Foveal avascular zone area analysis in juvenile-onset type 1 diabetes using optical coherence tomography angiography

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CLINICAL INVESTIGATION

Foveal avascular zone area analysis in juvenile-onset type 1 diabetes using optical coherence tomography angiography

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Abstract

Purpose Optical coherence tomography angiography (OCTA) was performed on patients with juvenile-onset type 1 diabetes (T1DM) but with no diabetic retinopathy to measure the foveal avascular zone (FAZ) area.

Study Design Retrospective single-facility study

Methods Twenty-nine patients (58 eyes) with juvenile-onset T1DM were studied. Images (3 mm x 3 mm cube centered on the fovea) were acquired using an OCTA device. Age at examination was 16.1 ± 8.7 years; onset age was 6.4 ± 3.5 years; duration of diabetes was 9.7 ± 8.3 years. Twenty-four age-matched healthy individuals were studied as controls.

Results FAZ area was significantly larger in T1DM patients than in controls (0.29 ± 0.09 vs. 0.25 ± 0.08 mm^2, $P = 0.0234$). Parafoveal vessel density was not significantly different between patients and controls (50.43 ± 4.24 vs. 50.07 ± 4.64, P = 0.8842). By generalized linear model analysis, annual HbA1c ($P = 0.0190$), number of serious hypoglycemic attacks ($P = 0.0210$), and onset age ($P = 0.0447$) were identified as variables significantly associated with FAZ area. Age, gender, duration of disease, total cholesterol, high or low-density lipoprotein, triglycerides, and body mass index were not significantly associated with FAZ area.

Conclusion Patients with juvenile-onset T1DM and no diabetic retinopathy had increased FAZ, but no significant difference in parafoveal vessel density compared to healthy controls. Larger FAZ area was associated with higher annual HbA1c, more episodes of severe hypoglycemic attacks, and older onset age.

Keywords foveal avascular zone · hypoglycemic attack · optical coherence tomography angiography · parafoveal vessel density · juvenile-onset type 1 diabetes

Introduction

A previous study of juvenile-onset type 1 diabetes mellitus (T1DM: patients aged under 16 years at diagnosis) reports that diabetic retinopathy occurred in 85% of the subjects and proliferative diabetic retinopathy in 1.5% after 16 years of observation, and the prevalence of the two increased to 85% and 18%, respectively, after 26 years of follow-up [1].

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Several clinical factors are related to the development of retinopathy after diagnosis of juvenile-onset T1DM. Male sex, age 5-14 years at onset, diabetes duration and HbA1c level are independently associated with non-proliferative diabetic retinopathy [2], while older onset age, higher HbA1c and higher triglyceride concentration are related to retinopathy development 10 years after diagnosis [3]. And, retinopathy at baseline, higher HbA1c and higher triglycerides are Significant predictors for developing proliferative diabetic retinopathy [4]. In juvenile-onset T1DM, it is important to detect the diabetes before onset of diabetic retinopathy, because non-proliferative retinopathy is a risk factor for incident proliferative retinopathy [2, 4, 5].

Optical coherence tomography angiography (OCTA) is a minimally invasive modality that can be performed in a short time without dye injection. This imaging modality is a potentially useful screening and follow-up tool, especially for childhood diabetes. Previous studies using OCTA

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in adult patients with diabetic retinopathy show that mean FAZ size was significantly larger and parafoveal vessel density was significantly lower compared to controls [6–8], but studies in adult patients with T1DM but no or mild diabetic retinopathy found no significant difference in FAZ area compared to controls [9, 10]. Recent studies evaluated FAZ and vessel density using OCTA in children with juvenile-onset T1DM but found no evidence of diabetic retinopathy. One study reports normal superficial and deep plexus vascular densities and FAZ area [11] in these children, while another found no significant difference in FAZ area but lower vascular length density [12], and yet another found significant differences in FAZ size and parafoveal vessel density [13] compared to healthy controls. New parameters such as FAZ and parafoveal vessel density could be sensitive imaging biomarkers that define early diabetic retinopathy [11–13]. Automated quantitative OCTA is useful for screening and disease monitoring of early-onset T1DM without retinopathy [12, 14]. Identifying the factors that affect FAZ is expected to help manage early-onset T1DM in pediatrics. In the present study, we used an upgraded model OCT device to measure FAZ area and parafoveal vessel density in cases of juvenile-onset T1DM without diabetic retinopathy and analyzed the factors affecting FAZ area.

Patients and Methods

Design

The retrospective study was conducted by reviewing the medical records at Nihon University Hospital between June 2016 and November 2018. This study was approved by the institutional review board/ethics committee of Nihon University Hospital. Inclusion criteria were T1DM patients with onset at 15 years of age or younger, not diagnosed with diabetic retinopathy that had undergone OCTA examination. The absence of diabetic retinopathy was defined as the absence of conventional retinopathy findings such as retinal hemorrhage and capillary aneurysm on fundus examination. Exclusion criteria were patients who had difficulties undergoing OCTA examination because of young age or inability to remain still during examination, patients with diabetic retinopathy or a history of intraocular surgery, patients with adult onset T1DM or type 2 diabetes mellitus, and patients with diabetic retinopathy or a history of intraocular surgery.

Ophthalmic examinations and OCTA

The following ophthalmic examinations were performed: slit-lamp biomicroscopy, indirect ophthalmoscopy, visual acuity, and intraocular pressure measurements. OCTA was performed using the Avanti RTVue XR AngioVue (Optovue), and 3 mm × 3 mm images of the retina centered on the fovea were obtained. The FAZ area was measured by the AngioVue software. With the upgraded model used in this study, it is no longer possible to measure the superficial and deep retinal layers separately. At the end of examination, the device generates a vascular image of the retinal slab from the internal limiting membrane to the outer plexiform layer plus 10 µm. This then was used to determine the area of FAZ. For parafoveal vessel density, the device automatically measured the density (%) of blood vessels in a 300 µm-wide ring surrounding the FAZ.

Other clinical parameters

Age at the time of examination, age at T1DM onset, duration of diabetes, annual HbA1c levels, and severe hypoglycemic events were extracted from the medical records. Severe hypoglycemia was classified according to the definitions of the International Society for Pediatric and Adolescent Diabetes (ISPAD) [15]. Clinically important or serious hypoglycemia was defined as glucose level ≤ 3.0 mmol/L (54 mg/dL), and severe hypoglycemia as an event associated with severe cognitive impairment (including coma and convulsions) requiring external assistance of another person to active administration of carbohydrates, glucagon, or any other necessary corrective actions.

