Sickle Cell Maculopathy: Microstructural Analysis Using OCTA and Identification of Genetic, Systemic, and Biological Risk Factors

SELIM FARES, SOPHIE HAJJAR, MARC ROMANA, PHILIPPE CONNES, MALIK ACOMAT, CORALIE ZOROBABEL, KEVIN ZUBER, ELISE BOULANGER-SCEMAMA, MARYSE ETIENNE-JULAN, THIERRY DAVID, AND LAURENCE BERAL

• PURPOSE: To identify genetic, systemic, and biological factors associated with the occurrence of sickle cell maculopathy (SCM). To evaluate microvascular macular alterations using optical coherence tomography angiography (OCTA) in sickle cell disease (SCD).

• DESIGN: Cross-sectional study.

• METHODS: One hundred fifty-one eyes of 78 adult SCD patients (43 HbSS, 30 HbSC, 4 S/β^+, and 1 HbS Lepore) and 40 eyes of 20 healthy controls underwent spectral-domain optical coherence tomography (SDOCT) and OCTA using Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). We analyzed the occurrence of SCM, the foveal avascular zone (FAZ) area, and the severity of macular ischemia and studied their relationships with genetic, systemic, and biological parameters using multivariate logistic regression analysis.

• RESULTS: Maculopathy occurred in 66 eyes (44%), and more frequently in HbSS patients (71%, $P = .004$). Multivariate analysis identified HbSS genotype and lower prothrombin ratio (PR) as independently associated with SCM ($P = .01$). Proliferative sickle cell retinopathy was also associated with SCM ($P = .02$). FAZ enlargement was associated with higher lactate dehydrogenase level ($P = .02$). Macular ischemia was more severe in patients with lower hemoglobin level ($P = .004$) and lower PR ($P = .01$). No flow areas were identified with OCTA even in eyes with no macular thinning (36 eyes, 42%) and appeared more frequently in the temporal superior subfield (36%).

• CONCLUSIONS: HbSS genotype, abnormal coagulation and hemolysis increase the risk of SCM. OCTA provides valuable criteria to identify potential risk factors of SCM. OCTA also improves detection of early microvascular changes before the onset of macular thinning. (Am J Ophthalmol 2021;224:7–17. © 2020 Elsevier Inc. All rights reserved.)

SICKLE CELL DISEASE (SCD) IS THE MOST COMMON GENETIC disease, affecting around 300,000 to 400,000 newborns every year.[1,2] SCD is caused by a mutation in the β-globin gene, resulting in the production of an abnormal hemoglobin called hemoglobin S (HbS). Hemoglobin S polymerizes under hypoxic conditions, which causes a mechanical distortion of red blood cells into a sickle-like shape. These sickled red blood cells are fragile and rigid, resulting in chronic hemolytic anemia and frequent vaso-occlusive-like complications in multiple organs, including the eyes.[3] SCD is a generic term encompassing different syndromes according to genotype: homozygotes (HbSS) or compound heterozygotes (HbS variant).

Sickle cell retinopathy (SCR) has been well described, with proliferative sickle cell retinopathy (PSR) being a major sight-threatening complication.[4] Since the development of spectral-domain optical coherence tomography (SDOCT), macular vascular abnormalities in SCD are now commonly detected.[5] Sickle cell maculopathy (SCM) is defined as patchy areas of severe retinal thinning in the temporal macula. The exact etiology of macular thinning remains unclear, but it may be related to the temporal macula ending along the horizontal raphe, making it a watershed zone between the vascular arcades of the retinal circulation and thus more susceptible to vascular occlusions.[6] By allowing depth-resolved visualization of macular vascular network with high resolution, optical coherence tomography angiography (OCTA) recently detected much more macular vascular alterations than previously and provided additional features, including enlargement and irregularities of the foveal avascular zone (FAZ), hairpin venular loops, and areas of flow loss responsible for irreversible macular thinning.[7,8]

AJO.com Supplemental Material available at AJO.com.
Accepted for publication Nov 23, 2020.

From the Service d'ophtalmologie, CHU de Pointe-à-Pitre/Abymes, Pointe-à-Pitre, Guadeloupe, France (S.F., S.H., M.A., C.Z., T.D., L.B.); Université de Paris, UMR_S1134, BIGR, INSERM, Paris, France (M.R., L.B.); Université des Antilles, UMR_S1134, BIGR, Pointe-à-Pitre, Guadeloupe, France (M.R., L.B.); Laboratoire d'Excellence GR-Ex, Paris, France (M.R., P.C.); Laboratoire Interuniversitaire de Biologie de la Motricité (LIBM) EA7424, Equipe "Biologie Vasculaire et du Globule Rouge," Université Claude Bernard Lyon 1, Université de Lyon, France (P.C.); Fondation Ophtalmologique Adolphe de Rothschild, Paris, France (K.Z., E.B.-S.); Unité Transversale de la Drépanocytose, CHU de Pointe-à- Pitre/Abymes, Pointe-à-Pitre, Guadeloupe, France (M.E.-J.); and Université des Antilles, Guadeloupe (FWI), France (T.D.,L.B.).

Inquiries to Selim Fares, Service d'ophtalmologie, CHU de Pointe-à-Pitre/Abymes, route de Chauvel, 97159, Pointe-à-Pitre, Guadeloupe, France; e-mail: selimfares8@gmail.com

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.11.019

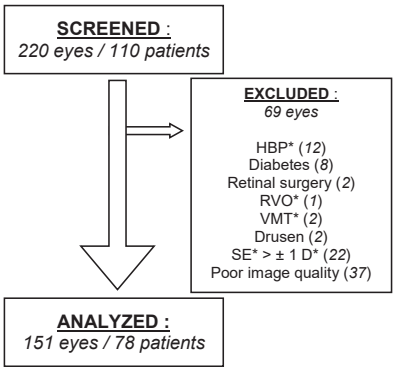

FIGURE 1. Flow chart of the study. Sixty-nine eyes of 32 patients were excluded because of 1 or multiple criteria of exclusion. *Abbreviations are as follows: D = diopter; HBP = high blood pressure; RVO = retinal venous occlusion; SE = spherical equivalent; VMT = vitreomacular traction.

