Retinal Microvascular Abnormalities in Children with Type 1 Diabetes Mellitus Without Visual Impairment or Diabetic Retinopathy

Tao Li,1,2 Yan Jia,3 Shanshan Wang,1,2 Anken Wang,3 Lu Gao,3 Chenzhao Yang,3 and Haidong Zou1,2

1Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
2Shanghai Eye Diseases Prevention & Treatment Center, Shanghai Eye Hospital, Shanghai, China
3Department of Ophthalmology, Children’s Hospital of Fudan University, Shanghai, China

Correspondence: Haidong Zou, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, No. 100 Haining Road, Shanghai 200080, China; zouhaidong@sjtu.edu.cn. Chenzhao Yang, Department of Ophthalmology, Children’s Hospital of Fudan University, No. 399 Wanyuan Road, Shanghai 201102, China; ychben@hotmail.com.

TL and YJ contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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PURPOSE. To study the characteristics and associated factors of retinal microvascular abnormalities in children with type 1 diabetes mellitus (T1DM) without visual impairment and diabetic retinopathy (DR).

METHODS. Case-control hospital-based study including children with or without DM. Optical coherence tomography angiography (OCTA; CIRRUS HD-OCT model 5000) was used to scan 6 × 6 mm square area of posterior retina and optic disc. The indexes analyzed mainly included vascular length density (VD), perfusion density (PD), and foveal avascular zone area, perimeter, and morphology. Independent risk factors were analyzed by multifactor linear regression.

RESULTS. A total of 47 children with T1DM and 44 healthy subjects were enrolled. Statistical analysis showed that VD within 1 to 3 mm (inner ring) of the macula in the case group was smaller than that in the control group (18.561 ± 1.151/mm; 19.161 ± 0.464/mm; P < 0.001), and mother’s excessive weight gain during pregnancy was an independent factor (P = 0.004); VD within 3 to 6 mm (outer ring) of the macula in the case group was smaller than that in the control group (19.044 ± 0.847/mm; 19.404 ± 0.496/mm, P = 0.029), while serum creatinine level was revealed to be an independent factor (P = 0.009); PD within 3 to 6 mm of the macula in the case group was higher than that in the control group (0.456 ± 0.015: 0.442 ± 0.030 (P = 0.003), with no independent factor observed in regression analysis.

CONCLUSION. Retinal microvasculopathy had already occurred in the parafoveal area of diabetic children without visual impairment and DR; early screening and close follow-up were recommended for children with high-risk factors.

Keywords: T1DM, children, macula, optic disc, perfuse density, vascular density, microvasculopathy

Diabetes mellitus (DM) has become a worldwide epidemic since the beginning of the 21st century.1 The number of people with DM worldwide reached 366 million in 20112 and is estimated to exceed 439 million by 2025.3 The prevalence of DM among children has also been increasing rapidly. The number of children with DM aged younger than 15 years was approximately 500,000 in 2014, and the number is growing by 3% every year.4

Retinopathy is a common ocular complication in DM patients and is associated with a high rate of blindness. According to Yau et al.5 in 2012, approximately 93 million people worldwide were diagnosed with diabetic retinopathy (DR), and 30% of these patients showed moderate or severe visual impairment and blindness (those with a best-corrected visual acuity [BCVA] < 20/63) because of DR. At present, there are few studies on the incidence of and the factors related to DR in children with DM. Due to their long lifespans, almost all children diagnosed with DM will show evolution to DR 20 years later. The Oulu cohort study of DR showed that 94% of type 1 DM (T1DM) children with an average onset age of 7 years would show evolution to DR at 17 to 34 years of age, with 35% of the cases showing proliferative DR (PDR).6 The Wisconsin Epidemiological Study of Diabetic Retinopathy showed that 98% of the children and teenagers with T1DM would show DR 15 years after their DM diagnosis.7 Thus, the vast majority of children with DM face a high risk of severe visual impairment due to DR when they grow into adults, which can cause long-term serious damage to the individuals and their families. Therefore, it is of great social significance to obtain reference data for the prevention and treatment of diabetic microvasculopathy in children with DM.

