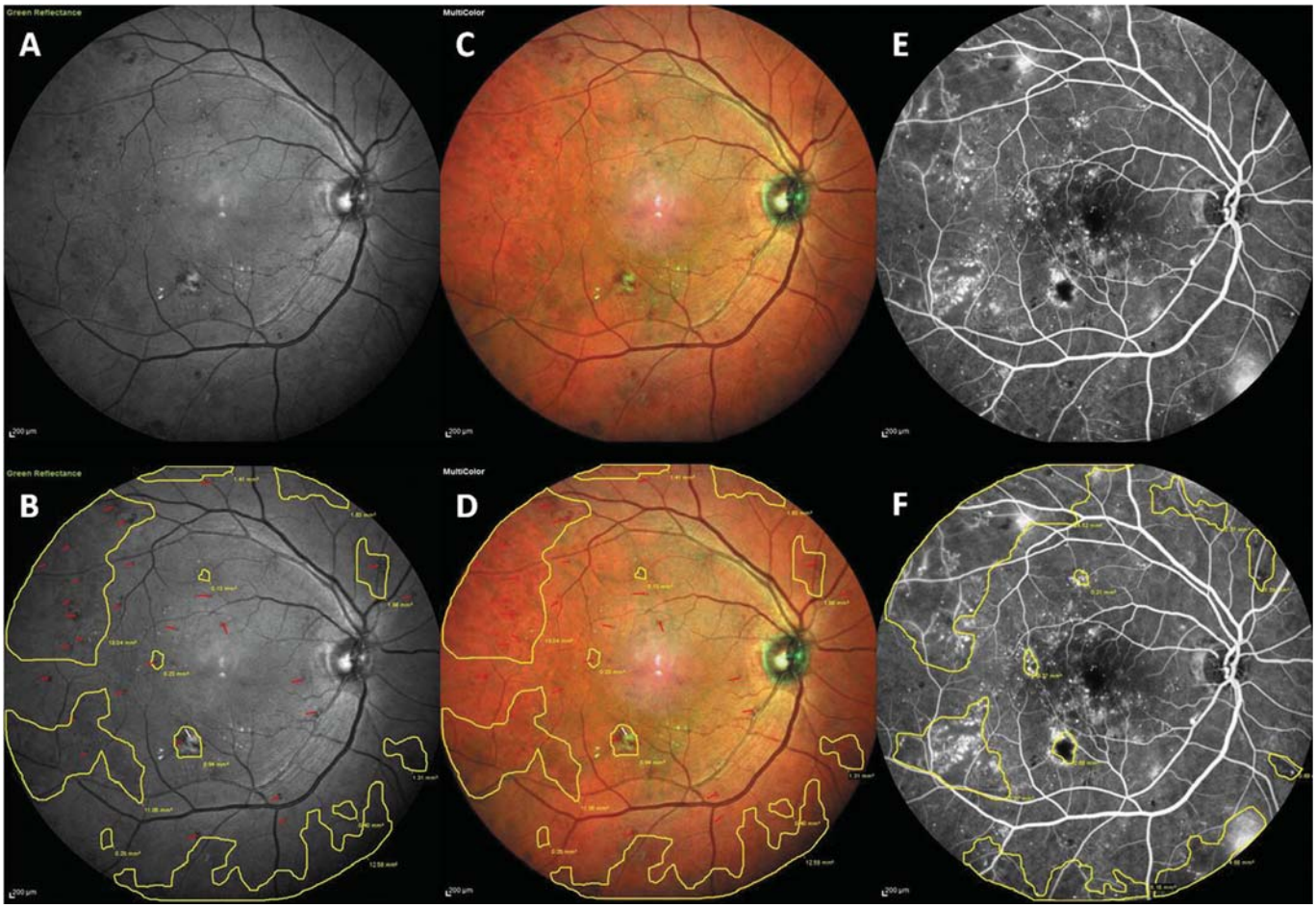

Figure 2: *Fifty-five degrees retinal images of a 57-year-old female patient with diabetic retinopathy: A-B, Green reflectance images; C-D, Multicolor images; E-F, Fluorescein angiography images. Detection of hypo-reflective–non-perfused–areas and measurement of their sizes in green reflectance and multicolor images (Areas surrounded by yellow line; B-D); Detection of non-perfused areas and measurement of their sizes in fluorescein angiography images (Areas surrounded by yellow line; F); Retinal hemorrhages (Red arrows; B-D); Soft exudates (White arrow; B-D).*