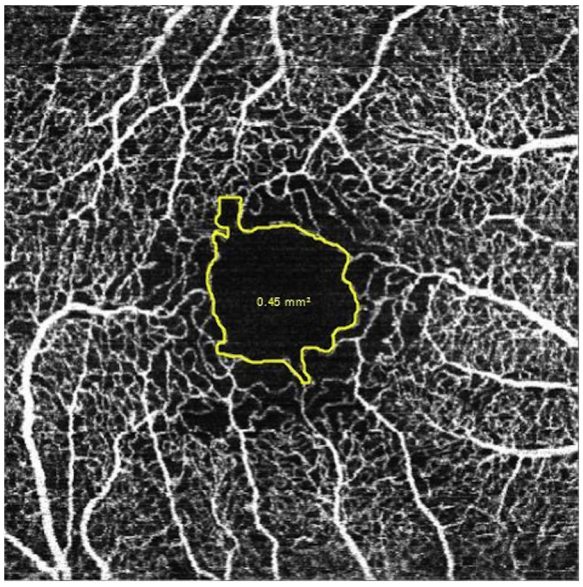

FIGURE 2. Manual delineation of foveal avascular zone area.

Previous studies showed that HbSC genotype, male sex, low fetal hemoglobin (HbF), high hemoglobin levels, and high blood viscosity (particularly in HbSS genotype) were associated with severe PSR.[9–11] However, no study has identified genetic or biological risk factors of SCM. Identifying risk factors could lead to reliable prediction models for SCM management and minimize the onset of irreversible macular scotoma, decreased retinal sensitivity, or vision loss described in eyes with severe macular thinning.[12,13]

The primary purpose of this study was to identify the systemic, biological, and genetic risk factors of SCM on OCT. The secondary purpose was to analyze macular microvascular network using OCTA and test if FAZ area and macular ischemia could be useful to identify more specific risk factors of SCM.

METHODS

• **PATIENTS:** This study received the ethical approval of the Regional Ethics Committee (CPP Sud-Ouest Outre-Mer III, Bordeaux, France; registration number: 2010-A00244-35) and adhered to the principles set by the Declaration of Helsinki. Written informed consent was obtained from each participant. This cross-sectional monocentric study concerned 110 patients with confirmed SCD diagnosis using standard methods and undergoing regular ophthalmologic evaluation at the Ophthalmology Department of the University Hospital of Guadeloupe (French West Indies) between January 1, 2019, and January 1, 2020. Exclusion criteria were age under 18; history of other confounding retinal vascular disease such as hypertension, diabetes, or retinal vein occlusion; prior retinal surgery; spherical equivalent $> \pm 1$ diopter; retinal involvement such as vitreomacular traction syndrome, epiretinal membrane, or drusen; poor-quality images; or ocular media opacities preventing detailed imaging. Sixty-nine eyes of 32 patients met these criteria and were excluded, so that 151 eyes of 78 patients were analyzed (Figure 1). An ethnic-matched control group of 20 unaffected African Caribbean controls were also recruited and underwent the same ocular procedures.

• **MULTIMODAL IMAGING:** Ophthalmic examination included best-corrected visual acuity (BCVA) measured with Snellen chart, slit-lamp evaluation, and dilated fundus examination. SCR was graded according to the Goldberg classification, including peripheral arteriolar occlusions (stage I), peripheral arteriovenous anastomoses (stage II), preretinal neovascularization (stage III), vitreous hemorrhage (stage IV), and retinal detachment (stage V).[14] Eyes with no retinopathy were graded as Goldberg stage 0. Patients with previously performed focal laser photocoagulation for peripheral neovascularization were classified as stage III, in accordance with the Department's protocol to treat all patients with peripheral neovessels by laser. Analyses were performed using 3 categories of retinopathy: no retinopathy (stage 0), nonproliferative retinopathy (stage I and II), and proliferative retinopathy (stage III, IV, and V).

All patients and unaffected controls underwent a macular SDOCT and OCTA using the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). For each eye, the standard scanning protocol included an automated "posterior pole" 30 × 25 cuboid volume scan of 61 high-resolution OCT scans and 10-degree and 20-degree OCTA scans centered on the fovea. Multiple scans were acquired as needed to secure adequate quality criteria and

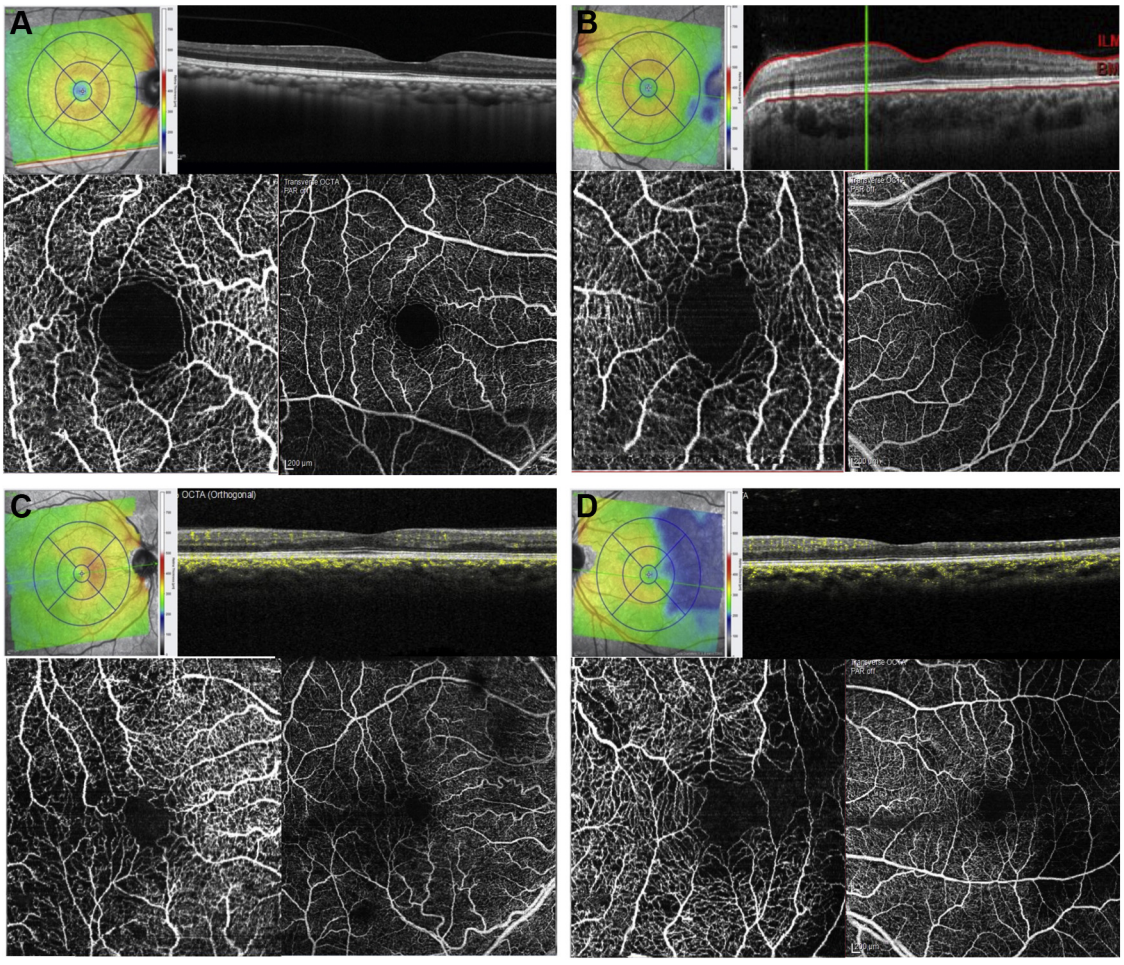

FIGURE 3. Subjective graduation of macular ischemia based on spectral-domain optical coherence tomography (OCT) and OCT angiography (OCTA) using the following criteria: extension of blue area in retinal thickness map, the areas of flow loss in OCTA 10° and 20°, the irregularity and enlargement of the foveal avascular zone. (A) No ischemia. (B) Mild ischemia. (C) Moderate ischemia. (D) Severe ischemia.