At present, there are a number of studies on the prevalence of and factors associated with DR in adults with DM, and long duration of DM, high HbA1C levels,7 hypertension, hyperlipidemia, and gene susceptibility are believed to be the major factors associated with DR in adults.8 However, there are few studies focused on DR in DM children, and only long disease duration was confirmed to be a risk factor for DR of children with T1DM. According to the study by Burger et al.9 in 1986, among 231 children with DM, DR was rarely seen in those with...
disease duration less than 5 years. They suggested that the median disease duration corresponding to the onset of early DR in children and teenagers is 9.1 years. The guidelines of the American Academy of Ophthalmology (AAO) released in 2017 recommended that children with T1DM for more than 5 years should undergo yearly fundus examinations and that children with T1DM who are older than 9 years should undergo screening every 3 to 5 years. For children younger than 9 years or with disease duration less than 5 years, no examination for DR has been officially recommended for now. However, we have recently confirmed DR diagnosis in some children with DM duration only 2 to 4 years or as young as 6 to 7 years old in our hospital. With the increase in the number of diabetic children, information regarding the characteristics and risk factors of retinal microvasculopathy in this population has become more and more valuable and of greater social significance.

The typical pathological alteration in DR is retinal microvasculopathy. The advancements in optical coherence tomography angiography (OCTA) technology over the past 5 years now allow direct and noninvasive assessments of the changes in the retinal microvasculature. Since studies have shown the presence of a foveal avascular zone (FAZ) and/or changes in the retinal vascular density in the macular area in adult diabetic patients with no DR, and described the acceptability and utility of OCTA examinations in children with DM, we aimed to determine the characteristics in the microvasculature in diabetic children without typical DR changes, thereby providing data and reference information for the prevention and treatment of visual impairment in young diabetic patients.

**Materials and Methods**

This was a hospital-based case-control study (clinicaltrials.gov identifier: NCT 03587948) approved by the ethics committee of both Shanghai General Hospitals affiliated to Shanghai Jiao Tong University School of Medicine (approval number: 2016KY005) and Children's Hospital of Fudan University in Shanghai (approval number: No. 01 (2018)). This study conformed to the guidelines proposed in the Declaration of Helsinki. It was a part of the Shanghai Children and Adolescent DM Eye study (SCADE). Between January 2018 and February 2019, children were diagnosed with T1DM at Children's Hospital of Fudan University in Shanghai were recruited for this study. We also recruited age- and sex-matched children without DM from this hospital as controls.

**Patient Selection**

The inclusion criteria were (1) provision of written informed consent by a guardian; (2) age over 5 years and less than 18 years; (3) diagnosis of T1DM based on the World Health Organization diagnostic criteria; (4) compliance with all the examinations of this study; and (5) BCVA of both eyes equal to or greater than 20/25.

The exclusion criteria were eye diseases or other systemic diseases that can affect the retina or vision. These included (1) clearly diagnosed DR according to the international DM classification criteria; (2) eyelid diseases, strabismus, corneal diseases, lens diseases, and other eye diseases that may affect the results of OCTA examinations; (3) glaucoma, maculopathy, and other eye diseases that may cause fundus retinal vasculopathy; (5) systemic diseases such as hypertension, respiratory system diseases, circulatory system diseases, and urinary system diseases in addition to DM; and (6) a history of eye surgery.

The research team consisted of three ophthalmologists at the Shanghai General Hospital and Children's Hospital of Fudan University in Shanghai; three optometrists; and 15 auxiliary staff. The project was headed by an ophthalmologist with extensive experience in the diagnosis and epidemiologic study of eye diseases. In this survey, the project leader trained the staff of the survey team prior to the formal survey, carried out the preliminary examinations for the study in the hospital after the end of the training, completed the consistency checks of the OCTA test results, and checked the measurement tools. The project leader reviewed all survey data after each survey.

**Ophthalmic Examination**

The examination was conducted in the ophthalmic clinic at Children's Hospital of Fudan University in Shanghai. First, baseline characteristics were surveyed using a questionnaire. The basic information included data regarding age, sex, history of DM, history of medications, family history of DM (which was defined by the presence of more than one immediate family member with DM or high blood pressure within three generations), parental ocular disease history; birth and feeding history; height of the children and their parents, current weight of the children and their parents, mother’s weight before pregnancy, mother’s maximum weight during pregnancy, and mother’s postpartum weight (weight of the mother within 6 months after childbirth). The survey questionnaires were filled by the guardians and the participants. The results of routine blood and urine tests performed in children within 6 months at the Children's Hospital of Fudan University in Shanghai were collected.