HbA1c was quantified by high-performance liquid chromatography, and expressed as National Glycohemoglobin Standardization Program (NGSP) units (%) (reference value; 4.6 – 6.1%) [16].

Total cholesterol levels, HDL levels, low-density lipoprotein (LDL) levels, triglycerides levels, and body mass indices were measured when OCTA was analyzed.

Statistics

Statistical analyses were performed using SPSS software version 21 (SPSS, Inc.). Data are expressed as mean ± standard deviation (SD) or percentage. Mann-Whitney was used to compare the two groups. Univariate analysis and generalized linear model analysis were used to analyze the factors for FAZ. *P* values less than 0.05 were considered to be statistically significant.

Results

Juvenile-onset T1MD

Twenty-nine patients with juvenile-onset T1MD who satisfied the selection criteria were identified. The patients were 15 girls and 14 boys with mean age at the time of examination of 16.1 ± 8.7 (range, 5 – 44) years, mean onset age of

6.4 ± 3.5 (range, 1 – 13) years, and mean disease duration of 9.7 ± 8.3 (range 1 – 37) years. Mean FAZ area OD was 0.29 ± 0.10 (range 0.07 – 0.51) mm^2, mean parafoveal vessel density was 50.54 ± 4.15 (range 42.11 – 57.69), and mean equivalent sphere was -1.6 ± 2.2 (range, -5.8 – 1.1) diopters (D). Mean FAZ area OS was 0.28 ± 0.09 (range 0.04 – 0.42) mm^2, mean parafoveal vessel density was 50.33 ± 4.39 (range 38.12 – 57.77), and mean equivalent sphere was -1.5 ± 2.4 (range, -7.0 – 1.1) diopters (D). There were no significant differences in FAZ area (P = 0.6939), parafoveal vessel density (P = 0.8033), and equivalent sphere (P = 0.7473) between right and left eyes.

Eleven of 29 patients were found to have severe hypoglycemic events with consciousness disturbance or convulsions, and blood glucose levels of 30-50 mg/dL. The mean number of severe hypoglycemic attacks during follow-up was 0.5 ± 0.6 (0 - 2). The mean annual HbA1c level was 8.1 ± 0.9 (range, 6.7 – 10.5)%, mean total cholesterol level was 176.1 ± 25.4 (range,133.0 – 230.0) mg/dL, mean HDL level was 59.9 ± 14.9 (range, 26.0 – 94.0) mg/dL, mean LDL level was 96.5 ± 26.3 (range, 44.0 – 158.0) mg/dL, mean triglyceride level was 125.6 ± 15.9 (range, 35 – 525.0) mg/dl, and mean body mass index was 20.4 ± 3.3 (range, 15.2 – 30.0) kg/m^2.

Healthy control

Healthy control subjects comprised 8 girls and 16 boys with mean age at the time of examination of 13.8 ± 7.0 (range, 5 – 29) years. Mean FAZ area OD was 0.25 ± 0.08 (range 0.07 – 0.40) mm^2, parafoveal vessel density was 49.46 ± 4.20 (range 37.22 – 55.33), and mean equivalent sphere was -1.6 ± 3.1 (range, -7.8 – 4.3) diopters (D). Mean FAZ area OS was 0.25 ± 0.07 (range 0.05 – 0.38) mm^2, parafoveal vessel density was 50.64 ± 5.05 (range 28.95 – 56.00), and mean equivalent sphere was -1.6 ± 2.8 (range, -7.5 – 4.3) diopters (D). There were no significant differences in FAZ area (P = 0.8609), parafoveal vessel density (P = 0.3645), and equivalent sphere (P = 0.1911) between right and left eyes.

Juvenile-onset T1MD vs. Healthy control

There was no significant difference in age between juvenile-onset T1DM patients and controls ($P = 0.1620$), neither was the mean equivalent sphere ($P = 0.5747$). The FAZ area in the eyes of pediatric T1DM patients was significantly larger than in control eyes (0.29 ± 0.09 vs. 0.25 ± 0.08 mm^2, $P = 0.0234$) (Table 1). The parafoveal vessel area was not significantly different (50.43 ± 4.24 vs. 50.07 ± 4.64, P = 0.8842).

In univariate analysis, larger FAZ area was associated with higher annual HbA1c ($P = 0.0266$), more episodes of severe hypoglycemic attacks ($P = 0.0133$), and older onset age ($P = 0.0474$) (Table 2). Age at the time of examination ($P = 0.3648$), gender ($P = 0.5878$), duration of disease ($P = 0.9174$), total cholesterol levels ($P = 0.4935$), HDL levels ($P = 0.2414$), LDL levels ($P = 0.9176$), triglyceride levels ($P = 0.7589$) and body mass indices ($P = 0.2803$) were not significantly associated with FAZ area. The number of severe hypoglycemic attacks was 0-2, and the statistics were analyzed including 0.

In generalized linear model analysis for FAZ area, annual HbA1c ($P = 0.0190$), number of severe hypoglycemic attacks ($P = 0.0210$), and onset age ($P = 0.0447$) were variables significantly associated with FAZ area (Table 2). Age ($P = 0.3732$), gender ($P = 0.4875$), duration of disease ($P = 0.9351$), total cholesterol ($P = 0.4473$), high ($P = 0.3095$) or low-density lipoprotein ($P = 0.9714$), triglycerides ($P = 0.9842$), and body mass index ($P = 0.1704$) were not significantly associated with FAZ area.

In generalized linear model analysis for parafoveal vessel density, age ($P = 0.0619$), gender ($P = 0.7381$), duration of disease ($P = 0.0619$), onset age ($P = 0.8407$), annual HbA1c ($P = 0.5787$), severe hypoglycemic attack ($P = 0.0614$), total cholesterol ($P = 0.7738$), high ($P = 0.3767$) or low-density lipoprotein ($P = 0.2447$), triglycerides ($P = 0.2229$), and body mass index ($P = 0.3214$) were not significantly associated with parafoveal vessel density.