the best-quality scans were selected by the lead grader for analysis. Images were anonymized and analyzed by 2 independent retina fellowship–trained graders in a masked fashion. In case of disagreement, scans were reviewed and arbitrary graded together. According to previous studies, SCM was identified as patchy retinal thinning areas on OCT (ie, blue areas of markedly decreased thickness on retinal thickness color-coded map).[5,15] The first step involved manual delineation of the full-thickness retina FAZ border taken from 10-degree OCTA scans using the "area" function of the software (Figure 2). Scans were then qualitatively graded by the investigators for macular ischemia from no (0) to severe (3) macular ischemia (including extension of flow loss areas, vessel density, enlargement of the FAZ, presence of perifoveal venular loops, and focal disruption of the perifoveal anastomotic capillary arcade). Figure 3 illustrates our macular ischemia graduation (additional examples are provided in the Supplemental Material, available at AJO.com).

• **CLINICAL AND BIOLOGICAL DATA COLLECTION:** Clinical data were retrospectively collected from patients' charts at the last-month routine clinical visit to the Sickle Cell Disease Center of Guadeloupe and included SCD type (genotype, α-thalassemia status), medical and surgery history (other systemic condition, spleen status, cholecystectomy), current treatment (hydroxyurea, chelation therapy, chronic transfusion, anticoagulants) and history of SCD systemic complications (acute chest syndrome, cerebrovascular accident, severe vaso-occlusive crisis frequency, osteonecrosis). Severe vaso-occlusive crises were defined as requiring acute Emergency Department visit or inpatient admission. Frequency of severe vaso-occlusive crises was defined using the method of Duan and associates; that is, as rare if once a year or less, occasional if twice a year, and frequent if 3 times a year or more, averaged over the 2 years prior to the ocular examination.[10]

We also collected laboratory results including HbF level; baseline hemoglobin level; hematocrit; C-reactive protein;

TABLE 1. Baseline Demographic Characteristics of the Sickle Cell Disease Population

	Total	HbSS	HbS Variant	*P* Values
Patients, n (eyes)	78 (151)	43 (85)	35 (66)	-
Age (years)	37 ± 11	35 ± 10	38 ± 12	.21
Female, n (%)	53 (68%)	29 (6)	24 (69)	.92
Systemic characteristics				
Hydroxyurea use, n (%)	27 (34.6)	27 (63)	0 (0)	<.001*
Anticoagulation, n (%)	12 (15.4)	9 (21)	3 (8,6)	.13
Regular phlebotomy, n (%)	24 (31)	7 (16)	17 (49)	<.01*
Chronic transfusion, n (%)	6 (7.7)	5 (12)	1 (3)	.22
Osteonecrosis, n (%)	24 (30.8)	16 (37)	8 (23)	.17
ACS history, n (%)	33 (42.3)	26 (60.5)	7 (20.0)	<.001*
VOC frequency/2 years, n (%)				
Rare	62 (79)	33 (77)	29 (83)	.51
Occasional	6 (7.7)	4 (9.3)	2 (5.7)	.69
Frequent	10 (13)	6 (14)	4 (11)	1
CVA, n (%)	10 (13)	10 (23)	0 (0)	<.01*
Cholecystectomy, n (%)	34 (44)	26 (60)	8 (23)	<.001*
Biological criteria				
Hemoglobin (g/dL)	9.2 ± 1.7 (6-13)	8.1 ± 1.2	10.7 ± 1.0	<.001*
Baseline HbF (%)	6.4 ± 7.0 (0-27.7)	9.0 ± 7.6	2.7 ± 3.9	<.001*
Hematocrit (%)	28 ± 5 (16-41)	24 ± 4	32 ± 3	<.001*
Platelets (g/L)	336 ± 155 (60-786)	388 ± 155	273 ± 130	<.001*
Reticulocytes (g/L)	198 ± 117 (7-582)	258 ± 120	124 ± 54	<.001*
Leukocytes (g/L)	8.1 ± 2.9 (3.9-16.2)	9.1 ± 3.0	6.8 ± 2.0	<.001*
CRP (mg/L)	5.27 ± 6.05 (0-32)	6.28 ±7.0	3.97 ± 4.4	.087
PR (%)	83 ± 14 (23-100)	78 ± 14	88 ± 13	<.01*
LDH (IU/L)	395 ± 178 (138-928)	507 ± 162	257 ± 60	<.001*
Total bilirubin (μmol/L)	36.4 ± 29.2 (6-184)	49.5 ± 32.9	20.6 ± 11.1	<.001*
GGT (U/L)	59 +- 52 (10-248)	74 ± 60	40 ± 33	<.01*
G6PD deficiency, n (%)	4 (5.3)	3 (7,.3)	1 (2.9)	.62
α-Globin gene number, n (%)	–	–	–	.47
2	2 (2.6)	2 (5.3)	0 (0)	–
3	20 (25.6)	12 (32)	8 (26)	–
4	47 (60.3)	24 (63)	23 (74)	–

ACS = acute chest syndrome; CRP = C-reactive protein; CVA = cerebrovascular accident; G6PD = glucose-6-phosphate dehydrogenase; GGT = gamma-glutamyl transferase; HbF = hemoglobin F; LDH = lactate dehydrogenase; PR = prothrombin ratio; VOC = vaso-occlusive crisis.

Asterisk indicates statistically significant *P* value.

prothrombin ratio (PR); reticulocyte, leukocyte, neutrophil, and platelet counts; lactate dehydrogenase (LDH); total bilirubin, serum ferritin, and fibrinogen levels; and G6PD deficiency. Biological parameters were obtained at steady state.