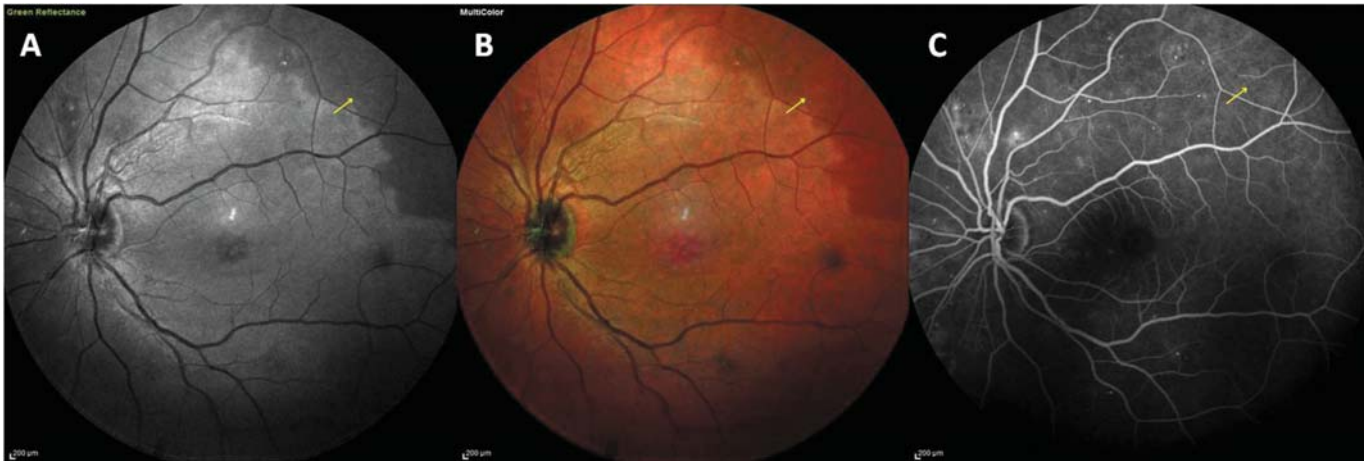

Figure 3: *Fifty-five degrees retinal images of a 60-year-old female patient with diabetic retinopathy: Hypo-reflective area due to hyper-pigmentation in the superior temporal retina in green reflectance image (Yellow arrow; A); Hypo-reflective area due to hyper-pigmentation in the superior temporal retina in multicolor image (Yellow arrow; B); Normal retinal perfusion in the superior temporal retina in fluorescein angiography image (Yellow arrow; C).*

image, false-positive evaluations were prevented by evaluating them together with MC images (Figure 2 and Figure 3). Hypo-reflectance due to hemorrhage is seen darker than that due to the non-perfused area (Figure 2). If a RNPA was present, its margins were drawn and its size was automatically measured by using the "draw region" tool on the SPECTRALIS HRA+OCT screen (Figure 2). The Acquisition software version was 6.5.2.0. Compliance with non-perfused areas in FA and MC–GR images was compared.

Even though there is RNPA in FA image, no RNPA in MC–GR image; the difference between the localizations of RNPA in MC–GR and FA images; or a difference of more than 25% between the size of RNPA in MC–GR and FA images, then these were considered as non-compliance. In MC–GR and FA images, the similar localization of RNPA and a difference of less than 25% between the sizes of RNPA were considered as compliance. Four retinal fields were evaluated separately.

Statistical analyses

Statistical analyses were carried out using The Statistical Package for the Social Sciences (SPSS; Inc., Chicago, IL, USA), V15. Results were given as frequency (percent) and mean ± standard deviation (minimum-maximum). MC–GR images were compared against FA in the detection of RNPAs. The number of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) results was calculated for MC–GR images. Sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and accuracy (AC) were calculated from these results. The rates of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated. Accuracy was calculated as the ratio of true positives and true negatives to the total number of cases. The kappa (κ) test was used to evaluate the agreement of two retina specialists. The κ test results were interpreted as follows: The values of 0.01-0.20 were considered none to slight; 0.21-0.40 were considered fair; 0.41-0.60 were considered moderate; 0.61-0.80 were considered substantial; and 0.81-1.00 were considered as almost perfect agreement.[7]

RESULTS

Fifty (50%) female and 50 (50%) male patients with a mean age of 61.3 ± 9.0 (37-84) years were included in the study. Two hundred eyes of 100 patients were prospectively evaluated. Twenty-two eyes with insufficient image quality were excluded from the study; 178 eyes were then analyzed.