Representative cases

Two representative cases with different onset ages are presented. Case 1 was a 15 year-old boy with onset of type 1 diabetes at 10 years of age and disease duration of 5 years. Severe hypoglycemic attacks occurred once at the age of 12. Laboratory findings at the time of examination were: annual HbA1c; 8.0%, total cholesterol; 150 mg/dL, high-density lipoprotein; 61 mg/dL, low-density lipoprotein; 75 mg/dL,

Table 1 Age of examination, equivalent sphere, and foveal avascular zone (FAZ) areas in patients with Juvenile-onset type 1 diabetes and aged-matched healthy controls

Parameter	Type 1 Diabetes (n=29)	Control (n=24)	*P*-value
Age (years)	16.1 ± 8.7	13.8 ± 7.0	0.1620[†]
Equivalent sphere (diopters)	−1.6 ± 2.3	−1.2 ± 2.8	0.5747[†]
FAZ area (mm^2)	0.29 ± 0.09	0.25 ± 0.08	0.0234[†]
Parafoveal vessel density (%)	50.43 ± 4.24	50.07 ± 4.64	0.8842[†]

[†]Mann-Whitney test

Table 2 Analysis of factors affecting the foveal avascular zone (FAZ) area of patients with type 1 diabetes

Parameter	Univariate analysis		Generalized linear model analysis *P*-value
	r	*P*-value	
Gender (female=1, male=2)	0.5878	0.073	0.4875
Onset age (years)	0.0474	0.261	0.0447
Age at the time of examination (years)	0.3848	0.121	0.3732
Duration of disease (years)	0.9174	0.014	0.9351
Annual HbA1c level (%)	0.0266	0.302	0.0190
Severe hypoglycemic attack (times)	0.0133	0.323	0.0210
Total cholesterol level (mg/dL)	0.4935	0.092	0.4473
HDL level (mg/dL)	0.2414	0.158	0.3095
LDL level (mg/dL)	0.9176	0.014	0.9714
Triglyceride level (mg/dL)	0.7589	0.041	0.9842
Body mass index (kg/m^2)	0.2803	0.144	0.1704

HDL = high-density lipoprotein; LDL = low-density lipoprotein

body mass index; 21.2 kg/m^2, and triglycerides; 32 mg/dL. The FAZ area was 0.35 mm^2 OD and 0.36 mm^2 OS (Fig. 1). The parafoveal vessel density was 52.15 OD and 55.18 OS.

Case 2 was a 15 year-old boy with onset of type 1 diabetes at one year of age and disease duration of 14 years. There was no history of severe hypoglycemic attack. Laboratory

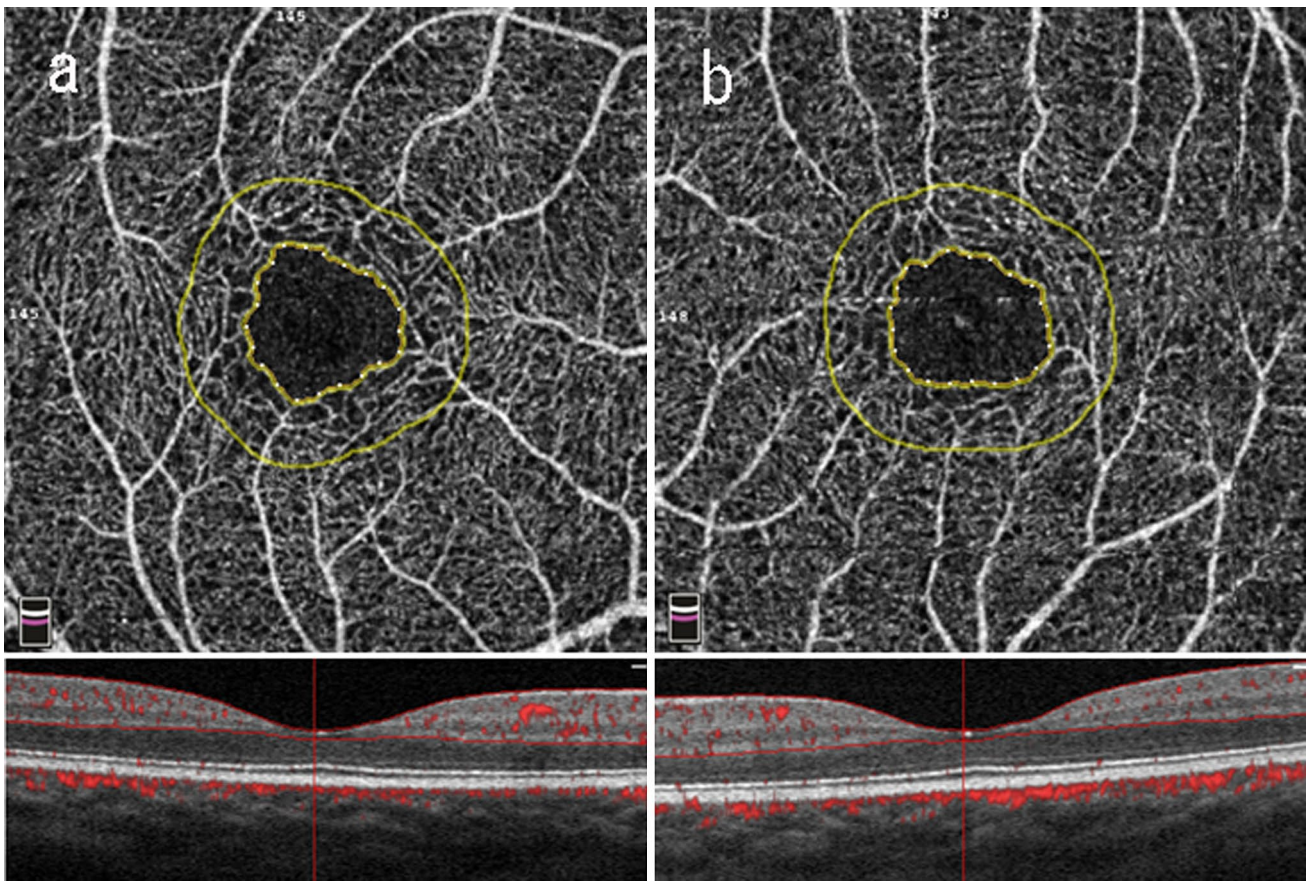

Fig. 1 Case 1: A 15 year-old boy with type 1 diabetes, onset at 10 years of age, disease duration of 5 years, annual HbA1c 8.0%, and severe hypoglycemic attacks at age 12. **a.** Right eye; foveal avascular zone area 0.35 mm^2 and parafoveal vessel density 52.15. **b.** Left eye; foveal avascular zone area 0.36 mm^2 and parafoveal vessel density 55.88

findings at the time of examination were: annual HbA1c; 7.4%, total cholesterol; 145 mg/dL, high-density lipoprotein; 60 mg/dL, low-density lipoprotein; 73 mg/dL, body mass index; 20.9kg/m^2, and triglycerides; 56 mg/dL. The FAZ area was 0.24 mm^2 in the right eye and 0.30 mm^2 in the left eye (Fig. 2). The parafoveal vessel density was 54.93 OD eye and 56.19 OS.