Subsequently, eye examinations covering the following aspects were performed: (1) Examinations of the eyelid, conjunctiva, cornea, anterior chamber, iris, pupil, and lens with ophthalmic slit lamp bio-microscopy (SL130; Zeiss, Germany), and examination of the fundus retina with 90D noncontact lens (90D, Ocular, Bellevue, WA, USA). (2) Macula and optic disc photography using digital nonmydriatic fundus photography (AFC-210; NIDEK, Tokyo, Japan) with the macula and optic disc as the centers. (3) Intraocular pressure measurement (NT-530P; NIDEK). (4) Measurement of the axial length (AL), anterior chamber depth, corneal thickness, corneal diameter, and lens thickness with IOL master 700 (Carl Zeiss Meditec, Jena, Germany), and examination of the fundus retina with 90D noncontact lens (90D, Ocular, Bellevue, WA, USA). (5) Assessment of the BCVA and the refractive power after administration of cyclopentolate drops (1%; Alcon, Fort Worth, TX, USA) three times at 5-minute intervals. Optometry examinations were performed 20 minutes or more after pupils were stably dilated; refractive index was assessed using computer automatic optics (KR-8900; Topcon, Tokyo, Japan) while BCVA was assessed using the international standard LogMAR visual acuity chart. (6) Examination of the posterior polar fundus using Swept-Source-OCTA (Triton; Topcon). (7) OCTA (CIRRUS HD-OCT model 5000; Carl Zeiss Meditec) to scan a 6 × 6 mm square area with an Angelus wavelength of 840 nm and a speed of 68,000 per second. The right eye was initially examined, followed by the left eye.

The OCTA scans were performed by three trained ophthalmologists with extensive imaging experience. The position of each child's head was carefully adjusted by the examiner before the test, and the parents were asked to keep their child calm during scanning. The tracking algorithm available on the CIRRUS device was used to minimize eye motion-related artifacts.
Study Index and Quality Control

The OCTA detection indicators analyzed in this study were as follows:

1. Vascular length density (VD): VD was defined as the total length of perfused vasculature per unit area in a region of measurement.
2. Perfusion density (PD): PD was defined as the total area of perfused vasculature per unit area in a region of measurement.
3. FAZ area (FAZA): The FAZA was the area of the foveal avascular zone, measured in \( \mu \text{m}^2 \).
4. FAZ perimeter (FAZP): The FAZP was the perimeter of the FAZ, measured in \( \mu \text{m} \). The larger the FAZP value, the more irregular or larger the area of the FAZ.
5. FAZ morphology index (FAZM) = \( 4\pi \times \text{FAZA}/\text{FAZP} \). It refers to the morphological changes in the nonvascular zone of the central fovea of the macula. The value ranged from 1 to 0: the closer the value was to 1, the more similar the shape was to a circle, while the closer the value was to 0, the more irregular the shape was.

These five detection indexes were generated automatically by the built-in OCTA analysis software (metrix 10.0; clearance by the US Food and Drug Administration pending) using principles described in the literature.16,17

At present, the software only reports values for the superficial vascular network, which is defined by the instrument to span from the internal limiting membrane to an estimated boundary of the inner plexiform layer. This inner plexiform layer boundary is calculated as 70% of the distance from the internal limiting membrane to an estimated boundary of the outer plexiform layer, which is determined as being 110 \( \mu \text{m} \) above the retinal pigment epithelium boundary automatically detected by the software.

In the present study, we evaluated the center, the inner ring (1–3 mm) on both scan patterns, and the outer ring (3–6 mm) on the 6 × 6 mm scan pattern (Fig.). If the children cooperated properly, then scanning images obtained over three consecutive attempts were analyzed and the mean value was obtained; if the children cooperated poorly with the examination, the clearest scanning images among the scanning results were analyzed. We used the index “signal strength” (SS) in the built-in software to assess the image quality. The images with signal quality (SQ) value < 0.8 were discarded. The average SS was 9.9 of all the images.

We manually checked the automated B-scan segmentation in pilot tests before proceeding with microvascular OCTA analysis and found that there were no obvious segmentation errors. In pilot tests, the intraclass correlation coefficient was calculated to be 0.958 for FAZA with the same examinee. The three ophthalmologists were not informed about the diagnosis of each child (DM or healthy) prior to evaluating the OCTA scans.