• **STATISTICAL ANALYSIS:** Mann-Whitney tests were used to compare the different demographic, clinical, and ocular characteristics between the 2 groups. A χ^2 test was used for qualitative data. For the 3-group comparisons, a Kruskal-Wallis test was used, followed by a Dunn post hoc test when appropriate. *P* value < .05 was considered as significant. Three ocular criteria were included in the analysis: the occurrence of maculopathy, the FAZ enlargement, and the severity of the macular ischemia. Because simple comparison of the distribution of systemic and biological factors between patients can be misleading as certain factors are interdependent or age related, we used multiple logistic regression analysis, adjusted by sex, genotype, and factors showing a *P* value < .1 in univariate analysis to identify risk factors independently associated with SCM, FAZ enlargement, and macular ischemia in an ordinal Generalized Estimating Equations model.

Complete data were available for 90% of the patients. For the 10% remaining, we used a simple imputation with a predictive mean matching method for the missing data. Matched controls were used to compare FAZ measures with SCD patients using a Mann-Whitney test. Statistical analyses were performed using R statistical software. Data are expressed in mean ± SD or percentages for quantitative and qualitative parameters, respectively.

TABLE 2. Univariate Analysis for Occurrence of Sickle Cell Maculopathy

	Maculopathy	No Maculopathy	*P* Value
Number of eyes (%)	66 (43.7)	85 (56.3)	–
Female, n (%)	42 (63.6)	60 (70,6)	.45
Age (years)	36.1 ± 9.5	36.8 ± 11.4	.63
Systemic characteristics			
HbSS genotype	47 (71.2)	38 (44.7)	.004*
Hydroxyurea use, n (%)	28 (42.4)	26 (30.6)	.19
Anticoagulation, n (%)	12 (18.2)	11 (12.9)	.45
Regular phlebotomy, n (%)	15 (22.7)	30 (35.3)	.15
Chronic transfusion, n (%)	8 (12.1)	3 (3.5)	.073
ACS history, eyes (%)	33 (50)	31 (36.5)	.17
VOC frequency/2 years, eyes (%)			
Rare	51 (77)	68 (80)	.68
Occasional	5 (8.2)	7 (7.6)	.88
Frequent	10 (15)	10 (12)	.54
Osteonecrosis	20 (30.3)	26 (30.6)	.95
Ocular characteristics			
FAZ area (mm^2)	0.56 ± 0.18	0.49 ± 0.13	.021*
Macular ischemia index, eyes (%)			
0	0 (0)	49 (58)	<.001*
1	18 (27)	34 (40)	–
2	33 (50)	2 (2)	–
3	15 (23)	0	–
Sickle cell retinopathy, eyes (%)			
Proliferative	20 (30.3)	14 (16.5)	.04*
Nonproliferative	46 (69.7)	71 (83.5)	–
Biological criteria			
Hemoglobin (g/dL)	8.8 ± 1.6	9.6 ± 1.7	.001*
HbF > 15%, n (%)	10 (15.2)	13 (15.3)	.97
Hematocrit (%)	26.1 ± 5.3	28.9 ± 5.0	.01*
Platelets (g/L)	373.0 ± 153.8	305.7 ± 147.9	.02*
Leukocytes (g/L)	8.3 ± 2.9	7.9 ± 2.9	.40
CRP (mg/L)	5.4 ± 4.9	6.2 ± 6.9	.45
PR (%)	79.2 ± 15.0	84.4 ± 12.9	.038*
LDH (IU/L)	449.7 ± 183.6	355.7 ± 161.0	.007*
Total bilirubin (μmol/L)	42.3 ± 30.6	32.9 ± 28.2	.12
GGT (IU/L)	57 ± 52.6	59.6 ± 51.8	.79
α-Globin gene number, n (%)	-	-	.85
2	3 (4.5)	2 (2.4)	–
3	19 (28.8)	26 (30.6)	–
4	44 (66.7)	57 (67.1)	–

ACS = acute chest syndrome; CRP = C-reactive protein; GGT = gamma-glutamyl transferase; HbF = hemoglobin F; LDH = lactate dehydrogenase; PR = prothrombin ratio; VOC = vaso-occlusive crisis.

Asterisk indicates statistically significant *P* value.

RESULTS

• **DEMOGRAPHIC RESULTS:** Table 1 summarizes patients' demographic and clinical characteristics. All participants were of African Caribbean origin. One hundred fifty-one eyes of 78 patients (25 men and 53 women) with a mean age of 37 years (range 18-61 years) were enrolled in this study. Five eyes were excluded owing to unilateral high myopia (1 eye), phthisis bulbi (1 eye), poor acquisition owing to no fixation (2 eyes), and a large foveal vascular pigmentary epithelium drusen (1 eye). Several sickle cell syndromes were analyzed, including homozygotes HbSS (43 patients, 55%) and compound heterozygotes HbS variant (35 patients, 45%), comprising HbSC (30 patients), HbS/β+ (4 patients), and HbS Lepore (1 patient). Visual acuity ranged from 20/25 to 20/20 for all patients.

The most frequent systemic events in our population were painful vaso-occlusive crisis (49%), acute chest syndrome (42%), and osteonecrosis (31%). There was no statistical difference in age, sex, functional α-globin gene number, vaso-occlusive crisis, osteonecrosis, anticoagulant medication, and chronic transfusion between HbSS and HbS variant groups. However, HbSS patients exhibited more ulcers ($P = .019$), more acute chest syndrome ($P < .01$), and more cerebrovascular accidents ($P < .01$). Lower hemoglobin and hematocrit ($P < .001$) and higher LDH and total bilirubin level ($P < .001$) were found in the HbSS group. Finally, HbSS patients seemed to exhibit a proinflammatory state: higher leukocyte and neutrophil levels ($P < .001$ and $P < .02$, respectively) and a trend to higher level of C-reactive protein ($P = .087$).

• **SICKLE CELL MACULOPATHY:** SCM was present in 66 eyes (43.7%), especially in HbSS patients (71.2%, $P = .004$). Intergrader agreement was 100% for the detection of SCM based on OCT. Univariate analysis (Table 2) showed that patients with SCM had lower hemoglobin ($P = .001$), lower hematocrit ($P = .01$), lower PR ($P = .038$), and higher LDH levels ($P = .007$) than those without. In addition, we observed higher occurrence of SCM in eyes with proliferative SCR ($P = .04$). Chronic transfusion tended to be associated with the occurrence of SCM ($P = .073$). As expected, maculopathy was associated with FAZ enlargement ($P = .021$) and more severe macular ischemia ($P < .001$). A cut-off level of HbF > 15% based on the prior studies about SCR did not show any association with maculopathy.[15] Multivariate regression analysis revealed that HbSS genotype and lower PR were the most predictive risk factors of SCM ($P = .01$).