Discussion

This study found that patients with juvenile-onset T1DM and no diabetic retinopathy had larger FAZ, but it was clear that the parafoveal vessel density was not different compared to healthy controls. The finding that higher annual HbA1c, more episodes of severe hypoglycemic attacks, and older onset age may affect the progression to diabetic retinopathy is expected to help in the management of early-onset T1DM. These results clearly demonstrate the benefit of OCTA since it provides high-quality images in detecting very early changes in T1DM before the onset of diabetic retinopathy.

This study shows that patients with juvenile-onset T1DM and no diabetic retinopathy had larger FAZ compared to healthy controls. A study in children with T1DM reports significant differences in superficial and deep plexus vascular density and FAZ area compared to healthy subjects [13]. In a study of diabetic patients aged 42-83 years without diabetic retinopathy, Takase et al. [17] report that FAZ areas in the superficial and deep plexus layers were significantly larger in eyes of diabetic patients than in healthy eyes. These results suggest that diabetic eyes show retinal microcirculation impairment in the macula even before retinopathy develops. In diabetes, retinal leukostasis and increased leukocyte-endothelial cell adhesion due to upregulation of ICAM-1 are early events associated with capillary occlusion and the development of diabetic retinopathy [18–20]. Expression of ICAM-1 is enhanced during the early stages of diabetes and the obstructed capillary vessels are temporally associated with leukocyte aggregation [18–20]. Injection of an anti-VCAM-1–neutralizing antibody reduces vascular endothelial growth factor-induced leukocyte plugging [21]. How

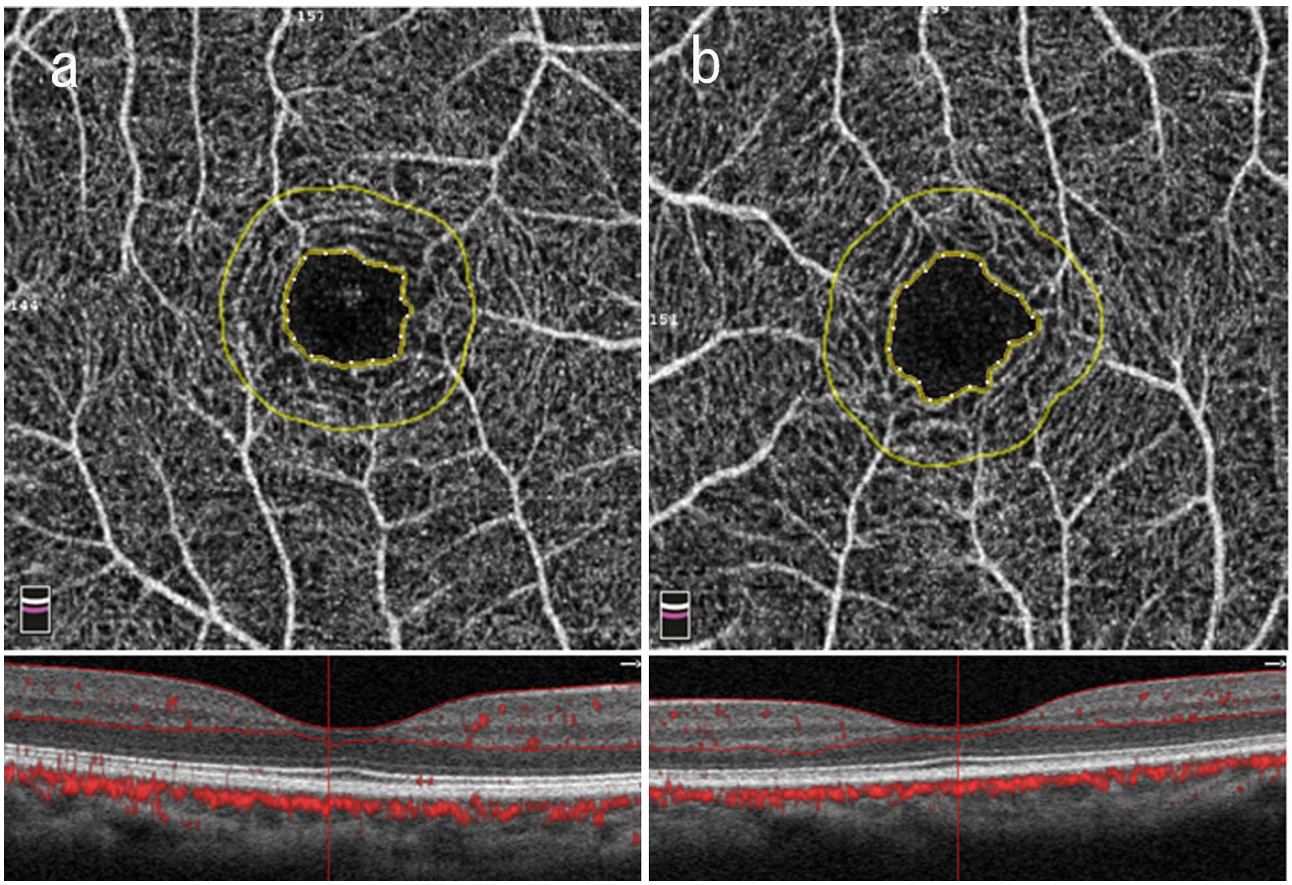

Fig. 2 Case 2: A 15 year-old boy with type 1 diabetes, onset at one year of age, disease duration of 14 years, annual HbA1c 7.4%, and no history of severe hypoglycemic attack. **a.** Right eye; foveal avascular zone area 0.24 mm^2 and parafoveal vessel density 54.93. **b.** Left eye; the foveal avascular zone area 0.30 mm^2 and parafoveal vessel density 56.19

these mechanisms relate to FAZ enlargement in eyes without diabetic retinopathy requires further study.

In this study, parafoveal vascular area did not increase significantly in T1DM patients compared to healthy controls. Gołębiewska et al. [11] used OCTA to compare parafoveal vessel density between children with T1DM and healthy controls, and report that the parafoveal deep vessel density in T1DM was significantly reduced when the serum creatinine levels, onset age of the disease, and diabetes duration increased. Using OCTA, Inanc et al. [13] observed that the parafoveal vessel density was smaller in children with T1DM than in healthy controls. In these studies, parafoveal vessel density was measured in various deep and superficial retinal layers, and significant findings were usually found only in some layers. Using an upgraded OCT in our study, it is not possible to obtain data of individual retinal layers, and only an overall parafoveal vessel density is provided. This may account for the discrepancy between our and previous findings. Further larger scale study using standardized methods to measure parafoveal vessel density is needed.