Statistical Analysis

Body mass index (BMI) was calculated as body weight (kg)/height (m\(^2\)). Based on the high correlation between both eyes of normal individuals,\(^10\) data for the left eyes were taken for analysis. The equivalent spherical power (ESP; in diopeters) was calculated as spherical power + 1/2 cylindrical power. Statistical analyses were performed using SPSS 22.0 software (IBM Corporation, Armonk, NY, USA). The measurement data were represented by mean plus or minus standard deviation, and the counting data were represented by frequency and percentage. Kolmogorov-Smirnov testing was used to determine whether the measurement data showed a normal distribution; if yes, then Student’s \( t \)-test was used to compare the differences in various values between the diabetic group and normal groups; otherwise, the Mann-Whitney \( U \) test was used. The statistical methods included the \( \chi^2 \) test, independent samples test, Pearson correlation analysis, etc. For correlation analysis between the OCTA detection index values and individual factors in the diabetic group, single-factor analysis was initially performed to identify the factors related to the density and FAZ changes in diabetic group, followed by multiple-factor linear regression analysis to analyze the independent risk factors—the significantly related factors in the single-factor analysis—with the density and FAZ index as the dependent variables. \( P < 0.05 \) represented statistical significance.

Results

A total of 47 children and adolescents with DM were admitted in the case group of this study. All of them were diagnosed with T1DM and were treated with insulin. Another 44 healthy participants were admitted to the control group of this study. The participant ages in the case group ranged from 6 to 17 years, while those in the control group ranged from 5 to 16 years. Basic information for the subjects in the two groups is shown in Table 1. Except for the difference in the maximum pregnancy weight and pre-pregnancy weight of the mothers, the two groups showed no statistically significant intergroup differences in age, sex, current BMI, BMI at birth, duration (weeks) of pregnancy at birth, current BMI of the father, current BMI of the mother, feeding mode, production mode, premature delivery, postpartum weight minus pre-pregnancy weight of the mother, number of children with a family history of DM (%), and number of children with a family history of hypertension (%).

The ages of the 47 children in the diabetic group ranged from 6 to 17 years, with a mean age of 11.1 ± 2.9 years. Among these, 18 children (38.298%) were diagnosed with T1DM before 5 years of age; 20 children (42.553%), at 5 to 10 years; and nine children (19.14%), at more than 10 years of age. The duration of DM ranged from 1 to 14 years (average, 4.3 ± 2.8 years). The age at onset ranged from 0 to 12 years (average, 6.8 ± 3.4 years). The blood HbA1C values ranged from 4.60 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum glucose level ranged from 0.45 to 7.09 mmol/L (average, 1.062 ± 0.232 mmol/L). The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L). The serum creatinine level ranged from 2.88 to 14.10 mmol/L (average, 8.115 ± 2.491 mmol/L), and 18 children (38.298%) had levels higher than 8 mmol/L. The total blood cholesterol level ranged from 2.88 to 8.28 mmol/L (average, 4.742 ± 1.085 mmol/L). The urine microalbumin level ranged from less than 2 to 263 mmol/L (average, 8.730 ± 1.085 mmol/L).
FIGURE 6×6 mm OCTA images of typical eyes of a diabetic child and a healthy child. OCTA (CIRRUS HD-OCT model 5000; Carl Zeiss Meditec) using AngioPlex was performed to scan a 6×6 mm square area with the fovea of the macula and the optic disc as the scanning centers. The image on the upper left (A) is a photo of a diabetic child aged 9 years with a DM duration of 5 years (original image size). The image on the upper right (A) is a photo of a normal child aged 10 years (original image size). The image on the middle left is a 6-fold magnified image of the central fovea of the macula (marked by Point C) in the upper left (B) image, VD = 4.2/mm, FAZA = 0.40 µm², FAZP = 2.89 µm, FAZM = 0.60. The image on the middle right is a 6-fold magnified image of the central fovea of the macula (marked by Point D) in the upper right image, VD = 9.8/mm, FAZA = 0.22 µm².
FAZP = 1.82 μm, FAZM = 0.84. The FAZA in the middle left image has increased obviously and the arch ring structure has begun to recede. The image on the bottom left is a 12-fold magnified image of the area of the temporal retina within 1 to 3 mm of the macular area (marked by Point E) in the upper left image. VD = 14.7/mm. The image on the bottom right is a 12-fold magnified image of the area of the temporal retina within 1 to 3 mm of the macular area (marked by Point F) in the upper right image. VD = 18.4/mm. The vascular density in the bottom left image shows an obviously decrease.

count group. The FAZA and FAZP in the diabetic group were all larger but not significantly than those in the control group. The FAZ morphological indexes showed similar values in both groups. In the single-factor statistical analysis, differences in the VD of the macular inner and outer rings and the PD in the diabetic and control groups were found to be statistically significant.