• **FOVEAL AVASCULAR ZONE AREA:** On OCTA, FAZ was measured in 142 eyes (94%) of SCD patients and in all unaffected control eyes. Intergrader agreement was 97% for the measurement of FAZ area. In 9 eyes, it was not detectable by the software used related to extensive macular ischemia (Figure 4) owing to central arterial occlusion (4 eyes) and extensive perifoveal capillary remodeling with a poorly defined FAZ (5 eyes). For these 9 eyes, the quantitative value of their surely enlarged FAZ could be misleading in the analysis. Accordingly, we used a censored statistical model that assigned 1.0 to larger FAZ area than 1.0. Univariate analysis (Table 3) showed that FAZ enlargement was associated with HbSS genotype ($P = .04$), hydroxyurea treatment ($P = .04$), anticoagulant medication ($P = .05$), positive history of acute chest syndrome ($P = .02$), occasional vaso-occlusive crises ($P = .01$), lower hematocrit and hemoglobin levels ($P = .01$), and higher LDH level ($P = .02$). Unexpectedly, we also found that male SCD patients had a smaller FAZ size ($P = .01$). In multivariate analysis only high LDH level was independently associated with FAZ enlargement (a rise of 0.03 μm^2 for every 100 units of LDH, $P = .02$). Interestingly, positive history for acute chest syndrome was marginally associated with FAZ enlargement ($P = .07$). Comparison between SCD patients with or without SCM and age-, ethnicity- and sex-matched controls revealed that the FAZ area was larger in our patients than in controls (respectively, 0.52 ± 0.19 mm^2 vs 0.38 ± 0.17 mm^2, $P < .01$).

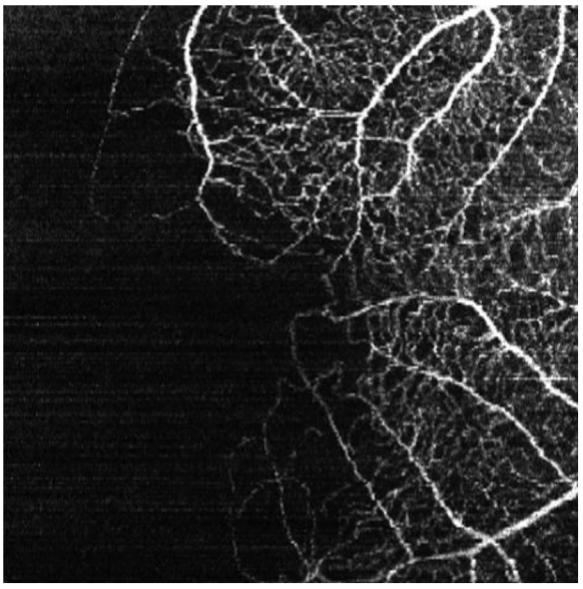

FIGURE 4. Example of difficulty to manually delineate foveal avascular zone area owing to extensive macular ischemia.

• **MACULAR ISCHEMIA:** Macular ischemia was subjectively identified using SDOCT and OCTA with quantitative and qualitative criteria: irregularity of the FAZ, the extension of no-flow areas, and the general vascular density. We graded ischemia from no (0) to severe (3) for all 151 eyes of the study. Intergrader agreement was 77% for the subjective grading of macular ischemia. The size of the grade 3 (severe) group of macular ischemia was not large enough to perform statistical analysis and was regrouped with the grade 2 (moderate) group for the analysis. Thus, we had 49 eyes (32.5%) with no ischemia, 52 (34.4%) with mild ischemia, and 50 (33.1%) with moderate or severe ischemia. Areas of no flow were more frequent in the temporal superior (36%) subfield then the temporal inferior (27%), nasal superior (9%), and nasal inferior (5%) subfields. Seventy eyes had irregularities of the FAZ borders (46%). Thirty-eight eyes had perifoveal venular loops (25%). Abnormal macular flow loss was visible even in SCD eyes with no macular thinning on OCT (36 eyes, 42%). To have sufficient numbers of patients in groups for the identification of potential risk factors of macular ischemia, we regrouped moderate (2) and severe (3) eyes. Univariate analysis (Table 4) revealed that 80% of the eyes with moderate and severe macular ischemia are

TABLE 3. Univariate Analysis of Foveal Avascular Zone Area

	FAZ Area			
	Estimator	Standard Error	t	*P* Value
Systemic characteristics				
Male	−0.09	0.03	7.30	.01*
HbSS genotype	0.07	0.03	2.07	.04*
Hydroxyurea use	0.07	0.03	2.05	.04*
Anticoagulation	0.09	0.04	1.97	.05*
Chronic transfusion	0.12	0.06	1.91	.06
Regular phlebotomy	−0.12	0.03	−3.53	<.01*
Osteonecrosis	0.04	0.04	1.28	.20
CVA	0.00	0.06	0.00	.98
ACS history	0.08	0.03	5.83	.02*
VOC frequency/2 years				
Rare	−0.01	0.03	0.10	.75
Occasional	0.09	0.03	6.75	.01*
Frequent	0.06	0.04	3.14	.08
Ocular characteristics				
Macular ischemia index				
1	0.001	0.018	0.00	.94
2	0.028	0.027	1.10	.29
3	0.036	0.037	0.94	.33
Proliferative retinopathy	−0.03	0.02	1.24	.27
Biological criteria				
Hemoglobin	−0.03	0.01	−2.73	.01*
HbF < 15%	−0.03	0.03	0.99	.32
Hematocrit	−0.01	0.00	6.84	.01*
Platelets	0.00	0.00	2.03	.15
PR	−0.0004	0.00	0.24	.62
LDH	0.0003	0.00	5.85	.02*
GGT	0.00	0.00	2.11	.15
α-Globin gene number	−0.01	0.03	−0.21	.83

ACS = acute chest syndrome; CVA = cerebrovascular accident; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; PR = prothrombin ratio; VOC = vaso-occlusive crisis.
Asterisk indicates statistically significant *P* value.

detected in HbSS patients ($P < .001$). We also showed a significant association between the severity of macular ischemia and hemoglobin level ($P < .001$), hematocrit and PR ($P = .01$ and $P < .001$, respectively), platelet level ($P = .01$), and LDH ($P = .003$). In multivariate analysis, low hemoglobin level ($P = .004$) and low PR ($P = .01$) remained independently associated with macular ischemia severity.