In our study, larger FAZ area was associated with older age of onset. Kang et al. [2] report that age 5–14 years at T1DM onset was a risk factor for non-proliferative diabetic retinopathy, and that a patient with T1DM onset at 5–14 years old may carry a risk comparable with four additional years of T1DM duration in a patients with onset age at <5 years or >14 years [2]. It could thus be hypothesized that patients with a higher age at onset of T1DM can become susceptible to microvascular complications at a later age causing altered set of cellular and immune responses to hyperglycemia induced cellular injury [22].

The FAZ area was associated with higher severe hypoglycemic attacks. Type 1 diabetes patients with more severe hypoglycemic attacks had higher prevalence of cardiovascular disease, diabetic nephropathy, and diabetic retinopathy [23]. The frequency of severe hypoglycemia is reported to be associated with longer diabetes duration, lower HbA1c, chronic renal disease, and QT interval prolongation on electrocardiogram [22]. The possible mechanisms underlying these hypoglycemia-induced effects include hemorrheological changes, white cell activation, vasoconstriction, and release of inflammatory mediators and cytokines [23]. Acute hypoglycemia may provoke upregulation and release of vasoactive substances in adults with and without T1DM [24]. This may be a putative mechanism for hypoglycemia-induced vascular injury. In T1DM, both CD40 expression and plasma soluble CD40 ligand concentrations' increase during hypoglycemia. Platelet-monocyte aggregation also increases significantly at 24 h after hypoglycemia. Future research is needed to examine the relationship between severe hypoglycemic attacks and diabetic retinopathy.

In this study the FAZ area was associated with higher annual HbA1c. Hyperglycemia remains the major instigator of the development of diabetic complications, including retinopathy. Due to sustained hyperglycemia, cellular metabolism is altered and the macromolecules undergo stable modifications. These acute and cumulative alterations in cellular metabolism and macromolecules result in structural and functional changes in the tissue [25]. Kang et al. [2] report that HbA1c level was a predictor of non-proliferative diabetic retinopathy in juvenile-onset T1DM, and that 1% increase of HbA1c may carry a similar risk to a 1-year increase in duration of juvenile-onset T1DM. Wang et al. [3] report that development of retinopathy 10 years after diagnosis of juvenile-onset T1DM was associated with older onset age, higher HbA1c, and higher triglyceride concentration. In childhood-onset T1DM, Skrivarhaug et al. [4] show that retinopathy at baseline, HbA1c, and triglycerides were significant predictors for developing proliferative diabetic retinopathy.

The duration of diabetes is known to be a direct risk factor [2]. The advantage of the present study on juvenile-onset T1DM is that the data of duration of diabetes was available from all the patients examined. Diabetic retinopathy was not detected in this study although retinopathy in patients with T1DM starts at an early age and continues throughout the disease duration. The present results illustrate the importance of appropriate diabetes control following early detection.

The limitations of the present study include a small number of patients and the retrospective study design.

In summary, OCTA was performed on patients with juvenile-onset T1DM and no diabetic retinopathy to measure the FAZ area. The FAZ area was larger in these patients than in healthy controls. In generalized linear model analysis, annual HbA1c, severe hypoglycemic attacks, and onset age were variables significantly associated with FAZ area. Our data suggest that diabetic eyes exhibit impairment of retinal microcirculation in the macula even before retinopathy develops, and that en face OCTA is a useful noninvasive screening tool for the detection of early microcirculatory disturbance in patients with juvenile-onset T1DM before the development of diabetic retinopathy.

Author contributions Conception and design (HO, TU, YK); analysis and interpretation (HO, YK, HS); writing the manuscript (HO, HS, YK); critical revision of the article (HO, YK, HS, AS, MA, UT); final approval of the article (HO, YK, HS, AS, MA, UT); data collection (HO, KY, MA); provision of materials (MA, UT); statistics (HS); literature search (HS, UT), administrative, technical or logistic support (HS, UT).

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論文の内容の要旨

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論文題名：Foveal avascular zone area analysis in juvenile-onset type 1 diabetes using optical coherence tomography angiography

（小児発症の 1 型糖尿病における OCT-Angiography を用いた中心窩無血管域の解析）

緒言：診断時 16 歳未満の小児発症 1 型糖尿病では、16 年の観察において 27%が糖尿病網膜症を発症し、1.5%が増殖糖尿病網膜症に至るとされている[1)]。小児発症 1 型糖尿病において、非増殖糖尿病網膜症は増殖糖尿病網膜症危険因子であり、糖尿病網膜症の発症前に潜在的な糖尿病網膜症を検出することは重要である。光干渉断層血管撮影（OCTA）は造影剤を用いず短時間で網膜血流を描出できる非侵襲的画像検査であり、この画像検査は特に小児糖尿病のスクリーニングやフォローアップに有用となると考えられる。OCTA では、網膜の黄斑部の中心窩に存在する無血管の網膜領域である中心窩無血管域（FAZ）、及びその周囲の血管密度を測定することができる。糖尿病網膜症のない成人の糖尿病患者の研究においては、表層と深層の FAZ は、健常対照より拡大していることは過去に報告があり[2)]、糖尿病網膜症発症前から網膜血流障害が生じていることが示唆されている。これらは初期の糖尿病網膜症を定義する高感度の画像バイオマーカーになる可能性がある。

目的および方法：本研究では、後ろ向きに糖尿病網膜症のない小児期発症 1 型糖尿病（T1DM）の FAZ と傍中心窩血管密度を測定して健常者と比較し、更に FAZ に影響を与える要因を検討した。対象は 2016 年 6 月から 2018 年 11 月の期間に日本大学病院眼科を受診した、16 歳未満で 1 型糖尿病を発症し、糖尿病網膜症を発症しておらず、OCTA を施行することができる症例で、内眼部手術の既往がある症例を除外した。健常患者は年齢と等価球面度数をマッチさせ、同意を取得できた患者とした。単変量分析では、Spearman 相関係数で検討し、単変量分析で解析した１１の変数について、一般化線形モデルで検討した。本研究は後ろ向き研究であり、2019 年 2 月 14 日に日本大学病院倫理委員会の承認を得た。（承認番号：20190201）