One hundred and forty-one (79.2%) eyes had non-proliferative DR and 37 (20.7) eyes had proliferative DR. In FA images, RNPAs were determined in 102 (57.3%) eyes, whereas RNPA was not detected in 76 (42.6%) eyes.

When MC–GR images were compared to FA images in terms of RNPAs in the entire 55º retina, without discrimination of the retina specialists it was detected that sensitivity was 87%; specificity was 89%; the positive predictive value was 92%; the negative predictive value was 83%; and the accuracy was 87% (Table 1). For the first retina specialist, these rates were 86%, 87%, 90%, 82%, and 86%, respectively (Table 1). For the second retina specialist, the rates were 88%, 91%, 93%, 84%, and 89%, respectively (Table 1). The rates of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy obtained by the comparison of MC–GR images with FA images in terms of RNPAs in the macular, nasal, superior temporal, and inferior temporal retinal fields by each retina specialist are shown in table 2. The highest accuracy rate was determined in the nasal field. The lowest accuracy rate was determined in the superior temporal field.

When the agreement between two retina specialists was evaluated, κ values were found to be 0.72, 0.77, 0.67, and

Table 1: *The comparison of multicolor-green reflectance and fundus fluorescein angiography images in terms of retinal non-perfused areas in the entire 55º retina.*

	Sensitivity	Specificity	Positive PV	Negative PV	Accuracy	κ
Total	87%	89%	92%	83%	87%	0.76
1st RS	86%	87%	90%	82%	86%	0.73
2nd RS	88%	91%	93%	84%	89%	0.79

κ= Kappa value; RS= Retina specialist; PV= Predictive value.

Table 2: *The comparison of multicolor—green reflectance and fundus fluorescein angiography images in terms of retinal nonperfused areas in the four retinal fields.*

Field		Sensitivity	Specificity	Positive PV	Negative PV	Accuracy	κ
Macular	Total	89%	87%	89%	87%	88%	0.77
	1st RS	90%	87%	89%	89%	89%	0.78
	2nd RS	88%	87%	89%	86%	88%	0.76
Nasal	Total	88%	94%	95%	85%	91%	0.81
	1st RS	94%	87%	90%	90%	91%	0.82
	2nd RS	86%	96%	96%	83%	90%	0.80
Superior temporal	Total	82%	86%	89%	79%	84%	0.68
	1st RS	79%	84%	87%	75%	81%	0.67
	2nd RS	86%	89%	91%	82%	87%	0.75
Inferior temporal	Total	88%	88%	93%	80%	88%	0.75
	1st RS	90%	75%	90%	75%	84%	0.67
	2nd RS	91%	95%	97%	85%	92%	0.84

κ=Kappa value; RS=Retina specialist; PV=Predictive value.

0.71 in macular, nasal, superior temporal, and inferior temporal fields, respectively. A substantial agreement was observed in all retinal fields.

DISCUSSION

DR is one of the causes of severe visual loss in adults ages 20 to 74 worldwide.[8] Retinal microangiopathy causes blindness of more than 10,000 people per year.[9] Macular ischemia is one of the causes of visual loss in patients with DR.[10] Non-perfused areas are formed when sufficient tissue perfusion cannot be achieved in the retina after capillary occlusion. It is important to identify small, non-perfused retinal areas in the early period.[11] FA is an imaging modality which has an important role in the management of DR by showing neovascular formation and capillary occlusion.[12] However, FA is an invasive examination that requires the use of a contrast agent. Mild side effects such as nausea (2.2-2.9%), vomiting (0.6-1.8%), and flushing, itching, and urticaria (0.1-0.5%) due to fluorescein are observed in approximately 5% of patients. Rarely, serious life-threatening side effects such as dyspnea, bronchospasm, laryngeal edema, cardiac arrest, syncope, and convulsions may be observed (0.04-0.4%).[12-15] Alternative methods to the FA are tried to be developed in the evaluation of ischemia. In the current study, the ability of MC-GR imaging in detecting RNPAs of eyes with DR was evaluated for the first time. MC–GR imaging was found to be consistent with the FA, which is the gold standard for imaging RNPAs in the eyes with non-proliferative and proliferative DR.

MC imaging captures the image by way of cSLO, using a laser scan comprising of three wavelengths. The laser wavelength enhances each separate layer, with the surface of the retina captured by the short wavelength (BR), the retinal vascular and inner retinal layers by the medium wavelength (GR), and the deep layers by the long wavelength (IR). We observed RNPAs as dark gray-colored hypo-reflective areas with distinct margins in the GR image. The confocal technology only captures reflected light. We thought that the loss and thinning of the inner layers in the RNPAs, where perfusion is impaired as a result of capillary dropout, increases the absorption and decreases the reflectance of short and medium wavelength laser. As a result, these areas are seen as hyporeflectant in BR and GR images. The appearance of the RNPAs in both GR and BR images is similar and compatible with each other. Although not used in our study, RNPAs can be highlighted by choosing the Green-Blue-Enhanced Color Balance setting.