DISCUSSION

THE CURRENT STUDY IS THE LARGEST STUDY TO DATE evaluating SCD patients with OCTA. The analysis revealed that HbSS genotype, lower PR, and PSR are independently associated with SCM. Correlation between PSR and SCM is an expected outcome. However, the association between genotype and SCM has been poorly described. Even though it is well accepted that SCR is more common and more severe in HbSC patients than in HbSS,[14,16] Mathew and associates reported a higher rate of retinal thinning in HbSS patients than in HbSC patients (48% vs 35%),[5] whereas other studies found no statistical difference.[8,15] Our study demonstrated that retinal thinning occurs more frequently in HbSS eyes compared to the other syndromes of SCD, especially in HbSC eyes. This result is in agreement with prior studies suggesting that higher rate of vaso-occlusive crisis in HbSS patients could lead to repeated ischemic episodes in the eyes and thus the development of extensive retinal atrophy.[9,17,18] On the other hand, milder degree of retinal ischemia in HbSC genotype would be not enough to infarct the retina but could result in the production of angiogenic factors and a higher risk of peripheral neovascularization.[19,20] Natural history of sickle cell retinopathy

TABLE 4. Univariate Analysis of Risk Factors in Macular Ischemia

	No Ischemia	Mild Ischemia	Moderate or Severe Ischemia	*P* Value
Number of eyes (%)	49 (32.5)	52 (34.4)	50 (33.1)	–
Female, eyes (%)	33 (67.3)	41 (78.8)	28 (56.0)	.40
HbSS genotype, eyes (%)	17 (34.7)	28 (53.9)	40 (80.0)	<.001*
Age	34.2 ± 11.2	41.0 ± 10.2	34.0 ± 9.0	.912
Systemic characteristics				
Hydroxyurea use, eyes (%)	11 (22.4)	21 (40.4)	22 (44.0)	.055
Regular phlebotomy, eyes (%)	17 (34.7)	18 (34.6)	10 (20.0)	.16
Chronic transfusion, eyes (%)	2 (4.1)	1 (1.9)	8 (16.0)	.09
Osteonecrosis, eyes (%)	11 (22.4)	22 (42.3)	13 (26.0)	.66
ACS history, eyes (%)	17 (34.7)	21 (40.4)	26 (52.0)	.16
Ocular characteristics				
Occurrence of maculopathy, eyes				
No maculopathy (%)	49 (100)	34 (65)	2 (4)	<.001*
Maculopathy (%)	0 (0)	18 (35)	48 (96)	–
Proliferative retinopathy, eyes (%)	24 (49.0)	28 (53.9)	37 (74.0)	.53
Photocoagulation, eyes (%)	12 (24.5)	19 (36.5)	21 (42.0)	.123
Biological criteria				
Hemoglobin (g/dL)	10.2 ± 1.7	9.0 ± 1.4	8.6 ± 1.6	<.001*
HbF > 15% (%)	5 (10.2)	10 (19.2)	8 (16.0)	.42
Hematocrit, mean ± SD	30.2 ± 5.1	27.3 ± 4.2	25.6 ± 5.6	.01*
Platelets (g/L)	298.8 ± 140.1	311.4 ± 141.4	395.3 ± 163.4	.01*
CRP (mg/L)	5.9 ± 6.1	6.0 ±7.0	5.6 ±5.2	.842
PR (%)	86.2 ± 8.9	82.7 ± 17.1	77.6 ± 13.5	<.001*
LDH (IU/L)	323.4 ± 140.5	399.8 ± 183.5	465.5 ± 176.9	.003*
Total bilirubin (μmol/L)	30.7 ± 27.1	33.4 ± 25.0	47.1 ± 33.7	.156
GGT (IU/L)	50.8 ± 46.5	68.7 ± 60.8	55.4 ± 46.2	.597

ACS = acute chest syndrome; CRP = C-reactive protein; GGT = gamma-glutamyl transferase; HbF = hemoglobin F; LDH = lactate dehydrogenase; PR = prothrombin ratio.

Asterisk indicates statistically significant *P* value.

is unique because peripheral neovessels can infarct over time.[16] In this study, we graded as PSR the few eyes with focal photocoagulation for a prior neovascularization, so PSR eyes may have been overrepresented in our analysis. However, our results suggest a strong relationship between maculopathy and retinopathy. This relationship may be explained by the fact that the temporal macular area and the retinal periphery are both supplied by small-caliber terminal arterioles; therefore vascular occlusion can easily damage these areas.[5] Interestingly, PR was detected at lower levels in patients with maculopathy. Low PR means a prolonged prothrombin time, which is common in SCD even if the reason is not well established.[21,22] It is likely that prolonged prothrombin time is a marker of chronic vascular inflammation, among others such as platelet activation and high plasma levels of adhesion molecules. Prolonged PR could be related to several conditions, such as liver dysfunction, that decrease the synthesis of clotting factors or dysfunctional coagulation factors on vitamin K deficiency owing to cholestasis, for example.

OCTA enabled us to determine valuable findings of maculopathy and to identify or confirm specific risk factors for macular damages in SCD. FAZ area and macular ischemia were correlated with several biological factors. Indeed, high serum LDH level was significantly associated with FAZ area enlargement in multivariate analysis, with a rise of 0.03 μm^2 for every 100 units of LDH. Also, lower hemoglobin level was significantly associated with more severe macular ischemia in multivariate analysis, with a rise of 0.36 μm^2 for every unit of hemoglobin. Two mechanisms have been proposed to be involved in the pathophysiology of SCD: chronic hemolysis, which could promote the development of several complications (such as leg ulcers, stroke, pulmonary hypertension, priapism); and blood hyperviscosity, which would increase the risks for vaso-occlusive-like complications.[23] Few studies found that severe SCR was more frequent in patients with high hemoglobin and hematocrit levels, suggesting blood hyperviscosity could be a potential risk factor.[9,24] These studies served as a basis to use phlebotomy to treat blood hyperviscosity and prevent retinopathy. In our study, we failed to

find any statistical association between SCM and complications belonging to the "blood hyperviscosity" phenotype. Moreover, the association found between hemolysis and FAZ area enlargement in our study suggests that hemolysis, and not blood hyperviscosity, could play a leading role in the development of SCM.

Concerning hydroxyurea use, clinical charts were retrospectively reviewed to assess the compliance in our study by comparing biological parameter expression affected by this drug before and after hydroxyurea treatment onset. This compound induces an increase of HbF, preventing HbS polymerization and red blood cells sickling, but its impact on the occurrence of ocular complications remains unknown.[23] It is possible that a high HbF level, by reducing the frequency of vaso-occlusive crises, could delay endothelial vascular injuries and the evolution to maculopathy or retinopathy. Prior studies suggest a protective effect of HbF on retinopathy, in particular when HbF is higher than 15%.[24,25] Even if Dell'Arti and associates showed a correlation between lower HbF and SCM, the current study failed to show any statistically significant association with hydroxyurea use.[15] However, other effects of hydroxyurea have been described, such as inhibition of proangiogenic factor expression, decrease of platelets and white blood cell counts, and decreased cytokine (TNF-α and IL-10) levels.[26,27] Because hydroxyurea has been given to pediatric patients for a few decades, it could be interesting to assess the onset of ocular complications over time (ie, prospectively) and compare with patients without hydroxyurea treatment.