結果：T1DM は 29 例 58 眼で、健常対照は 24 例 48 眼であった。T1DM の FAZ は 0.29±0.09mm^2、傍中心窩血管密度は 50.43±4.24%、等価球面度数は-1.6±2.3D であり、右眼と左眼の FAZ（P = 0.6939）、傍中心窩血管密度(P = 0.8033)、等価球面度数(P = 0.7473)に有意差は認めなかった。年齢は 16.1±8.7 歳、発症年齢は 6.4±3.5 歳、病期は 9.7±8.3 年であった。29 人の患者のうち 11 人は、フォローアップ中は意識障害またはけいれんを伴う重度の低血糖発作を認めた。重度の低血糖発作の回数は 0.5±0.6 回、血糖値は 30-50 mg / dL であった。1 年平均 HbA1c は 8.1±0.9%、総コレステロール値は 176.1±25.4mg / dL、HDL 値は 59.9±14.9mg / dL、LDL 値は 96.5±26.3mg / dL、中性脂肪値は 125.6±15.9mg / dl、BMI は 20.4±3.3kg / m2 であった。健常対照は、FAZ は 0.25±0.08mm2、傍中心窩血管密度は 50.07±4.64%、等価球面度数は-1.2±2.8D であり、平均年齢が 13.8±7.0 歳のであった。右眼と左眼の FAZ（P = 0.8609）、傍中心窩血管密度（P = 0.3645）%、等価球面度数（P = 0.1911）に有意差は認めなかった。小児発症 1 型糖尿病患者と対照の間に年齢、平均等価球面度数（P = 0.5747）の有意差はなかった。小児発症 1 型糖尿病患者の眼の FAZ は、対照眼よりも有意に大きかった（P = 0.0234）。傍中心窩血管面積に有意差はなかった（P = 0.8842）。単変量解析では、FAZ が大きいほど、1 年平均 HbA1c が高値であり（P = 0.0266）、重度の低血糖発作の回数が多く（P = 0.0133）、発症年齢

が高かった（P = 0.0474)。また、これらの項目は一般化線形解析でもそれぞれ1年平均HbA1c（P = 0.0190)、重度の低血糖発作（P = 0.0210)、発症年齢（P＝0.0447）の項目が関連した。その他項目は有意な関連を認めなかった。

考按： FAZが拡大する機序としては、糖尿病においてはその初期からICAM-1の発現が増強され、網膜白血球停滞と白血球内皮細胞接着の増加の結果、毛細血管の閉塞が起こるとされており、この機序によりFAZの拡大が起こった可能性がある[3)－5)]。高血糖は、網膜症を含む糖尿病合併症の発症の主な原因であり、高血糖が持続するため、細胞の代謝が変化し、高分子は安定した修飾を受ける。これらの累積的な変化は、組織の構造的および機能的変化をもたらすとされており[6)]、高血糖の持続により、網膜毛細血管レベルの障害も生じた可能性があると考えられた。1型糖尿病では、マクロファージや樹状細胞によるサイトカイン放出を誘導するCD40発現と血漿可溶性CD40リガンド濃度の両方が低血糖時に増加し、血小板単球凝集が、低血糖後24時間で著しく増加するとされている[7)]。低血糖発作は、1型糖尿病のある成人とない成人において血管作動性物質のアップレギュレーションと放出を引き起こす可能性があり、これにより低血糖症による血管損傷を起こす原因となる可能性がある。

結語：OCTAは非侵襲的に糖尿病網膜症発症前の小児発症1型糖尿病患者のFAZの拡大という潜在的網膜変化を検出でき、スクリーニング器機として有用であった。1年平均HbA1cの上昇、低血糖発作の増加、発症年齢の高齢化がFAZの拡大と相関していた。

文献

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補足文章

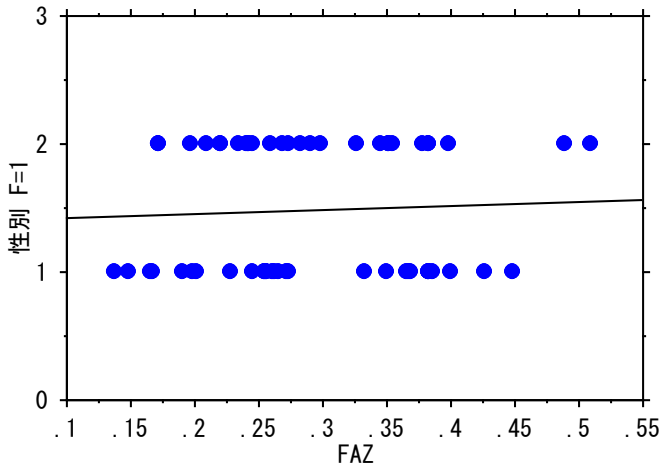
【補足目的】

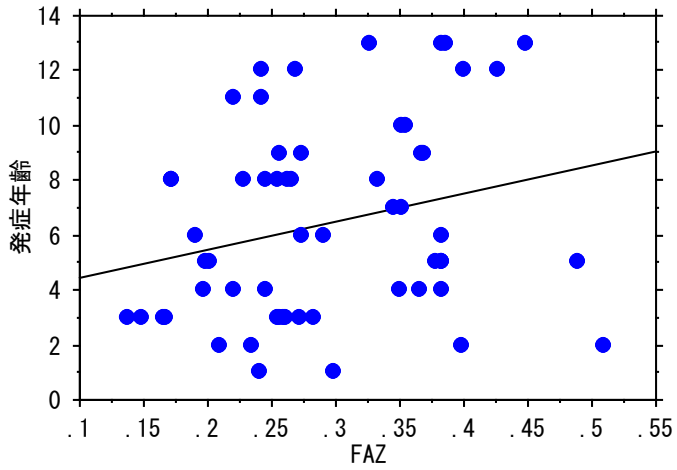
主論文「Foveal avascular zone area analysis in juvenile-onset type 1 diabetes using optical coherence tomography angiography」(Japanese Journal of Ophthalmology, 64 巻, P.271-277)を 2020 年 3 月に発表した。本論文での学位申請をするにあたり、予備審査会でご指摘いただいた点につき、補足を行った。