We further performed stepwise multivariate linear regression analysis. Using the data from 47 cases of diabetic children, we performed regression analysis with the VD of the inner and outer rings of the macular area and the PD of the inner ring of the macular area as the dependent variables, and age, sex, current BMI, BMI at birth, birth weight, weeks of pregnancy at birth, BMI of the father, feeding method, type of labor (vaginal delivery or cesarean section), premature delivery, maximum pregnancy weight minus pre-pregnancy weight of the mother, postpartum weight minus pre-pregnancy weight of the mother, current BMI of the mother, number of people with a family history of DM (%), number of people with a family history of hypertension (%), number of children whose mothers had hyperglycemia during pregnancy, number of children whose mothers had high blood pressure during pregnancy, IOP, AL, ESP, and HbA1C, serum creatinine, and serum creatinine levels as the independent variables. The analysis indicated that there were no independent factors influencing the average blood flow density in the region within 1 to 3 mm of the macular area; the maximum weight during pregnancy minus the pregancy weight of the mother was an independent factor influencing the average vascular density in the region within 1 to 3 mm of the macular area; and serum creatinine, serum triglyceride, serum cholesterol, and urine microalbumin levels were independent factors. The VD increased with mother's maximum pregnancy weight minus weight before pregnancy; however, higher serum creatinine values were associated with lower average VD. The results of regression analysis are shown in Table 3.

**DISCUSSION**

A PubMed search indicated that there is no worldwide study that has investigated retinal microvasculopathy among children and adolescents with childhood-onset T1DM without typical DR and visual impairment. This study showed that the VD of the macular inner and outer rings and the PD of the macular inner ring of children with DM were significantly different from those in healthy children, indicating that VD and PD may be the candidates to predict the onset of DR in future cohort studies. In addition, independent factors related to the VD/PD of the macula were also found in this study, which could be considered as candidates in studies on prevention of DR.

The “gold standard” method for the diagnosis of DR is fluorescein angiography (FA). However, since it is an invasive procedure, FA is not an appropriate choice for diabetic children without visual impairment or healthy children. Studies in adult populations have proved that OCTA and FA have good correlation and repetition. As a noninvasive and repeatable investigation technique, OCTA naturally suited this population. The built-in analysis software of CIRRUS HD-OCT used in this study could quantify the length and the width of

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**Table 1. Clinical Information of the 47 Children in the Diabetic Group and the 44 Children in the Control Group Included in This Study**