On OCT, the occurrence of maculopathy in patients with SCD was 43.7%, which is in agreement with prior studies showing that maculopathy frequency varied between 44% and 60%.[5,28,29] A great advantage of OCTA is to detect microvascular abnormalities before retinal thinning occurs. For instance, Minvielle and associates already detected perifoveal abnormalities in fluorescence angiography in only 50% of eyes, whereas OCTA detected in 100% of cases.[7] Our results are in agreement with prior studies. Areas of no flow on OCTA were present in 36 of 85 eyes without maculopathy on OCT. This observation suggests that OCTA may detect early signs of macular changes in patients and can likely be considered as a clinically useful marker in the screening and the evaluation of SCD progression or even for preventive treatment.

As expected, FAZ area was found larger in SCD patients than in controls. However, this finding should be read with caution because the loss of capillaries may have been the result of blood flow velocity below the detection threshold of the OCTA rather than no perfusion. In addition, it is important to acknowledge that FAZ area can vary with ethnicities.[30] An enlarged FAZ area is not specific to patients with SCD but is also a physiological variation for unaffected subjects from African origin. To avoid any bias related to ethnicity effect, the control group was selected in the same population studied and the foveal splaying owing to FAZ enlargement was not considered as severity criteria in the grading of macular ischemia. We also noticed a substantial variability in FAZ area and irregularity between patients and controls. Some studies performed on unaffected eyes suggest that this parameter can be ambiguous and thus may be insufficient for discriminating the occurrence and severity of maculopathy in SCD.[31,32] Because of the extended disruption in the FAZ of 9 concerned eyes beyond the borders of the OCTA scans, causing misleading quantitative values with clinical implications, we did not exclude them from analysis but used a censored statistical model instead. As such, eyes with more severe macular vascular pathology have been included in this study in order to establish association with potential risk factors. We did not detect statistical difference between male and female subjects in the occurrence of maculopathy and the macular ischemia, but men exhibited a lower FAZ area than women. Interestingly, some studies have questioned a protective female effect in SCR. Indeed, Fox and associates found that HbSS men had a 2.5-fold higher risk of developing severe SCR than women.[9] They attributed the female protective effect to estrogen levels through a vascular protective effect by increasing endothelial nitric oxide synthase activity. Further studies are warranted to address this issue.

Finally, our analysis also revealed that the occurrence of the maculopathy or the severity of macular ischemia can vary significantly between 2 eyes of the same patient. Future prospective studies may elucidate the ocular risk factors that may predispose the injury of 1 eye compared to the other.

Our study had several limitations. First, it was a cross-sectional study, so we could not estimate the incidence and the evolution of the SCM. Further studies are warranted to evaluate macular microvascular changes with OCTA in the same patients over time in a longitudinal manner. Secondly, we used strict inclusion and exclusion criteria resulting in a modest cohort of patients, which limited the multivariate analysis for potential risk factors. Yet, we did not exclude patients with prior photocoagulation because we believe that it reflects a real-life situation and their exclusion can underestimate a number of severe patients in the present analysis. Moreover, recent studies demonstrated that macular perfusion and FAZ did not change in diabetic retinopathy after panretinal photocoagulation.[33,34] Another limitation concerned image acquisition and the software used. OCTA has the advantage to be a noninvasive dye-free technique and to provide structural microvascular information. However, it requires a precise and stable ocular fixation from the patients for several seconds. This technical limitation had probably led to exclude patients with low visual acuity owing to extensive macular ischemia or vitreous hemorrhages, and thus to low numbers of stage 3 or higher retinopathy in our studied population. Furthermore, the software we used could not provide any vascular density, so we could

not have any quantitative criteria for ischemia. For this reason, we subjectively graded macular ischemia because we suspect that OCTA is a better tool to reveal maculopathy than OCT. However, the study may underestimate the frequency of macular abnormalities in patients with more severe systemic events. The last limitation concerned the grading of SCR based on clinical fundus examination rather than based on fluorescein angiography, which is the gold standard. We decided to stage retinopathy based on clinical examination alone for 2 reasons. First, fluorescein angiography is an invasive procedure with a risk of anaphylactic shock and other severe side effects specific to our studied population, such as triggering painful vaso-occlusive crises.[35] Secondly, its findings are not always clinically relevant, especially in case of peripheral neovessels, which can auto-infarct over time.[16] Nevertheless, we acknowledged that the lack of fluorescein angiography in our study could have led us to underestimate the number of stage 1 or 2. To limit any bias, we combined data in only 2 categories (proliferative or nonproliferative retinopathy), as has been previously done.[10]

In conclusion, the identification of systemic, genetic, and biological factors associated with the occurrence and the severity of maculopathy confirms the complexity of this disease. Association between SCM and other organ damages in SCD is a significant question. OCTA is a highly sensitive method to detect microvascular macular abnormalities before the onset of macular thinning that commonly define the maculopathy status. Avoiding the occurrence of these abnormalities is critical to minimize and to manage the functional implication of retinal thinning in these young patients. Prospective studies are needed to determine the natural history of SCM and its relevance regarding preventive strategies for SCD patients. Furthermore, the emergence of novel technology such as OCTA could provide valuable information to contribute in a public health screening and follow-up of patients with SCD.

FUNDING/SUPPORT: THIS RESEARCH DID NOT RECEIVE ANY SPECIFIC GRANT FROM FUNDING AGENCIES IN THE PUBLIC, commercial, or not-for-profit sectors. Financial Disclosures: The authors have no financial disclosures. None of the authors have any proprietary interests or conflicts of interest related to this submission. All authors attest that they meet the current ICMJE criteria for authorship.