Some previous studies[5,16] have investigated the detection of several posterior segment pathologies using MC imaging in DR. Li et al.[17] compared the visualization of the lesions of DR using MC imaging and conventional colour fundus photography. They determined that the positive numbers of microaneurysms, diabetic macular edema and epiretinal membranes were higher with MC imaging compared with conventional colour fundus photography.[17] Roy et al.[18] demonstrated that hard exudates, cotton-wool spots, and hemorrhages were seen better on MC and in GR images as compared to colour fundus photography, BR, and IR images, respectively. However, retinal ischemia was not evaluated with MC imaging in previous studies. We demonstrated that MC-GR imaging may have a role in the evaluation of ischemia in eyes with DR. In detecting RNPA's, the sensitivity and specificity of MC-GR imaging are 87% and 89%, respectively. Since MC imaging did not

require the use of a contrast agent, it was thought that it could be used as an alternative to FA in the evaluation of RNPAs in diabetic patients with a history of allergy and in whom we could not use contrast agents especially due to renal and hepatic problems or pregnancy. Additionally, other important advantages of MC imaging are that it is non-invasive and non-contact, has short processing times, with no side effects.

Recently, OCT angiography (OCTA) has been used in the diagnosis and treatment follow-up of DR. OCTA is a fast, non-invasive imaging technique. It is a successful technique to demonstrate the retinal RNPAs and neovascularization of the retina and optic disc in detail. OCTA clearly visualizes retinal and choroidal capillary networks and quantifies capillary dropout using en-face visualization of separate layers. However, the limitations of OCTA are its inability to show vascular leakage; presentation of a relatively small field of view; and the occurrence of errors in the presence of media opacity.[19] Garcia et al.[20] reported that OCTA could be an alternative to FA in detecting diabetic macular ischemia. Byeon et al.[21] detected a correlation between foveal ganglion cell layer damage on OCT and foveal avascular zone (FAZ) damage in FA in the eyes with DR. They suggested that FA was more sensitive than OCT in detecting vascular damage and that OCT provides objective results and seems to be a non-invasive substitute for FA.

The standard FA shows 30º area of the retina. By combining seven fields from standard FA images, a retinal area of approximately 75º can be scanned. With the advancement of technology, new fundus angiographies have been developed. Ultra-wide-field angiographies allow the imaging of approximately 200° area of the retina by using ellipsoidal mirror technology. Peripheral RNPAs that cannot be determined by standard angiography can be detected with these angiographies.[22] Thirty-degree and 55º field images can be captured with MC imaging. Therefore, MC imaging is insufficient according to wide-angle angiographies in the evaluation of peripheral RNPAs. This limitation will be eliminated in the future if MC imaging is made possible with wider angles. Sim et al.[23] observed a relationship between ischemia and vascular leakage in the central macula and retinal periphery with ultra-wide-field FA in patients with DR. Evaluation of ischemia in the central retina contributes to understandings about ischemia in the peripheral retina. It is also useful to detect only macular ischemia, as it is an important cause of visual impairment in patients with DR.

During MC imaging, light is scattered in the central area due to the curved structure of the lens and an artifact is formed such as a bright light in the retina. This artifact prevents evaluation in the images of some patients. Pang et al.[24] detected an imaging artifact in the form of a hyperreflective spot on the macula that may be mistaken for true chorioretinal pathology predominantly in pseudophakic patients. They have termed this artifact as ghost maculopathy. Pseudophakic patients were not included in this study in order to avoid this artifact.

Current study showed that ischemia in the posterior pole of the retina can be detected by green laser wavelength scanning of MC imaging. However, the image is affected by media opacities. The possibility of an artifact should be considered when evaluating MC imaging. In addition, in some patients, the differences in retinal pigmentation may be mistakenly evaluated as RNPAs. It is important for the person who makes the evaluation to gain experience because the color tones are different from those seen in the fundus examination. In this study, 55º MC–GR images were used. However, studies using 30º MC–GR images may be useful in evaluating macular ischemia, as the central retina can be evaluated in more detail on 30-degree images. This study is the first known to explore this issue and needs to be supported and developed with new investigations.

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