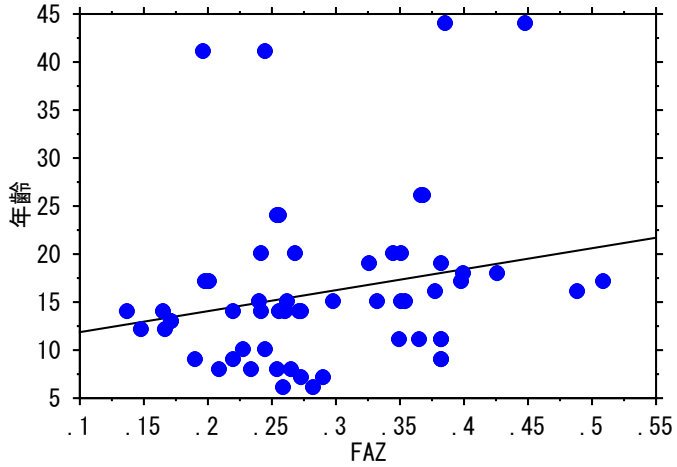
【主論文の補足】

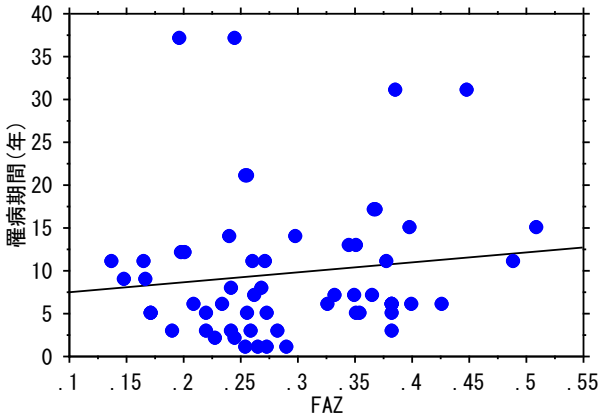
本論文では、糖尿病網膜症のない小児期発症 1 型糖尿病（T1DM）と健常対照での FAZ 面積の比較を行った。平均年齢は T1DM は 16.1 歳、健常対照は 13.8 歳であったが、年齢に有意差はなく、小児期において FAZ は拡大しないという報告がある [1) 2)]。また、BMI は FAZ は関連していないという報告もあり [2)]、年齢、BMI による FAZ への影響は考慮しなくてよいと考えた。

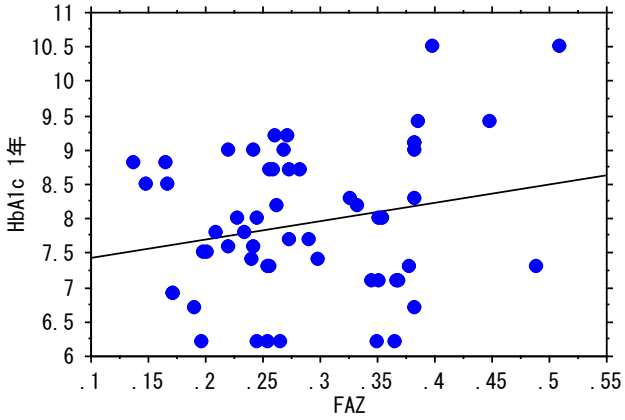
主論文 Table2 について、それぞれの項目の作図を行った。

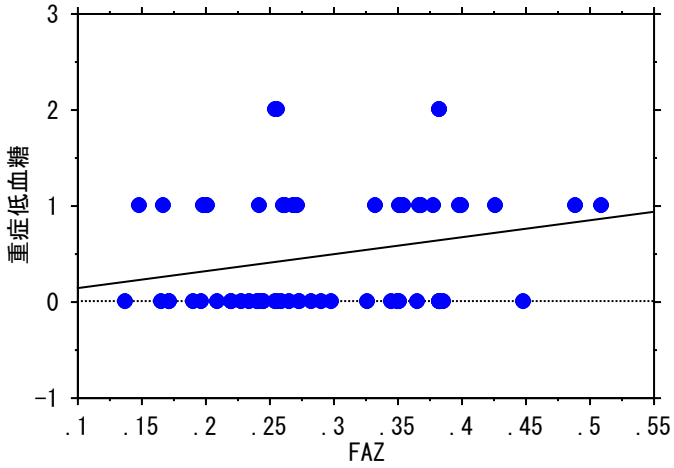


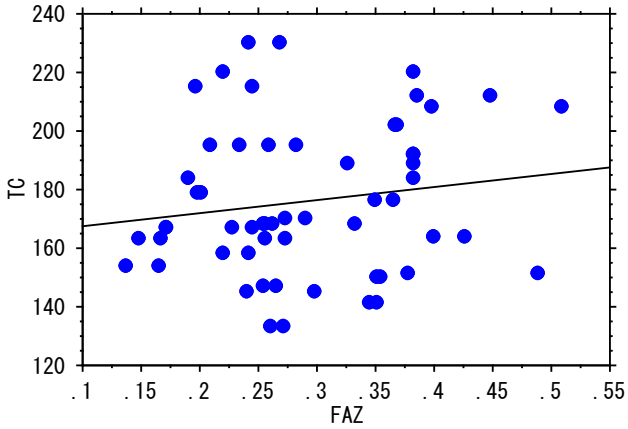

発症年齢
FAZ
0
2
4
6
8
10
12
14
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55

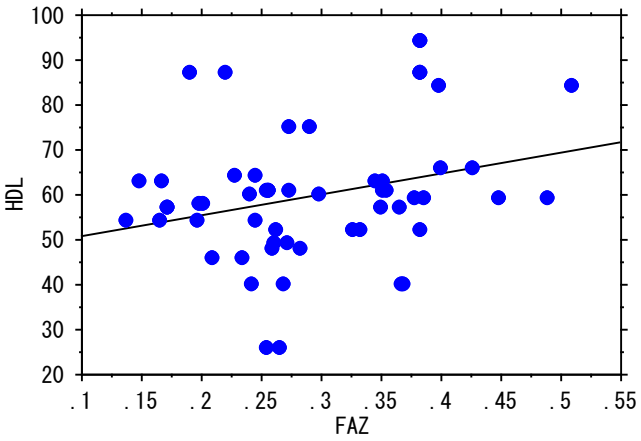

年齢
FAZ
5
10
15
20
25
30
35
40
45
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55

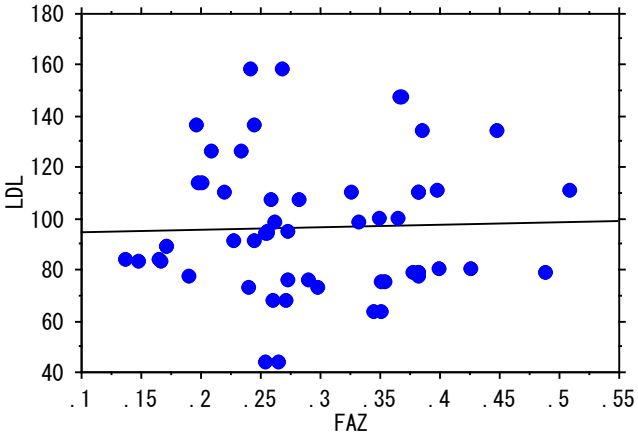

罹病期間(年)
FAZ
0
5
10
15
20
25
30
35
40
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55