Acknowledgments: The authors thank Unity of Sickle Cell Disease–Pointe à Pitre, Guadeloupe, for providing the clinical, genetic, and biologic data; and the patients who participated in the study.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008;86(6):480–487.
2. Elagouz M, Jyothi S, Gupta B, Sivaprasad S. Sickle cell disease and the eye: old and new concepts. *Surv Ophthalmol* 2010;55(4):359–377.
3. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 2010;376(9757):2018–2031.
4. Abdalla Elsayed MEA, Mura M, Al Dhibi H, et al. Sickle cell retinopathy. A focused review. *Graefes Arch Clin Exp Ophthalmol* 2019;257(7):1353–1364.
5. Mathew R, Bafiq R, Ramu J, et al. Spectral domain optical coherence tomography in patients with sickle cell disease. *Br J Ophthalmol* 2015;99(7):967–972.
6. Stevens TS, Busse B, Lee C, et al. Sickling hemoglobinopathies: macular and perimacular vascular abnormalities. *Arch Ophthalmol* 1974;92(6):455.
7. Minvielle W, Caillaux V, Cohen SY, et al. Macular microangiopathy in sickle cell disease using optical coherence tomography angiography. *Am J Ophthalmol* 2016;164:137–144.
8. Han IC, Tadarati M, Pacheco KD, Scott AW. Evaluation of macular vascular abnormalities identified by optical coherence tomography angiography in sickle cell disease. *Am J Ophthalmol* 2017;177:90–99.
9. Fox PD, Dunn DT, Morris JS, Serjeant GR. Risk factors for proliferative sickle retinopathy. *Br J Ophthalmol* 1990;74:172–176.
10. Duan XJ, Lanzkron S, Linz MO, Ewing C, Wang J, Scott AW. Clinical and ophthalmic factors associated with the severity of sickle cell retinopathy. *Am J Ophthalmol* 2019;197:105–113.
11. Lemaire C, Lamarre Y, Lemonne N, et al. Severe proliferative retinopathy is associated with blood hyperviscosity in sickle cell hemoglobin-C disease but not in sickle cell anemia. *Clin Hemorheol Microcirc* 2013;55(2):205–212.
12. Martin GC, Dénier C, Zambrowski O, et al. Visual function in asymptomatic patients with homozygous sickle cell disease and temporal macular atrophy. *JAMA* 2017;135(10):1100.
13. Chow CC, Genead MA, Anastasakis A, Chau FY, Fishman GA, Lim JI. Structural and functional correlation in sickle cell retinopathy using spectral-domain optical coherence tomography and scanning laser ophthalmoscope microperimetry. *Am J Ophthalmol* 2011;152(4):704–711.
14. Goldberg MF. Classification and pathogenesis of proliferative sickle retinopathy. *Am J Ophthalmol* 1971;71(3):649–665.
15. Dell'Arti L, Barteselli G, Riva L, et al. Sickle cell maculopathy: identification of systemic risk factors, and microstructural analysis of individual retinal layers of the macula. *PLoS One* 2018;13(3):e0193582.
16. Downes SM, Hambleton IR, Chuang EL, et al. Incidence and natural history of proliferative sickle cell retinopathy. *Ophthalmology* 2005;112(11):1869–1875.
17. Serjeant BE, Mason KP, Condon PI, et al. Blood rheology and proliferative retinopathy in sickle cell-haemoglobin C disease. *Br J Ophthalmol* 1984;68(5):325–328.
18. Serjeant BE, Mason KP, Acheson RW, Maude GH, Stuart J, Serjeant GR. Blood rheology and proliferative retinopathy in homozygous sickle cell disease. *Br J Ophthalmol* 1986;70(7):522–525.

19. Ballas SK, Kesen MR, Goldberg MF, et al. Beyond the definitions of the phenotypic complications of sickle cell disease: an update on management. *Scientific World Journal* 2012;2012:1–55.
20. Lopes FCM, Traina F, Almeida CB, et al. Key endothelial cell angiogenic mechanisms are stimulated by the circulating milieu in sickle cell disease and attenuated by hydroxyurea. *Haematologica* 2015;100(6):730–739.
21. Raffini LJ, Niebanck AE, Hrusovsky J, et al. Prolongation of the prothrombin time and activated partial thromboplastin time in children with sickle cell disease. *Pediatr Blood Cancer* 2006;47(5):589–593.
22. Nilesh T, Deepti J, Ns I, Nitin G. Haemostatic alterations in patients of sickle cell trait and homozygous sickle cell disease – a hospital based case control study. *Indian J Med Res* 2014;3(3):264–274.
23. Kato GJ, Hebbel RP, Steinberg MH, Gladwin MT. Vasculopathy in sickle cell disease: biology, pathophysiology, genetics, translational medicine, and new research directions. *Am J Hematol* 2009;84(9):618–625.
24. Dembélé AK, Toure BA, Sarro YS, et al. Prevalence and risk factors for sickle retinopathy in a sub-Saharan comprehensive Sickle Cell Center. *Rev Med Interne* 2017;38:572–577.
25. Estepp JH, Smeltzer MP, Wang WC, et al. Protection from sickle cell retinopathy is associated with elevated HbF levels and hydroxycarbamide use in children. *Br J Haematol* 2013;161(3):402–405.
26. Lopes FCM, Ferreira R, Albuquerque DM, et al. In vitro and in vivo anti-angiogenic effects of hydroxyurea. *Microvasc Res* 2014;94:106–113.
27. Lanaro C, Franco-Penteado CF, Albuqueque DM, et al. Altered levels of cytokines and inflammatory mediators in plasma and leukocytes of sickle cell anemia patients and effects of hydroxyurea therapy. *J Leukoc Biol* 2009;85(2):235–242.
28. Hoang QV, Chau FY, Shahidi M, Lim JI. Central macular splaying and outer retinal thinning in asymptomatic sickle cell patients by spectral-domain optical coherence tomography. *Am J Ophthalmol* 2011;151(6):990–994.
29. Leitão Guerra RL, Leitão Guerra CL, Bastos MG, de Oliveira AHP, Salles C. Sickle cell retinopathy: what we now understand using optical coherence tomography angiography. A systematic review. *Blood Rev* 2019;35:32–42.
30. Wagner-Schuman M, Dubis AM, Nordgren RN, et al. Race- and sex-related differences in retinal thickness and foveal pit morphology. *Invest Ophthalmol Vis Sci* 2011;52(1):625–634.
31. Linderman RE, Muthiah MN, Omoba SB, et al. Variability of foveal avascular zone metrics derived from optical coherence tomography angiography images. *Trans Vis Sci Tech* 2018;7(5):20.
32. Magrath GN, Say EAT, Sioufi K, Ferenczy S, Samara WA, Shields CI. Variability in foveal avascular zone and capillary density using optical coherence tomography angiography machines in healthy ayes. *Retina* 2017;37(11):2102–2111.
33. Mirshahi A, Ghassemi F, Fadakar K, et al. Effects of panretinal photocoagulation on retinal vasculature and foveal avascular zone in diabetic retinopathy using optical coherence tomography angiography: a pilot study. *J Curr Ophthalmol* 2019;31(3):287–291.
34. Lorrusso M, Milano V, Nikolopoulou E. Panretinal photocoagulation does not change macular perfusion in eyes with proliferative diabetic retinopathy. *Ophthalmic Surg Lasers Imaging Retina* 2019;50(3):174–178.
35. Acheson R, Serjeant G. Painful crises in sickle cell disease after fluorescein angiography. *Lancet* 1985;1(8439):1222.