<table>
<thead>
<tr>
<th></th>
<th>Diabetic Group (n = 47)</th>
<th>Control Group (n = 44)</th>
<th>Statistics*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, %</td>
<td>19 (40.425)</td>
<td>20 (45.454)</td>
<td>$\chi^2 = 0.235$</td>
<td>0.675</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>11.1 ± 3.9</td>
<td>10.2 ± 2.2</td>
<td>t = 1.616</td>
<td>0.110</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>18.103 ± 5.478</td>
<td>17.669 ± 3.111</td>
<td>t = 0.626</td>
<td>0.533</td>
</tr>
<tr>
<td>Birth BMI, mean ± SD, kg/m²</td>
<td>13.103 ± 1.771</td>
<td>12.817 ± 1.635</td>
<td>Z = −0.950</td>
<td>0.342</td>
</tr>
<tr>
<td>Birth weight, mean ± SD, kg</td>
<td>340.500 ± 517.252</td>
<td>330.818 ± 410.976</td>
<td>Z = −0.457</td>
<td>0.648</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>38.2 ± 2.3</td>
<td>38.6 ± 1.9</td>
<td>Z = −0.091</td>
<td>0.928</td>
</tr>
<tr>
<td>Father’s current BMI, mean ± SD, kg/m²</td>
<td>24.825 ± 3.777</td>
<td>24.487 ± 2.775</td>
<td>t = 0.520</td>
<td>0.604</td>
</tr>
<tr>
<td>Mother’s current BMI, mean ± SD, kg/m²</td>
<td>22.303 ± 2.868</td>
<td>21.409 ± 2.225</td>
<td>t = 1.653</td>
<td>0.102</td>
</tr>
<tr>
<td>Type of feeding</td>
<td></td>
<td></td>
<td>$\chi^2 = 3.215$</td>
<td>0.200</td>
</tr>
<tr>
<td>Artificial milk, %</td>
<td>9 (19.149)</td>
<td>3 (6.818)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed feeds</td>
<td>13 (27.660)</td>
<td>16 (36.364)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
<td>$\chi^2 = 0.008$</td>
<td>1.000</td>
</tr>
<tr>
<td>Spontaneous vaginal delivery, %</td>
<td>25 (53.191)</td>
<td>23 (52.723)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean delivery, %</td>
<td>22 (46.809)</td>
<td>21 (47.727)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premature delivery, %</td>
<td>6 (12.766)</td>
<td>2 (4.454)</td>
<td>$\chi^2 = 3.068$</td>
<td>0.216</td>
</tr>
<tr>
<td>Mother’s maximum weight during pregnancy minus weight before pregnancy, mean ± SD</td>
<td>14.543 ± 5.800</td>
<td>12.000 ± 3.971</td>
<td>t = 2.343</td>
<td>0.018</td>
</tr>
<tr>
<td>Mother’s postpartum weight minus</td>
<td>5.686 ± 5.451</td>
<td>4.53 ± 3.193</td>
<td>Z = −0.214</td>
<td>0.831</td>
</tr>
<tr>
<td>weight before pregnancy, mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of DM, %</td>
<td>17 (36.170)</td>
<td>14 (31.818)</td>
<td>$\chi^2 = 0.192$</td>
<td>0.825</td>
</tr>
<tr>
<td>Family history of hypertension, %</td>
<td>24 (51.064)</td>
<td>24 (54.545)</td>
<td>$\chi^2 = 0.286$</td>
<td>0.676</td>
</tr>
<tr>
<td>Maternal hyperglycemia, %</td>
<td>1 (2.127)</td>
<td>1 (2.273)</td>
<td>$\chi^2 = 0.002$</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* P refers to the nonparametric independent sample test; $\chi^2$ refers to the chi-square test; Z refers to Mann-Whitney U nonparametric independent sample test.
vasculature, and the quantification results could enable analysis of small changes in the retinal vasculature or early-stage perfused vessels in children with diabetes. Although some studies showed that the 3×3 mm scanning mode could provide higher resolution for detecting the linear density of the vasculature in comparison with the 6×6 mm scanning mode,21 this study still adopted the 6×6 mm scanning mode. The reasons were as follows: (1) the 6×6 mm scans covered a larger examination area and were more sensitive to early DR17; (2) the results of this study were obtained by comparing the differences between the case and control groups, and the same scanning mode was adopted for both groups; therefore, the results were not affected by the resolution.

DR is a disease mainly characterized by recombination, degeneration, and destruction of the capillaries.22,23 Our study found that the VD of the inner and outer rings of the macula in children and adolescents with DM showed a statistically significant decrease. We considered that this change may be caused by cellular degeneration in the retina and the thickening of the basal membrane and endothelial cell proliferation due to hyperglycemia, which eventually destroyed the capillary integrity, resulting in destruction of the capillary structure. In the eyes of adults with early DM, the OCTA image also showed a significant decrease in the VD of the macula.24–26

A study involving 94 T1DM patients in Poland with an average age of 15.3±2.1 years and duration of 6.4±3.3 years found there were no statistically significant differences between adolescents with T1DM and healthy participants in vessel densities.27 This was different from our study. The OCTA used in our study was the CIRRUS HD-OCT Model 5000 (Carl Zeiss Meditec), while the OCTA used in the Polish study was the RTVue XR Avanti with AngioVue (Optovue, Inc., Fremont, CA, USA). None of the results were comparable between the two studies because of different scanning areas and calculation methods. We believe these differences led to the different trend observed in the two studies.

Some studies have shown that both the VD and PD of the macula decreased in adults with DR.12,20,28,29 However, this study found a slight but significant increase in PD instead of a decrease within the inner ring of the macula in diabetic children. We believe that the relatively short disease duration (the average disease duration of DM in this study was only 4.3±4.0 years) and the preclinical-phase DR are two of the major factors responsible for these results. In preclinical DR, hyperglycemia and oxygen deficit led to the expansion of retinal capillaries, resulting in relatively high perfusion; the high perfusion, in turn, interfered with the retinal self-adjustment mechanism and resulted in further expansion of retinal capillaries.30 Another possibility is that the hyperglycemia and adjustment mechanisms could cause the VD of the macula to increase and the PD to decrease; however, in the adult group, the OCTA images showed a decrease in both VD and PD.25