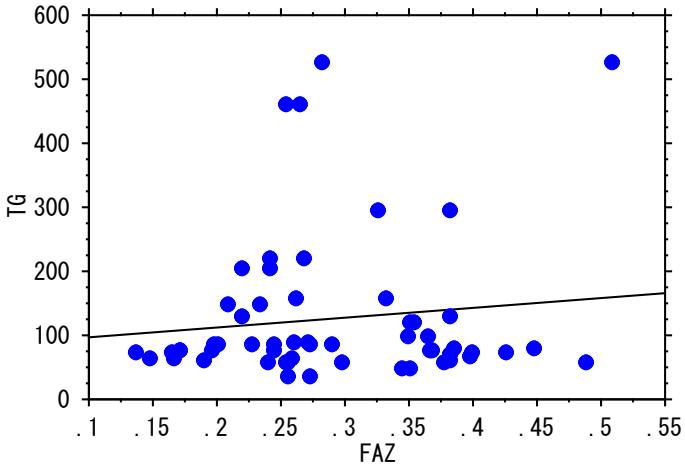

HbA1c 1年
FAZ
11
10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55

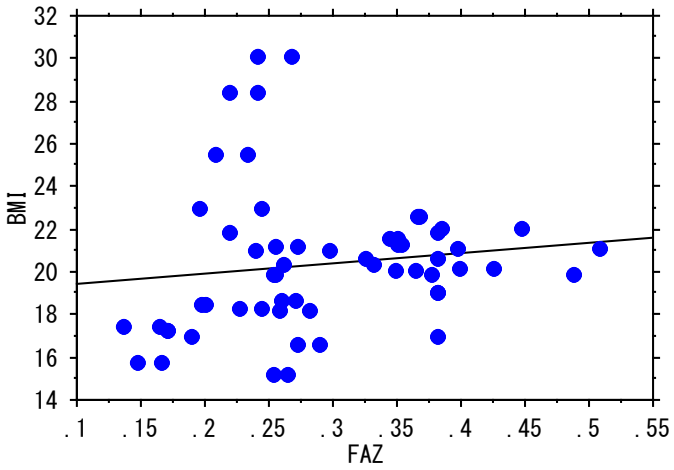

重症低血糖
FAZ
3
2
1
0
−1
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55


TC
FAZ
240
220
200
180
160
140
120
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55

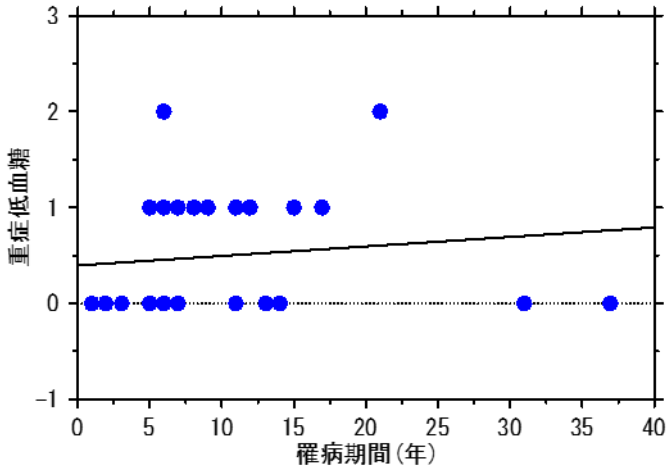

HDL
100
90
80
70
60
50
40
30
20
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55
FAZ


LDL
180
160
140
120
100
80
60
40
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55
FAZ


TG
600
500
400
300
200
100
0
.1
.15
.2
.25
.3
.35
.4
.45
.5
.55
FAZ



本研究発表時より、これまで（2021 年 9 月 2 日現在）重症低血糖発作と FAZ の関連を指摘した論文は調べられた範囲では本論文しかない。今回、重症低血糖発作と罹病期間とは相関しなかったが、本研究における重症低血糖発作の回数は発症からの累積であり、現在の 1 症例当たりの 0.1 回/年という平均回数よりは多くなっていることについては、罹病期間が長い症例も含まれるので頻度としては高いものになっていると考えられる。



P=0.3288　r=0.131

本論文で、発症年齢が高かったほど FAZ 面積が大きくなったことについては、糖尿病の発症年齢が 40 歳以下ではより年齢が高いことが、発症年齢が 40 歳以上では HbA1c が高値であることが、糖尿病網膜症発症のリスクファクターであるという報告がある [3)]。また、

2 型糖尿病による末期腎臓病は糖尿病発症年齢が高くなると、年齢が上がることに伴う因子が増加する可能性があり、年齢が上がることによる原因がそれのみであるのかを同定することが困難である可能性があるという報告もある[4)]。本研究においても、年齢が上がったことにより、その他の交絡因子が関わってしまった可能性は否定できない可能性がある。発症年齢とその他血液検査データとの相関係数については、1 年平均 HbA1c 0.9418、総コレステロール値 0.4040、HDL コレステロール値 0.1095、LDL コレステロール値 0.1338、中性脂肪値 0.3160 であり、発症年齢と 1 年平均 HbA1c は強い相関を認めていた。

本論文では FAZ 面積の拡大と 1 型糖尿病罹病期間との相関を証明できなかった。FAZ 拡大と罹病期間の相関は、単回帰分析では P=0.014 で有意差はみられているため影響する要因と考えられる。一般線形化モデルでは、有意差は認められなかったが、発症年齢、重症低血糖発作、年間 HbA1c といった要因がより強く影響したために相関を証明できなかったと推測した。

【主論文の今後への展望】

本論文からは、小児発症 1 型糖尿病患者においても糖尿病網膜症発症前から既に潜在的に網膜血管に変化を来していることが示唆された。OCTA は非侵襲的に短時間で施行できるため、小児患者にも使用することができる。また、FAZ を長期間定期的に確認することにより、糖尿病網膜症発症前であっても網膜血管障害が生じているため、眼科から小児科、内科に治療の厳格化を求めることができ、結果として、患者の視機能低下につながる糖尿病網膜症発症を遅らせることができる可能性がある。

【文献】

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主論文の原文

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