Table 2. The Eye Examination Results for 47 Children in the Diabetic Group and 44 Children in the Control Group Included in This Study (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Diabetic Group (n = 47)</th>
<th>Control Group (n = 44)</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP, mm Hg</td>
<td>17.870 ± 3.696</td>
<td>18.145 ± 2.873</td>
<td>0.461</td>
</tr>
<tr>
<td>AL, mm</td>
<td>23.942 ± 1.268</td>
<td>24.324 ± 1.444</td>
<td>*0.187</td>
</tr>
<tr>
<td>ESR, diopter</td>
<td>−1.135 ± 2.16</td>
<td>−1.554 ± 1.75</td>
<td>*0.336</td>
</tr>
<tr>
<td>VD of the macular center, /mm</td>
<td>11.242 ± 3.300</td>
<td>11.804 ± 2.540</td>
<td>*0.310</td>
</tr>
<tr>
<td>VD of the macular inner ring, /mm</td>
<td>18.561 ± 1.151</td>
<td>19.184 ± 0.458</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VD of the macular outer ring, /mm</td>
<td>19.044 ± 0.847</td>
<td>19.404 ± 0.496</td>
<td>0.029</td>
</tr>
<tr>
<td>PD of the macular center</td>
<td>0.258 ± 0.082</td>
<td>0.269 ± 0.064</td>
<td>*0.523</td>
</tr>
<tr>
<td>PD of the macular inner ring</td>
<td>0.456 ± 0.015</td>
<td>0.445 ± 0.029</td>
<td>0.003</td>
</tr>
<tr>
<td>PD of the macular outer ring</td>
<td>0.479 ± 0.010</td>
<td>0.471 ± 0.022</td>
<td>0.132</td>
</tr>
<tr>
<td>VD of the optic disc center, /mm</td>
<td>5.826 ± 4.802</td>
<td>6.742 ± 4.869</td>
<td>0.323</td>
</tr>
<tr>
<td>VD of the optic disc inner ring, /mm</td>
<td>18.072 ± 1.826</td>
<td>18.429 ± 1.355</td>
<td>0.559</td>
</tr>
<tr>
<td>VD of the optic disc outer ring, /mm</td>
<td>18.744 ± 2.578</td>
<td>19.202 ± 1.133</td>
<td>0.364</td>
</tr>
<tr>
<td>PD of the optic disc center</td>
<td>0.152 ± 0.131</td>
<td>0.177 ± 0.137</td>
<td>0.395</td>
</tr>
<tr>
<td>PD of the optic disc inner ring</td>
<td>0.464 ± 0.050</td>
<td>0.475 ± 0.055</td>
<td>0.327</td>
</tr>
<tr>
<td>PD of the optic disc outer ring</td>
<td>0.464 ± 0.068</td>
<td>0.479 ± 0.051</td>
<td>0.298</td>
</tr>
<tr>
<td>FAZA, μm²</td>
<td>0.245 ± 0.009</td>
<td>0.236 ± 0.012</td>
<td>*0.647</td>
</tr>
<tr>
<td>FAZP, μm²</td>
<td>1.953 ± 0.441</td>
<td>1.896 ± 0.419</td>
<td>*0.511</td>
</tr>
<tr>
<td>FAZM</td>
<td>0.787 ± 0.065</td>
<td>0.787 ± 0.059</td>
<td>0.755</td>
</tr>
</tbody>
</table>

*P refers to the P value in the independent sample t-test. The other values were from the Mann-Whitney U nonparametric independent sample test.

Table 3. Results of Multivariate Linear Regression Analysis of 47 Children in the Diabetic Group Included in This Study

<table>
<thead>
<tr>
<th>Model</th>
<th>Independent Factor</th>
<th>Unstandardized Coefficients</th>
<th>SC</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>SD</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>VD of the macular inner ring, /mm</td>
<td>Constant</td>
<td>17.268</td>
<td>0.443</td>
<td>38.994</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Mother’s max pregnancy weight minus weight before pregnancy</td>
<td>0.088</td>
<td>0.029</td>
<td>0.431</td>
<td>3.056</td>
</tr>
<tr>
<td>VD of the macular outer ring, /mm</td>
<td>Constant</td>
<td>20.485</td>
<td>0.533</td>
<td>38.436</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine</td>
<td>−0.036</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SC, standardized coefficient.
mia and oxygen deficit caused destruction and reduction of local capillaries, leading to compensatory dilatation of the microvasculature.\textsuperscript{37} As the disease progressed, capillary occlusion increased and the VD dropped continuously and obviously; however, the PD showed dilatation over a certain period in early diabetic eyes but no eventual decrease.\textsuperscript{12,26,28,29}

Previous studies have shown that in adults with DM, the more serious the DR, the larger the FAZA, and that the FAZ significantly enlarged even before the appearance of typical DR.\textsuperscript{31–35} In this study, we noted enlargement of the FAZA and elongation of the FAZP without statistical significance in children with T1DM with no vision impairment and typical DR, which is consistent with the findings of previous studies in adults and be attributable to the young age and relatively short duration. Therefore, we believed that significant changes in the VD and PD within 1, 1 to 3, and 3 to 6 mm of the macular area as well as the FAZA or FAZP were worthy of attention; thus, these parameters were all candidate predictors of DR in children with DM and should be closely followed up in our future cohort studies.

Previous OCTA studies regarding the alterations in the VD and PD of the peripapillary regions mainly focused on the new vessels around the optic disc in cases of PDR.\textsuperscript{34} This study showed that in the eyes of children with early DM, both blood flow density and vascular density within 1, 1 to 3, and 3 to 6 mm of the peripapillary regions decreased, which, however, was not statistically significant. We believe that in comparison with the macula, which is densely populated by photoreceptor cells and consumes large quantities of oxygen that are mainly provided by the choroid, the oxygen consumption of the peripapillary region was relatively small. Therefore, in the eyes of children with early DM, the changes in the peripapillary region were also relatively less.

Interestingly, in comparison with the control group, our study showed a significant decrease in the VD of the macular outer ring in the case group, and only a slight decrease in the peripapillary region in the case group. In theory, these two detection areas partially overlapped at the nasal region of the macula/temporal section of the peripapillary region (Fig.). Such differences may be related to the differences in the distribution of nutrition-providing vessels and high-consumption retinal cells between the optic disc and the macula. The findings also indicate the need to pay more attention to the temporal side of the macular outer ring in early-stage DM patients.

DM duration is the only acknowledged predictor of DR in children in AAO’s guidelines. However, we did not find any link between the VD/PD/FAZ index and duration, which may suggest that in early diabetic eyes, hyperglycemia and hypoxia instead of duration are the major pathogenic factors underlying microvasculopathy. The regression analysis model used in this study showed that the maximum pregnancy weight minus the pre-pregnancy weight of the mother and the serum creatinine value were independent risk factors for the VD of the macular inner and outer rings. Mother’s maximum weight during pregnancy minus weight before pregnancy can affect the outcome of pregnancy in a very complex way, and had long-term effects on the metabolic status of the neonates, mainly by affecting visceral fat accumulation, lipid metabolism, and glucose tolerance in the mother and the fetus.\textsuperscript{35–37} Our study found for the first time that this weight change may lead to an increase in the VD of the macular inner ring in children with T1DM. We doubt that excessive amniotic fluid influences retinal vascular development in children. In the former study, human amniotic fluid exhibits strong stimulatory effect on human corneal endothelial cell growth,\textsuperscript{38} but until now, no report of amniotic fluid effect on the retinal vasculature has been published. Future studies may help to explain the effect from mother’s weight gain on macular microvascular density in the T1DM children. Serum creatinine was a marker of glomerular filtration rate, and could also be used to monitor the progression of diabetic nephropathy.\textsuperscript{39} In adults with DM, the retinal damage was proportional to the extent of kidney damage, and kidney damage usually occurred before retinal damage.\textsuperscript{40} In children with DM, the increase of serum creatinine levels also indicated early systemic microvascular damage.\textsuperscript{41,42} Therefore, we believe that for children diagnosed with T1DM at less than 7 years of age and low gestational weight gain and children with significantly increased serum creatinine levels, glycaemia control and follow-up eye examinations should be performed more frequently in the early stage, and that the maximum pregnancy weight minus pre-pregnancy weight of the mother and the serum creatinine value should receive attention as the main candidate factors related with DR onset and independent of the disease course in future cohort studies.

This study had several limitations. First, the study was a single-center study based on the Chinese Han population, and contained only a small number of subjects. Second, previous studies have shown that VD changes in the deep capillaries occurred earlier than those in the superficial capillaries of adults with DM,\textsuperscript{13,34} which is beyond the scope of the built-in software. Thus, the data for deep capillaries of the retina were not included.

In summary, we employed OCTA to quantify microvasculopathy in the eyes of children with T1DM and found that the VD of the macular inner and outer rings and the PD of the inner ring were significantly different from those in healthy children. The maximum pregnancy weight minus the pre-pregnancy weight of the mother and the serum creatinine levels were independent risk factors. Our research group will study the prevention of DR in children with DM in a subsequent large-scale multicenter follow-up cohort study.

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**References**


